76° Convegno SISVET

Bari, 21-23 giugno 2023

AISMEVEM AIPVET AIVI AMV ANIV

ARNA RNIV SICLIMVET SICV SIFTVET

SIRA SOFIVET SOIPA

IL CODICE ISBN ASSOCIATO AGLI ATTI DEL 75° CONVEGNO SISVET È

978-88-909092-5-2

Si ringrazia la collaborazione non condizionante di:

biosurgex

ACV TRIGGIANO
Analisi Cliniche Veterinarie

Dechra
Veterinary Products

esaote

Elanco

Foschi

medvet
prodotti medicali professionali

innovet
Veterinary Innovation
member of SWEDENCARE

MIGLIORI service

MYLAV INDUSTRY

MSD
Animal Health

SIVOMIXX
slab51®
10 DROP 200 800
In forma, naturalmente

vetoquinol
ACHIEVE MORE TOGETHER

niMED
LIFE TO TECH, TECH TO LIFE

zoetis

MEGASTORE ZOO QUARK

***Comitato Scientifico* della FEDERAZIONE SISVET**

PRESIDENTE SISVET: *Adriana Ianieri*

VICE PRESIDENTE: *Paolo De Girolamo*

SEGRETARIO GENERALE: *Laura Rinaldi*

RAPPRESENTANTI DELLE SOCIETA' SCIENTIFICHE

AIPVET: *Francesca Tiziana Cannizzo*

AISMeVeM: *Annamaria Grandis*

AIVI: *Pierluigi Di Ciccio*

AMV: *Alessia Di Giancamillo*

ARNA: *Andrea Formigoni*

ANIV: *Maurizio Mazzei*

RNIV: *Katia Cappelli*

SICLIM-VET: *Micaela Sgorbini*

SICV: *Francesco Staffieri*

SIFTVet: *Marco De Liguoro*

SIRA: *Maria Cristina Veronesi*

SOFIVet: *Fiammetta Berlinguer*

SOIPA: *Giuseppe Cringoli*

Coordinatore Conferenza dei direttori: *Domenico Bergero*

Direzione generale della sanità animale e dei farmaci veterinari: *Pier Davide Lecchini*

Direzione generale per l'igiene e la sicurezza degli alimenti e la nutrizione: *Massimo Casciello*

FNOVI: *Gaetano Penocchio*

IZZSS: *Antonio Limone*

Comitato Organizzatore Locale

***Antonio Crovace**, Past President SISVET*

Società Federazione SISVET

AIPVET: Associazione Italiana di Patologia Veterinaria
AISMEVEM: Associazione Italiana di Storia della Medicina Veterinaria e della Mascalciac
AIVI: Associazione Italiana dei Veterinari Igienisti
AMV: Associazione Italiana dei Morfologi Veterinari
ANIV: Associazione Nazionale Infettivologi Veterinari
ARNA: Associazione Ricercatori Nutrizione Alimenti
RNIV: Rete Nazionale di Immunologia Veterinaria
SICLIMVET: Società di Clinica Medica Veterinaria
SICV: Società Italiana di Chirurgia veterinaria
SIFTVET: Società Italiana Farmacologia e Tossicologia Veterinaria
SIRA: Società Italiana Riproduzione Animale
SOFIVET: Società di Fisiologia Veterinaria
SOIPA: Società Italiana di Parassitologia

ABSTRACT

Workshop e Main Lecture

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

Workshop 1 - ECM
Mercoledì, 21 giugno 2023
Chronic pain in animals: clinical and comparative approach

Moderatori:
Prof.ssa Noemi Romagnoli [Università degli Studi di Bologna]
Dott. Domenico Ventrella [Università degli Studi di Bologna]

11.00	Comparative approach to chronic pain **Lynne Sneddon** *University of Gothenburg*
11.40	Recognition and clinical signs of chronic pain in small animals **Federico Corletto** *University of Nottingham*
12.10	Osteoarthritis as a clinical model of chronic pain: new guidelines for staging and treatment **Francesco Staffieri** *Università degli Studi di Bari*
12.30	New pharmacologic strategies for treatment of pain in animals **Giorgia della Rocca** *Università degli Studi di Perugia*
13.00	Interventional analgesia for treatment of neuropathic pain **Filomena Puntillo** *Università degli Studi di Bari*
13.30	**Chiusura dei lavori**

Workshop 2 - ECM
Mercoledì, 21 giugno 2023
Opportunities offered by natural substances in the veterinary field

Moderatori:
Prof.ssa Anna Zaghini [Presidente SIFTVET]
Prof. Pierluigi Aldo Di Ciccio [Università degli Studi di Torino]
Prof. Luciano Pinotti [Università degli Studi di Milano]

14.45	Using natural extracts for sustainable control of parasites **Vincenzo Musella** *Università degli Studi di Catanzaro*
15.10	Is there a potential of polyphenols for gut health and pig production? **Giuseppe Bee** *Agroscope, Berna*
15.35	Antibacterial activity of natural substances: focus on their synergistic actions **Filippo Fratini** *Università degli Studi di Pisa*
16.00	Natural substances in the food industry: perspectives and limits **Filippo Giarratana** *Università degli Studi di Messina*
16.25	Preparations of botanical origin when used as feed additives: the approach developed by EFSA for the risk assessment of complex mixtures **Paola Manini** *Autorità Europea per la Sicurezza Alimentare*
16.50	Discussione
17.00	Chiusura dei lavori

Workshop 3 - ECM
Giovedì, 22 giugno 2023
Responsible reproduction in small animals: tomorrow is now

Moderatori:
Prof. Stefano Romagnoli [Università degli Studi di Padova]
Prof.ssa Maria Cristina Veronesi [Università degli Studi di Milano]

08.45	The moral status of the animal as a sentient being in the bioethical debate **Prof.ssa Luisella Battaglia** *Università degli Studi di Genova,* *Comitato Nazionale per la Bioetica, Presidenza del Consiglio dei Ministri*
09.10	Breeding for aesthetics: a growing challenge for canine welfare **Giulia Rubini** *AUSL Bologna*
09.35	Moulding man's best friend: how has selective breeding impacted canine behaviour and welfare? **Rowena Packer** *RVC*
10.00	Breeding and breeding soundness: a combination that must adapt and evolve **Magdalena Schrank** *Università degli Studi di Padova*
10.25	Brachycephalic airway respiratory syndrome after more than 40 years from the first description: the modern surgical philosophy in the management of possible corrections **Stefano Romussi** *Università degli Studi di Milano*
10.50	**Discussione**
11.00	**Chiusura dei lavori**

TAVOLA ROTONDA
Mercoledì, 21 giugno 2023
Innovazione della didattica nelle Scienze Veterinarie

Moderatori:
Prof. Domenico Bergero
Prof.ssa Monica Forni
Prof. Giuseppe Crescenzo

17.30	La valutazione del ricercatore tra ricerca e didattica **Menico Rizzi** *ANVUR*
17.50	Ruolo del Faculty development nella qualificazione didattica dei docenti universitari **Antonella Lotti** *Università degli Studi di Foggia*
18.00	I Clinical Skill lab nel curriculum di medicina veterinaria, primi tentativi di integrazione **Tiziana Cannizzo** *Università degli Studi di Torino*
18.10	**Ruolo dell'AI nell'apprendimento delle hard e delle soft-skill** **David Lembo** *Università degli Studi di Torino*
18.25	Ridefinizione del Logbook delle competenze in medicina veterinaria **Andrea Barbarossa** *Università degli Studi di Bologna*
18.35	Il Tirocinio Pratico Valutativo: l'esperienza dei corsi di medicina **Bruno Moncharmont** *LUM- Libera Università del Mediterraneo*
18.45	Il Tirocinio Pratico Valutativo in medicina veterinaria **Domenico Bergero** *Università degli Studi di Torino*
18.55	**Discussione**
19.00	**Chiusura dei lavori**

SIMPOSIO FEDERALE
Venerdì, 23 giugno 2023
Innovative and precision technologies in veterinary medicine

Moderatori:
Prof.ssa Alessia Di Giancamillo
Prof. Giuseppe Cringoli
Prof.ssa Katia Cappelli

10.30	Saluti dalle Autorità **On. Marcello Gemmato** *[Viceministro alla Salute]*
11.00	Imaging based in situ tissue profiling: combination of highplex immunofluorescence and spatially resolved trascriptomic **Francesca Ravanetti** *[AMV - Università di Parma]*
11.20	Exploring different alternatives to the cryopreservation of cells and gametes **Pasqualino Loi** *[SOFIVET -Università degli Studi di Teramo]*
11.40	Artificial intelligence and digital pathology: application in veterinary pathology **Massimo Salvi** *[AIPVET - Politecnico di Torino]*
12.00	Applications of computer vision systems for meat safety assurance in abattoirs **Marianne Sandberg** *[AIVI -Denmark Technical University]*
12.20	Extracellular vesicles as innovative immunomodulatory agents **Elisabetta Razzuoli** ***[****RNIV IZSPLV]*
12.40	Precision technologies for the diagnosis and control of parasitic diseases **Maria Paola Maurelli** **Antonio Bosco** *[SOIPA – Università degli studi di Napoli]*
13.00	Pharmacogenetics in veterinary precision medicine: dream or reality? **Mery Giantin** *[SIFTVET - Università degli Studi di Padova]*
13.20	The technological revolution in pet medicine: artificial intelligence, precision medicine and the Internet of Things (IoT) **Paolo Ciaramella** **Francesco Porciello** *[SICLIMVET - Università degli Studi di Perugia]*
13.30	**Discussione**
13.40	**Chiusura dei lavori**

SIMPOSIO FEDERALE
Venerdì, 23 giugno 2023
Innovative and precision technologies in veterinary medicine

Moderano:
Prof. Francesco Staffieri
Prof.ssa Annamaria Grandis

14.40	New technologies in the medical and surgical field **Claudio Marchetti** *[SICV -Università degli Studi di Bologna]*
15.00	Extracellular vesicles from reproductive tissues: innovative technology for clinical applications or regenerative medicine in animal reproduction **Anna Lange Consiglio** *[SIRA - Università degli Studi di Milano]*
15.20	Milk infrared spectroscopy to assess health disorders in dairy cows **Alessio Cecchinato** *[ARNA - Università degli Studi di Padova]*
15.40	Proteomics as a tool for the development of companion diagnostics in Veterinary Public Health **Paola Roncada** *[ANIV - Università degli Studi di Catanzaro]*
16.00	From paper to digital: a historical excursus of the cataloguing of veterinary collections **Rosiana Schiuma** *[AISMEVEM - Università degli Studi di Bologna]*
16.20	**Discussione**
16.30	**Chiusura dei lavori**

AIPVET

21 giugno 2023 [Sala Levante (Giulia Centre)]
14.30-15.30

Moderatore:
Prof.ssa Valentina Zappulli

MAIN LECTURE:
Recent update in liver pathology
Dr. John M. Cullen

AIPVET

22 giugno 2023 [Sala Grecale (Giulia Centre)]
16.30-17.30

Moderatore:
Prof.ssa Paola Roccabianca

TEACHING COURSE
Tips and tricks in diagnostic liver pathology
Dr. John M. Cullen

AIVI

22 giugno 2023
16.40-17.10

[Sala Levante (Giulia Centre)]

Moderatori:
Prof.ssa Tiziana Civera
Prof. Sergio Ghidini

LECTIO MAGISTRALIS:
Application of Computer Vision System for meat safety assurance in chicken *abattoirs*: VetInspector
Dr.ssa M. Sandberg

AMV
21 giugno 2023
17.30-18.00

[Sala Scuderia]

MAIN LECTURE:
Il cervello dei grandi erbivori
Prof. B. Cozzi

AMV

22 giugno 2023
16.30-17.00

[Sala Libeccio (Giulia Centre)]

MAIN LECTURE:
Legislazione nazionale sull'utilizzo di animali ai fini scientifici ed educativi: Direttiva Europea 63/2010 vs Dlgs 26/2014
Prof. P. De Girolamo

RNIV

22 giugno 2023
16.30-17.15

[Sala Carducci (Villa Rachele)]

Moderatori:
Prof.ssa Katia Cappelli
Prof.ssa Elisabetta Razzuoli

MAIN LECTURE:
EVs between challenges and achievements: a flow cytometric approach
Prof.ssa Barbara Canonico

SIRA

21 giugno 2023
14.30-15.00

[Sala Grecale (Giulia Centre)]

Moderatori:
Prof. Giovanni Michele Lacalandra

MAIN LECTURE:
Assisted reproductive technologies in dromedary camel in Saudi Arabia
Dr. Taher Kamal Osman

SOFIVET

21 giugno 2023
14.30-14.50

[Sala Libeccio (Giulia Centre)]

Moderatori:
Prof. Domenico Ventrella
Prof.ssa Federica Pirrone

MAIN LECTURE:
Implications of pain in fishes
Prof.ssa Lynne Sneddon

SOFIVET

21 giugno 2023
15.10-15.30

[Sala Libeccio (Giulia Centre)]

Moderatori:
Prof. Domenico Ventrella
Prof.ssa Federica Pirrone

MAIN LECTURE:
Breeding for better canine health: Is outcrossing the future?
Prof.ssa Rowena Packer

SOIPA

22 giugno 2023
16.30-17.00

[Sala D'Annunzio (Villa Rachele)]

MAIN LECTURE:
Updating on epidemiology of trichinellosis: the Italian situation
Prof. Fabrizio Bruschi

MAIN LECTURE:

Prof. Fabrizio Bruschi
Leishmania tarentolae vs. *Leishmania infantum* in the Mediterranean basin: future challenges and opportunities
Prof. Domenico Otranto

COMPARATIVE APPROACH TO CHRONIC PAIN

Lynne U. Sneddon (1)

(1) University of Cothenburg, Sweden

Corresponding author: **Lynne U. Sneddon -** lynne.sneddon@bioenv.gu.se

Acute pain is short term and is considered an adaptive mechanism that promotes healing. In humans chronic pain is defined as pain that lasts more than 3–6 months. This arbitrary cut off point has no clearly delineated switch from acute to chronic pain at 3 or 6 months. Instead, pain is a complex phenomenon which varies over time. While the temporal component is important to determine chronic pain the underlying mechanisms are far more important. Adaptive versus maladaptive pain may be better terms to describe pain. This presentation will use the term chronic pain to refer to long-term pain as opposed to short term acute pain. Chronic pain affects humans in complex ways including physiological, sensory, affective states, cognitive ability, behavioral changes, and sociocultural and we can infer that animals may also be affected in a comparable manner. A variety of measures have been applied to animals to discern their pain state and information from veterinary studies as well as from scientific experiments can be used to investigate chronic pain and its treatment. Most research has been focused on chronic joint pain, other areas of chronic pain, such as neuropathic and cancer pain, neglected. Further much of what we know comes from mammals with other animals less well studied. This presentation will provide an overview of what we know from different animal groups on the existence and extent of chronic pain.

ASSESSMENT OF CHRONIC PAIN IN DOGS AND CATS: TOOLS FOR FOOLS?

Federico Corletto (1)

(1) University of Nottingham

Corresponding author: **Federico Corletto** - freddie73@me.com

Identification and quantification of chronically pain is one of the cornerstones for its management. While we have made significant progress in identifying chronic pain and the conditions most commonly associated to it, semi-objective quantification of chronic pain is still a major source of frustration for veterinarians and owners. Chronic pain often does not manifest itself with specific behaviours, but it is more often associated with fine behavioural changes, in many occasions noticed only by the owner. The role of osteoarthritis on quality of life in cats has been identified linking what many were considering as age related behavioural changes, to consistent radiological findings demonstrating existence of the disease. The extremely subjective and waxing and waning nature of chronic pain is hindering assessment and making treatment particularly challenging. Can we objectively measure something that is, by definition, subjective? Quality of life scoring systems, mostly completed by the owner, are now used in the attempt to see the "greatest picture", and not to focus on what could be simply considered regression to the mean, or simply a biased assessment of pain intensity by the owner, whose judgement may be affected by a new therapy. Objective measures of sensation, use of limbs (for osteoarthritis) are used to produce repeatable numbers, to assess efficacy of treatments, yet we cannot be categorically sure that we are measuring what we intend to assess. A more holistic approach is therefore indicated, where the veterinary surgeon, with the essential input of the owner, assess quality of life of the animal.

OSTEOARTHRITIS AS A CLINICAL MODEL OF CHRONIC PAIN: NEW GUIDELINES FOR STAGING AND TREATMENT IN DOGS

Francesco Staffieri (1)

(1) Università degli Studi di Bari "Aldo Moro", Dipartiemento di Medicina di Precisione e Rigenerativa e Area Jonica, Bari, Italia

Corresponding author: **Francesco Staffieri** - francesco.staffieri@uniba.it

Osteoarthritis is a chronic inflammatory and degenerative disease of the synovial joints and is the most common cause of chronic pain in dogs. Accurate diagnosis requires a complete clinical assessment of the animal including the characterization of the abnormal behavior and/or lifestyle. The Canine OstheoArthritis Staging Tool (COAST) has been recently developed by an international board of experts to give a criterion for staging of this multifactorial disease (1). COAST is based on 2 fundamentals approaches: 1) to combine the owner and veterinarian evaluation and 2) to assess non only the joint but also the effects of the disease on the whole patient. COAST aims to combine the changes in behavior and quality of life together with the alterations of the posture and gait as well as the specific articular modifications, including the radiographic assessment, in a score which stage the severity of OA in preclinical (0/1), mild (2), moderate (3) and severe (4). COAST provides a functional and anatomical assessment of OA (2). Management of OA is becoming more and more advanced thanks to the discovery of news pharmacological and not pharmacological options. The main goal is to reduce the clinical signs associated with joint inflammation and pain and in some instances to provide an adequate support to the joint cartilage. New anti-inflammatory and analgesic drugs are available our days together with several nutraceutical supports as well as physical and instrumental therapies (i.e.: laser, acupuncture). However, basic interventions on the lifestyle, environment modification, owner education, as well as adequate weight control are also very important in the management of the disease. The COASTeR is a specific tool derived from the COAST, with the exclusion of the radiographic assessment, specifically developed for the guidance of the management of the disease. COASTeR has a base and build approach, considering a proportional management of the disease based on the severity of the clinical signs. Moreover, it includes the monitoring of the patients based on which the management can be adapted to the specific needs of the case. In this way the OA management become tailored on the severity of the diseases in the individual case and can be adapted based on its evolution. The aims of the COAST and COASTeR is to provide a criterion to stage a complex disease and provide a more rational approach to its management from a clinical but also scientific point of view.

[1] Cachon, T., Frykman, O., Innes, J. F., Lascelles, B. D. X., Okumura, M., Sousa, P., Staffieri, F., Steagall, P. V., Van Ryssen, B., & COAST Development Group (2018). Face validity of a proposed tool for staging canine osteoarthritis: Canine OsteoArthritis Staging Tool (COAST). Veterinary journal (London, England: 1997), 235, 1–8. https://doi.org/10.1016/j.tvjl.2018.02.017

[2] Stabile, M., Van Ryssen, B., Minei, S., Coppieters, E., Crovace, A., Lacitignola, L., & Staffieri, F. (2022). Observational study of the clinical value of the canine osteoarthritis staging tool. Veterinary journal (London, England: 1997), 283-284, 105832. https://doi.org/10.1016/j.tvjl.2022.105832

NEW PHARMACOLOGIC STRATEGIES FOR THE TREATMENT OF PAIN IN ANIMALS

Giorgia Della Rocca (1)

(1) Department of Veterinary Medicine, University of Perugia, Italy

Corresponding author: **Giorgia Della Rocca -** giorgia.dellarocca@unipg.it

In the last two decades, pain management in animals has come a long way, with many strategies, both pharmacological and non-pharmacological, to be put in place for the treatment of acute and chronic painful conditions.
Besides NSAIDs, which remain one of the mainstream approaches for inflammatory pain, leading to a continuous release on the market of new molecules belonging to this class of analgesics (the last of them being enflicoxib, a COX-2 selective inhibitor characterized by a 7-days phasic pharmacokinetic), new drugs acting along the various steps of the pain pathway are nowadays possibly included within a multimodal approach.
One of the newest drugs that has obtained marketing authorization is the anti-nerve growth factor monoclonal antibody (mAb anti-NGF). Specifically, mAb anti-NGF appears to be particularly promising in managing ostroarthritic (OA) pain, due to its ability to reduce neurogenic inflammation as well as nerve endings sprouting induced, at list partly, by the NGF.
A couple of years before the release of the mAb anti-NGF, an oral formulation based on grapiprant (a drug acting on the EP4 receptor subtype for prostaglandins) has been marketed for the long-term treatment of OA pain. Being the EP4 receptor responsible for PGE2-mediated sensitization of sensory neurons, the specific EP4 antagonism by grapiprant is characterized by an analgesic efficacy comparable to NSAIDs.
Adjuvant analgesics such as paracetamol (a COX-3 selective inhibitor), NMDA-antagonists (ketamine, amantadine and memantine), voltage-gated calcium channels blockers (gabapentin and pregabalin), and serotonin/norepinephrine reuptake inhibitors, such as SSRIs, SNRIs and TCAs, find application in the multimodal management of chronic pain.
The antiemetic drug maropitant has also been evaluated for possible analgesic properties due to its antagonism toward the NK1 receptors, strongly implicated in the peripheral and central sensitization induced by Substance P.
The last few years have seen a growing interest in the use of compounds based on Cannabis sativa derivatives, due to their capability to modulate the nociceptive threshold at peripheral, spinal and supraspinal levels.
Recently, preclinical studies in animal models and clinical studies in humans have pointed the attention toward L-acetyl-carnitine and its analgesic properties, mainly due to its capability to provoke an over-expression of a specific glutamate receptor subtype (mGlu2) by an epigenetic mechanism.

INTERVENTIONAL ANALGESIA FOR TREATMENT OF NEUROPATHIC PAIN

Filomena Puntillo (1)

(1) Department of Interdisciplinary Medicine, University of Bari, Aldo Moro

In patients with refractory neuropathic pain, interventional neuromodulation can be considered, in the context of an interdisciplinary approach and according to the model of "pain treatment conutinuum". Neuromodulation is defined by the International Neuromodulation Society as "the alteration of nerve activity through targeted delivery of a stimulus, such as electrical stimulation or chemical agents to specific neurological sites in the body" and according to the applied stimulus, neuromodulation is called electrical or chemical. About the former, a lot of electrical interventional neuromodulation techniques are available; these include an array of invasive and non-invasive approaches that can be grouped by modality waveform, or anatomical target. I will focus of on the mechanism of action of Spinal Cord Stimulation (SCS), which has undergone exponential growth and development, dorsal root ganglion stimulation (DRG), and percutaneous peripheral nerve stimulation (PNS), highlighting their new indications and evidence from the literature. The same overview will be done for intrathecal analgesia which is the last option for refractory pain. The latest recommendation from the Polyanalgesic Consensus Conference (PACC) for safe and effective intrathecal infusion of opioids, anesthetics and ziconotide, alone or in combination, will be discussed.

USING NATURAL EXTRACTS FOR SUSTAINABLE CONTROL OF PARASITES

Vincenzo Musella (1)

(1) Department of Health Sciences, University "Magna Graecia" of Catanzaro, Italy

Corresponding author: **Vincenzo Musella** - musella@unicz.it

Leading humanitarian organisations and a large body of scientific literature report that parasites play a major role in human and animal health, welfare and the quality and quantity of animal production. Often underestimated, they have a major impact, with economic losses exceeding 30% of GDP. Given the absence of vaccines, the synthetic drug is the main tool for controlling these infections. In daily practice, the use/abuse of drugs is widespread without an accurate diagnosis, with the real risk of residues in milk, meat and derivatives, as well as the often-underestimated dispersion in the environment and the emergence of drug resistance phenomena. The growing prevalence of these phenomena is increasing the demand for alternative solutions, such as the use of phytotherapies or natural mixtures without environmental residues.

Prior to the creation of synthetic anthelmintics by pharmaceutical companies in the mid-20th century, humans relied on plants to control intestinal parasites in livestock, and in Europe, medieval herbals and printed books from the 17th-19th centuries are full of descriptions of plants fed to livestock to expel parasites.

In Italy, many small farmers and shepherds continue to use traditional plant mixtures to treat GI nematode infections in sheep or to control ectoparasites. The use of medicinal plants derived from traditional medicine, or veterinary ethnobotany, has been handed down from generation to generation for centuries, but has been partly lost to technological development in the chemical-pharmaceutical field.

Based on this experience, numerous studies have been published that in fact confirm the efficacy of certain plants traditionally known for their anti-parasitic properties. However, these methods still need to be studied and perfected, and there is a lack of adequate studies on standardisation techniques that guarantee the composition of the extracts while avoiding the natural alterations that could occur over time. Furthermore, it is necessary to develop systems for evaluating anti-parasitic efficacy other than those used for synthetic products, as many other factors must be included in the analyses to assess the cost-benefit of anti-parasitic treatment. There will never be similar efficacy values, but one solution today could be to alternate these methodologies to significantly reduce the use of systemic drugs and maximise the efficacy of natural extracts.

IS THERE A POTENTIAL OF POLYPHENOLS FOR GUT HEALTH AND PIG PRODUCTION?

Giuseppe Bee (1), Marco Tretola (1)

(1) Agroscope (Switzerland)

Corresponding author: **Giuseppe Bee** - giuseppe.bee@agroscope.admin.ch

Polyphenols have received a lot of attention in recent years due to their potential beneficial impact on gut health. Among the groups of polyphenolic molecules, tannins are one of the most widely studied in pig production. They are a heterogeneous group of astringent polyphenols and can be classified into three classes: condensed, hydrolysable and complex tannins. Owing to their hydroxyl and phenolic groups, tannins interact with and precipitate macromolecules, such as proteins and polysaccharides. These properties result in antiviral, antifungal, antibacterial and antioxidant activities that ultimately give the tannins several interesting biological applications. One application, closely linked to their potential use as antimicrobial, antioxidant and anti-inflammatory agents, has gained great interest in recent years, especially in post-weaning piglets' nutrition, due to the ban of using pharmacological doses of ZnO to reduce the incidence of post-weaning diarrhea caused by enterotoxigenic *Escherichia coli* (ETEC). Relevant is the fact that some tannins reduce the growth of pathogenic bacteria but at the same time promote that of beneficial bacteria. Supplementation of a starter diet for weaned pigs with hydrolysable tannins originating from chestnut wood extract for 1 month tended to increase the viable counts of Lactobacilli in the jejunum, although the bacterial counts in the cecum were not affected. We recently showed that the amount of supplied hydrolysable tannins plays a crucial role in their effect on fighting ETEC-induced diarrhea. The severity of diarrhea was reduced upon 0.5% supplementation with hydrolysable tannins, whereas ETEC F4 shedding in the feces was unaltered. However, doubling their inclusion level in the starter diet of weaned piglets lowered ETEC F4 shedding and the number of piglets with diarrhea and at the same time increased their growth rate. In addition, the relative abundance of *Clostridium spp.* in the jejunum was also lowered, whereas the abundance of *Lactobacillus spp*. was unaffected. These examples illustrate the potential of this class of polyphenols to positively affect pig health and by that minimize the use of medication. Further studies should explore the potential of combining different classes of tannins and some functional nutrients like amino acids, the latter being relevant for gut integrity, to further develop nutritional strategies for improving piglet health during critical periods like the post-weaning period.

ANTIBACTERIAL ACTIVITY OF NATURAL SUBSTANCES: FOCUS ON THEIR SYNERGISTIC ACTIONS

Filippo Fratini (1)

(1) Department of Veterinary Sciences University of Pisa

Corresponding author: **Filippo Fratini** – filippo.fratini@unipi.it

In light of the worrying and growing phenomenon of antibiotic resistance, it becomes increasingly desirable to direct research toward the use of substances with antibacterial activity as alternatives to traditional synthetic antibiotics. There are many natural substances, mainly of plant origin, but not only, that could potentially find application in veterinary medicine for the topical and, in some cases also systemic, treatment of certain conditions such as dermatitis, pyoderma and mastitis. However, the fact that a substance is of natural origin does not necessarily mean that it has no side effects or should not be treated in the same way as any other synthetic drug. Essential oils, for example, are excellent alternatives because of the presence of active ingredients that are effective against many microbial agents but are often histolesive, irritating, or even toxic when used pure or at certain dosages. It therefore becomes essential to evaluate the possibility of using them in synergy with other essential oils or other natural substances that dilute their concentration by diluting their irritating action, reduce their side effects and in some cases even enhance their efficacy in association with the compounds contained in the substances used in association with them. A branch that is still very little explored is in fact represented by the combined action of natural substances, which instead could be exploited precisely to obviate these drawbacks and to enhance the potential synergies or additive actions that may occur, while also averting possible antagonistic actions. Although *in vivo* studies directed at these specific aspects are still very few, *in vitro* studies are fortunately multiplying just in recent years. On this occasion I intend to briefly present the results of some of our work that evaluated the *in vitro* combined antibacterial action of essential oils among themselves and of essential oils in combination with natural peptides against both collection microorganisms and field isolates.

NATURAL SUBSTANCES IN THE FOOD INDUSTRY: PERSPECTIVES AND LIMITS

Filippo Giarratana (1)

(1) Department of Veterinary Science - University of Messina

Corresponding author: **Filippo Giarratana** - fgiarratana@unime.it

The growing consumer demand for foods free of chemical additives has pushed the scientific community and food industries to search for alternative natural substances. In this regard, in the last decade, several natural substances obtained from plants, algae, fungi, bacteria, insects, etc., have been tested and proposed as natural preservative agents in foods.
These natural substances can be used as extracts, exploiting the synergistic effects of the different constituents, or as single molecules, after their purification, thus adding only the most bioactive ones to food. Given the many different biological properties such as antibacterial, antifungal, antiparasitic, and antioxidant activity, natural substances have been proposed in foods to extend their shelf-life, or for safety purpose against foodborne zoonotic agents (such as *Listeria monocytogenes*, *Clostridia*, *Salmonella sp.*, *Anisakis*) but also as flavoring -coloring -texturing agents or for their nutritional value.
Although the results of scientific research in this sector are very promising and the food industries increasingly use these substances, there are still several limitations and drawbacks. First of all, the definition of "natural" substance in relation to the extraction methods which are not always so "green". In this regard, according to ISO/DIS 19657:2017, food can be defined as "natural" on the basis of its ingredients, which must be obtained from one or more basic materials deriving from plants, algae, fungi, animals, and microorganisms exclusively through physical and/or enzymatic and/or microbiological processes (no chemical synthesis). Other important limitations to the use of these substances are related to their influence on the organoleptic characteristic of foods; the strong variability of the composition and of the effects in relation to the geographic origin, physiological state, season, etc.; the possible phenomena of resistance acquisition especially in the bacteria.

PREPARATIONS OF BOTANICAL ORIGIN WHEN USED AS FEED ADDITIVES: THE APPROACH DEVELOPED BY EFSA FOR THE RISK ASSESSMENT OF COMPLEX MIXTURES

Paola Manini (1)

(1) European Food Safety Authority (EFSA), FEEDCO Unit, ASSESS Department

Corresponding author: **Paola Manini** - Paola.MANINI@efsa.europa.eu

The EFSA Panel on Additives and Products or Substances used in Animal Feed (FEEDAP) is currently assessing the safety of about 200 preparations of botanical origin (essential oils, extracts, mixtures, etc.) when used in animal nutrition as feed additives (feed flavourings). These botanical preparations are complex mixtures characterised by the presence of hundreds of components, including substances that are genotoxic and/or carcinogenic (e.g., methyleugenol, estragole, furocoumarins, etc.).

In 2019 the EFSA Scientific Committee (EFSA SC) developed a new methodology for the evaluation of the combined exposure to multiple chemicals (mixtures), which is based on the assessment of the individual components (component-based approach). This approach allows the use of existing toxicological data for the individual components, avoiding the need to generate new animal data for each of the mixtures under assessment.

The FEEDAP Panel has further developed the approach to assess the safety for the target animal species (food-producing animals and companion animals) of feed additives of botanical origin. The approach requires: (i) an extended characterisation of the mixture, aimed at identifying substances of concern, particularly substances which are genotoxic and/or carcinogenic; (ii) an evaluation of the structural and metabolic similarity of the components of the mixture, aimed at identifying groups of substances (assessment groups) characterised by the same toxicological profile; (iii) the identification of a toxicological reference point (e.g., a NOAEL) for each component and/or assessment group; (iv) the estimate of animals' exposure for each component and/or assessment group; (v) the comparison of animal exposure with the reference point by calculating the combined margin of exposure (MOET) for each assessment group. The magnitude of the MOET for the different assessment groups allows to conclude if the additive is safe under the proposed conditions of use.

For botanical preparations which contain compounds that are genotoxic and/or carcinogenic, the approach developed by the FEEDAP Panel considers the likelihood that the different target species could develop cancer as a result of the exposure to genotoxic and/or carcinogenic substances in the diet during their life. A different approach has been developed for short-living animals (targets species for fattening) and for long-living and reproductive animals.

THE MORAL STATUS OF THE ANIMAL AS A SENTIENT BEING IN THE BIOETHICAL DEBATE

Luisella Battaglia (1)

(1) Università di Genova; Comitato Nazionale per la Bioetica – Presidenza del Consiglio dei Ministri

Corresponding author: **Luisella Battaglia** - battaglialuisella@gmail.com

One of the most significant novelties in the philosophical field in recent decades has been the emergence of the animalist theme--also solicited by studies of cognitive ethology and comparative neurophysiology--which has been extending to the wider public with relevant modifications in terms of ethics social, helped also by a growth of sensitivity for ecological problems.
At the center of the animal bioethics debate is the question—of particular importance for veterinary medicine—relating to the moral status of animals: do they enter the ethical sphere or not? And, if so, under what title? Different answers are possible: from those who recognize, within an interspecific egalitarianism, in non-humans full-fledged moral subjects, endowed with rights or bearers of interests of consideration, to those who prefer to speak of man's responsibility towards beings considered as moral patients, passive recipients of our duties. Beyond the variety of theoretical perspectives, the intent to arrive at an ethics that realistically takes into account the requests, in different contexts, of the different subjects involved, human and non-human, is relevant, in order to arrive at a bioethical paradigm unitary, broad enough to cover the range of different types of relationships but, together, flexible enough to grasp the specificity of individual relationships. A point of reference is the concept of One Health, at the heart of veterinary medicine. for which human health is inextricably linked to animal health and the environment, Equally relevant is the effort to arrive at a shared juridical codification, witnessed by the Universal Declaration of Animal Rights (1978) in 14 articles which finds its most direct antecedents, as well as in the International Animal's Charter (1953) in the *Déclaration des Droits de l'Animal* (1924) written by *André Géraud*. Among its most qualifying points it is necessary to remember at least the art.1—All animals are born equal before life and have the same rights to existence'—and the art. 2-' a. Every animal has the right to respect. c. Every animal has the right to human consideration, care and protection'--'. The Barcelona Declaration (1998) should also be mentioned, which foreshadows the possibility of extending bioethical values such as integrity and dignity and the Lisbon Treaty (2007), which recognizes animals as sentient beings.

BREEDING FOR AESTHETICS: A GROWING CHALLENGE FOR CANINE WELFARE

Giulia Rubini (1), Eleonora Nannoni (1), Giovanna Martelli (1)

(1) Azienda AUSL, Bologna
(2) Department of Veterinary Medical Sciences
(3) University of Bologna

Corresponding author: **Giulia Rubini** - giulia.rubini@ausl.bologna.it

Canine breeding aimed at satisfying extreme aesthetic needs and expectations of owners is an increasingly evident trend that has severe consequences on the welfare of dogs.
A classic example is the well-known brachycephalic syndrome that characterizes some breeds, such as the French Bulldog. The shortness of the muzzle, the roundness of the skull and the eyes (neotenic characteristics) communicate a sense of cuteness and the need for protection and care. Tiny dogs with bulging eyes, like Chihuahuas, elicit similar feelings. Unfortunately, these hyper-types often have severe physical and behavioral problems. However, in a paradox difficult to explain, owners of these animals show loyalty to the breed, which they tend to repurchase in the event of their dog's death. Such a choice would depend on owners' preference for the breed personality rather than its aesthetics. In this context, the veterinarian's role should be decisive in making those who intend to buy these dogs at least aware of the consequences of their choice. To this end, breed-related issues should be given vital importance during studies in Veterinary Medicine.
Another more recent trend are the so-called designer dogs, hybrids obtained from the mating of individuals of different breeds and marketed as new breeds, often at higher prices than the parent breeds. These dogs are sought after for aesthetic and fashion reasons and because it is believed (but without solid scientific evidence) that some of them are hypoallergenic (for example, the Labradoodle, which derives from the cross between a Labrador and a Poodle). However, despite the phenomenon called hybrid vigour, increased disease resistance has not been demonstrated in designer dogs, probably due to the absence of selection in the parental lines, as parents are only used to obtain the desired phenotype of hybrids. Conversely, designer dogs appear to have higher risks for some pathologies than their parent breeds. However, the literature on designer dogs is still very scarce, and more studies are needed to come to firmer conclusions.
From a regulatory point of view, according to Legislative Decree 30 December 1992 n. 529, the sale of hybrids cannot be equated to that of purebred individuals. Therefore, the diffusion of designer dogs could become a shortcut for marketing and spreading animals without any ethical, legal or medical guarantee. It will, thus, once again, be the task of veterinarians to provide adequate support to future owners.

MOULDING MAN'S BEST FRIEND: HOW HAS SELECTIVE BREEDING IMPACTED CANINE BEHAVIOUR AND WELFARE?

Rowena MA Packer (1)

(1) Royal Veterinary College

Corresponding author: **Rowena MA Packer** - rpacker@rvc.ac.uk

Extensive artificial breeding by man has resulted in the domestic dog becoming the most phenotypically diverse mammalian species, with extraordinary variation in both morphological traits (e.g., body mass/proportions, coat type/coloration) and behavioural traits (e.g., abilities such as herding, pointing, tracking, hunting). Early selection focused upon creating 'types' of dog to fulfil functional roles for humans, e.g., hunting, guarding; and thus 'form followed function'. Given that early physical roles heavily relied upon functional morphology, selectively breeding from dogs who performed best in their role likely resulted in health being selected for by proxy. However, the predominant role of the modern dog is companionship. In 2023, the *Fédération Cynologique Internationale* recognises 356 breeds internationally, many of which now primarily fulfil a companion role. Desirable characteristics of companion dogs largely focus upon behavioural traits such as friendliness with humans and other animals. As such, form and function have become uncoupled for many breeds, with appearance and behaviour the strongest selection forces. Genetic mutations such as brachycephaly (foreshortened facial bones) and chondrodystophy (disproportionately short legs) have been captured by artificial selection to create morphologically distinctive breeds such as the English Bulldog and Dachshund, respectively. Celebration of these characteristics in both the pet owning and show-breeding communities has resulted in 'extreme', exaggerated versions of such breeds (e.g., flatter faces, shorter legs), that are associated with elevated risks of a range of health disorders. Alongside this, efforts to create and maintain morphologically distinct breeds has led to widespread inbreeding, reducing genetic diversity within breeds, and resulting in the dog possessing more known inherited disorders than any other non-human mammal. Awareness of conformation-related disorders in dogs has peaked over the past decade, with a plethora of scientific evidence documenting the often-severe disease burden associated with extreme body shapes. Paradoxically, some extreme breeds with the most profound health problems have become the most popular breeds internationally, such as the brachycephalic (short-muzzled) French Bulldog. This talk will explore how human selection has resulted in detrimental changes to dog behaviour and welfare and offer solutions for a healthier future for man's best friend.

[1] PACKER RMA, HENDRICKS A, & BURN CC (2015) Impact of facial conformation on canine health: Corneal ulceration. PLoS ONE 10(5): e0123827.
[2] PACKER RMA; O'NEILL DG; FLETCHER F; FARNWORTH MJ (2019) Great expectations, inconvenient truths, and the paradoxes of the dog-owner relationship for owners of brachycephalic dogs. PLoS ONE. 14 (7): e0219918.
[3] O'NEILL D; PEGRAM C; CROCKER P; BRODBELT D; CHURCH D; PACKER RMA (2020) Unravelling the health status of brachycephalic dogs in the UK using multivariable analysis. Scientific Reports 10, 17251.
[4] PACKER RMA; O'NEILL DG; FLETCHER F; FARNWORTH M (2020) Come for the looks, stay for the personality? A mixed methods investigation of reacquisition and owner recommendation of Bulldogs, French Bulldogs and Pugs. PLOS ONE 15(8): e0237276.

BREEDING AND BREEDING SOUNDNESS: A COMBINATION THAT MUST ADAPT AND EVOLVE

Magdalena Schrank (1), Antonio Mollo (1)

(1) Department MAPS, University of Padua, Legnaro, Italy

Corresponding author: **Magdalena Schrank -** magdalena.schrank@unipd.it

Dog breeding is very well regulated in most western countries, although control and repercussions differ and ethical concerns are raised more frequently. Indications on health criteria a dam or a stud should satisfy to be eligible for breeding are provided in a breed specific manner. Reproductive health, if not for obvious conditions (e.g., cryptorchidism), does not play a role and a breeding soundness exam is rarely performed and never requested. The capacity to mate, to be mated and to give birth naturally are not considered as criteria for eligibility. The requests for artificial insemination (AI) and elective Cesarean sections (ECS) have increased over the last decades, which led to an improvement of techniques and an increase of studies on the subject. Although AI, with frozen semen, offers possibilities to maintain genetic diversity, fresh semen AI in dams which are unable to being mated or use of fresh semen from males which are unable to mate allows reproduction of animals lacking the very basics every species needs to have to persist. Certain breeds are at a very high risk for dystocia, which leads to very high incidences of ECS in these breeds. ECS have a reportedly, lower anesthesiologic and surgical risk for the dam as well as a higher survival rate of the pups compared even to eutocic parturitions. Regardless, ECS should be considered a treatment for a problem. In cases of a singleton pregnancy, an ECS may be considered the best choice for the dam and the pup. In cases of incapacity for labor instead, breeding of these individuals most likely results in the following generation having the same or at least similar problems.
Maternal behavior of a dam has received lesser attention in the past. Considering the social nature of the canine species and its role as “man’s best friend”, correct maternal behavior and its impact on the pup and the adult dog are of great importance. Although differently regulated in different countries, the eligibility for breeding does not include any of the above considerations. The veterinarian practitioner is in a difficult position as techniques (e.g., AI) are available, yet their use and therefore the breeding of these animals is not always ethically correct. A change in the way of reasoning is needed and a breeding soundness exam as intended in the veterinarian profession and evaluation of maternal behavior of dams should go along with what is requested by kennel clubs for eligibility for breeding.

BRACHYCEPHALIC AIWAYS RESPIRATORY SYNDROME AFTER MORE THAN 50 YRS FROM THE FIRST DESCRIPTION:THE MODEN SURGICAL PHILOSOPHY IN THE MANAGEMENT OF POSSIBLE CORRECTIONS

Stefano Romussi

(1) Dipartimento di Medicina Veterinaria e Scienze animali- UNIMI

Corresponding author: **Stefano Romussi -** stefano.romussi@unimi.it

The upper airways obstructive respiratory syndrome of brachycephalic dogs, also known by the acronym BAOS, (Brachycephalic Airway Obstructive Syndrome) represents a serie of morphological abnormalities of the upper airways, determining an obstruction in the air passage.
The first published description of surgery to correct the cause of obstructive dyspnea in a bulldog dates back to 1964 by Leonard. The surgeon recognized the cause of the obstruction in a redundant portion of the soft palate in an English Bulldog and proposed a treatment based on simple palatoplasty able to correct the alteration and to solve the dyspnoea.
Starting from the first description, the best surgical technique to obtain good results has been the goal of the philosophy that guided surgeons in the initial management of severe dyspnea in brachycephalic patients.
Most recent reviews clearly underline that the results of corrective surgery, even when performed with standard techniques by skilled surgeons, are closely related to the morphological characteristics of the single subject. Important limitations directely related to morphological selection have been identified. The size of the pharyngeal space, the diameter of the cricoid cartilage, the extension of the pterygopalatine processes represent insurmountable limits able to interfere with the surgical effect.
The modern philosophy in BAOS corrective surgery has changed and the scientific experiences acquired represent the base for a multidisciplinary work with the aim to identify the skull aberrations which could be modified only considering a change in the breed standard. Future research could compare the predisposition of disorders between dogs with more moderate physical features compared to those with extreme physiques to assess potential welfare gains from breeding for less drastic characteristics. Royal Veterinary College experts said urgent action was needed to reshape the breed back to how it looked in the 1800s.. This way could represent the only possibility to avoid drastic decisions like that adopted in late 2022 by a court in Norway which banned the breeding of bulldogs.

The aim of the lecture is to discuss on the evolution of the surgeons' aproach in the management of the Brachycephalyc Airways Obstructive Syndrome

IMAGING BASED IN SITU TISSUE PROFILING: COMBINATION OF HIGHPLEX IMMUNOFLUORESCENCE AND SPATIALLY RESOLVED TRANSCRIPTOMIC

Francesca Ravanetti (1)

Department of Veterinary Science - University of Parma

Corresponding author: **Francesca Ravanetti** - francesca.ravanetti@unipr.it

Understanding the organization of cells and tissues and how it influences their function is a fundamental pursuit in life sciences research. The spatially resolved transcriptomics combines microscopy and next-generation sequencing to empower scientists in measuring gene expression within a specific tissue or cellular context [1]. This approach overcomes the limitation of scRNA-seq by retaining the spatial information of gene expression within the tissue context. The spatial transcriptomic can be combined with highplex immunofluorescence allowing to target a single cell istotype. This represents a powerful tool to characterize the mechanistic understanding of tissue organization, development, and pathogenesis, as well as to identify prognostic or therapeutic targets. Starting from a formalin fixed paraffin embedded (FFPE) histological slide, the spatial molecular omics mainly define positional relationship and interactions among cells within a specific tissue and reveal the impact of spatial cell distribution on expression profiles and morphologic phenomes [2]. The here presented results described the morphology-driven, in situ high-plex profiling of normal mouse lung and its modification during the fibrogenesis induced by Bleomycin instillation. A Tissue Microarray was designed and prepared starting from archive FFPE blocks of normal and BLM treated mice at four time points 7, 14, 21 and 28 days. At least 6 cases from each group were considered into the TMA. Histological TMA section was immunostained for epithelial marker (PanCK), smooth muscle marker (α-SMA) and nucleated hematopoietic cells (CD45). Cell-type specific segmentation were achieved through the use of binary masks based on the fluorescent markers. Oligos from the selected regions were released upon focused exposure to UV light and aspirated via a microcapillary tube to obtain the transcriptomic profiling [2]. Hierarchical clustering analysis based on differentially expressed RNA transcripts revealed clear clustering of fibrogenesis during time course; the role of each cell type in both normal lung and BLM induced fibrosis were subsequently revealed trough the pathway analysis and statistically significance gene expression.

[1] Merritt CR et al, Nat Biotechnol. 2020; 38(5):586-599.
[2] Zollinger DR et al, Methods Mol Biol. 2020; 2148:331-345.

EXPLORING DIFFERENT ALTERNATIVES TO THE CRYOPRESERVATION OF CELLS AND GAMETES

Pasqualino Loi (1)

(1) Dipartimento di Medicina Veterinaria, Università di Teramo

Corresponding author: **Pasqualino Loi** - ploi@unite.it

Cryopreservation in liquid nitrogen (LN) is currently the only method of long-term storage of cells and sperm. However, storage in LN is expensive, requires dedicated facilities, equipment and personnel, is potentially dangerous and could be a vehicle for pathogens; finally, its production leaves a high carbon dioxide footprint.
In recent years, my research group, in collaboration with other European, Thai and Japanese partners, has been exploring methods towards the storage of cells and germplasm, sperm in particular, in an anhydrous state. The logic of our goal is based on water removal techniques which induce a reversible block of cellular metabolism, a survival strategy already available in nature defined "anhydrobiosis". In my report, I shall present the results obtained in our trials of reversible dehydration of cells (fibroblasts) and spermatozoa (ram), previously exposed to natural compounds that work as "xeroprotectors" (xero=dry), such as Late Embryogenesis Abundant proteins (LEAp, expressed by vegetable seeds during drying), and trehalose, a disaccharide produced by organisms physiologically prepared for dehydration (anhydrobionts).

ARTIFICIAL INTELLIGENCE AND DIGITAL PATHOLOGY: APPLICATION IN VETERINARY PATHOLOGY

Massimo Salvi (1)

(1) Department of Electronics and Telecommunications, Politecnico di Torino, Italy

Corresponding author: **Massimo Salvi** - massimo.salvi@polito.it

Artificial intelligence (AI) has emerged as a powerful tool in healthcare, including pathology. Digital pathology is a rapidly evolving field, with advances in whole slide imaging and machine learning algorithms, that has the potential to transform the practice of veterinary pathology. Digital pathology is the process of converting glass slides into digital images that can be viewed, analyzed, and stored on a computer. These images can then be analyzed using AI algorithms to detect patterns and anomalies that may be difficult for human pathologists to identify. This can provide significant benefits in terms of speed, accuracy, and objectivity.

In veterinary pathology, AI-based tools can be used for various applications, including the detection and classification of tumors, identification of infectious agents, and evaluation of tissue changes in response to drugs or toxins. AI-based tools can also improve the efficiency of pathology laboratories by automating routine tasks such as tissue segmentation and classification. This can reduce the workload of veterinary pathologists, enabling them to focus on more complex and challenging cases.

However, there are also some challenges to the implementation of AI in veterinary pathology. One of the major challenges is the lack of standardized protocols for the generation of digital images, which can affect the accuracy of the AI algorithms. Additionally, there is a need for large-scale datasets of high-quality digital images to train the algorithms, which can be difficult to obtain.

In conclusion, AI-based tools have the potential to revolutionize veterinary pathology by improving the accuracy, speed, and objectivity of diagnoses. While there are still some challenges to be addressed, continued research and development in this area hold great promise for the future of veterinary pathology.

APPLICATIONS OF COMPUTER VISION SYSTEMS FOR MEAT SAFETY ASSURANCE IN *ABATTOIRS*

Marianne Sandberg (1), Sergio Ghidini (2), Lis Alban (1), Andrea Capobianco Dondona (2), Bojan Blagojevic (1), Martijn Bouwknegt (1), Len Lipman (1), Jeppe Seidelin Dam (1), Ivan Nastasijevic (1), Dragan Antic (1)

(1) National Food Institute, Technical University of Denmark, 2800, Kgs. Lyngby, Denmark
(2) Department of Food and Drug, University of Parma, Via del Taglio 10, 43126, Parma, Italy

Corresponding author: **Marianne Sandberg** - marsan@food.dtu.dk

There has been attempts to develop computer based vision system (CVS) for veterinary meat inspection for over two decades. In 2017-2019, new EU legislation was introduced on official controls in food production, opening for the use of CVS as a complementary tool in meat inspection as a part of a Risk Based Meat Safety Assurance System (RB-MSAS). A systematic review was conducted to map the number of CVS's developed for detection of carcass contamination and gross pathological lesions in the overall meat safety assurance of bovine, pigs and broiler chicken. In this review, 62 articles were found and analysed. There were 35 articles reporting on CVS performance in the detection of carcass/organ lesions and 27 in the detection of contamination on the carcass. CVS for broiler chickens, pig and bovine in MSAS were reported in 53, 5 and 4 articles, respectively. To what extent the developed CVS's were validated varied. Only three articles reported results from on-site and in real-time evaluation of CVS performance in abattoir vs performance of the official veterinarian. Most of the reported CVS performance measures (i.e., sensitivity and specificity) were >80%. The challenges reported were related to achieving high sensitivity for detection of the food safety and animal health/welfare related conditions. Moreover, there were challenges with acquiring high specificity, minimising false positives, with the purpose of minimising food waste as well as to maintain CVS economically viable for abattoirs. While conditions that could be of importance food safety wise should be detected for the individual carcasses, animal health and welfare conditions might only need to be documented on flock level. For most of the identified CVS's, there is still a need for more training to achieve better performance. In a RB-MSAS, a CVS should interact with a Food Chain Information (FCI) database, which would allow for easy forward and backward flow of information. An advanced CVS would be capable of detecting and recording several lesions concurrently, and hence generate more accurate FCI. The data captured in such a digitalised system could, improve studies of association between diseases and their management, biosecurity/overall heard health plans leading to improved animal health and welfare. A fully digitalised RB-MSAS could also increase traceability, favouring e.g., foodborne outbreak investigations and monitoring of antibiotic usage/presence of antimicrobial resistance.

EXTRACELLULAR VESICLES AS INNOVATIVE IMMUNOMODULATORY AGENTS

Elisabetta Razzuoli (1)

(1) National Reference Center of Veterinary and Comparative Oncology (CEROVEC), Istituto Zooprofilattico Sperimentale del Piemonte, Liguria e Valle d'Aosta, Piazza Borgo Pila 39-24, 16129 Genova, Italy.

Corresponding author: **Elisabetta Razzuoli -** elisabetta.razzuoli@izsto.it

The term extracellular vesicles (EVs) encloses all lipid bilayer cell-derived particles which can be distinguish based on diameter in: small EVs (50–150 nm), the most enriched in biological fluids; medium-sized EVs (200–800 nm); and large EVs (diameter ≥1 μm). The heterogeneity of EVs is a consequence of the variety of types and functional states of the releasing cells as well as of the different biogenetic routes. EVs are involved in several homeostatic processes and can modulate receiving cell functions by delivering intercellular signals through their surface proteins and encapsulated cargo molecules (such as proteins, RNAs, lipids and glycans). Cytokines and EVs share several functions as mediators of intercellular communication and cytokines can associate with EVs as either internal or external cargo. Given the main feature of EVs as signaling mediators between cells and organs, they have the potential to function as biomarkers or as therapeutic tools. EVs are involved in fundamental immune mechanisms and immune-mediated disease processes, having a role in inflammation, antigen presentation, and the development and activation of B and T cells. Indeed, EVs can directly present antigen on their surface MHC molecules, or cross-dressing present when attached to the surface of dendritic cells (DCs) or being internalized by DCs. Moreover, numerous molecules known to participate in immune regulation have been identified on the surface of EVs, including the immune-checkpoint molecules programmed death ligand 1 (PDL1), cytotoxic T lymphocyte antigen 4 (CTLA4) and the apoptosis-inducing ligands FASL and TNF-related apoptosis inducing ligand (TRAIL).
EVs, and the miRNA enclosed, play a pivotal role also in in the tumour microenvironment between tumour and immune cell cross-talk, promoting both immunosuppression and antitumour immunity. EVs are also implicated in host immune response against microbial stimuli due to the evolutionarily conserved release of EVs and in allergic responses both as carriers of allergens and as modulators of the allergic response. Moreover, EVs carry numerous autoantigens that are implicated in autoimmune diseases. EV-based immunotherapeutic approaches are gaining interest especially for mesenchymal stem cell-derived EVs as well as milk-derived EVs, which have immunomodulatory functions and a broad immunosuppressive potential.

PRECISION TECHNOLOGIES FOR THE DIAGNOSIS AND CONTROL OF PARASITIC DISEASES

Maria Paola Maurelli (1), Antonio Bosco (1)

(1) Department of Veterinary Medicine and Animal Production - University of Naples Federico II

Corresponding author: **Maria Paola Maurelli** - mariapaola.maurelli@unina.it

Parasitic infections may influence animal health, welfare and productivity, in terms of reduced growth rate, meat, milk and wool quantity and quality, and reduced fertility. In recent years, technological innovations have been rapidly developed and applied also to parasitology field aiming to perform accurate epidemiological studies, diagnosis, as well as efficient control approaches and treatment strategies [1, 2]. Indeed, thanks to use of new electronic devices, e.g., biometric sensors, Global Positioning System (GPS), dataloggers, camera traps (CT) and drones, a continuous real-time monitoring and data collection related to macro and micro epidemiology of parasites in pet and farm animals can be performed, rationalizing the control of parasitic infections and reducing the risks of drug abuse and environmental contamination. In this way, Geographical Information Systems (GIS) and Disease Mapping tools are indispensable to manage all the spatiotemporal data, allowing to plan an integrated approach of precision veterinary medicine from sampling strategies to prevention and prediction of parasitic diseases [3].
Moreover, remarkable progress has been performed in diagnostic field thanks to development of new digitized tools (semi-) automated for a rapid, user-friendly, standardized and accurate recognition of parasitic elements and assessment of Faecal Egg Count/Faecal Egg Count Reduction (FEC/FECR) for both pen-side and laboratory use. This new generation of smartphone-based methods and digital microscopes (e.g., Kubic FLOTAC Microscope) combined with use of artificial intelligence (AI) algorithms, including machine learning and deep learning, has been developed to overcome gaps and limitations i.e., human errors and time for analysis, of the traditional microscopy [4, 5].
Finally, innovative approaches, e.g., App, immersive virtual reality, can be used also for the dissemination and training activities to stakeholders and end-users [6].
Therefore, all the abovementioned strategies will be very important to assist a new generation of operators (i.e., technicians, physicians, veterinarians and farmers) in veterinary parasitology.

[1] Vercruysse et al., 2018. Parasitology, 145,1655-1664;
[2] Charlier et al., 2022. Adv Parasitol, 115,171-227;
[3] Rinaldi et al., 2006. Geospat Health, 1(1), 33-47;
[4] Cringoli et al., 2021a. Parasitology, 148,427-434;
[5] Rinaldi et al., 2022. Adv Parasitol, 118,85-176;
[6] Cringoli et al., 2021b. Vet Parasitol, 290,109347

PHARMACOGENETICS IN VETERINARY PRECISION MEDICINE: DREAM OR REALITY?

Mery Giantin (1), Mauro Dacasto (1)

(1) Department of Comparative Biomedicine and Food Science - University of Padua

Corresponding author: **Mery Giantin** - mery.giantin@unipd.it

The term pharmacogenetics (PGx) integrates the field of genetics and pharmacology with the aim of investigating the effect of an individual genotype on the pharmacokinetics and pharmacodynamics (PK/PD) of drugs. This discipline, fuelled by recent cutting-edge sequencing technologies, led to the gradual shift from the traditional approach of "one drug fits all" to a more personalized, patient-oriented treatment, defined as precision medicine [1]. At present, 68 clinically important human pharmacogenes, including phase I and II drug-metabolizing enzymes, transporters, drug targets and oncogenes, have been identified [2]. Human cytochromes P450 (CYPs), the most studied pharmacogenes, are highly polymorphic, and CYP polymorphisms are associated to significant adverse drug reactions, lack of response to treatment and inter-individual variability in drug response [3].

PGx is a somewhat new discipline in veterinary medicine (VM); even though significant strides have been made in the past 20 years, it is still in its infancy compared to the human counterpart [4]. Most of data concern the canine species for its importance in pre-clinical and clinical sciences. A well-known example is the ABCB1 deletion polymorphism in Collie dogs and other herding dog breeds causing fatal reactions to many P-glycoprotein substrates [5]. Moreover a CYP1A2 stop codon polymorphism, whose prevalence varies considerably between and within dog breeds, has been described [6]. Conversely, few examples of PGx application in food-producing species are available. Apart from polymorphisms influencing PK (e.g., ABCG2 Tyr581Ser in dairy cows and CYP3A28 Gly197Ser in meat cattle [7-8]), most of identified variants are usually associated to productivity traits.

Since PGx shows a wide range of applications and undoubted benefits, more efforts have to be made to develop its use in VM and promote an individualized therapy approach; to reach this goal, large scale genetic data are needed and the functional impact of pharmacogene variants must be characterized.

[1] Primorac, D et al (2020). Pharmacogenomics 21, 141.
[2] Caspar, SM et al (2021). Pharmacogenomics 22, 177.
[3] Sim, SC et al (2013). Pharmacogenomics J 13, 1.
[4] Mealey, K et al (2019). Hum Genet 138, 467.
[5] Mealey, K et al (2003). J Am Vet Med Ass 223, 1453.
[6] Court, MH (2013). Vet Clin Small Anim 43, 1027.
[7] Mahnke, H et al (2016). Drug Metab Dispos 44, 700.
[8] Giantin, M et al (2019). Sci Rep 9, 19672.

THE TECHNOLOGICAL REVOLUTION IN PET MEDICINE: ARTIFICIAL INTELLIGENCE, PRECISION MEDICINE, AND THE INTERNET OF THINGS (IOT)

Paolo Ciaramella (1), Antonio Di Loria (1), Francesco Porciello (1)

(1) Società Italiana di Clinica Medica Veterinaria SICLIMVET

Corresponding author: **Paolo Ciaramella** - paociara@unina.it

In recent years there has been a sudden development of new information technologies applied to the clinic of pets, on a par with what has been observed in human medicine. Machine learning and/or artificial intelligence (AI) are currently an interesting reality used in diagnostic support in common clinical practice to make individual or group work easier, more efficient, and sometimes more effective. In particular, the potential of AI is particularly interesting and can help veterinary surgeons acquire greater security in the practice of the profession. In the near future, it will be normal to entrust AI with the task of interpreting diagnoses automatically and generating reports in real-time which will be subsequently examined and verified by the veterinary specialist, even with the use of intelligent software capable of comparing and comparing images from by various instrumental examinations, especially in cardiovascular medicine of small animals.

NEW TECHNOLOGIES IN THE MEDICAL AND SURGICAL FIELD

Claudio Marchetti (1)

(1) Università degli Studi di Bologna

Corresponding author: **Claudio Marchetti -** claudio.marchetti@unibo.it

New technologies have now revolutionized not only our lifestyles but also the world of medicine and surgery. Indeed, research has made a lot of progress in the electronic, robotic, and technological fields, which have led to the creation of equipment and devices capable of helping medicine and surgery in improving diagnoses and therapies. This report intends to focus on the state of the art of the use of new technologies in the predominantly surgical field, eminently maxillofacial surgery:

- 3D printing
- custom-made devices
- intraoperative navigators
- robotics
- virtual reality
- augmented reality
- Artificial intelligence

EXTRACELLULAR VESICLES FROM REPRODUCTIVE TISSUES: INNOVATIVE TECHNOLOGY FOR CLINICAL APPLICATIONS OR REGENERATIVE MEDICINE IN ANIMAL REPRODUCTION

Anna Lange Consiglio (1)

(1) Department of Veterinary Medicine and Animal Science, Università degli Studi di Milano

Corresponding Author: **Anna Lange Consiglio –** anna.langeconsiglio@unimi.it

Intercellular communications can be mediated by direct links between cells through intercellular cytoplasmic connection, or without direct contact through secretion of signaling molecules acting as paracrine factors. A recently known mechanism of cell communication is the release and uptake of extracellular vesicles (EVs) that are released into extracellular fluid from all kinds of cells, including mesenchymal stromal/stem cells (MSCs). These EVs contain a variety of bioactive molecules, including messenger RNAs, microRNAs (miRNAs), lipids, proteins, and nucleic acids for long-distance communication between cells.
MiRNAs are responsible for regulating many genes and their pathways with the result of controlling cell differentiation, proliferation, apoptosis, metabolism, secretion, and regeneration.
Extracellular vesicles are involved also in the intercellular communication at each stage of the reproductive system in both male and female reproductive tracts. They play a significant role in the transmission of specific cargo molecules to modulate granulosa cell proliferation and differentiation, cumulus expansion, gametogenesis, follicular growth, oocyte and sperm maturation, fertilization rate, embryo development, blastocyst formation and implantation, pregnancy out comes, and fertility.
The function of EVs in reproduction depends on the load of EVs and their ability to interact with receptor cells to deliver various types of cargo. Physiological and pathological conditions influence EV concentration, cargo, and function. Then, alterations in EV composition could impair fertility, but also influence fetal development and lead to long-term consequences for offspring health.
The study of EVs in reproduction has the potential for expanding current understanding of the normal physiology of reproduction, identifying high or low-quality sperm and oocytes or pathological conditions such as implantation failure. Then, EVs may be used as non-invasive diagnostic biomarkers in female/male fertility, risk of abortion, fetal growth, and placental function. In addition, EVs derived from reproductive system or from MSCs could be used as therapeutic agents able to maintain or to restore the reproductive success, in case of disorders or pathologies. These nanoparticles may also be engineered for tissue-specific transfers, such as the transfer of selected compounds (for example miRNA) into gametes and embryos to increase reproductive success.

MILK INFRARED SPECTROSCOPY TO ASSESS HEALTH DISORDERS IN DAIRY COWS

Alessio Cecchinato (1), Diana Giannuzzi, Erminio Trevisi

(1) Department of Agronomy, Food, Natural Resources, Animals and Environment (DAFNAE), University of Padua, Legnaro, Padua, Italy
(2) Department of Animal Science, Food and Nutrition - DIANA, Università Cattolica del Sacro Cuore, 29122, Piacenza, Italy

Corresponding author: **Alessio Cecchinato –** alessio.cecchinato@unipd.it

There is an ever-growing interest in research oriented towards the improvement of animal health condition as it affects the efficiency and sustainability of dairy farms as well as the quality of derived products. Several serum metabolites are known to be valuable indicators of cows' health status. However, despite the advantages of metabolic profile testing, blood sampling on a regular basis is invasive, logistically challenging, and costly. Given the metabolic relevance of the mammary gland and the availability of milk, its use as a biofluid to monitor the health and nutritional status of dairy cows has been widely investigated. For instance, the well-known fat-to-protein ratio has been widely described as indicator of negative energy balance and subclinical ketosis, while the lactose content has been reported to be an effective indicator of mammary gland inflammation. Additional fine compounds, like the milk fatty acid (FA) profile and milk minerals could represent a fingerprint of the cow's nutritional and metabolic status and could be suitable indicators of metabolic disorders. However, although interesting, all these fine compounds present a strong limitation related to their impossibility of being used at the farm level and on a large scale. The advent of fully automated data recording technologies and high-throughput phenotyping systems has opened up a myriad of opportunities to predict health disorders in dairy cattle. Indeed, the use of infrared (IR) techniques, as the in-line near-infrared (NIR) and the Fourier-transformed infrared (FTIR) spectroscopy, have been proven to be powerful precision phenotyping tools for high-throughput milk quality and animal health assessment. Overall, the in-line NIR instrument has less predictive ability respect to the FTIR instrument, which is commonly used within milk recording schemes for breeding purposes, but it has the enormous advantage to provide an in-line monitoring over lactation for each animal. The IR techniques require sophisticated statistical and computational approaches for efficient data management and appropriate data mining, as they involve large datasets with many covariates and complex relationships among themselves. Some applications of these tools will be presented in the context of precision livestock farming, boosting animal health and welfare, environmental footprint, and overall sustainability of animal production.

This review was carried out within the Agritech National Research Center and received funding from the European Union Next-Generation EU (PIANO NAZIONALE DI RIPRESA E RESILIENZA (PNRR)–MISSIONE 4 COMPONENTE 2, INVESTIMENTO 1.4–D.D. 1032 17/06/2022, CN00000022).

PROTEOMICS AS A TOOL FOR THE DEVELOPMENT OF COMPANION DIAGNOSTICS IN VETERINARY PUBLIC HEALTH

Paola Roncada (1)

(1) Università degli Studi di Catanzaro

Corresponding author: **Paola Roncada** – roncada@unicz.it

Current strategies applied to control and/or eradicate most of the One-Health-relevant infectious diseases rely on diagnostic tests that suffer from either limited specificity, sensibility or hinder the fair discrimination between naturally-infected and vaccinated animals. Altogether, this results in severe bugs in the monitoring and control campaigns performed, with an impressive effort, by diverse regulatory levels of both national and international orders. Along, the diffusion of zoonotic agents over time and space, and the concurrent spread of their antimicrobial resistance traits, keep active the yet warning phenomenon of antimicrobial treatment failure, of relevant interest in all the spheres of life including humans, animals and the environment. To overcome these issues, a steadily growing number of studies are being performed integrating the contribution of multiple disciplines to provide a multifaced solution to such a complex matter. Proteomics is one of the major omics sciences aimed at the thorough and systemic study of both the zoonotic agents and the host biology, providing the knowledgebase for unprecedented advances in diverse fields of biomedicine, ranging from biomarker discovery until drug development.
With regard to the companion diagnostics, the deep characterization of the pathogen proteome and subproteomes, along with the detailing of the protein tridimensional structure provide essential inputs to feed bioinformatics algorithms specifically tailored for the accurate and reliable prediction of a variety of biologically relevant information suitable for the design of novel prophylactic measures and the optimization of innovative companion diagnostics. Examples of studies employing proteomics-based biomarker discovery, immunoproteomics and immunoinformatics are already made available by this research group, highlighting the potential of the proteomics discipline as a powerful tool to provide per se suitable candidates for the companion diagnostics besides serving, or being combined with further disciplines, for the companion diagnostics and the design of innovative prophylactic strategies. Altogether, the above examples underline the power of this omics discipline with the provision of accurate and reliable data, enabling the development of novel companion diagnostics at unprecedented effectiveness levels as compared with the traditional approaches.

[1] https://doi.org/10.1016/j.vetimm.2023.110548
[2] doi: 10.1016/j.onehlt.2021.100253
[3] DOI: 10.1021/acs.jproteome.0c00553
[3] DOI: 10.3390/pathogens9090705

FROM PAPER TO DIGITAL: A HISTORICAL EXCURSUS OF THE CATALOGUING OF VETERINARY COLLECTIONS

Rosiana Schiuma (1)

(1) Dipartimento di Storia Culture Civiltà, Alma Mater Studiorum Università di Bologna

Corresponding author: **Rosiana Schiuma –** rosiana.schiuma2@unibo.it

How has the way tangible items of cultural heritage are catalogued changed over time? What is the most advanced technology in the world of museum cataloguing? These are the questions that will be addressed in this historical exploration of the cataloguing of scientific and naturalist collections, with specific reference to the Alessandrini-Ercolani Anatomy of Domestic Animals collection and Pathological Anatomy and Veterinary Teratology collection held by the Department of Veterinary Medical Sciences - University of Bologna. The analysis, which begins by looking at the techniques from the nineteenth century, i.e., when the collections were put together, ends with the present day and a presentation of the digitisation of the university's museum system (SMA), performed employing sophisticated computer programs. The document also examines not only accounts of methods, but also the exciting prospects generated by the project.

MAIN LECTURE - AIVI

APPLICATION OF COMPUTER VISION SYSTEM FOR MEAT SAFETY ASSURANCE IN CHICKEN *ABATTOIRS*: VETINSPECTOR

Marianne Sandberg (1), Anders Dong Jørgensen (2), Jens Fagertun (2)

(1) National Food Institute, Technical University of Denmark, 2800, Kgs. Lyngby, Denmark,
(2) IHFood A/S, Titangade 9C, 1. 2200 Copenhagen N, Denmark

Corresponding author: **Marianne Sandberg** - marsan@food.dtu.dk

Computer vision systems (CVS) has been used in chicken slaughter houses for quality sorting for more than two decades. In 2017-2019, new EU legislation was introduced on official controls in food production, opening for the use of CVS as a complementary tool in chicken meat inspection as a part of a Risk Based Meat Safety Assurance System (RB-MSAS). *Post mortem* inspection (PMI) of chickens is, today, manually conducted by the official veterinarians (OVs) – sometimes at a slaughter speed of 12,000 chickens per hour. Even higher slaughter-speeds, might imply that the quality of the manually conducted PMI will be utterly challenged resulting in a rise in the intra- and inter-rater variation. VetInspector is an image-analysis tool to support PMI in chicken – currently consisting of two camera-stations. In the development process of VetInspector, feasibility, agreement and performance studies were conducted. About 30% disagreement between OV's conducting the grading and rating of pictures of carcasses with different lesions was observed. Both grading of lesion severity and whether the carcass qualified for approval for human consumption or not, were done. The performance study revealed that among the carcasses and viscera that the OV's approved, 1 % of had lesions and should not been approved. Similarly 2 % of the approved viscera should not been approved. Clearly, the "wrongly-classified" in the manual conducted PMI were not 0 %. Cut-offs between approved/not approved for carcasses with different lesions/severity of lesions should be based upon the most updated scientific evidence and be managed by the National Food Safety authorities in the implementation process of VetInspector. Implementation of VetInspector will improve feedback to the farmers on flock health status. In the new version of VetInspector, the aim is to record several lesions concurrently, and hence to provide a more precise evaluation of abnormalities and generate more accurate Food Chain Information's in the RB-MSAS. Moreover, there will be included a VetInspector-station for inspection of the slaughter hygiene.

MAIN LECTURE - AMV

THE BRAIN OF LARGE HERBIVORE

Bruno Cozzi (1)

(1) Department of Comparative Biomedicine and Food Science , Università degli Studi di Padova

Large domestic and wild herbivores brains' haven not been studied much. Their appearance and conformation are those of other mammals, however the cortical columns have a different organization from that of man and laboratory rodents. Veterinarians have a duty to safeguard animal welfare, including animals in production. Are we certain that nothing have been overlooked?

MAIN LECTURE - AMV

NATIONAL LEGISLATION ON THE USE OF ANIMALS FOR SCIENTIFIC AND EDUCATIONAL PURPOSES: EUROPEAN DIRECTIVE 63/2010 VS DLGS 26/2014

Paolo De Girolamo (1)

(1) DIPARTIMENTO DI MEDICINA VETERINARIA E PRODUZIONI ANIMALI, Univeristà degli Studi di Napoli

Since the 1980s, the harmonization of the use of animals for scientific purposes in the Member States has been on the agenda of the European legislation. When the regulatory process began, ending with the issue of Directive 2010/63/EU. It aims to protect the welfare of the animals. In Italy, the Directive has been transposed with the *D.lgs. n. 26* of 2014 and it introduced greater restrictions compared to those set by the European standard. It imposed a necessary reflection on the prospects that may arise on animal experimentation at a national level. Nonetheless, it is necessary to consider the heavy bureaucratic burden required to the scientific community that deals with in vivo research. On the other hand, the rigorous fulfilments required by the legislator ensure greater protection of animal welfare and a meticulous methodological approach to the advantage of the reproducibility and translatability of scientific data. Furthermore, it contributes decisively to the reduction of the use of animals in research.

MAIN LECTURE - RNIV

EVS BETWEEN CHALLENGES AND ACHIEVEMENTS: A FLOW CYTOMETRIC APPROACH

B. Canonico, M. Montanari, D. Lopez, G. Panza, F. Grandoni, S. Papa

(1) Department of Biomolecular Sciences, University of Urbino "Carlo Bo", Urbino, Italy
(2) CREA-Consiglio per la Ricerca in Agricoltura e l'Analisi dell'Economia Agraria, Centro di ricerca Zootecnia e Acquacoltura (Research Center for Animal Production andAquaculture), Monterotondo, Italy

Corresponding author: **Barbara Canonico –** barbara.canonico@uniurb.it

Extracellular vesicles (EVs) are particles released by cells and have high potential as disease biomarkers. The original classification distinguished between exosomes (30-120 nm), originating from the formation of multivesicular bodies, microvesicles (120-1000 nm), which are formed by cell membrane budding, and apoptotic bodies (1-5 µm), developed from dying cells. This classification has been recently revised by the International Society of Extracellular Vesicles (ISEV) as follows: EVs < 200 nm are "small EVs" and > 200 nm are "large EVs" [1]. The differences in size could represent an indication of the specific biogenesis processes. Furthermore, EVs exhibit properties making them preferable drug delivery carriers compared to synthetic vehicles or nanoparticles: they can escape immune clearance and stably circulate in the blood.

Even if flow cytometry (FC) has been mainly applied for EV studies, the absence of agreement on protocols for FC detection of EVs generated controversy. The standard FC procedures, using only scatter parameters, allow the detection of the "tip of the iceberg" of all EVs [2]. Two acquisition approaches are mainly used: placing the threshold on the side scatter (SSC) channel or using a trigger threshold on a fluorescence (FL) channel. EVs are studied by flow cytometers, which measure scattering in arbitrary units. With Mie's theory, arbitrary units can be related to diameter when the particle refractive index (RI) is known: new calibration systems convert the arbitrary units of light scattering signals measured by flow cytometry to the particle size in nanometers [3]. Furthermore, different immunocapture platforms exist to allow detection and characterization of exosomes [4]. These procedures avoid the problems of the nanodimensions, conversely, they reduce the possibility of characterizing the EV heterogeneous pool. Finally, FC can differentiate EV types, by avoiding any pre-analytical manipulation, detailing and completing data by other techniques (TEM; NTA; DLS etc.). Unfortunately, not all the EV subtypes can be identified by recognized markers. Still, the use of generic EV probes, combined with the more recent FC approaches, may improve the FC detection of EVs, opening new ways to study EVs in vitro, ex vivo, in any human or veterinary clinical setting.

[1] Biagiotti, S.; Abbas, F.; Montanari, M.; Barattini, C.; Rossi, L.; Magnani, M.; Papa, S.; Canonico, B. Extracellular Vesicles as New Players in Drug Delivery: A Focus on Red Blood Cells-Derived EVs. Pharmaceutics 2023, 15, doi: 10.3390/pharmaceutics15020365.
[2] Simeone, P.; Celia, C.; Bologna, G.; Ercolino, E.; Pierdomenico, L.; Cilurzo, F.; Grande, R.; Diomede, F.; Vespa, S.; Canonico, B.; et al. Diameters and Fluorescence Calibration for Extracellular Vesicle Analyses by Flow Cytometry. 2020, 21, doi:10.3390/IJMS21217885.
[3] van der Pol, E.; van Leeuwen, T.G.; Yan, X. Misinterpretation of Solid Sphere Equivalent Refractive Index Measurements and Smallest Detectable Diameters of Extracellular Vesicles by Flow Cytometry. Sci. Rep. 2021, 11, 1–6, doi:10.1038/s41598-021-03015-2.
[4] Carrillo-Rodríguez, P.; Robles-Guirado, J.Á.; Cruz-Palomares, A.; Palacios-Pedrero, M.Á.; González-Paredes, E.; Más-Ciurana, A.; Franco-Herrera, C.; Ruiz-de-Castroviejo-Teba, P.A.; Lario, A.; Longobardo, V.; et al. Extracellular Vesicles from Pristane-Treated CD38-Deficient Mice Express an Anti-Inflammatory Neutrophil Protein Signature, Which Reflects the Mild Lupus Severity Elicited in These Mice. Front. Immunol. 2022, 13, 1–29, doi:10.3389/fimmu.2022.1013236.

MAIN LECTURE - SIRA

ASSISTED REPRODUCTIVE TECHNOLOGIES IN DROMEDARY CAMEL IN SAUDI ARABIA

Taher Kamal Osman

(1) Department of Advanced Biotechnology and Research, Salam Veterinary Group, Saudi Arabia

Corresponding author: **Taher Kamal Osman** - taher.kamal@svg.sa

The dromedary camel (DC) is a central element of the Bedouin, nomadic, rural identity; it is the object of special consideration, even though, in Arab culture, the horse prevails in the animal hierarchy. The dromedary camel is venerated by the Quran, in which several verses are dedicated to it; camels were used as payment of the annual portion of a Muslim's personal fortune given as charity to people in need. Although now urban dwellers, many Arabs in the Gulf countries spend weekends in tents among the camels that they still possess (Faye 2016). Camel racing is a traditional cultural heritage sport in contemporary Arab Gulf Countries since it preserves and promotes national cultural identity (Khalaf, 1999). It is even part of 'beauty contests': the most beautiful subjects are determined and can therefore worth very high prices, although still lower than prices achieved by the best racing animals (several million of USD) (Faye 2022). Given the high value of beauty subjects, fraudulent beauty treatments have increased in recent years and thus diagnostic procedures for detecting such treatments have been developed as well (Tharwat et al., 2021). The total number of camels recorded in the world was 35,525,270; in Saudi Arabia and Oman, camels represents the 15-35% of the total livestock whereas in United Arab Emirates and Qatar such percentage is higher than 80% (Faye, 2020). Opened in June 2020, the Salam Veterinary Group camel hospital is the first camel hospital in Saudi Arabia and the largest veterinary hospital in the world. The hospital provides several services (laboratory analysis, internal medicine, surgery, obstetric and gynaecology) and is now a reference point for all dromedary camel breeders in Saudi Arabia and of some elite breeders of other gulf countries. Some of the provided reproductive services are: diagnosis and treatment of male and female infertility, reproductive surgery, on farm female reproductive evaluation, neonatology clinic.

Dromedary camels are seasonal breeders and induced ovulators, the gestation last about 13 months and females produce a single calve every two-three years (Monaco et al., 2015). The embryo transfer (ET) technique increases the reproductive efficiency and speed up the genetic selection of elite animals. Due to the commercial interest in racing and beauty camels, great efforts have been implemented for developing the multiple ovulation technique in camels, from 1990 onward. Most of the services provided by the SVG camel hospital are, indeed, related with the ET. There are five SVG ET units in Saudi Arabia with around 600 donors and 2000 recipients enrolled in the program. The centre provides also clinical and reproductive management of customers' elite bulls used for the program. Overall, an average of about 300-400 animals are evaluated by ultrasonography every day and around 10 superovulated donors are flushed. The target for the 2023-2024 breeding season will be the doubling the ET units and thus the number of animals enrolled in the program. After an initial evaluation of the ovarian activity, the donors' ovarian stimulation protocol begins at the early stage of the follicular wave or after a progesterone based synchronization protocol: the dominant follicle is induced to ovulate and 2500 IU eCG are injected on day 4 after ovulation, 400 mg pFSH are then administered in decreasing doses (2 x 80 mg, 2 x 60 mg, 2 x 40 g 2 x 20 mg) over a period of 4 days. The dams can produce between 4–30 follicles and are mated at around 7–10 days, when the majority

of follicles are of 1.3–1.7 cm in diameter. A more practical soperovulation treatment with eCG (3000–4000 IU) has shown to produce a comparable embryo yield to FSH (Manjunatha, et al., 2020). Also the use of slow release hyaluronan preparation of Folltropin-V and Pluset in a split-injection protocol (2 IM injection 48 hours apart) has been shown to produce satisfactory results (Manjunatha et al., 2019). A high incidence of superstimulation failure, premature luteinization of follicles, variability in ovarian response among females, and apparent refractoriness to repeated superstimulation treatments are, however, common with dromedary donors and major challenges are encountered particularly when dealing with aged heifers: a careful monitoring and understanding of the ovarian response to the superovulation regime is therefore necessary. For maximum embryo recovery, flushing is performed 7–8 days after mating by using a18-22 gauge Foley catheter with the cuff placed at the base of each horn. Embryo recovery rates (embryo recovered/ovulations) are highly variable and depend on many factors including superovulation treatment, fertility of the donor and the male, management, collection date and technician experience. Embryos are transferred into recipients on day 5 or day 6 after ovulation; in our daily routine recipients are randomly selected after serial ultrasound examination and those with a mature follicle are injected with GnRH 24–48 h after the donor is mated. Other synchronization methods (use of progesterone or meclofenamic acid) are also available but they are not practical when dealing with a large number of animals such as in our reality. Pregnancy rates are highly variable (from 50 to 70% in the first month of pregnancy) and several factors could be involved in the success of the program (Karen and Mansour 2020). From our observation it seems that even severe weather conditions could significantly affect the early embryo mortality and the abortion rates.

Embryo cryopreservation could facilitate a wider application of ET in camelids. The embryo could be transported, thawed and transferred after timing of natural ovarian cycle thus without the need to synchronize recipients with donors. Greater success combining ethylene glycol and glycerol as cryoprotectants and replacing sucrose with galactose in the warming media have been reported (Herrid et al., 2017); even if not still commercially appliable, at SVG camel hospital, embryo are frozen by vitrification when a large number of embryo is recovered and good quality recipients are not available.

The technology of production by in vitro fertilization (IVF), intracytoplasmic sperm injection (ICSI) or somatic cell nuclear transfer (SCNT) has been slow in camelids when compared with other domestic animal species (Wani, 2021). There is great interest of the SVG camel hospital for the development of ICSI and SCNT; somatic cells are collected and frozen as well as epididymal spermatozoa in case of sudden death of high value donors or bulls, waiting for the full development of the ICSI and SCNT for obtaining elite offspring. Regarding the male germoplasm conservation there are considerable efforts for the development of efficient semen preservation techniques but despite the satisfactory post thaw motility of spermatozoa, the results of artificial insemination are still poor. Appropriate technique for semen processing, investigation about the role of seminal plasma in semen preservation are currently performed at the SVG reproduction laboratories. In addition, studies on sperm sorting for AI in superovulated donors, with fresh semen, have been planned for the next breeding season. Concluding: the dromedary camel agrobusiness sector represent an important market in Saudi Arabia and the management of dromedary camel reproduction represent a great opportunity for providing veterinary services as well as for research studies in assisted reproductive technologies.

Bibliography :

Faye, B. (2016). Des dromadaires et des hommes au moyen-orient: Identité et modernité. Anthropology of the Middle East, 11(1), 51-65. doi:10.3167/ame.2016.110106. Faye B. (2022). Is the camel conquering the world? Animal Frontiers, Volume 12, Issue 4, August 2022, Pages 8–16, https://doi.org/10.1093/af/vfac034. Khalaf S (1999). Camel Racing in the gulf, notes onthe evolution of a traditional cultural sport. Antropos, 94, 85-106. Tharwat, M., Al-Hawas, A., & Alboti, Y. (2021). infrared thermography in dromedary camels with injected and stretched lips in camel beauty pageants. Journal of Camel Practice and Research, 28(3), 355-359. doi:10.5958/2277-8934.2021.00054.0. Faye, B. (2020). How many large camelids in the world? A synthetic analysis of the world camel demographic changes. Pastoralism, 10(1) doi:10.1186/s13570-020-00176-z. Monaco D, Padalino B, Lacalandra GM. 2015 Distinctive features of female reproductive physiology and artificial insemination in the dromedary camel species. Emirates Journal of Food and agric ulture, 27, 328-337. Manjunatha, B. M., Al-Hosni, A., & Al-Bulushi, S. (2020). Superovulation of dromedary camels with eCG: An effective and practical technique. Theriogenology, 151, 112-118. doi: 10.1016/j.theriogenology.2020.04.017. Manjunatha, B. M., Al-Hosni, A., & Al-Bulushi, S. (2019). Simplified superovulation protocols in dromedary camels (camelus dromedarius). Theriogenology, 126, 214-221. doi: 10.1016/j.theriogenology.2018.12.006. Anouassi A. & Tibary A. 2013. Development of a large commercial camel embryo transfer program: 20 years of scientific research. Animal Reproduction Science, 136, 211-221.Karen A &Mansour N. 2020. Factors affecting pregnancy rates and pregnancy losses after embryo transfer in dromedary camels, Animal Reproduction Science, 221. H errid M, Billah M, Skidmore JA. Successful pregnancies from vitrified embryos in the dromedary camel: Avoidance of a possible toxic effect of sucrose on embryos. Anim Reprod Sci 2017; 187:11623. Wani N.A., 2021 In vitro embryo production (IVEP) in camelids: Present status and future perspectives, Reproductive Biology, 21, 2021.

MAIN LECTURE - SOIPA

UPDATING ON EPIDEMIOLOGY OF TRICHINELLIDS: THE ITALIAN SITUATION

Fabrizio Bruschi, Michele Corritore, Maria Angela Gomez-Morales

(1) Department of Translational Research, N.T.M.S., School of Medicine, Università di Pisa, 56123 Pisa, Italy
(2) Struttura di Malattie Infettive, IRCCS "Casa Sollievo della Sofferenza", San Giovanni Rotondo(FG), Italy
(3) Department of Infectious Diseases, National Institute of Health, 00161 Rome

Corresponding author: **Fabrizio Bruschi** - fabrizio.bruschi@unipi.it

The parasitic nematode *Trichinella* is characterized by an extremely wide host range and geographical distribution [1]. At present, ten different species have been described, *Trichinella spiralis, Trichinella nativa. Trichinella nelsoni, Trichinella britovi, Trichinella murrelli, Trichinella patagoniensis, Trichinella chanchalensis* (included in the clade of encapsulating species) and *Trichinella pseudospiralis, Trichinella papuae* and *Trichinella zimbabwensis* (belonging to the clade of not encapsulated species) plus three different genotypes [1]. Trichinellosis, the disease caused by this parasite, is controlled in countries in Europe and U.S.A., but it is emerging in both the industrialised and medium-low income countries, such as China, Argentina and some eastern European ones [1]. As regards Italy, in the last decade 164 human cases occurred, mostly of them caused by T. britovi, the only autochtonous species in our Country, being the wild boar the most frequent source meat [2]. In three cases the aetiological agent was identified as T. spiralis, but the pork meat was consumed in Romania. Of particular interest was the outbreak occurred closed to Genoa in 2015, which involved 30 individuals, in fact despite the fact that the meat (presumably wild boar) responsible for the outbreak was not available for analyses, by serological and cellular immunology evaluation it was possible to attribute the outbreak to T. pseudospiralis [3]. This Trichinella species circulates in Italy in the wildlife, in particular it was detected in red fox (Vulpes vulpes), birds (Strix aluco, Athene noctua, Milvus milvus) and wild boars (Sus scrofa), and more recently in a wolf [4], but the Genoa outbreak was the first observation of human trichinellosis cases caused by T. pseudospiralis in Italy. In 2023, an outbreak of trichinellosis occurred in San Marco in Lamis (municipality of the Gargano National Park, Apulia Region). Although the source of infection was not traced back, twelve persons were diagnosed by clinical and laboratory investigations.

[1] Pozio, E., 2021. Epidemiology. In: Bruschi, F. (Ed.), Trichinella and Trichinellosis. Academic Press, London, pp. 185–263.
[2] Pozio E., 2023. Capillariasis and trichinellosis. In: Bruschi F., Pozio E. (Eds.) De Carneri Parassitologia Generale ed umana, CEA, Milan, pp. 439-447.
[3] Gómez-Morales MA, Mazzarello G, Bondi E, Arenare L, Bisso MC, Ludovisi A, et al.. Second outbreak of Trichinella pseudospiralis in Europe: clinical patterns, epidemiological investigation and identification of the etiological agent based on the western blot patterns of the patients' serum. Zoonoses Public Health. 2021 Feb;68(1):29-37.
[4] Ricchiuti L., Petrini A., Interisano M., Ruberto A., Salucci S., Marino L., et al. First report of Trichinella pseudospiralis in a wolf (Canis lupus italicus). Int J Parasitol Parasites Wildl. 2021, 24: 195-198.

MAIN LECTURE - SOIPA

LEISHMANIA TARENTOLAE VS. *LEISHMANIA INFANTUM* IN THE MEDITERRANEAN BASIN: FUTURE CHALLENGES AND OPPORTUNITIES

Jairo Alfonso Mendoza-Roldan, Andrea Zatelli, Domenico Otranto

(1) Dipartimento di Medicina Veterinaria, Università degli Studi di Bari, Valenzano, Italy
(2) Faculty of Veterinary Sciences, Bu-Ali Sina University, Hamedan, Iran

Corresponding author: **Domenico Otranto –** d.otranto@veterinaria.uniba.it

The genus *Leishmania (Kinetoplastida, Trypanosomatidae*) infect mostly mammals, except for the subgenus *Sauroleishmania*, associated to reptiles. *Leishmania tarentolae* is a protist isolated from geckoes in the Old World where it occurs in sympatry with *Leishmania infantum*, the causative agent of canine leishmaniasis (CanL) [1]. Though, L. tarentolae is considered non-pathogenic and is transmitted by herpetophilic Sergentomyia spp. sand flies, the high similarity in gene composition (i.e., 90%) with *L. infantum*, has made this protist a model for recombinant protein production and vaccine candidate [2]. On the other hand, the finding of Sergentomyia spp. sand flies feeding mainly of human and other mammals' blood has spurred our attention toward investigating its circulation in a canine population [1,3]. In addition, the prevalence and distribution of *L. tarentolae* and *L. infantum* in reptilian hosts was also investigated [4]. Hence, we will provide an overview about the research being carried out. In particular, the finding of dogs and lacertid lizards infected with *L. tarentolae* suggest their sympatric circulation, with a potential overlap in vertebrate hosts [1,3]. Meanwhile, the finding of *L. infantum* infection in European reptiles raises new questions on the epidemiology of *L. infantum* [4]. The interactions between these two species should be further investigated in both vectors and hosts in endemic areas of CanL, especially where control programs are established.

[1] Mendoza-Roldan, J. A., Latrofa, M. S., Iatta, R., R S Manoj, R., Panarese, R., Annoscia, G., Pombi, M., Zatelli, A., Beugnet, F., & Otranto, D. (2021). Detection of Leishmania tarentolae in lizards, sand flies and dogs in southern Italy, where Leishmania infantum is endemic: hindrances and opportunities. Parasites & vectors, 14(1), 461.
[2] Pombi M, Giacomi A, Barlozzari G, Mendoza-Roldan J, Macrì G, Otranto D, Gabrielli S. Molecular detection of Leishmania (Sauroleishmania) tarentolae in human blood and Leishmania (Leishmania) infantum in Sergentomyia minuta: unexpected host-parasite contacts. Med Vet Entomol. 2020 Dec;34(4):470-475.
[3] Iatta R, Mendoza-Roldan JA, Latrofa MS, Cascio A, Brianti E, Pombi M, Gabrielli S, Otranto D. Leishmania tarentolae and Leishmania infantum in humans, dogs and cats in the Pelagie archipelago, southern Italy. PLoS Negl Trop Dis. 2021 Sep 23;15(9):e0009817.
[4] Mendoza-Roldan JA, Votýpka J, Bandi C, Epis S, Modrý D, Tichá L, Volf P, Otranto D. Leishmania tarentolae: A new frontier in the epidemiology and control of the leishmaniases. Transbound Emerg Dis. 2022 Sep;69(5):e1326-e1337.

AIPVET

IMMUNOHISTOCHEMICAL CHARACTERIZATION OF CELLS OF THE MONONUCLEAR PHAGOCYTE SYSTEM IN THE MOUSE

Luca Bertola (1,2), Andrea Gaspare Valenti (3), Simone Canesi (1,2), Andrea Cappelleri (1,2), Eugenio Scanziani (1,2), Lucia Minoli (4), Camilla Recordati (1,2)

(1) Department of Veterinary Medicine and Animal Sciences, University of Milan, Via dell'Università, 6 26900 Lodi (LO) – Italy
(2) Mouse and Animal Pathology Laboratory (MAPLab), Fondazione Unimi, Viale Ortles, 22/4 20139 Milano (MI) – Italy.
(3) Department of Infectious Diseases and Pathobiology, Institut für Tierpathologie, University of Bern. 4. Aptuit Srl, an Evotec Company, Via Alessandro Fleming, 4 37135 Verona – Italy

Corresponding Author: **Luca Bertola** - luca.bertola@unimi.it

The mononuclear phagocyte system (MPS) represents a heterogeneous system of cells comprising circulating monocytes, classically and alternatively activated macrophages, and dendritic cells, involved in several physiologic and pathologic processes.1 Immunohistochemistry has been proven useful to detect MPS cells and their distribution in murine tissues in different experimental settings. However, no comprehensive references exist in literature for tissue MPS cells at steady state.2,3 Therefore, the aim of the study was to immunohistochemically characterize MPS cells in a selected set of murine healthy tissues and pathological conditions.

Formalin-fixed and paraffin embedded sections of liver, spleen, renal and mesenteric lymph nodes, kidneys, and lungs from 25 C57BL/6 mice were examined. Sections from inflammatory (necro-suppurative hepatitis and *Pneumocystis murina pneumonia*) and neoplastic (human fibrosarcoma xenograft and mammary carcinoma syngraft) conditions were also included. All sections were immunostained for Iba1, F4/80, MARCO, CD206, Ym1, HO1, Arginase-1, iNOS and MHC-II. A qualitative (morphology and distribution) and a semi-quantitative (number and staining intensity) analysis of positive cells was carried out for each organ and marker.

Our analyses confirmed the morphological and immunophenotypic heterogeneity of murine MPS cells, in both healthy and pathological tissues. The combination of morphology, location and immunohistochemical positivity allowed us to precisely define MPS subsets in specific cases (i.e., Kupffer cells in the liver, marginal zone macrophages and marginal metallophilic macrophages in the spleen) and to speculate on specific MPS cell functions (i.e., pro-tumoral CD206+ and Arginase+ tumor associated macrophages).

This study provides useful insights into the actual role of the various MPS cell subsets in different organs and conditions in the mouse and intends to be an immunohistochemical reference of murine MPS cells.

1. Gordon S and Pluddemann A. The mononuclear phagocytic system. Generation of diversity. Front Immunol. 10:1893, 2019.
2. Guilliams M, et al. Dendritic cells, monocytes and macrophages: a unified nomenclature based on ontogeny. Nat Rev Immunol. 14(8): 571-578, 2014.
3. Rheg J, et al. The Utility of Immunohistochemistry for the Identification of Hematopoietic and Lymphoid Cells in Normal Tissues and Interpretation of Proliferative and Inflammatory Lesions of Mice and Rats. Tox Path. 40: 345-374, 2012.

TISSUE-RESIDENT MACROPHAGES AND YOLK SAC ERYTHRO- MYELOID PROGENITORS' ACTIVITY: THE EFFECT OF IN OVO ADMINISTRATION OF A LIVE AND INACTIVE MIXTURE

Lucia Biagini (1), Livio Galosi (1), Sara Berardi (1), Alessandra Roncarati (1), Subeide Mari (1), Valentina Grifantini (1), Giacomo Rossi (1)

(1) Università degli Studi di Camerino, Scuola di Bioscienze e Medicina Veterinaria

Corresponding Author: **Lucia Biagini** - lucia.biagini@unicam.it

The yolk sac is the oldest extraembryonic membrane supporting embryogenesis whose original role was related to the absorption of nutrients from the yolk. Although the yolk is absent in the eggs of placental mammals, this continues to represent a fundamental organ for embryonic development [1,2]. Among various functions, it has been observed that tissue-resident macrophages originate from erythro-myeloid progenitors (EMP) derived from the yolk sac [3]. EMP cells after the onset of the circulation, through the blood vessels, colonise various organs starting from the liver, main embryonic hematopoietic site, giving rise to tissue-resident monocytes. We previously observed that in the chicken the in ovo administration of a probiotic blend (SLAB51®) or a patented tyndallized mixture, are able to stimulate both embryonic macrophages maturation and neo-angiogenesis in the yolk sac [4].The aim of this study is to detect whether the expansion of the hematopoietic line in the yolk sac is able to increase the number of monocyte – macrophages cells in different embryonic organs before hatching. 120 Ross 308 eggs were incubated and divided into three groups: on day 18 of incubation group P received $1x10^5$ CFU of probiotic bacteria, diluted in 0.05 ml of saline sterile solution; group E received a mixture composed by 850 CFU of dry extract enriched with $1x10^5$ CFU of probiotic bacteria in 0.05 ml of sterile saline solution; remaining eggs, as control group (C), were inoculated exclusively with saline sterile solution. For each group, after 8, 12, 24 and 36 hours from administration, the yolk sac was collected in 10 eggs. For the last sampling also the different embryo organs were collected, and samples were subsequently processed for histological and immunohistochemical evaluation. The organs evaluated were liver, lungs, spleen, Bursa of Fabricius and intestinal tract. The analysis of these tissues showed a stronger presence of Iba1-positive macrophages in P and E, compared to C especially in liver, lungs and intestinal tract. The same tissues were also tested with CD204 and MHC class II and TLR4. This cellular pattern indicates a marked induction in the number of tissue resident macrophages after the in ovo stimulation by different live and inactive PAMPs and especially in the liver, confirming the hypothesis that the stimulation already observed in the yolk sac has direct effect also in the maturation of the innate immune system in other organs.

[1] Ross, C., & Boroviak, T. E. (2020). Origin and function of the yolk sac in primate embryogenesis. Nature communications, 11(1), 3760. https://doi.org/10.1038/s41467-020-17575-w

[2] Garceau, V., Balic, A., Garcia-Morales, C., Sauter, K. A., McGrew, M. J., Smith, J., Vervelde, L., Sherman, A., Fuller, T. E., Oliphant, T., Shelley, J. A., Tiwari, R., Wilson, T. L., Chintoan-Uta, C., Burt, D. W., Stevens, M. P., Sang, H. M., & Hume, D. A. (2015). The development and maintenance of the mononuclear phagocyte system of the chick is controlled by signals from the macrophage colony-stimulating factor receptor. BMC biology, 13, 12. https://doi.org/10.1186/s12915-015-0121-9

[3] Gomez Perdiguero, E., Klapproth, K., Schulz, C., Busch, K., Azzoni, E., Crozet, L., Garner, H., Trouillet, C., de Bruijn, M. F., Geissmann, F., & Rodewald, H. R. (2015). Tissue-resident macrophages originate from yolk-sac-derived erythro-myeloid progenitors. Nature, 518(7540), 547–551. https://doi.org/10.1038/nature13989

[4]Biagini L., Galosi L., Berardi S., Roncarati A., Mari S., Grifantini V., Rossi G. Early macrophage recruitment and increased neoangiogenesis in the yolk sac of chicken embryos inoculated with a probiotic blend. Atti LXXV CONVEGNO SISVET, Lodi 15-18 Giugno 2022, pag. 97.

PRECLINICAL ASSESSMENT OF THE CNS TOXICITY IN PAEDIATRIC SUBJECTS: HISTOLOGICAL, HISTOCHEMICAL AND IMMUNOHISTOCHEMICAL EVALUATION IN SPRAGUE DAWLEY NEWBORN RATS

S. Canesi (1,2), D. Bianchi (2), M. Longo (3), E. Tamborini (3), M. Messina (3), V. Zanzottera (3), M. Russo (3), C. Bernardi (3) and C. Recordati (1,2)

(1) Department of Veterinary Medicine and Animal Sciences, University of Milan, Lodi, Italy
(2) Mouse and Animal Pathology Laboratory, UniMi Foundation, Milan, Italy
(3) Accelera S.r.l, Nerviano, Italy

Corresponding Author: **Simone Canesi** - simone.canesi@unimi.it

Preclinical toxicity studies in pups and juvenile animals are required by the Regulatory Authorities to identify possible adverse effects of new drugs to support their paediatric development (1). Among these drugs, anaesthetics administered in the perinatal period can cause neurotoxic effects. The sequence of key events in the brain development is considered highly conserved between humans and rodents (2). In particular, the postnatal period between days 7-10 in rats is the most adequate to investigate toxic effects induced by drugs to be administered to a paediatric population ranging in age from birth to the first months of life (3). The preclinical assessment of the brain development includes behavioural trials, learning and memory tests. However, these evaluations cannot be performed in non-completely grown animals. Thus to study the potential neurotoxic effects particularly during the first week of life, the best approach remains brain histopathology. The aim of this study was to develop a histopathological method to evaluate the potential neurotoxicity of new anaesthetics in paediatric population. 7 day-old Sprague Dawley rats were intraperitoneally injected with Vehicle (4.34% lactose solution 8 ml/kg) or Ketamine (KET) (20 mg/kg at 2.5 ml/kg) five times every 90 minutes. This treatment schedule allowed to maintain the sedation for 6 consecutive hours to mimic the paediatric clinical situation. Two hours and 16 hours post-treatment 2 animals per group were sacrificed, the brain was harvested, formalin-fixed and processed for histology (H&E), histochemistry (Fluoro-Jade) and immunohistochemistry (Cleaved Caspase-3 (CC3), yH2AX, Iba-1 and GFAP) to evaluate neuronal damage and glial response. The number of positive cells (CC3; yH2AX) and the % area of positive cells (Iba-1 and GFAP) were evaluated through digital image analysis (QuPath) in the cortex and hippocampus. The routine H&E staining, and the immunostaining with CC3 and yH2AX revealed that KET induced neuronal apoptosis in neonatal rats, and that apoptosis was more prominent at 2h than at 16h post-treatment, accompanied by a mild activation of the Iba1+ microglia. No consistent results were obtained with Fluoro-Jade. In conclusion, we set up a histological and immunohistochemical method to evaluate KET-induced neuronal damage that could be applied to evaluate the neurotoxicity of new paediatric anaesthetics.

1 ICH guideline S11 on nonclinical safety testing in support of development of paediatric pharmaceuticals – September 26th 2020.
2 Semple D. et al. (2013). Brain development in rodents and humans: identifying benchmarks of maturation and vulnerability to injury across species. Prog Neurobiol.0:1-16.
3 Buelke-Sam J. (2003). Comparative schedules of development in rats and humans: implications for developmental neurotoxicity testing. Paper presented at: 2003 Society of Toxicology Annual Meeting; Salt Lake City, UT.

DEVELOPMENT OF A STANDARDIZED PROTOCOL FOR ASSESSING IMMUNODEFICIENCY IN MOUSE MODELS

Andrea Cappelleri (1)(2), Simone Canesi (1)(2), Luca Bertola (1)(2), Valentina Capo (3)(4), Alessandra Zecchillo (3)(4), Valeria Martini (1), Pierangelo Moretti (1), Anna Villa (3)(4), Camilla Recordati (1)(2), Eugenio Scanziani (1)(2)

(1) Università degli Studi di Milano, Dipartimento di Medicina Veterinaria e Scienze Animali
(2) Fondazione UniMi, Mouse and Animal Pathology Laboratory
(3) IRCCS San Raffaele Scientific Institute, San Raffaele Telethon Institute for Gene Therapy
(4) National Research Council, Institute of Genetic and Biomedical Research, Milan Unit

Corresponding Author: **Andrea Cappelleri** - andrea.cappelleri@unimi.it

Immunodeficient mouse strains are used in several fields of biomedical research, including organ transplantation, oncology, stem cell research and therapy. (1,3) The immunodeficient status of the models needs to be thoroughly characterized to guarantee reliable experimental outcomes. However, no standardized system for assessing immunodeficiency in mice currently exists, and an objective comparison of the immunodeficiency of various immunodeficient mouse strains is therefore difficult. (2)

The aim of the study was to develop a standardized multi-approach protocol for the morpho-phenotypical assessment of the immunodeficiency in immunodeficient mouse models.

Four immunodeficient mouse strains on C57BL/6 background were selected: CD40L, Was, Rag1, and Rag1 R972Q. The main lymphoid organs belonging to 5 mice for each strain were weighed and analyzed by means of histology, immunohistochemistry, and flow cytometry and compared to 5 C57BL/6 wild-type controls. Hematology and bone marrow cytology were also performed. The main immune cell populations were investigated, including lymphocytes, monocytes/macrophages, neutrophils, and NK cells.

Histology revealed the main morphological differences between controls and immunodeficient strains, which were particularly evident in the most severe immunodeficient models (i.e. lack of follicles in lymph nodes and spleen of Rag1 mice). Immunohistochemistry allowed to examine the distribution of the immune cell populations and to identify subtle differences (i.e. discrimination between germinal centers and poorly cellular PALS in the spleen of Was mice). Flow cytometry was useful to quantify the immune cell populations. Hematology highlighted the main differences between strains in the circulating immune cells. No differences were found in the bone marrow myeloid:erythroid ratio measured on cytological smears.

Our protocol revealed to be an effective tool for characterizing the morpho-phenotypical immunodeficient features in different mouse strains, successfully combining the quantification of the immune cell populations with their spatial distribution within the lymphoid organs. Besides confirming immunodeficient phenotypes in well-known mouse models, this protocol might be useful for a standardized characterization of immunodeficient mouse models of new development, and of those with possible unintended immune alterations.

[1] Belizário JE. Immunodeficient mouse models: an overview. Open Immunol J. 2009;2.
[2] Ye W et al. Quantitative evaluation of the immunodeficiency of a mouse strain by tumor engraftments. J Hematol Oncol. 2015;8:59.
[3] Zhang B et al. Mouse models with human immunity and their application in biomedical research. J Cell Mol Med. 2009;13:1043–1058.

SUBCLINICAL MASTITIS REGULATION BY EXTRACELLULAR VESICLES MIRNAS IN DAIRY COWS

Cuccato Matteo (1), Divari Sara (1), Giannuzzi Diana (2), Moretti Riccardo (1), Leroux Christine (3,4), Sacchi Paola (1) and Francesca Tiziana Cannizzo (1)

(1) Department of Veterinary Sciences (DSV), University of Turin, Grugliasco (TO), Italy
(2) Department of Agronomy, Food, Natural Resources, Animals and Environment (DAFNAE), University of Padua, Legnaro (PD), Italy
(3) INRAE, Université Clermont Auvergne, VetAgro Sup, UMR Herbivores, Saint Genes-Champanelle, France (4) Department of Food Science and Technology, University of California, Davis, UC Davis, CA, USA

Corresponding Author: **Matteo Cuccato** - matteo.cuccato@unito.it

Mastitis is a severe inflammation of the mammary gland of dairy cows. Typically, mastitis monitoring is based on measuring the somatic cell count (SCC) on milk samples. However, diagnosis of subclinical infection is problematic since the milk and the udder appear normal, reducing the possibilities of early treatments [1]. Recently, several studies revealed that miRNAs vehiculated by extracellular vesicles (EVs) have a fine-tuned role in the regulation of bovine mastitis [2]. The main aim of this study was to investigate the milk EVs miRNome profile during subclinical mastitis. Milk samples were collected from a total of 174 dairy cows during routine monitoring tests. EVs were isolated from milk by size exclusion chromatography (SEC) columns and their characterization was done by western blot using TSG101 and CD9 markers. Then, miRNAs were extracted from EVs using an automated extractor and a smallRNA-sequencing protocol was performed on selected samples. The differential analysis was conducted using an SCC threshold value of 200,000 cells/ml to allocate samples in each group. Finally, the functional analysis to predict miRNAs role was performed using miRWalk, OmicsNet and Cytoscape. A total of 1997 differentially expressed microRNAs were identified, and the majority of them were downregulated. A total of 68 miRNAs was obtained by false discovery rate (FDR) value < 0.05. Finally, the functional analysis highlighted miR-455-5p, miR-1301-3p and miR-503-5p, which were the main downregulated microRNAs. These miRNAs are related to innate immunity and inflammation processes. In particular, miR-1301-3p has already been reported as regulating mastitis in bovine experimentally infected with *Staphylococcus aureus* [3], but no studies investigating the role of miRNAs in spontaneous bovine mastitis are present in literature. Intriguingly, miR-455-5p seems to have anti-inflammatory activity in humans and its downregulation in our samples could be explained by the onset of subclinical mastitis [4]. In conclusion, investigating miRNAs role in subclinical mastitis could allow a better understanding of the disease and an improvement in its diagnosis and management.

[1] Ruegg PL. 2017. A 100-Year Review: Mastitis detection, management, and prevention. Journal of Dairy Science, 100:10381–10397. [2] Ji Z-H et al. 2022. Exosomes in mastitis - research Status, opportunities, and challenges. Animals, 12:2881.
[3] Luoreng ZM et al. 2021. Expression profiling of microRNA from peripheral blood of dairy cows in response to *Staphylococcus aureus*-infected Mastitis. Frontiers in Veterinary Science 8:691196.
[4] Torabi S et al. 2019. miR-455-5p downregulation promotes inflammation pathways in the relapse phase of relapsing-remitting multiple sclerosis disease. Immunogenetics 71:87–95.

PATHOLOGICAL FINDINGS IN CAPTIVE SNAKES FROM PRIVATE ENCLOSURES IN THE NORTH-EAST OF ITALY

Luca Spadotto (1), Laura Cavicchioli (1), Diego Cattarossi (2), Erica Marchiori (3), Cinzia Centelleghe (1)

(1) Dept. of Comparative Biomedicine and Food Science, University of Padova (PD)
(2) Clinica veterinaria "Casale sul Sile", Casale sul Sile (TV)
(3) Dept. of Animal Medicine, Production and Health, University of Padova (PD)

Corresponding Author: **Luca Spadotto** - luca.spadotto@unipd.it

Recently, the public interest in snakes is drastically increased, in parallel with the number of exotic animals housed as pets. A variety of snake species are kept under human care and the most common species are Boas and Pythons. Whereas these animals' pathology isn't fully investigated, the main causes of disease rely on infections, coming from bacteria (Gram-negative, but also *Mycoplasma* and *Clamydophila*), viruses (Herpesvirus, Adenovirus, Arenavirus, Paramyxovirus), fungi (*Ophidiomyces*, *Chrysosporium*), parasites such as Protozoa (*Cryptosporidium serpentis*), Metazoa (*Rhabdias* spp.), ticks and mites (*Ophionyssus natricis*; *Entonyssus* and *Hamertonia*) (1). Regarding non-infectious causes, nutritional imbalances or deficiencies causing gout or obesity are very common, while neoplasia is rarely reported (2).
This study aims to investigate the causes of mortality in 12 captive boids and 8 pythons dead between 2019 and 2023 in zoological gardens and households in Veneto. A complete and standardized post-mortem investigation was performed, and all organs were sampled for further histological, microbiological, and parasitological examinations.
Of all the 20 animals examined, 9 snakes showed signs of severe inflammatory diseases; the target organs were equally distributed between lungs (3/9) and kidneys (3/9), while liver (2/9) and skin (1/9) were less affected. Three animals presented end-stage kidney disease, while 11 snakes were affected by degenerative hepatic conditions, such as lipidosis (9/11), glycogenosis (1/11) and cysts (1/11). In 1 python multiple masses of a rectal carcinoma with hepatic metastases were observed, while 1 boa showed a severe encephalitis. To date, 14 snakes were RT-PCR-tested for Reptarenavirus: 5 showed a multiorgan positivity, while 4 showed a single organ positivity. Histologically, the viral inclusion bodies related to inclusion body disease were clearly and diffusely visible in 5 of these animals. *Cryptosporidium serpentis* was detected in the feces of one boa and its presence was confirmed at histology through observation of the protozoan within the gastric epithelium. Oocysts referable to *Caryospora* spp. were found in three animals.
In conclusion, from our observations, Reptarenavirus remains an endemic disease in many snakes and needs continuous diagnostic attention. Infection remains an important cause of disease in snakes, but the importance of dietary conditions should be underlined to address owners to proper maintenance.

[1] Mitchell MA, CHAPTER 7 - SNAKES, Editor(s): Mitchell MA, Tully TN, Manual of Exotic Pet Practice, W.B. Saunders, 2009, Pages 136-163, ISBN 9781416001195.
[2] Ossiboff RJ, CHAPTER 37 - SERPENTES, Editor(s): Terio KA, McAloose D, St. Leger J, Pathology of wildlife and zoo animals, 2018, Pages 891-913, ISBN 9780128053065

COMPARISON AND ASSESSMENT OF 3D IN-VITRO MODELS OF BOTTLENOSE DOLPHIN (*Tursiops Truncatus*) CELL LINES ON NOVEL COLLAGEN-FREE SCAFFOLDS AND ON MATRIGEL

Lucrezia Ferretti (1), Valentina Moccia (1), Cinzia Centelleghe (1), Andrea Venerando (2), Monica Dettin (3), Elisabetta Sieni (4), Federico Caicci (5), Massimo Castagnaro (1), Valentina Zappulli (1), Sandro Mazzariol (1)

(1) Department of Comparative Biomedicine and Food Science, University of Padova, Legnaro, Italy
(2) Department of Agrifood, Environmental and Animal Sciences, University of Udine, Udine, Italy
(3) Department of Industrial Engineering, University of Padova, Padova, Italy
(4) Department of Theoretical and Applied Sciences, Insubria University, Varese, Italy
(5) Department of Biology, University of Padova, Padova, Italy

Corresponding Author: **Sandro Mazzariol** – sandro.mazzariol@unipd.it

Several species of cetaceans are increasingly threatened worldwide by multifactorial causes, including natural and anthropogenic stressors, which often act synergistically (Fossi et al., 2007). Among cetaceans, dolphins, as apex predators, represent perfect sentinels of the marine ecosystem in terms of pollution and viral spread (Wallach et al., 2015). The creation of in-vitro complex systems to assess the interaction of their organ systems with environmental conditions, is therefore of very considerable interest.
Two-dimensional (2D) cell cultures are considered relatively poor models to mimic the natural structure of tissues (Polson et al., 2012). This disadvantage has led to the establishment of a variety of three-dimensional (3D) culture systems in which cells grow in complex interactions with the extracellular matrix (ECM), better mimicking the condition of cells in-vivo.
However, to date, only few attempts have been carried out for the establishment of cetacean 3D culture models.
Thus, in this study, different 3D systems of bottlenose dolphin (*Tursiops truncatus*) skin fibroblasts have been developed. Particularly, two novel scaffolds (hyaluronic acid and ionic-complementary self-assembling peptides such as: RGD-EAbuK and EAbuK-IKVAV) have been compared to Matrigel.
Histological (Hematoxylin and Eosin and Masson Trichrome) and fluorescent staining with both nuclear and membrane dyes (Hoechst, and Red CellBrite), electron microscopy (TEM) analyses and viability assays (CellTiter-Glo 3D Cell) have been performed on the models. RT-PCR has instead been used to detect ECM components produced by cells into the 3D systems.
Results showed that Matrigel induced cells to form complex spheroids while the scaffolds allowed single cells to grow producing a collagenous ECM containing collagen1a1, laminin B1 and elastin. Moreover, comparing the two novel scaffolds, the EAbuK-IKVAV one resulted the most suitable for the cells growth in term of quantity and viability.
The development of this innovative approach is the first step towards the possibility to create 3D in-vitro models of dolphin cells, that will enable the study of several pathological processes such as chronic exposure to low doses of chemical stressors resembling the in-vivo conditions.

[1] Fossi, M.C., Casini, S., Marsili, L., 2007. Potential toxicological hazard due to endocrinedisrupting chemicals on Mediterranean top predators: state of art, gender differences and methodological tools. Environ Res. 104: 174–82.
[2] Wallach, A.D., Izhaki, I., Toms, J.D., Ripple, W., J, Shanas, U., 2015. What is an apex predator? Oikos 124: 1453–1461.
[3] Polson, A.G., Fuji, R.N., 2012. The successes and limitations of preclinical studies in predicting the pharmacodynamics and safety of cell-surface-targeted biological agents in patients. Br J Pharmacol. 166: 1600-2.

DOLPHIN AND SEA TURTLE NECROPSY AND VIRTUAL REALITY: EXPLORING OPEN SOURCE AND FREE 3D SOFTWARE TOOLS

Jean Marie Graic (1), Giorgia Corazzola (1), Tommaso Gerussi (1), Ksenia Orekhova (1), Cinzia Centelleghe (1), Steffen De Vreese (2), Antonio Di Bello (3), Stefano Ciccarelli (3), Carla Grattarola (4), Antonio Petrella (5), Giuseppe Lucifora (6), Sandro Mazzariol (1)

(1) Dept. of Comparative Biomedicine and Food Science, University of Padova (PD)
(2) Laboratori d'Aplicacions Bioacústiques (LAB) - Universitat Politécnica de Catalunya (Es)
(3) Dept. of Veterinary Medicine, University of Bari (BA)
(4) Centro di Referenza per le Indagini Diagnostiche sui Mammiferi marini spiaggiati (C.Re.Di.Ma.); Istituto Zooprofilattico Sperimentale del Piemonte Liguria e Valle d'Aosta
(5) Istituto Zooprofilattico Sperimentale di Puglia e Basilicata
(6) Istituto Zooprofilattico Sperimentale del Mezzogiorno

Corresponding Author: **Sandro Mazzariol** - sandro.mazzariol@unipd.it

In studying anatomy, physiology, pathology and related scientific branches, the classical methods used in education are 2D images, drawings, or videos. Since they are used to explain 3- or 4-dimensional processes, it often requires creative approaches to teaching and visualization that inherently leave an amount of uncertainty in the understanding of in-vivo structures and processes. Therefore, these classical methods are often accompanied by hands-on learning, such as in the veterinary necropsy room, also requested by EAEVE accreditation. On one side, the use of animal carcasses gives to the student a direct contact with real cases studies giving also the opportunity to enter in contact with practical activities. On the other hand, it is difficulty repeatable lacking of standardization and it is limited by the challenges of carcass retrieval.
The rapid development of immersive technologies in various industries, together with communities involved in developing free and open-source software, provides an opportunity to try and fill this gap. With the support of the Project ECCE AQUA, funded under the Project of Excellence call by MUR in 2017, we have developed and implemented a virtual reality necropsy experience that looks at conveying knowledge on complex morphology, physiology and pathology of marine mammals. The experience allows the user to perform a postmortem evaluation of a dolphin and sea turtle. It is a completely immersive experience using Virtual Reality goggles and controllers that takes the user through the necropsy protocol in a virtual necropsy environment. This experience also guide students to samples and data collection and reporting besides the use of standardized procedures.
Besides the presented results, the developed tools can be applied in education and training to various audiences, from students to experts, to create visually stunning, real-time virtual educational platforms to acquire knowledge and experience before applying it in real-world conditions. We also explain the workflow that has given rise to the application, from obtaining anatomical data from wild stranded animals through the software workflow and the final output as a VR experience, and show how it can be applied in other situations and institutions at almost no cost, and we highlight the many opportunities that virtual reality provides for versatile and interactive education in person and remotely.

HISTOPATHOLOGICAL FINDINGS IN *Orthonychiurus Folsomi* FOLLOWING ORAL EXPOSURE TO BRAKE SYSTEM WEAR PARTICULATE MATTER

Karen Power (1), Giulia Papa (2), Ilaria Negri (2), Paola Maiolino (1)

(1) Department of Veterinary Medicine and Animal Productions, University of Naples "Federico II"
(2) Department of Sustainable Crop Production DI.PRO.VE.S., University "Cattolica del Sacro Cuore", Piacenza.

Corresponding Author: **Karen Power** - karen.power@unina.it

Collembola are among the most widespread and abundant soil-dwelling arthropods. They contribute to the well-being of soil ecosystems by participating to plant litter decomposition, regulating soil fertility and flow of energy through above- and below-ground food webs, helping to maintain biodiversity. To date, soil ecosystems are experiencing great pressure due to pollution deriving from anthropocentric activities, among these there is surely road traffic. In urban areas non-exhaust particulate matters (PM) (abrasion of vehicle components, e.g., braking system, tires, and friction with road surfaces) represent an important source of heavy metal contamination and roadside soils are nowadays becoming an important reservoir of heavy metals, potentially causing great harm to its biodiversity. In this study we investigated morphological alterations of the midgut, ovary and abdominal fat body of *Orthonychiurus folsomi* (*Collembola:Onychiuridae*) fed low (LC) and high concentration (HC) dose of metal-based particulate matter (PM) from brake systems (brake pad and brake disc).
Histopathological examination of LC-treated samples did not show any significant alterations, compared to the control samples; while HC-treated samples revealed alterations of the examined organs: the midgut showed numerous basophilic vacuoles of variable size in the cytoplasm of the epithelial cells while the lumen contained colorless e reflective material and scattered small hemocytes, ovaries showed different degrees of alteration ranging from inflammation to necrosis, while the fat body presented signs of degeneration and necrosis. Moreover, focal areas of melanisation were observed in all analyzed organs. Our results suggest that oral exposure *of O. folsomi* to brake wear PM can cause dose-dependent effects on the midgut, ovaries, and fat body, which can impair their functionality and lead to reduction to reproductive capacity, humoral immunity and survival rate. However, currently the pathogenic mechanism underlying histological alterations is unknown and more studies are needed.

[1] Callejas-Chavero, A., Reyes-Lechuga, G., García-Gómez, A., Palacios-Vargas, J.G., Flores-Martínez, A., Castaño-Meneses, G., 2022. Diesel effects on some population attributes of Orthonychiurus folsomi Schäffer 1900 (Collembola: Onychiuridae) under laboratory conditions. Environmental Monitoring and Assessment 194. https://doi.org/10.1007/s10661-022-10385-1
[2] Filser, J., 2002. The role of Collembola in carbon and nitrogen cycling in soil. Pedobiologia 46, 234–245. https://doi.org/10.1078/0031-4056-00130 OECD, 2020. Non-exhaust Particulate Emissions from Road Transport: An Ignored Environmental Policy Challenge. OECD Publishing, Paris. https://doi.org/10.1787/4a4dc6ca-en
[3] Papa, G., Capitani, G., Capri, E., Pellecchia, M., Negri, I., 2021.
Vehicle-derived ultrafine particulate contaminating bees and bee- products. Science of The Total Environment 750, 141700. https://doi.org/10.1016/j.scitotenv.2020.14170

PLATELET RICH PLASMA (PRP) INDUCES ACTIVATION OF NRF2-MEDIATED ANTIOXIDANT DEFENSE RESPONSE IN EQUINE TENOCYTES EXPOSED TO HYDROGEN PEROXIDE (H2O2): AN IN VITRO STUDY

Alessia Tognoloni (1), Desirèe Bartolini (2), Ilaria Porcellato (1), Antonio Di Meo (1), Marco Pepe (1), Francesco Galli (2), Elisabetta Chiaradia (1)

(1) Dipartimento di Medicina Veterinaria, Università degli Studi di Perugia
(2) Dipartimento di Scienze Farmaceutiche, Università degli Studi di Perugia

Corresponding Author: **Alessia Tognoloni** - alessia.tognoloni@gmail.com

The etiology of tendinopathies, that represent common disabling conditions occurring in equine athletes, is still unclear. Growing evidences suggested the involvement of reactive oxygen species (ROS) and oxidative stress (OS) in the onset and progression of tendon disorders [1]. Cells can counteract the deleterious effect of ROS by activating the intracellular antioxidant response regulated by the erythroid nuclear factor 2-related factor 2 (Nrf2) [2]. This transcription factor represents also a possible target of exogenous antioxidant which can help cells to cope OS. In the last few years, the platelet rich plasma (PRP) has emerged as a promising therapeutic approach in the treatment of tendon injury. In addition to the well-known high regenerative potential demonstrated on various tissues including tendons, ligaments, muscles and cartilage, important antioxidant effects are also attributed to PRP [3].

In this study the effects of PRP on equine tenocytes exposed to OS were investigated, to better understand the different PRP biological outcomes under OS conditions. First of all, a possible cytoprotective effect of 10% PRP on tenotoxicity induced by H2O2 (0, 0.1, 0.5, 1mM) was tested through MTT reduction assay. To test the antioxidant effect of PRP, the protein oxidation levels, as protein carbonyls and protein-4HNE adducts, were measuring by Oxyblot analysis, as well as, the nuclear translocation of Nrf2 and the levels of some antioxidant enzymes as superoxide dismutase 2 (SOD-2), catalase (CAT), heme oxygenase 1 (HO-1), NAD(P)H oxidoreductase quinone-1 (NQO1), glutamate cysteine ligase catalytic (GCLC) and glutathione S-transferase subunit (GSTs) were analysed by western blotting. Furthermore, the enzymatic activity of CAT and GSTP was also evaluated.

Results indicated the cytoprotective effect of PRP against H2O2-induced cytotoxicity on tenocytes. PRP was resulted also able to reduce protein oxidative damage and induced activation of antioxidant cellular response through Nrf2 nuclear translocation. In addition, the levels of analysed antioxidant enzymes and the enzymatic activity of GST and CAT were increased.

This study adds new findings on molecular mechanisms underlying the tendon regenerative properties of PRP, that could be the result of activation of different cellular pathways, aimed to restore the tenocytes homeostasis to promote the repair of tendon tissue, whose poor healing and high relapse rate is one of the main features.

[1] Lui P.P.Y., et al. Roles of Oxidative Stress in Acute Tendon Injury and Degenerative Tendinopathy—A Target for Intervention. Int. J. Mol. Sci. 23, 2022.
[2] Kovac S. et al. Nrf2 regulates ROS production by mitochondria and NADPH oxidase. Biochimica et Biophysica Acta - General Subjects 1850 (4): 794–801, 2015.
[3] Pauly S., et al. The effect of autologous platelet rich plasma on tenocytes of the human rotator cuff. BMC Musculoskeletal Disorders 19 (1): 1–10, 2018.

Bartonella SPP. DISTRIBUTION ASSESSMENT IN RED FOXES (*Vulpes vulpes*) COUPLING GEOSPATIALLY-BASED TECHNIQUES

Annalisa Viani (1), Tommaso Orusa (2,3,4), Sara Divari (5), Stella Lovisolo (5), Stefania Zanet (5), Enrico Borgogno - Mondino (2), Riccardo Orusa (1), Enrico Bollo (5)

(1) Istituto Zooprofilattico Sperimentale del Piemonte, Liguria e Valle d'Aosta (IZS PLV) SC Aosta-CeRMAS (National Reference Center for Wildlife Diseases), Località Amerique 7/C, 11020 Quart (AO), Italy
(2) Department of Agricultural, Forest and Food Sciences (DISAFA), GEO, 4Agri DISAFA Lab, Università degli Studi di Torino, Largo Paolo Braccini 2, 10095 Grugliasco, Italy
(3) Earth Observation Valle d'Aosta - eoVdA, Località L'Île-Blonde 5, 11020 Brissogne, Italy
(4) IN.VA spa, GIS Unit, Località L'Île-Blonde 5, 11020 Brissogne, Italy; 5 Department of Veterinary Sciences (DSV), Università degli Studi di Torino, Largo Paolo Braccini 2, 10095 Grugliasco, Italy

Corresponding Author: **Enrico Bollo** – enrico.bollo@unito.it

Nowadays, reports on the presence of *Bartonella* spp. in Italy, especially in wild canids, are few in literature. In this study, the prevalence of Bartonella spp. was investigated with reference to fox populations (*Vulpes vulpes* L.) culled or found dead in the provinces of Cuneo and Biella (Piedmont) and Aosta Valley (NW Italy). The analysis was carried out at municipality level by coupling molecular diagnostic techniques and satellite remote sensing with the aim of testing possible relationships between pathogen presence and environmental conditions. The DNA was extracted from the spleen of 114 sampled animals; 7.9% (9/114) of samples yielded *Bartonella* spp. DNA by means qPCR (ssrA gene). Positive samples were further analyzed by end-point PCR for ssrA, gltA and rpoB genes of *Bartonella* spp.: PCR products of eight samples were sequenced and, based on the results, *B. schoenbuchensis* R1 was found to be the most abundant *Bartonella* species (62.5%, 5/8). Candidatus "Bartonella gerbillinarum" was found in 25% (2/8) of samples.
Concerning remote sensing contribution, data from NASA USGS Landsat 4-9 missions (TOA collection), ranging from 2011 to 2022, were processed in Google Earth Engine. Assuming (as reported in literature) that pathogens, especially in rangelands, can be influenced by humidity, the Tasseled Cap Wetness index (TCW) was computed (Baig & DeVries, 2020) for each date temporal profile consisting of composite images for each meteorological season. Seasonal TCW was positively associated to Bartonella spp. infection in foxes as infection was always associated to TCW>0.7. This threshold seems to be an important parameter for the *Bartonella* spp. risk mapping.
To verify the relationship between TCW and *Bartonella spp*. presence, the Moran's index I was calculated per each composite imagery to test the existence of a spatial correlation, and LISA (Local Indicators of Spatial Autocorrelation) maps at the municipal scale were created. Moran I proved to be always ≥0.90, and therefore statistically significant. Moreover, Canonical Corresponding Analysis between pathogen prevalence and municipal-based TCW show a strong link between positivity and TCW, demonstrating the possible use of TCW as a parameter to facilitate disease management and control.
In conclusion, a simultaneous spatial correlation between *Bartonella* spp. DNA presence in foxes of the municipalities and the TCW was detected.

SWINE CONJUNTIVITIS SUSTAINED BY *Mycoplasma* SP. 1654_15. A NOVEL SPECIES CLOSELY RELATED TO *Mycoplasma hyorhinis*

Cornaggia M. (1), Merenda M. (1), Vio D. (2), Gastaldelli M. (1), Conci V. (1), Righetti V.(1), Bottinelli M. (1), Sandri G. (3), Catania S. (1)

(1) Istituto Zooprofilattico delle Venezie, Sezione Territoriale di Verona, Laboratorio di Diagnostica Clinica e Sierologia di Piano, Buttapietra (VR), Italia.
(2) Istituto Zooprofilattico delle Venezie, Sezione Territoriale di Pordenone, Laboratorio di Patologia e Benessere della Specie Suina, Cordenons (PN), Italia
(3) Soc.Ag. La Pellegrina Spa, Verona (VR), Italia

Corresponding Author: **Matteo Cornaggia** - mcornaggia@izsvenezie.it

Conjunctivitis is an uncommon finding in commercial swine herds, usually considered to be a secondary symptom of respiratory or viral systemic disease, or a result of irritation by dust or ammonia.
In 2020 in Germany a new species of *Mycoplasma*, so called *Mycoplasma* sp. 1654_15, strictly related to *Mycoplasma hyorhinis*, was isolated in an outbreak of swine conjunctivitis.
We investigated cases of conjunctivitis in 4 wean-to-finish swine farms (A, B, C, D).
Lesions were characterized by a bilateral conjunctivitis with oedema and redness of the palpebral surfaces in addition, a mild rhinitis was described.
Eye, nasal and lungs swabs were collected and *Mycoplasma hyorhinis* was isolated and characterized from all samples analyzed.
A sequencing was conducted on all the isolated obtained by eye swabs and resulted positive for *Mycoplasma* sp. 1654_15, assuming that the pathogen is widespread also in Italy.
The common diagnostic tools available are not able to discern between *Mycoplasma* sp. 1654_15 and *Mycoplasma hyorhinis*. Therefore, new diagnostic tools are required to gain the correct diagnosis of swine conjunctivitis.
The inflammation of the conjunctiva represents a distress source that leads to a poor animal welfare condition, considered to be a cause of aggressive behavior and a risk factor for diseases developement and cause of economic loss for the farm.

[1] Hennig-Pauka I., Sudendey C., Kleinschmidt S., Ruppitsch W., Loncaric I., Spergser J. (2020) Swine conjunctivitis associated with a novel mycoplasma species closely related to *Mycoplasma hyorhinis*. Pathogens 10(1):13.
[2] McAuliffe L., Ellis R.J., Lawes J.R., Ayling R.D., Nicholas R.A. (2005) 16S rDNA PCR and denaturing gradient gel electrophoresis; a single generic test for detecting and differentiating *Mycoplasma* species. J.Med.Microbiol. 54(8):731-739.
[3] Resende T.P., Pieters M., Vannucci F.A. (2019) Swine conjunctivitis outbreaks associated with *Mycoplasma hyorhinis*. Journal of Veterinary Diagnostic Investigation 31(5):766-769.

THE ROLE OF FELINE LEUKOCYTES ANTIGENS (FLAS) IN CATS' SUSCEPTIBILITY TO CORONAVIRUSES INFECTION: A BIOINFORMATIC INVESTIGATION

Michela Buonocore (1, 2), Davide De Biase (1), Domenico Sorrentino (1), Anna Maria D'Ursi (1), Antonio Giordano (3), Orlando Paciello (2)

(1) Department of Pharmacy, University of Salerno, Fisciano, Salerno, Italy.
(2) Department of Veterinary Medicine and animal production, University of Naples Federico II, Napoli, Italy
(3) Sbarro Institute for Cancer Research and Molecular Medicine, Center for Biotechnology, College of Science and Technology, Philadelphia, USA
(4) Department of Medical Biotechnologies, University of Siena, I-53100 Siena, Italy University, Philadelphia, PA 19122, USA

Corresponding Author: **Davide De Biase** - ddebiase@unisa.it

Several studies suggested that the Feline Leukocyte Antigen (FLA), a homolog of the human HLA, plays a pivotal role in the transmission of viruses to cats (1). Recently, an Italian study demonstrated that different Class I/II human leukocyte antigen (HLA) alleles might define an individual susceptibility to SARS-CoV-2 spreading, contributing to the differences in the distribution of the infection through different populations (2). SARS-CoV-2 is a highly transmissible and pathogenic virus for humans, yet its infectivity has also been reported in domestic animals (3). To date, the mechanisms underlying the susceptibility of humans and several animals to SARS-CoV-2 infection are still largely elusive. With these premises, this study aimed to explore a novel bioinformatic approach to predict the role of FLAs in susceptibility to SARS-CoV-2 and two distinct Feline Coronaviruses (FCoV) infection (namely, Feline enteric Coronavirus, FeCV and Feline Infectious Peritonitis Virus, FIPV) in cats. We performed an epitope mapping of 9 residues long amino acids deriving from SARS-CoV-2, FeCV and FIPV glycoproteins and predicted their affinities for different alleles of the three main loci in class I FLAs (4) as deposited on UniProt database. The predicted complexes with the most promising affinities were then subjected to molecular docking and molecular dynamics simulations to understand the contribution of each residue to the binding energy in the pocket. Results showed that the FLA-I H locus (alleles H-*00401, H-*008012 and H-*00701) is largely responsive to many epitopes deriving from spike and replicase proteins of the analyzed coronaviruses. Moreover, a sequence alignment of all predicted epitopes for the three loci reveaLed that repeated patterns of amino acids are preserved between the sequences with the best interaction scores. Even though certain epitopes were uniquely expressed by the proteins in exam, other epitopes were found on glycoprotein belonging to coronaviruses targeting other animals, suggesting a cross-reactivity to different antigens in cats. These preliminary findings can be exploited as a tool for the prediction of CoVs' sensibility in a wide number of species and also as a future perspective to further explore FCoVs pathogenesis and for the development of peptide vaccines able to activate the immune systems for untreatable diseases, like FIP.

[1] Liang, R. et al. Major Histocompatibility Complex Class I (FLA-E*01801) Molecular Structure in Domestic Cats Demonstrates Species-Specific Characteristics in Presenting Viral Antigen Peptides. J. Virol. 92, (2017).
[2] Correale, P. et al. HLA-B*44 and C*01 Prevalence Correlates with Covid19 Spreading across Italy. Int. J. Mol. Sci. 21, 5205 (2020).
[3] Buonocore, M. et al. New putative animal reservoirs of SARS-CoV-2 in Italian fauna: a bioinformatic approach. Heliyon 6, e05430 (2020).
[4] Holmes, J. C. et al. Polymorphisms and tissue expression of the feline leukocyte antigen class I loci FLAI-E, FLAI-H, and FLAI-K. Immunogenetics 65, 675–689 (2013).

FELINE CORONAVIRUS-ASSOCIATED INFLAMMATORY MYOPATHY IN CAT: A PRELIMINARY STUDY

Emanuela Vaccaro (1), Valeria Russo (1), Giovanni Valerio Salanti (1), Gianluca Miletti (2) , Pasquale Santoro (3) , Serenella Papparella (1) , Orlando Paciello (1)

(1) Department of Veterinary Medicine and Animal Production, University Federico II of Naples, Napoli, Italy
(2) Istituto Zooprofilattico Sperimentale del Mezzogiorno, Portici, Italy
(3) Veterinary Diagnostic Laboratory (Di.Lab.), Napoli, Italy.

Corresponding Author: **Emanuela Vaccaro** - emanuela.vaccaro@unina.it

Inflammatory myopathies (IM) are a heterogeneous group of acquired muscle disorders that are underestimated in human and veterinary medicine. They can be associated with bacterial, parasitic, and viral infections, but in many cases, they are still unknown. Most inflammatory myopathies are considered autoimmune disorders in which skeletal muscle is inappropriately targeted by the immune system. In animals, IM has been described in association with several infectious diseases, such as leishmaniasis in dogs, piroplasmosis in horses and feline immunodeficiency virus (FIV) in cats. The morphological and molecular features of these IMs support an autoimmune pathogenesis, as circulating anti-muscle autoantibodies have been found in each of these infectious diseases. The aim of this study is to investigate the presence of inflammatory infiltrates and circulating autoantibodies to skeletal muscle in feline Coronavirus (FCoV)-positive cats. The study was conducted in 10 FCoV-positive cats and 10 control cats. A necropsy examination was performed, and skeletal muscle samples from quadriceps femoris and triceps brachii were taken. For each muscle specimen, a complete histological study was performed to evaluate the morphological changes. An immunohistochemical and immunofluorescence study was performed to identify inflammatory cells and the presence of feline Coronavirus in muscle tissues. In addition, sera from 5 serologically FCoV-positive cats and 3 controls were tested for circulating anti-skeletal muscle autoantibodies by indirect immunofluorescence (IIF). Preliminary results showed inflammatory myopathies in 8/10 (80%) skeletal muscle samples from FCoV-infected cats. The inflammatory cells were mainly B lymphocytes (CD79+) and fewer T cells (CD3+) and several FCoV-positive cells. The MHC class I and II were also overexpressed in skeletal muscle fibers of FCoV-positive cats. The sera of FCoV-positive cats showed a dilution-dependent immunofluorescent staining intensity on muscle fibers in up to 1:1000 dilutions compared with controls. In conclusion, our results showed that natural FCoV infection is frequently associated with IM and a B cell-dominated inflammatory response. Muscle fibers positivity to sera of FCoV-positive cats suggests the possible presence of circulating autoantibodies. Further studies are needed to better elucidate the pathogenetic mechanisms underlying FCoV-associated IM in cats.

[1] Dalakas, M. C. Inflammatory myopathies: update on diagnosis, pathogenesis and therapies, and COVID-19-related implications. Acta Myol. 39, 289–301 (2020).
[2] Hussein HM, R. E. The role of viral infections in the development of autoimmune diseases. Crit Rev Microbiol. 45, 394–412.
[3] Kapsogeorgou EK, T. A. Autoantibodies in Autoimmune Diseases: Clinical and Critical Evaluation. Isr Med Assoc J. 18, 519–524.

REPLACING SOY AND CORN WITH BAKERY-BY-PRODUCT: IS FORMER FOODSTUFFS RECYCLING SUSTAINABLE FOR POULTRY HEALTH AND PRODUCTION?

Ippolito Dorotea (1), Srikanthithasan Karthika (1), Colombino Elena (1), Diaz Vicuna Elena (1), Miniscalco Barbara (1), Bergagna Stefania (2), Gariglio Marta (1), Fiorilla Edoardo (1), Schiavone Achille (1), Forte Claudio (1), Capucchio Maria Teresa (1)

(1) Department of Veterinary Science, University of Turin, Largo P. Braccini 2, 10095, Grugliasco, TO.
(2) Istituto Zooprofilattico Sperimentale del Piemonte, Liguria e Valle d'Aosta, via Bologna, 148, 10154, Torino.

Corresponding Author: **Maria Teresa Capucchio** - mariateresa.capucchio@unito.it

Sustainability is an imperative for feed and food resources. Replacing unsustainable dietary ingredients with former foodstuffs, such as bakery by-products (BBP), has been proposed for livestock [1], but their use in poultry diet has not been studied recently.
The present work explores the effects of BBP inclusion levels as a substitute for corn and soy in broilers.
In total, 200 one-day-old male ROSS-308 chicks were assigned into 4 groups (5 replicates and 10 birds/pen). The dietary treatments were: Control (CTRL: commercial feed), L-BBP (6.25% BBP), M-BBP (12.5% BBP) and H-BBP (25% BBP). Growth performance, leukogram and blood serum biochemistry were assessed. At 33 days of age, 15 birds/group were slaughtered, and duodenum, jejunum, ileum, liver, spleen and Fabricius's bursa (FB) were sampled, formalin-fixed and paraffin-embedded. The intestinal morphometry and histology of the organs were assessed as per previously published parameters [2]. Depending on Shapiro-Wilk test results, morphometrical differences were tested by One-way and Two-way ANOVA with Tukey post hoc or Kruskall-Wallis test, while Fisher's exact test compared degrees of inflammation (significant $p<0.5$, R 4.2.2. software).
The groups showed no differences in final weight, while average daily feed intake (g/d) and feed conversion ratio showed a linear decrease ($p<0.05$) following the increasing levels of BBP inclusions (CTR: 62.5 g/d, 1.57 and H-BBP: 57.5 g/d, 1.39 respectively).
Blood cholesterol, triglycerides and glutamic-pyruvic transaminase increased ($p<0.05$) with increasing BBP inclusion, but no differences in histological liver structure were observed. Diet influenced gut development, as birds fed H-BBP and L-BBP had lower ($p<0.05$) (length) villi and mucosa and wider ($p<0.008$) (width) villi. These effects were more marked in the duodenum of L-BBP ($p=0.02$). Mild/moderate enteritis was found in all groups with more severe pictures in L-BBP than H-BBP and M-BBP (OR 13.2, $p=0.006$ and OR=27.5, $p=0.001$), explaining the morphometric results. A systemic response was not evident, since the spleen/FB reactivity or leukogram did not vary among the groups.
The inclusion of BBP in the broiler diet showed overall desirable effects on production, that were not so straightforward on gut health. Further investigations, such as characterization of gut microbiota and metabolites, are, underway to provide a complete insight into the effects of BBP inclusion on gut health and development.

1. Pinotti, L.; Luciano, A.; Ottoboni, M.; Manoni, M.; Ferrari, L.; Marchis, D.; Tretola, M. Recycling Food Leftovers in Feed as Opportunity to Increase the Sustainability of Livestock Production. Journal of Cleaner Production 2021, 294, 126290, doi:https://doi.org/10.1016/j.jclepro.2021.126290.
2. Colombino, E.; Karimi, M.; Nu, M.A.T.; Tilatti, A.A.; Oddon, S.B.; Calini, F.; Bergamino, C.; Fiorilla, E.; Gariglio, M.; Gai, F.; et al. Effects of Feeding a Thermomechanical, Enzyme-Facilitated, Coprocessed Yeast and Soybean Meal on Growth Performance, Organ Weights, Leg Health, and Gut Development of Broiler Chickens. Poultry Science 2023, 102, 102578, doi:https://doi.org/10.1016/j.psj.2023.102578.

INNOVATIVE DIGITAL TEACHING TECHNIQUES IN VETERINARY PATHOLOGY: REVIEW OF THE LITERATURE AND PERSPECTIVE STUDIES

M. Giacomazzo (1), M. Gelain (1), L. Cavicchioli (1), S. Mazzariol (1), V. Zappulli (1), M. Castagnaro (1)

(1) Department of Comparative Biomedicine and Food Science (BCA), University of Padova, Legnaro (PD), Italy.

Corresponding Author: **Marta Giacomazzo** - marta.giacomazzo@phd.unipd.it

The global deployment of COVID-19 has accelerated the integration of digital and online resources into veterinary education [1]. As a result, there is a growing interest in the use of innovative digital techniques (DTs) as an integrative learning method to provide students with new educational experiences.
Moreover, according to EAEVE, during undergraduate education, there is an urgent need to prepare the veterinary students to meet the challenges of DTs and artificial intelligence (AI) [2].
The teaching of Veterinary Pathology and Pathological Anatomy deals with various critical aspects such as performing necropsies [3] and describing and interpreting morphological lesions. In most cases, gross pathology relies on the use of images and specimens from the necropsy room while histopathology and cytopathology have been taught using the light microscope and providing slides to each student [4]. Recently, the teaching mode has changed and digital pathology (DP) has been identified as an excellent alternative teaching method along with online digital resources [4], such as videos and serious games, or in other words, student-centered activities.
Here we present a review of 50 papers from international peer-reviewed journals analyzed to understand the learning impact and satisfaction on veterinary students in relation to the use of DTs in Veterinary Pathology. The results highlight that DTs and AI may improve the quality and efficiency of learning and teaching processes, through the dissemination of online resources and can be particularly useful when practical training is reduced [2]. The choice of the best type of DTs to use should be based on specific educational objectives and contexts.
The results of our review will be used to set up new tools directed toward the development of the best DT-integrated teaching model aimed at supporting classical education. The integrated learning process will be analyzed subjectively through the acquisition of student opinions (written survey) and objectively through comparative evaluation (exam grade).
In summary, the current cohort of veterinary students requires integrative DTs to acquire the Day One Competences [2] at their best. However, the extent to which new DTs must be integrated into classical teaching methods needs to be carefully evaluated to achieve the adequate teaching balance. Thus, the use of DTs and AI should aim at enhancing theoretical and practical training rather than replacing it [2].

[1] Muca, E.; Cavallini, D.; Odore, R.; Baratta, M.; Bergero, D.; Valle, E. Are Veterinary Students Using Technologies and On line Learning Resources for Didactic Training? A Mini-Meta Analysis. Educ. Sci. 2022, 12, 573. https://doi.org/10.3390/educsci12080573
[2] Avignon, D.et al. Report of the ECCVT expert working group on the impact of Digital Technologies & Artificial Intelligence in veterinary education and practice final report, Adopted by ECCVT on 28 September 2020
[3] Munson, L., et al. "Elements of good training in anatomic pathology." Veterinary pathology 47.5 (2010): 995-1002
[4] Bertram CA, Klopfleisch R. The Pathologist 2.0: An Update on Digital Pathology in Veterinary Medicine. Vet Pathol. 2017 Sep;54(5):756-766. doi: 10.1177/0300985817709888. Epub 2017 Jun 5. PMID: 28578626

HEMOSIDERIN-LADEN MACROPHAGES IN CANINE MAMMARY TUMOURS

Giada Giambrone (1), Cecilia Vullo (2), Roberto Puleio (3), Claudia Rifici (1), Gabriele Marino (1), Alessandra Sfacteria (1)

(1) Department of Veterinary Sciences, University of Messina.
(2) Department of ChiBioFarm, University of Messina.
(3) Histopathology and Immunohistochemistry Laboratory, Istituto Zooprofilattico Sperimentale della Sicilia.

Corresponding Author: **Giada Giambrone** - giada.giambrone@studenti.unime.it

Macrophages are among the main actors in "cancer immunoediting" with several functions including recycling iron and packaging it in hemosiderin (1). However, even though tumour-associated macrophages (TAMs) are widely studied in breast cancer and canine mammary gland tumours (2), hemosiderin-laden macrophages (HLMs) have not received as much attention. It has been suggested that HLMs have a positive correlation with angiogenesis, as reported in some pathological processes like the atherosclerotic vascular disease (3). Considering the growing interest in iron metabolism in cancer, this study aims to evaluate the presence of HLMs in canine mammary tumours.
Canine mammary tumours presenting aggregates of pigmented macrophages were chosen, including simple and non-simple carcinomas. Prussian blue and Meguro stain were performed to assess the presence of iron. Immunohistochemistry was carried out for Macrophage Marker (MAC/387), VEGF and VEGFR.
Evaluation in H&E sections showed that these groups of cells were variously localized. Some of them were in areas of dysplasia, others in large aggregates in the peritumoral stroma, often interspersed in areas of lymphocyte infiltration, or as groups of few cells in the intratumoral stroma or intermingled with the neoplastic epithelium. Some conspicuous groups were also located near necrotic or degenerated areas. Several mast cells, detected by the immunostain for mast cell tryptase, were admixed within the larger aggregates. These pigmented cells were variably stained with Prussian blue and reacted strongly with DAB in the Meguro staining method thus confirming the presence of iron within them. At the immunohistochemistry, HLMs were negative for the MAC/387 but strongly positive for VEGFR and VEGF.
Considering their presence in different histotypes and different grades of carcinoma, the hypothesis is that HLMs may protect cancer cells from hypoxia acting as angiogenic promoters by contributing iron to both the neoplastic cells and the tumour inflammatory microenvironment (4). This latter hypothesis is supported by the mingling with mast cells, M1 macrophages and lymphocytes. Furthermore, the negativity for MAC/387 might underline that they are not M1 macrophages and that they could represent, also in canine mammary gland tumours, a novel subtype of macrophage that deserves to be better investigated for the potential role in cancer biology.

[1] Shen L et al. Crosstalk between Macrophages, T Cells, and Iron Metabolism in Tumor Microenvironment. Oxid Med Cell Longev., Feb 2;2021:8865791, 2021.
[2] Monteiro LN et al. Tumour-associated macrophages: Relation with progression and invasiveness, and assessment of M1/M2 macrophages in canine mammary tumours. Vet J., Apr;234:119-125, 2018.
[3] Guo L et al. Linking Hemorrhage, Angiogenesis, Macrophages, and Iron Metabolism in Atherosclerotic Vascular Diseases. Arterioscler Thromb Vasc Biol., Apr;37(4):e33-e39, 2017.
[4] Jung M et al. Macrophage iron homeostasis and polarization in the context of cancer. Immunobiology, Feb;220(2):295-304, 2015.

EXPRESSION OF CARNITINE PALMITOYL TRANSFERASE 1A (CPT1A) IN EQUINE SARCOID: A PRELIMINARY STUDY OF METABOLIC REPROGRAMMING

Manuela Martano (1), Karen Power(1), Gennaro Altamura (1), Brunella Restucci (1)

(1) Department of Veterinary Medicine and Animal Production, University of Naples Federico II, Via Delpino, 1 80137 Naples, Italy.

Corresponding Author: **Manuela Martano** - manuela.martano@unina.it

Equine sarcoid is the most common skin neoplasia of fibroblastic origin in horses. Delta Bovine papillomavirus (BPV type 1, 2 and 13) are considered the main etiologic agent, however a multifactorial aetiology has been proved involving genetic risk factors and skin trauma (Borzacchiello et al., 2019). We have previously shown an overexpression of VEGF in sarcoid fibroblasts associated with abnormal vessel structures which lead to a hypoxic condition, confirmed also by an upregulation of HIF-1α (Martano et al., 2020). Despite such hypoxic condition, sarcoid fibroblasts progressively produce excessive connective tissue, leading to excessive accumulation of extracellular matrix (ECM) due to an impaired degradation (2). There is growing evidence that in the case of hypoxic conditions, neoplastic cells adjust their metabolism to utilize different energy sources which allow them to survive and proliferate. One of these alternative pathways is fatty acid oxidation (FAO) mediated by the carnitine system (CS), where Carnitine Palmitoyl transferase 1A (CPT1A) has a central role in controlling the entrance of fatty acids into the mitochondria for oxidation (Qu et al.,2016). Even if metabolic dysregulation appears to be a hallmark of neoplastic diseases, this aspect has not yet been studied in equine sarcoid. Therefore, 20 samples of BPV positive equine sarcoids and 5 normal skins were processed for immunohistochemical (IHC) and biochemical analysis to evaluate the expression levels of CPT1A. 12/20 sarcoid samples (60%) showed a strong IHC staining for CPT1A in more than 75% of sarcoid fibroblasts, 4/20 sarcoid samples (20%) were moderately immunostained in 50-75% sarcoid fibroblasts, and the remaining 4/20 sarcoid samples (20%) were weakly immunostained in 25-50% of sarcoid fibroblasts. All normal skin samples showed weak IHC staining in basal epidermis and few dermal fibroblasts. Results of Western blotting were consistent with IHC. Our results share similarities with those showed in human papillomavirus associated tumours, which reported an upregulation of CPT1A expression in tumour samples compared to control samples, suggesting that virus could plays a role in metabolic regulation of tumours, thought the manipulation of metabolism in infected host cells for its better adaptation (Li et al., 2021). It may be worthwhile to explore if BPV could have a role in metabolic regulation of equine sarcoid to better understand the pathogenesis of this tumour and to explore the possibility of use the metabolism as target for future therapy.

[1] Borzacchiello, G., Corteggio, A. Equine Sarcoid: State of the art. Ippologia 2009, 20, 7–14.
[2] Martano, M; Altamura, G., Power, K., Restucci, B., Carella, F., Borzacchiello, G., Maiolino, P.Evaluation of Hypoxia-Inducible Factor-1 Alpha (HIF-1α) in Equine Sarcoid: An Immunohistochemical and Biochemical Study Pathogens 2020, 14;9(1),58.
[3] Qu, Q., Zeng, F., Liu, X., Wang, Q.J., Deng, F. Fatty acid oxidation and carnitine palmitoyl transferase I: Emerging therapeutic targets in cancer. Cell Death Dis. 2016, 7, e2226.
[4] Li, B., Sui, L. Metabolic reprogramming in cervical cancer and metabolomics perspectives. Nutr Metab 2021, 18(1):93.

A PRELIMINARY EVALUATION OF THE PROGNOSTIC ROLE OF HER-2 AND HER-3 IMMUNOHISTOCHEMICAL EXPRESSION IN CANINE MELANOMA

Francesca Parisi (1), Arianna Pecorari (1), Luigi Aurisicchio (2), Alessandro Poli (1), Francesca Millanta (1)

(1) Dipartimento di Scienze Veterinarie Università di Pisa, viale delle Piagge 2, Pisa.
(2) Evvivax Srl, via Castel Romano 100, Roma.

Corresponding Author: **Francesca Parisi** - francescaparisi@vet.unipi.it

Canine melanoma is a malignant and aggressive neoplasm showing clinical, histological, and molecular features similar to the human counterpart. In human medicine, Epidermal Growth Factor Receptors (EGFR) have already been suggested as prognostic markers and potential therapeutic targets in cutaneous melanoma [1]. The aim of this study was to evaluate the immunohistochemistry expression of HER-2 and HER-3 in canine melanomas through immunohistochemistry and correlate their expression with clinicopathological parameters of the examined tumors.
Twenty-one canine melanoma samples were recruited (10 cutaneous, 9 oral/mucosal and 2 digital/foot pad melanomas). Data regarding signalment and clinical parameters were also collected. Histopathological investigations through hematoxylin and eosin staining were carried out to analyze histological type, presence of atypia, ulceration and mitotic count. On each sample, immunohistochemistry using an anti-HER-2 and anti-HER-3 antibodies was performed. HER-2 and HER-3 positivity was classified using already established scoring criteria [2] and a statistical analysis was carried out. Results highlighted that 47% of samples were epithelioid melanomas, 19% spindle-cell melanomas and 34% showed a mixed pattern. HER-2 was expressed in 62% of cases and HER-3 in 38% of cases, while coexpression of the two receptors has been detected in 30% of samples. A statistically significative association ($P<0.05$) was observed between the expression of HER-2 and HER-3 and the presence of ulceration in oral/mucosal and digital/footpad melanomas. This work confirmed the expression of HER-2 and HER-3 in canine melanomas and their association with negative prognostic parameters such us ulceration. Further studies are necessary to confirm these data by increasing the samples size and combining pathological investigations with molecular biology in the investigation of EGFR family receptors.

[1] Liu, S., Geng, R., Lin, E., Zhao, P., & Chen, Y. (2021). ERBB1/2/3 Expression, Prognosis, and Immune Infiltration in Cutaneous Melanoma. Frontiers in genetics, 12, 602160. https://doi.org/10.3389/fgene.2021.602160
[2] Veloso, E. S., Gonçalves, I. N. N., Silveira, T. L., Oliveira, F. S., Vieira, D. S., Cassali, G. D., Del Puerto, H. L., & Ferreira, E. (2020). Diverse roles of epidermal growth factors receptors in oral and cutaneous canine melanomas. BMC veterinary research, 16(1), 24. https://doi.org/10.1186/s12917-020-2249-2.

EFFECTS IN VITRO OF SIRT1 INIBHITION IN CANINE MAMMARY TUMORS

Sgadari M. (1), Martano M. (1), Ferrara G. (1), Restucci B. (1)

(1) Department of Veterinary Medicine and Animal Productions, University of Naples "Federico II".

Corresponding Author: **Mariafrancesca Sgadari** - mariafrancesca.sgadari@unina.it

Sirtuin1 (SIRT1) is a NAD + -dependent class III of histone deacetylases and is involved in many physiological and pathological processes. The role of SIRT1 is controversial, particularly in cancer, as it could act either as a tumor suppressor or tumor promoter depending on the cellular context or specific tumors. In our pilot study (Sgadari M, 2019) of SIRT1 expression in canine mammary neoplastic tissues and cell lines (CMT), immunohistochemistry and Western blot analysis revealed decreased expression of SIRT1 in less differentiated malignant phenotypes. These results allowed us to hypothesize an oncosuppressive role of SIRT1 in CMT. Therefore, the aim of the present study is to clarify the function of SIRT1 by investigating the cytotoxic effect of a selective SIRT1 inhibitor, Selisistat or EX -527, on five CMT cell lines (P-114, CMT-U309, CMT-U27, CMT-U131, and CMT-U229). Cytotoxicity studies were performed using the 3-(4,5-dimethylthiazol-2-yl)- 2,5-diphenyltetrazolium bromide (MTT) assay. CMT cells were treated with increasing concentrations of EX -527 (0-0.1 µM-0.25 µM-0.5 µM-1 µM-1.5 µM-2 µM-2.5 µM) for 72 hours. The viability of neoplastic cells was also assessed using the clonogenic assay. Pharmacological treatment with increasing concentrations of EX -527 (5 µM to 10 µM) was performed for 14 days. In both assays, the percentage of surviving cells was calculated by measuring the intensity of spectrophotometric absorbance at 570 nm. Statistical analyses were performed using GraphPad Prism v8.0 software. Results with $p < 0.05$ were considered statistically significant. The MMT and clonogenic assays showed an increased percentage of surviving cells at concentrations of 1 µM EX -527 up to 2.5 µM and at 5 µM up to 10 µM, respectively. Cell survival was different for the different cell lines used. However, all data were statistically significant. Our results indicate that selective inhibition of SIRT1 was associated with increased cell viability and clone proliferation. Therefore, our results seem to confirm the previously hypothesized tumor suppressive effect of SIRT1. Our results are consistent with those of Kang Y.Y. (2018), who found an association between downregulation of SIRT1 and malignant transformation, invasion, and metastasis in oral cancer. However, the controversial role of SIRT1 in cancer remains open, and further studies are needed to elucidate the role and neoplastic pathways in which SIRT1 is involved.

[1] Sgadari M, Cacciola NA, Restucci B. Sirtuin 1 expression in canine mammary carcinomas: preliminary investigations. Proceedings of the 74th Convegno Sisvet, pag. 42, 2021.
[2] Kang YY, Sun FL, Zhang Y, Wang Z. SIRT1 acts as a potential tumor suppressor in oral squamous cell carcinoma. J Chin Med Assoc. 2018 May;81(5):416-422. doi: 10.1016/j.jcma.2017.09.004. Epub 2017 Oct 16. PMID: 29050728.

MACROSCOPIC AND MICROSCOPIC ASSESSMENT OF GUNSHOT RESIDUES IN VETERINARY FORENSIC PATHOLOGY

Lorenzo Riccio (1); Giuseppe Piegari (1); Ilaria d'Aquino (1); Giovanni Valerio Salanti (1); Gianluca Miletti (2), Paride Minervini (3), Orlando Paciello (1)

(1) Department of Veterinary Medicine and Animal Production, University of Naples Federico II, Naples, Italy
(2) Istituto Zooprofilattico Sperimentale del Mezzogiorno, Portici, Italy.
(3) Ballistic Technical Consultant,Siena (Italy)

Corresponding Authors: **Giuseppe Piegari** - giuseppe.piegari@unina.it; **Lorenzo Riccio** - lorenzo.riccio@unina.it

Gunshot residues (GSR) are particles produced during the discharge of a firearm. The typical composition of GSR is made of lead (Pb), barium (Ba) and antimony (Sb) (1). In gunshot trauma Rhodizonate Sodium (NaR) staining is used to detect GSR on the gunshot lesions; this method, although widely used in human forensic pathology, do not find a direct application in animals; this is due to anatomical differences of animals compared to human anatomy (such as tissue resistance and presence of fur) that could influence the intensity and distribution patterns of gunshot residues on animal cadaver. Considering these observation, the aim of our study was to investigate the sensitivity and specificity of macroscopic and microscopic application of Rhodizonate Sodium (NaR) staining in veterinary forensic pathology. To this aim, the heads of 2 adult pigs and the limbs of 4 adult dogs (died from causes independent from the experiment) were selected for the study and subdivided into two groups: group A was composed by 2 pig heads and 8 dog limbs shot at a firing ground at different distances (near-contact and 1 m for pig heads; near-contact, 1 m and 6 m for limbs dog) using different firearm and bullets. Group B (control group) was composed by 8 dog limbs on which it was simulated single-edge Knife wound. The tissue holes were excised and divided into two aliquots for macroscopic and microscopic lead detection using Na-R methods. Aliquots for macroscopic Na-R chromograph staining were rapidly analyzed using the Bullet Hole Testing Kit (BTK). Aliquots for histopathological examination were fixed in formalin and embedded in paraffin; 3 µm tissue sections were cut and stained with Na-R staining. The BTK was strongly positive in all dog limbs shot at near-contact; in contrast, the positivity at 1m and 6m distance was observed in 4 out of 8 cases (50%). However, Na-R histochemical staining on dog limbs tissues was detected at near-contact in all assessed samples and in 6 out of 8 cases (75%) at 1m and 6m distance (4/4 at 1m of distance and 2/4 at 6m of distance). The hole lesions from group B did not show lead positivity on both macroscopic and microscopic tests. Our results suggest that the joint evaluation of macroscopic and microscopic Rhodizonate Sodium (NaR) chromogenic tests may be used as valid tools to characterize the traumatic injuries and identify the gunshot residues in veterinary forensic pathology.

[1] Charles, Sebastien, Bart Nys, and Nadia Geusens; Primer composition and memory effect of weapons—Some trends from a systematic approach in casework.

AIVI

RABBIT SLAUGHTER HYGIENE: EVALUATION OF PROCESS HYGIENE CRITERIA FOR THE SUPPOSED FOOD CATEGORY CARCASSES OF RABBIT

Maria Parigi (1), Laura Fiorentini (2), Laura Prandini (3), Claudio Milandri (1), Mauro Rosetti (1), Giovanni Tosi (1), Simona Perulli (2), Linda Formenti (3), Federico Tomasello (3), Federica Savini (3), Valentina Indio (3), Alessandra De Cesare (3), Andrea Serraino (3), Federica Giacometti (3)

(1) Azienda USL della Romagna, Dipartimento Sanità Pubblica, distretto Forlì, U.O. Igiene Alimenti di origine animale
(2) Istituto Zooprofilattico Sperimentale della Lombardia e dell'Emilia-Romagna (IZSLER), Sede Territoriale di Forlì
(3) Università di Bologna, Dipartimento di Scienze Mediche Veterinarie (DIMEVET), Via Tolara di Sopra 50, Ozzano Emilia (Bologna, Italia)

Corresponding Author – **Laura Prandini -** laura.prandini3@unibo.it

Rabbit, being part of the category of lagomorphs, is defined by Regulation 853/2004 as "meat from rabbits and hares, as well as meat from rodents" and included in Chapter IV (Slaughter hygiene) in which the inspection of rabbit meat is regulated, in relation to the hygienic methods of slaughter and ante and postmortem requirements [1]. However, this commodity is not considered in Regulation 2073/2005 as process hygiene criteria related to meat and products thereof [2]. This study presents data, in relation to carcasses of rabbit, on aerobic plate count, *Enterobacteriacae*, *Pseudomonas* spp. counts and for the presence of *Listeria monocytogenes*, *Salmonella* spp. and *Campylobacter* spp.. A total of 89 samples were collected by Veterinary Authority in an industrial slaughterhouse located in Forlì during 15 different days of slaughtering. For each day of sampling, 5 carcasses (from the same slaughtered batch) and one sample of washing liquid were collected before chilling and after skinning, respectively. Results showed a level of contamination for aerobic colony count and *Enterobacteriacae* respectively in the range from 2.00 to 5.28 (mean 3.30, SD 0.85) and from 0 to 3.85 (mean 1.50, SD 0.98) Log CFU/cm2, with statistically significant differences between the different days of slaughtering. *Pseudomonas* spp. were isolated in 27.40% of carcasses with a contamination level ranging between 0.12 and 2.67 Log CFU/cm2. No pathogenic bacteria were detected in all the examined carcasses and washing liquid samples. Few data were available in literature on microbiological quality and safety of rabbit carcasses and meat and in comparison to those a lower level of contamination was observed in our study. Even if comparisons with hygiene criteria established for other mammalian species could be not considered appropriate, a total of 6.85%, 20.55% and 15.07% of the carcasses with contamination levels >4.9 Log CFU/cm2 for aerobic colony and >2.4 and 2.9 Log CFU/cm2 for *Enterobacteriacae* were identified, respectively, considering a level of contamination reduced of 1/5 as proposed for non-destructive sampling by Conferenza Stato Regioni 41/2016 [3]. Given our results, the affinity with poultry slaughtering could not be supported, at least for the process hygiene criteria for *Campylobacter* spp.. Further analysis in several industrial slaughter plants are necessary to formulate process hygiene criteria potentially applicable to rabbit slaughtering.

[1] REGULATION (EC) No 853/2004. *Official Journal of the European Union* L 139/55.
[2] REGULATION (EC) No 2073/2005. *Official Journal of the European Union* L 338/1.
[3] 41/CSR del 3 marzo 2016: Linee guida relative all'applicazione del Regolamento (CE) n. 2073/2005 e successive modifiche ed integrazioni sui criteri microbiologici applicabili agli alimenti. *Conferenza Permanente per i rapporti tra lo Stato, le Regioni e le Province Autonome di Trento e Bolzano*

HEPATITIS E VIRUS DETECTION IN LIVER AND MUSCLE TISSUES SAMPLED FROM HOME SLAUGHTERED DOMESTIC PIGS IN CENTRAL ITALY

Gianluigi Ferri (1), Giorgia Giantomassi (2), Daniele Tognetti (3), Alberto Olivastri (3), Alberto Vergara (1)

(1) Food Inspection Research Unit, Department of Veterinary Medicine, University of Teramo
(2) Veterinary Practitioner, Ascoli Piceno, (3) Veterinary Service I.A.O.A., AST Ascoli Piceno

Corresponding Author: **Gianluigi Ferri** - gferri@unite.it

Hepatitis E virus is a cosmopolitan emerging foodborne pathogen, and raw or undercooked liver and pork products can cause infection through the orofecal route. The domestic pig (*Sus scrofa domesticus*) represents the main "urban reservoir" both for humans and wild animals (Priemer et al., 2022). In Central-Southern Italy, the small traditional farming method, and the possibility of environmental sharing with wild species can represent crucial aspects for HEV diffusion and persistence. The aim of this study was to detect HEV RNA (genotype and subtype) from home slaughtered domestic pigs in the Ascoli Piceno province (Central Italy) and more specifically from Monti Sibillini National Park. For this purpose, a total of 236 liver and muscle tissue samples were collected from subjects with a final age of 1 year and half and weight of 200±20 Kg. Each tissue aliquot (15g) was conferred at slaughterhouse level. Laboratory workflow started from omogenization, followed by the RNA extraction using the Trizol LS method. The last analytical steps were bio-molecular screenings: nested RT-PCR and qRT-PCR amplifying specific genetic determinants belonging to the HEV ORF-1, ORF-2, and ORF-3 genes. Sanger sequencing and phylogenetic analysis were performed. The IBM® SPSS® Statistics Software was used for the statistical data analysis calculating the chi-square value (with Yates's correction). A total of 42/236 (17.79% CI 95%: 12.92-22.66%) liver samples resulted positive for the HEV RNA detection (ORF-1 and 2); while 8/42 (19.04% CI 95%: 7.17-30.91%) of positive subjects (liver organ) detected HEV RNA from diaphragm specimens too. Sequences presented high nucleotide similarities (over than 98.00%) with genotype 3. The discovered HEV-3 was the same amplified from wild boars' populations screened in the same province (Ferri et al., 2022). Basing on the farming systems and on the possible environmental sharing with wildlife, the scientific hypothesis of cross-infection possibility was supported by the obtained data. This study wants to focus further attention on the importance of HEV environmental surveillance that represents a crucial public health concern (following a One-health approach).

[1] Priemer et al. Co-Circulation of Different Hepatitis E Virus Genotype 3 Subtypes in Pigs and Wild Boar in North-East Germany. Pathogens. 6,11:773, 2022.
[2] Ferri et al. Hepatitis E Virus Detection in Hunted Wild Boar Liver and Muscle Tissues in Central Italy. Microorganisms. 11,10:1628, 2022.

EVALUATION OF BACTERIAL TRANSLOCATION IN ROUTINE AND EMERGENCY SLAUGHTERING

Daniele Pattono (1), Gaia Muratore (1), Angela Maria Catania (1), Ugo Ala (1), Alessandra Dalmasso (1), Paolo Rossetto (1), Maria Teresa Bottero (1)

(1) Veterinary Sciences Department, Turin University (TO), Italy.
*A.S.L. TO3, Pinerolo (TO), Italy.

Corresponding Author: **Daniele Pattono** - daniele.pattono@unito.it

According to community regulations, in the case of animals unable to be transported, slaughter outside the slaughterhouse (On Farm Emergency Slaughtering, OFES) is allowed. Even in this case the veterinarian must ensure animal welfare and suitability for human consumption within the Community (EC Reg. 853/2004). To date, there is much debate about the hypothesis that the stress resulting from the state of suffering may cause the phenomenon of bacterial translocation from the intestinal lumen with related deep contamination of the carcass (ANSES, 2010). The present work aims to compare the microflora of regularly slaughtered animals (8) and emergency slaughtered animals (8). Lymph node tissue and liver tissue samples were taken as first dissemination stations. For this purpose, traditional microbiological evaluation was complemented by the 16S metabarcoding approach. In particular, quantitative culture techniques were applied to count Total Mesophilic Bacterial Count (TMBC), *Enterobacteriaceae* (ENT) and *Escherichia coli* beta-glucuronidase +. In addition, the absence of Salmonella has been verified (Reg.CE 2073/2005). In parallel on the total DNA extracted from the samples, 16S metabarcoding was performed (Biolcati et al., 2020) in order to identify the entire microbial flora present, including Viable But Non-Culturable microorganisms (VBNC). The results of the traditional analyzes did not show the presence of *E.coli* beta-glucuronidase + and Salmonella in any of the samples tested. Regarding the other microbial counts, only TMBC and ENT in lymph nodes were statistically higher in emergency slaughtered animals. The application of 16S metabarcoding has highlighted results in agreement with traditional methods. From these early data, it can be inferred that emergency slaughtering does not imply an increase in bacterial translocation. Therefore, the EC decision appears justified and does not seem to create higher levels of risk. However, it would be desirable to expand the sampling to confirm the results obtained with greater statistical certainty.

[1] Reg. CE n. 853/2004 del Parlamento Europeo e del Consiglio, del 29 aprile 2004, che stabilisce norme specifiche in materia di igiene per gli alimenti di origine animale, 2004.
[2] ANSES; AVIS de l'Agence Nationale de Sécurité Sanitaire de l'Alimentation, de l'Environnement et du Travail relatif aux contaminations microbiologiques des viandes à l'abattoir, 2010: 1-62.
[3] Reg. CE n. 2073/2005 della Commissione, del 15 novembre 2005, sui criteri microbiologici applicabili ai prodotti alimentari, 2005.
[4] Biolcati, Ferrocino, Bottero, Dalmasso. High-throughput sequencing approach to investigate Italian artisanal cheese production. J. Dairy Sci. 103, 2020

GROWTH POTENTIAL OF LISTERIA MONOCYTOGENES IN STEAK TARTARE: APPLICATION OF PREDICTIVE MICROBIOLOGY

Erica Tirloni (1), Cristian Bernardi (1), Viviana Fusi (1), Simone Stella (1)

(1) University of Milan, Department of Veterinary Medicine and Animal Science

Corresponding Author: **Erica Tirloni** - erica.tirloni@unimi.it

The aim of the present study was to estimate the growth potential of *Listeria monocytogenes* in several brands of bovine steak tartare marketed in Northern Italy, applying mathematical models included in a predictive microbiology software. The study included steak tartare samples belonging to 13 different brands (three batches for each brand); the samples were submitted to microbiological (enumeration of the Total Viable Count, Lactic Acid Bacteria, *Enterobacteriaceae*, *Pseudomonas* spp., yeasts and moulds) and chemical-physical characterization (water activity-Aw, moisture, pH, salt, organic acids, nitrites). This characterization was fundamental to obtain the inputs for the application of the Food Spoilage and Safety Predictor software which allows the estimation of the growth of L. *monocytogenes* during the shelf-life. Lactic acid bacteria, the main component of the microflora, were variable among the brands (from 3.38 to 6.24 log cfu/g) (Bohaychuck et al., 2006). pH and aw values were always higher than 5.3 and 0.96, respectively, thus they could not be considered as single efficient hurdles to prevent the growth of *L.* monocytogenes (Regulation (EC) No. 2073/2005*)*; the same was observed for salt content (constantly < 2%) and nitrites (not quantifiable in all the samples). Nevertheless, the combination of all the hurdles, evaluated by predictive microbiology (based on the characteristics of the products and using critical development factors) resulted in an estimated growth always < 0.5 log cfu/g throughout the shelf life; these output allowed us to consider all the steak tartare typologies analysed as unfavourable substrate for *L. monocytogenes* growth.

[1] Bohaychuck VM, Gensler GE, King RK, Manninen KI, Sorensen O, Wu JT, Stiles ME, McMuller LM, 2006. Occurrence of pathogens in raw and ready-to-eat meat and poultry products collected from the retail marketplace in Edmonton, Alberta, Canada. J Food Protect 69: 2176-82.
[2] European Commission (EC) (2005). European Commission Regulation (EC) No. 2073/2005 of 15 November 2005 on microbiological criteria for foodstuffs. Official Journal of the European Union, L338, 22/12/2005.

IN VITRO ANTIBACTERIAL ACTIVITY OF SILVER NANOPARTICLES AGAINST MOST COMMON PATHOGENIC AND SPOILAGE FOOD BACTERIA

Marica Egidio (1), Yolande Thérèse Rose Proroga (2), Luca Scotti (3), Andrea Mancusi (2), Marika Di Paolo (1), Rosa Luisa Ambrosio (1), Anna Balestrieri (2), Raffaele Marrone (1)

(1) Department of Veterinary Medicine and Animal Production, University of Naples Federico II, 80137 Naples, Italy
(2) Department of Food Inspection, Istituto Zooprofilattico Sperimentale del Mezzogiorno, 80055 Portici (Naples), Italy
(3) Department of Medical, Oral and Biotechnological Sciences, "G. d'Annunzio" University of Chieti-Pescara, Chieti, Italy

Corresponding Author: **Marika Egidio** - marika.dipaolo@unina.it

Microbial contamination is one of the main problems of the food industry, considering the implications to public health due to foodborne diseases [1]. For this reason, food quality assurance systems applied to production processes are essential to generate products that are free of microbiological hazards. In this context, different types of antibiotic agents in food or in packaging have been used; however, the spread of multi-antibiotic-resistant bacteria [2] has highlighted the need to find alternatives. The introduction of nanotechnology in the food industry may offer potential solutions for this challenge. Silver nanoparticles (AgNPs) are antimicrobial agents that have a wide spectrum of action, including against pathogenic bacteria and spoilage fungi [3]. The aim of the present study was to evaluate the antibacterial activity of AgNPs (8–14 nm; synthetized by patented methods, EU-Pathent; Department of Medical, Oral and Biotechnological Sciences, "G. d'Annunzio" University of Chieti-Pescara, Chieti, Italy) on the bacteria most commonly found in food: *Staphylococcus aureus* (ATCC 25923), *Pseudomonas aeruginosa* (ATCC 27853), *Escherichia coli* (ATCC 25922), *Listeria monocytogenes* and *Salmonella typhimurium* (strains isolated from food). In this regard, *in vitro* study was carried out by assessing the effectiveness of the AgNPs on four different concentrations of each tested microbial strain (150x105 CFU/portion test, 150x104 CFU/ portion test, 150X102 CFU/ portion test and 150x10 CFU/ portion test) and at three different time intervals (15 minutes, 1 hour, 6 hours). The antimicrobial activity of AgNPs was already detected after 15 minutes and it was observed greater effectiveness against Gram-negative bacteria than Gram-positive bacteria probably due to their membrane structure, allowing them to be more resistant [4]. Moreover, data showed that antimicrobial effect was directly related to microbial concentration. These preliminary results provided important information on the silver nanoparticles spectrum of action, and this is an aspect that appears particularly promising to obtain a viable alternative to traditional antimicrobials to be used against pathogens and spoilage agents most commonly found in the food chain, harmful both to health and quality aspects.

FOUNDING
Ricerca Corrente IZS ME 01/21 RC: "Utilizzo di ARGIRIUM SUNc per uso alimentare e ambientale per le detersioni delle superfici di lavoro".

[1] Su, Z., Du, T., Liang, X., Wang, X., Zhao, L., Wang, J., ... & Zhang, W. (2022). Nanozymes for foodborne microbial contaminants detection: Mechanisms, recent advances, and challenges. Food Control, 109165.
[2] Dugassa, J., & Shukuri, N. (2017). Review on antibiotic resistance and its mechanism of development. Journal of Health, Medicine and Nursing, 1(3), 1-17.
[3] Elechiguerra, J. L., Burt, J. L., Morones, J. R., Camacho-Bragado, A., Gao, X., Lara, H. H., & Yacaman, M. J. (2005). Interaction of silver nanoparticles with HIV-1. Journal of nanobiotechnology, 3, 1-10.
[4] Kim, J. S., Kuk, E., Yu, K. N., Kim, J. H., Park, S. J., Lee, H. J., ... & Cho, M. H. (2007). Antimicrobial effects of silver nanoparticles. Nanomedicine: Nanotechnology, biology and medicine, 3(1), 95-101.

IMPACT OF CURING ON MICROBIAL PARAMETERS AND *LISTERIA MONOCYTOGENES* GROWTH IN FISH FILLETS

Federica Savini (1), Valentina Indio (1), Yitagele Terefe Mekonnen (1), Federico Tomasello (1), Federica Giacometti (1), Lia Bardasi (2), Roberta Taddei (2), Sean Cuomo (3), Alessandra De Cesare (1), Andrea Serraino (1)

(1) Department of Veterinary Medical Sciences, University of Bologna, Italy
(2) Zooprophylactic Institute of Lombardia and Emilia Romagna regions (IZSLER), Bologna province, Italy
(3) Arredo inox S.r.l., Crotone, Italy

Corresponding Author: **Federica Savini** - federica.savini3@unibo.it

Fish curing allows to add value to the product. The process entails a loss of weight due to the evaporation of the water and determines a more tender texture and a stronger flavour to the product. Fish fillets, after curing, are directly exposed to air into the cabinets for up to 10 days where specific combinations of temperature, air speed and relative humidity are applied during time depending on the desired final product, which is in intended as ready to eat (RTE).
During the production of fish fillets, we enumerated Total Bacteria Count (TBC) and *Enterobacteriaceae* as well as performed contamination with *Listeria monocytogenes* to estimate its growth potential.
The analyses were performed on three different fish species: salmon (*Salmo salar*), tuna (*Thunnus thynnus*) and swordfish (Xiphias gladius) during the processing period. TBC counts ranged from 3.55 to 2.74 log CFU/g in salmon, from di 4.22 log CFU/g to 4.94 log CFU/g in tuna and 5.69 to 5.93 log CFU/g in sword fish at the beginning and at the end of the cycle, respectively. On the contrary, *Enterobacteriaceae* were not detected in any of the sample neither at the beginning nor at the end of the experimental trial. *L. monocytogenes* counts remained stable throughout the production cycle with a slight reduction towards the final stages. Our results underline how *L. monocytogenes* eventually present on raw material does not grow during processing, but we didn't test the behavior of the pathogen along the product shelf life. In addition, even though TBC counts reached almost 6 log CFU/g in sword fish, neither were the *Enterobacteriaceae* ever detected, nor was evident spoilage of fillets.

[1] "Seafood Processing: Technology, Quality and Safety; Boziaris, I.S., Ed.; 1st ed.; Wiley, 2014; ISBN 978-1-118-34621-1"

DEVELOPMENT OF A DNA BARCODING AND METABARCODING METHOD FOR A RAPID IDENTIFICATION OF SPECIES IN SEAFOOD PRODUCTS

Del Sambro L. (1), Bianco A. (1), Capozzi L. (1), Caldarola G. (1), Normanno G. (2), Miccolupo A. (1), Giannico A. (1), Ridolfi D. (1), Toce M. (1), Parisi A. (1)

[1] Istituto Zooprofilattico Sperimentale Della Puglia E Della Basilicata. Via Manfredonia, 20, 71121 Foggia FG
[2] Dipartimento di Scienze Agrarie, Alimenti, Risorse Naturali e Ingegneria (DAFNE). Università di Foggia, Via Napoli, 25, 71122 Foggia FG

Corresponding Author: **Laura Del Sambro** - laura.delsambro@hotmail.it

Seafood products are characterized by a very wide diversity of supply, ranking as one of the foods most susceptible to species substitution fraud (1). The identification is critical both for the related economic implications and for the multiple ecological and health consequences associated with incorrect labeling. In this regard, our study aims to standardize molecular analysis based on the Sanger method and the metabarcoding approach using Next Generation Sequencing (NGS) for the identification of fish species. In order to identify multispecies fish foods, the metabarcoding approach was tested by preparing some mixes of samples previously identified by Sanger method. This technique was able to identify all the species present in the analyzed samples and to assess representativeness of each DNA species in total DNA.
During 2019-2021, a total of 77 samples belonging to national and international seafood products were collected in local stores in the Apulia Region.
The purified DNA extracted was amplified using the universal primer targeted COI, and cytB genes (2,3) and Sanger sequencing was used to perform the analysis of PCR products. For metabarcoding analysis, DNA was amplified by primers designed on a hypervariable region of the mitochondrial 12S gene (4); libraries were prepared and sequenced on Illumina MiSeq platform. Fish species in collected samples were detected based on sequencing results using BOLD and GenBank databases. The identified species were matched with labeling on products for detecting food fraud.
Seventy-seven samples were analyzed by Sanger sequencing; for 43 of them the metabarcoding approach was used. Mislabeling were detected in 9% (n=7) of the samples analyzed by Sanger method; the same samples analyzed in NGS were found to be subject to fraud with the exception of two samples, which had too low sequencing coverage. In 4 samples declared as swordfish (Xiphias gladius), haddock (Melanogrammus aeglefinus) were detected. For three samples the reported species (Merluccius merluccius, Engraulis encrasicolus and Mustelus mustelus) were different from the identified species (Merluccius hubbsi, Engraulis ringens and Mustelus asterias), although belonging to the same genus.
The genetic strategies proposed in this study showed efficient results for species detection. Identification based on DNA barcoding and metabarcoding is a relatively simple and efficient tool for food authentication.

[1] Johnson, R. (2014). Food fraud and economically motivated adulteration of food and food ingredients. Washington, DC: Congressional Research Service
[2] Pepe T, Trotta M, di Marco I, Cennamo P, Anastasio A, Cortesi ML. (2005). Mitochondrial cytochrome b DNA sequence variations: an approach to fish species identification in processed fish products. J Food Prot. Feb;68(2):421-5. doi: 10.4315/0362-028x-68.2.421. PMID: 15726991.
[3] Asiah N, Junianto J, Yustiati A, Sukendi S, Fahmi MR, Muchlisin ZA, Kadapi M, Windarti W. (2019). Biometric and genetic differences in kelabau (Osteochilus spp.) as revealed using cytochrome c oxidase subunit 1. F1000Res. Feb 12;8:177. doi: 10.12688/f1000research.17319.3. PMID: 32089821; PMCID: PMC7008605.
[4] Dunn N., Savolainen V., Weber S., Andrzejaczek S., Carbone C., Curnick D. (2022). Elasmobranch diversity across a remote coral reef atoll revealed through environmental DNA metabarcoding, Zoological Journal of the Linnean Society, Volume 196, Issue 2, October 2022, Pages 593–607, https://doi.org/10.1093/zoolinnean/zlac014

IMPLEMENTATION OF A DNA METABARCODING WORKFLOW FOR SEAFOOD AUTHENTICATION

Alice Giusti (1), Chiara Malloggi (1), Viola Lonzi (1), Riccardo Forzano (2), Beatrice Meneghetti (2), Antonio Solimeo (2), Lara Tinacci (1), Andrea Armani (1)

(1) Fishlab, Dipartimento di Scienze Veterinarie, Università di Pisa;
(2) Fiorital S.p.A., Localita' Marittima Fabbricato, 114, 30135 Venezia

Corresponding Author: **Alice Giusti** - alice.giusti183@outlook.it

The DNA metabarcoding, based on the Next Generation Sequencing (NGS) technologies, is a promising analytical method to authenticate complex seafood products. However, it is still scarcely applied in the context of the offical control and FBOs self-control systems, mainly due to the lack of standardized workflow protocols (from the sampling to the final data analysis).
In this study, the fish burger was selected as experimental model to implement a DNA metabarcoding workflow for the analysis of complex seafood products.
A short DNA fragment (≈200 bp) of the 16Sr RNA gene, was selected as molecular target. The sequencing was performed on Illumina platform, and the data were processed using DADA2. The taxonomic assignment was performed using Blastn against GenBank (identity value ≥99%). The inclusion of experimental samples (positive controls, analytical blanks, replicates, samples processed in dirty laboratory environment) throughout the entire workflow was especially taken into account to assess the presence of artefacts/false positive and filter the final data. The PCR protocol, during the library preparation phase, was properly adjusted to limit the presence of primer dimers, probably due to the Illumina overhang adapter sequences. The selected primer pair was proved as good candidate for metabarcoding protocols applied to seafood authentication, although additional performance tests on targeted species should be performed.
Sequences of sea bass (Dicentrarchus labrax) were highly predominant in all the analyzed fish burgers,with percentages ≥99.34% except for one, considered as mislabelled, where also many sequences of Atlantic salmon (Salmo salar) were found (12.41%).
A threshold value was fixed to remove false-positive sequences (FP) linked to sample cross-contaminations and/or environmental contaminations (e. g. human DNA) at various level of the seafood chain. Overall, the laboratory procedures seemed to minimally contribute to the presence of FP.
DNA metabarcoding, beside being confirmed as efficient method to authenticate complex seafood products, was also proved as effective for the evaluation of FBOs management respect to hygiene and safety criteria. However, it is still pivotal to harmonize protocols by adopting measures to be assumed as quality control of the process. This, allowing a more extensive use of metabarcoding in the context of both official controls and FBOs self-control, could increase the capability to reduce food frauds.

[1] Ficetola, G. F., Taberlet, P., & Coissac, E. (2016). How to limit false positives in environmental DNA and metabarcoding?. Molecular Ecology Resources, 16(5), 604-607.
[2] Giusti, A., Tinacci, L., Sotelo, C. G., Marchetti, M., Guidi, A., Zheng, W., & Armani, A. (2017). Seafood identification in multispecies products: assessment of 16SrRNA, cytb, and COI Universal Primers' efficiency as a preliminary analytical step for setting up metabarcoding next-generation sequencing techniques. Journal of agricultural and food chemistry, 65(13), 2902-2912.
[3] Haynes, E., Jimenez, E., Pardo, M. A., & Helyar, S. J. (2019). The future of NGS (Next Generation Sequencing) analysis in testing food authenticity. Food Control, 101, 134-143.
[4] Mottola, A., Piredda, R., Catanese, G., Giorelli, F., Cagnazzo, G., Ciccarese, G., ... & Di Pinto, A. (2022). DNA metabarcoding for identification of species used in fish burgers. Italian Journal of Food Safety, 11(3).

DETERMINATION OF PERSISTENT ORGANIC POLLUTANTS IN FISH TISSUES BY ACCELERATED SOLVENT EXTRACTION TECHNIQUE (EXTREVA ASE™) AND GC-MS/MS TO SUPPORT FOOD SAFETY

Giacomo Mosconi (1), Renato Malandra (2), Luca M. Chiesa (1), Sara Panseri (1)

(1) Università degli Studi di Milano, DIVAS
(2) ATS Milano

Corresponding Author: **Giacomo Mosconi** - giacomo.mosconi@unimi.it

Halogenated hydrocarbons as polychlorinated biphenyls (PCBs), organochlorine pesticides (OCPs), and polybrominated diphenyl ethers (PBDEs) are, together with polycyclic aromatic hydrocarbons (PAHs), part of the persistent organic pollutants (POPs) family (Lallas, 2001). The primary source of POPs exposure is food, especially fish (Schlummer et al., 1998). Lake fishes from Northern Italy, such as Mediterranean shad (*Alosa agone*), represent niches in terms of consumption even if they play an added value product for typical food preparation as well. The aim of this study was to develop and validate an analytical method for the determination of POPs in fish tissues using the new EXTREVA ASE™ to assess its capability to be used for monitoring plans supporting food safety official controls. Overall, 30 shad were provided by the fish market of Milan. 6 PCBs, 16 OCPs, 7 PBDEs and 4 PAHs were determined in fish tissues. Two key improvements of the method were introduced: freeze-drying and the use of Supel QuE Z-Sep for the cleanup. The method validation parameters were defined by SANTE 2021. The method showed good linearity (R2>0.99), repeatability (4-19%) and recovery (84-109%) for all the compounds. The one-step accelerated solvent extraction method using Z-Sep as fat retainer is both rapid and cost-effective and minimizes waste generation compared to the classic methods. PCBs were detected in all the samples (1.09-11.8 ng/g). PBDE 47 was detected in 100% of the samples. PBDE 99, 28 and 100 were detected in over 70% of the samples. PBDE 153, 33 and 154 were detected in less than 40% of the samples. The concentrations ranged from 1.05 to 5.72 ng/g. Despite his ban, DDT was detected in 70% of the samples, while its reductive dechlorination products DDD and DDE were detected in 100% of the analyzed samples. With 87% and 70% in terms of revealed detection frequency hexachlorobenzene and Endosulfan I were the two other more OCPs found. The concentrations for OCPs ranged from 1.03 to 14.81 ng/g. The most frequently detected PAH was anthracene (83%), followed by chrysene (63%), fluoranthene (13%), and pyrene (7%). Fortunately, none of the PAHs was present in concentration over the LOD. This is the first reported example of an in-line cleanup on the EXTREVA ASE system. All results obtained confirm the efficacy of the method for the determination of multiresidue pollutants in fish tissues that could be pivotal to support food safety controls involved in monitoring plans.

[1] Lallas, P.L. The Stockholm Convention on persistent organic pollutants. Am. J. Int. Law 2001, 95, 692 – 708.
[2] Schlummer, M.; Moser, G.A.; McLachlan, M.S. Digestive tract absorption of PCDD/Fs, PCBs, and HCB in humans: Mass balances and mechanistic considerations. Toxicol. Appl. Pharmacol. 1998, 152, 128 –137. European Commission. Analytical Quality Control and Method Validation Procedures for Pesticide Residues Analysis in Food and Feed (SANTE 11312/2021). 2022. Available online: https://www.eurl-pesticides.eu/docs/public/tmplt_article.asp?CntID=727

INNOVATIVE APPROACH TO WELFARE MONITORING OF THE EUROPEAN EEL (*ANGUILLA ANGUILLA*) AS A TOOL TO ENSURE FOOD SAFETY

Rosa Luisa Ambrosio (1), Ennio Cocca (2), Carlotta Ceniti (3,4), Valeria Vuoso (1), Marika Di Paolo (1), Gianna Palmieri (2), Mauro Esposito (5), Aniello Anastasio (1)

(1) Department of Veterinary Medicine and Animal Production, University of Naples Federico II, 80137 Naples, Italy
(2) Institute of Biosciences and BioResources, National Research Council (IBBR-CNR), 80131 Napoli, Italy
(3) Department of Health Sciences, University "Magna Gracia" of Catanzaro, Campus Universitario "S. Venuta", Viale "S. Venuta", 88100 Catanzaro, Italy
(4) Interdepartmental Center Veterinary Service for Human and Animal Health, CISVetSUA, University of Catanzaro Magna Grecia, 88100 Catanzaro, Italy
(5) Istituto Zooprofilattico Sperimentale del Mezzogiorno, 80055 Portici (NA), Italy

Corresponding Author: **Rosa Luisa Ambrosio** - rosaluisa.ambrosio@unina.it

The interconnections between humans, animals and the environment have always been at the basis of natural events that affect global health. Aware of the close relationship between animal welfare and exposure to contaminants, it is advisable to identify new solutions for the sensitive and fast detection of the states of malaise attributable to habitat [1]. The system to be developed should allow rapid checks on a greater number of samples, without having to attack tissues intended for human consumption, such as muscles. In this context, the aim of this study was to investigate how specific genes could be involved in studies on animal exposure to different degrees of water pollution in 4 rivers in Salerno province (Calore, Picentino, Bussento, and Sele), in Campania Region (south Italy) [2]. The goal was to identify specific biomarkers to be proposed as sensitive tools in risk assessment analyses. European eels (*Anguilla anguilla*) were used in this research, due to their role as sentinel animals. A total of 42 sub-adult individuals were fished with electric stunner in 6 points of rivers. Livers and gills were collected to investigate the expression of genes involved in oxidative metabolisms and metal detoxification, such as superoxide dismutase (*sod*), cytochrome P4501A (*cyp1A*), metallothionein (*mt*), and acylpeptide hydrolase 2 (*apeh2*) (b-*actin* gene expression level was the internal control for normalization). Muscles were collected to measure the levels of 21 metals. Results highlighted a heterogeneous picture among rivers. Transcriptomic studies seem to suggest that subjects sampled in the Sele river have recently been exposed to high levels of contaminants, because they presented an upregulation of both liver and gill genes involved in the first mechanisms of defense (*sod*, *cyp*). Reversely, eels from Bussento showed an up-regulation of the *apeh2* gene, involved in the repair phases of irreversible and chronic damage to proteins, and the highest concentrations of arsenic and mercury in muscles. The PCA analysis highlighted a correlation between these heavy metals with the transcriptomic data of the *apeh2* gene, despite the low absolute levels of heavy metals in tissues [3].
In conclusion, the study lays the foundations for the identification of biological markers from liver and gills that could be introduced in an early warning system to monitor the exposure of fishery products even to very low metal concretions.

[1] G.E. Maes, J.A.M. Raeymaekers, B. Hellemans, C. Geeraerts, K. Parmentier, L. De Temmerman, F.A.M. Volckaert, C. Belpaire, 2013. Gene transcription reflects poor health status of resident European eel chronically exposed to environmental pollutants. Aquatic Toxicology 126 (2013) 242–255.
[2] L. Baillon, F. Pierron, P. Pannetier, E. Normandeau, P. Couture, P. Labadie, H. Budzinski, P. Lambert, L. Bernatchez, M. Baudrimont, 2016. Gene transcription profiling in wild and laboratory-exposed eels: Effect of captivity and in situ chronic exposure to pollution. Science of the Total Environment 571 (2016) 92–102.
[3] L. Baillon, F. Pierron, J. Oses, P. Pannetier, E. Normandeau, P. Couture, P. Labadie, H. Budzinski, P. Lambert, L. Bernatchez, M. Baudrimont, 2016. Detecting the exposure to Cd and PCBs by means of a non-invasive transcriptomic approach in laboratory and wild contaminated European eels (Anguilla anguilla). Environ Sci Pollut Res (2016) 23:5431–5441

USE OF ESSENTIAL OIL VAPORS IN MODIFIED ATMOSPHERE PACKAGING FOR SHELF-LIFE MANAGEMENT OF FRESH FISH

Maria Nobile (1), Davide Pessina (2), Sara Panseri (1), Luca Maria Chiesa (1)

(1) Dipartimento di Medicina Veterinaria e Scienze Animali, Università degli Studi di Milano, Via dell'Università 6, 26900, Lodi; (2
(2) Supermercati Il Gigante Spa

Corresponding Author: **Maria Nobile** - maria.nobile1@unimi.it

In recent years, the nutritional, economic and commercial importance of the fresh and processed fish consumption, as well as that of raw fish, has become consistently, considering a whole series of critical issues to maintain the freshness, the nutritional and sensory characteristics and hygienic-sanitary safety. Some studies evaluated the use of essential oils in packaging to improve the shelf life of food (Doğan et al. 2017; Asik et al. 2014; Vatavali et al. 2013). Their use is linked to their antimicrobial, antioxidant properties, allowing the color maintenance and modulating the appearance of unpleasant sensory notes without altering the original aromatic food identity (Bakkali et al. 2008). This is important if we consider that halving of global food waste is one of the 2030 Agenda goals. This study evaluated the effects of 2 different essential oil vapors by using Aroma+ patented system (water with rosemary extract and lemon essential oil) on 3 different fishery products (bluefin tuna steaks, swordfish steaks and Argentine prawns), packaged in a protective atmosphere. The essential oil vapors were added individually to each product with the aim of monitoring the shelf life of these fish at 4 different time points (T0, T4, T7 and T8) through microbiological, chemical (determination of color, pH, redox potential, biogenic amines, volatile compounds) and sensory analyses. Finally, the results obtained from the fish products packaged with the inclusion of essential were compared with those of the same products packaged in a classic protective atmosphere. The obtained results demonstrated that the products preserved with essential oil inclusion reached the end of the shelf-life in compliance with the process hygiene and food safety criteria, receiving at the end a higher appreciation judgment from the panel of consumers in the case of shrimp and swordfish samples with the addition of rosemary essential oil. The addition of the latter, on the other hand, highlighted the worst result at the end of tuna shelf life. In particular, the color evaluation, which represents an influencing parameter for the consumer's first choice, did not show statistically significant differences compared to the classic packaging, keeping the color suitable during the entire shelf life. In conclusion, the obtained results can be considered encouraging to support the inclusion of essential oils as a valid alternative to the classic modified atmosphere for fish packaging.

[1] Bakkali, F., Averbeck, S., Averbeck, D., & Idaomar, M. (2008). Biological effects of essential oils–a review. Food and chemical toxicology, 46(2), 446-475.
[2] Asik, E., & Candoğan, K. (2014). Effects of chitosan coatings incorporated with garlic oil on quality characteristics of shrimp. Journal of Food Quality, 37, 237e246.
[3] Doğan, G., & İlzci, L. (2017). Effects on quality properties of smoked rainbow trout (Oncorhynchus mykiss) fillets of chitosan films enriched with essential oils. Journal of Food Processing and Preservation, 41(1), e12757.
[4] Vatavali, K., Karakosta, L., Nathanailides, C., Georgantelis, D., & Kontominas, M. G. (2013). Combined effect of chitosan and oregano essential oil dip on the microbiological, chemical, and sensory attributes of red porgy (Pagrus pagrus) stored in ice. Food and Bioprocess Technology, 6, 3510-3521.

BACTERIA POPULATION DYNAMICS IN ARTISANAL FERMENTED SOFT CHEESES AS PREDICTORS OF THE PRESENCE OF FOODBORNE PATHOGENS

Valentina Indio (1), Chiara Oliveri (2), Alex Lucchi (2), Ursula Gonzales Barron (3), Fouad Achemchem (4), Antonio Valero (5), Frederique Pasquali (2), Federica Savini (1), Gerardo Manfreda (2), Alessandra De Cesare (1)

(1) Department of Veterinary Medical Sciences, University of Bologna, Italy
(2) Department of Agricultural and Food Sciences, University of Bologna, Italy
(3) Polytechnic Institute of Braganza, Portugal
(4) Ibn Zohr University, Marocco
(5) Dpt. Food Science and Technology, University of Cordoba, Spain

Corresponding Author: **Valentina Indio** - valentina.indio2@unibo.it

The dynamics between beneficial and pathogenic bacteria in foods are often under investigated because foodborne pathogens are both detected and enumerated with target analytical methods, as ISO cultural methods and PCR. The aim of this study was to investigate the bacterial population dynamics in artisanal cheeses using an untargeted sequencing method, represented by shotgun metagenomics. A total of 4 types of artisanal soft cheeses, produced in different Mediterranean areas (i.e., Italy, Portugal, Spain and Morocco), were analysed. The genomes of pathogenic bacteria listed in the "EU One Health Zoonoses Report 2020"[1], including foodborne pathogens, were detected in all samples. The highest relative abundance of pathogens as Escherichia coli, Salmonella enterica, Klebsiella pneumoniae and several species of Shigella was highlighted in the Moroccan cheese, while the Italian cheese almost exclusively host Streptococcus ssp. The relative abundance of these bacteria, together with other less represented pathogens (i.e., Enterococcus faecalis, Enterococcus faecium, Yersinia ssp., Clostridium ssp. and Bacillus cereus) allowed to cluster all samples based on their origin. Moreover, the results showed that the overall abundance of pathogens negatively correlate with that of beneficial microorganisms, such as Lactococcus and Lactobacillus. These results highlight that the output of shotgun metagenomics can be used to map the microbial populations associated to artisanal cheeses to both detect the presence of foodborne pathogens and predict their colonization potential according to the abundance of beneficial microbes.

[1] Martínez A, Montes de Oca N, Armenteros M, Uffo O, Riverón Y, González D, Remón D, Benone Paes S, Adrião M, Farías de Andrade S, Villoch A. Identification of bacterial hazards in the production of artisan fresh cheese in Cuba. J Dairy Res. 2020 May;87(2):263-265.
[2] Stefanou CR, Bartodziejska B, Gajewska M, Szosland-Fałtyn A. Microbiological Quality and Safety of Traditional Raw Milk Cheeses Manufactured on a Small Scale by Polish Dairy Farms. Foods. 2022 Dec 4;11(23):3910.
[3] European Food Safety Authority; European Centre for Disease Prevention and Control. The European Union One Health 2021 Zoonoses Report. EFSA J. 2022 Dec 13;20(12):e07666.

PRELIMINARY RESULTS ON DETECTION OF EXTENDED-SPECTRUM BETA-LACTAMASE (ESBL)-PRODUCING ESCHERICHIA COLI FROM RED FOXES, CORVIDS, AND WATERFOWL IN THE EMILIA-ROMAGNA REGION, NORTHERN ITALY

Bardasi Lia (1), Massella Elisa (1), Sampieri Maria (1), Carfora Virginia (2), Conter Mauro (3), Lamperti Luca (3), Marra Federica (3), Rega Martina (3), Bacci Cristina (3), Bonardi Silvia (3)

(1) Experimental Zooprophylactic Institute of Lombardy and Emilia Romagna, 40127 Bologna, Italy
(2) Experimental Zooprophylactic Institute of Lazio and Tuscany - National Reference Laboratory for Antimicrobial Resistance, 00178 Rome, Italy
(3) Department of Veterinary Science, University of Parma, Strada del Taglio 10, 43126 Parma, Italy

Corresponding Author: **Mauro Conter** - mauro.conter@unipr.it

In the context of the One Health approach, antimicrobial resistance (AMR) is considered one of the main challenges. Various studies have reported how wildlife populations may act as sentinels for AMR in the environment, especially in highly anthropized landscape or where zootechnical activities are intensive. Furthermore, predators may acquire AMR bacteria through consumption of prey.
Escherichia coli can acquire AMR genes, including those encoding resistance to Highest Priority Critically Important Antimicrobials (HPCIAs) for human medicine as Extended-spectrum β-lactamases (ESBL) genes, thus representing a good bioindicator for AMR (1-3).
In the study period January 2020-April 2022, 370 E. coli strains were isolated from faecal samples of 347 wild animals (115 red foxes, 183 corvids, and 49 waterfowl) in the Emilia-Romagna region (Northern Italy) to assess the prevalence of ESBL E. coli faecal shedders. Following Decision (EU) 2020/1729 (4), the isolates were tested for AMR towards 15 antimicrobials, most of them ranked as Critically Important Antimicrobials (CIAs) by the WHO. By MIC test, 31/370 (8.4%) phenotypically ESBL-producing E. coli were detected as resistant to cefotaxime and/or ceftazidime. ESBL E. coli were isolated from red foxes (n= 13), corvids (n= 11) and waterfowl (n= 7). By PCR, phenotypically-resistant E. coli harboured ESBL-plasmidic related genes; in particular, blaSHV, blaTEM and blaCTX genes were carried by 2 (6.5%), 12 (38.7%) and 30 (96.8%) isolates, respectively. The co-presence of all genes was never found, but 38.7% of ESBL-producing E. coli harboured blaTEM and blaCTX –genes simultaneously.
Overall, the genotypically-confirmed ESBL E. coli tested by MIC test were found resistant to cefotaxime (100%), ampicillin (96.9%), sulfamethoxazole (87.1%), ceftazidime (87.1%), ciprofloxacin (51.6%), tetracycline (48.4%), trimethoprim (35.5%), nalidixic acid (16.1%), chloramphenicol (16.1%), gentamycin (6.5%), colistin (3.2%). All isolates were susceptible to amikacin, meropenem, tigecycline and azithromycin.
The prevalence of ESBL-producing E. coli found in our study shows the potential role of wild animals, especially waterfowl (7/49; 28.6%) and red foxes (13/115; 11.3%), as carriers of MDR isolates. This phenomenon is worsened by co-resistances to CIAs, thus representing a potential public health risk and suggesting the use of wild animals as sentinels for the analysis of the AMR spread in the environment.

[1] Gambino, D.; Vicari, D.; Vitale, M.; Schirò, G.; Mira, F.; Giglia,M.L.; Riccardi, A.; Gentile, A.; Giardina, S.; Carrozzo, A.; et al. Study on Bacteria Isolates and Antimicrobial Resistance in Wildlife in Sicily, Southern Italy. Microorganisms 2021, 9, 203. https://doi.org/10.3390/microorganisms9010203
[2] Homeier-Bachmann, T.; Schütz, A.K.; Dreyer, S.; Glanz, J.; Schaufler, K.; Conraths, F.J. Genomic Analysis of ESBL-Producing E. coli in Wildlife from North-Eastern Germany. Antibiotics 2022,11, 123. https://doi.org/10.3390/antibiotics11020123
[3] Athanasakopoulou, Z.;Reinicke, M.; Diezel, C.; Sofia, M.;Chatzopoulos, D.C.; Braun, S.D.; Reissig, A.; Spyrou, V.; Monecke, S.;Ehricht, R.; et al. Antimicrobial Resistance Genes in ESBL-Producing Escherichia coli Isolates from Animals in Greece. Antibiotics 2021,10, 389. https://doi.org/10.3390/antibiotics10040389
[4] Commission Implementing Decision (EU) 2020/1729 on the monitoring and reporting of antimicrobial resistance in zoonotic and commensal bacteria and repealing Implementing Decision 2013/652/EU. OJ L 387, 2000, p. 8–21

AN UPDATE ON THE PREVALENCE OF LISTERIA SPP. AND L. MONOCYTOGENES IN SHEEP'S MILK CHEESEMAKING FACILITIES

Carlo Spanu (1), Sara Salza (2), Gabriella Piras (2), Rita Melillo (2), Monica Molotzu (2), Lucia Giagnoni (1), Luisa Doneddu (1), Alessandra Tondello (3), Alessio Cecchinato (3), Piergiorgio Stevanato (3), Andrea Squartini (3), Tiziana Tedde (2), Alessandro Graziano Mudadu (2)

(1) University of Sassari, Department of Veterinary Medicine, Via Vienna 2, 07100 Sassari, Italy.
(2) Veterinary Public Health Institute of Sardinia "G. Pegreffi, Via Duca degli Abruzzi, 8, 07100 Sassari SS.
(3) University of Padova, DAFNAE, Viale dell'Università 16, 35020, Legnaro, Italy

Corresponding Author: **Carlo Spanu** - cspanu@uniss.it

Listeria monocytogenes and Listeria spp. are frequently isolated from the sheep's milk cheesemaking environment, with an overall prevalence ranging from 10% to 20% [1]. The aim of the present study was to provide updated information on the occurrence of Listeria monocytogenes and Listeria spp. contamination in the processing environment of Sardinian sheep's milk cheesemaking facilities. Environmental samples were collected from 12 industrial plants during production runs. Each facility was visited once for the collection of approximately twenty swab samples. Environmental testing was conducted using commercial sponge sampling kits which were dragged on food contact (drainage and working tables, shelves, conveyor belts and inner part of equipment) and non-food contact surfaces (floor, floor drains and outer part of equipment). Samples were collected from curd production, whey heating, salting, cold storage, and ripening areas. Sponges were also used to scrub the surface of Pecorino Romano cheeses. Detection of Listeria was conducted using ISO 11290:2017. Molecular confirmation was carried out using real-time PCR [2]. Overall were collected 73, 148 and 30 sponges respectively from food contact, non-food contact surfaces and food samples. The presence of L. monocytogenes or other Listeria spp. was observed in all the facilities. In 8 facilities Listeria spp. was recovered in combination with L. monocytogenes, in 3 facilities only Listeria spp. was detected, while in one facility was detected the sole presence of L. monocytogenes. Overall, the genus Listeria was observed in 55 samples (21.9%) while in 36 (14.3%) and 34 (13.5%) samples was observed respectively the presence of other Listeria spp. and L. monocytogenes. L. monocytogenes, other Listeria spp. and their simultaneous presence accounted respectively for 19 (34.5%), 21 (38.2%) and 15 (27.3%) of contaminated samples. Samples positive for the genus Listeria were collected respectively from 14 (25.5%) food contact surfaces, 36 (65.5%) non-food contact surfaces and 5 (9.1%) food samples. The greater prevalence was observed in product washing (30.9%), product salting (10.9%) areas while the sampling sites most frequently contaminated were floors (36.4%) and floor drains (29.1%). Our study confirms the need of continuous monitoring of Listeria in cheesemaking facilities and the role of non-food contact surfaces as an early warning for the possible transmission of contamination to foods.

[1] Spanu et al. (2015). Occurrence and traceability of Listeria monocytogenes strains isolated from sheep's milk cheese-making plants environment. Food Control, 47:318-325.
[2] Cremonesi et al. (2014). Development of 23 individual TaqMan® real-time PCR assays for identifying common foodborne pathogens using a single set of amplification conditions. Food Microbiology, 43:35-40.

SOY, RICE AND OAT DRINKS: INVESTIGATING CHEMICAL AND BIOLOGICAL SAFETY IN PLANT-BASED MILK ALTERNATIVES

Roberta Giugliano (1), Noemi Musolino (1), Valentina Ciccotelli (1), Valentina Savio (1), Barbara Vivaldi (1), Carlo Ercolini (1), Daniela Manila Bianchi (1), Lucia Decastelli(1)

(1) NRL Pesticides in Cereals and Feed – Istituto Zooprofilattico Sperimentale del Piemonte, Liguria e Valle D'Aosta, Genoa, Italy

Corresponding Author: **Roberta Giugliano** - roberta.giugliano@izsto.it

Due to cow milk allergy, lactose intolerance, calories and hypercholesterolemia concerns, consumption of milk alternatives such as plant-based drinks have increased, especially soya drink consumption. This trend seems to be common in all developed countries [1] [2].
The aim of our study is to grow folder knowledge of the security of plant-based drinks [3] helping competent authorities to issue a risk assessment and to apply national plans about consumer safety. The research was conducted on a representative selection of beverages available on the local markets of metropolitan Turin area (North Italy).
We have indagated chemical hazard looking for pesticide residues. Samples were treated using our validated method in accordance with SANTE/2019/12682 protocol. The method consists in extracting samples using SweEt method and in analysing in GC-MS/MS (Thermo SCIENTIFIC TRACE 1300 coupled with TSQ 8000 Evo) equipped with an AS 3000 autosampler. Results show that plant-base beverage do not present chemical risks, we have detected very low pesticides concentrations (fipronil sulfone, piperonyl butoxide and pirimiphos-methyl residue) in analysed matrices. In addition, no correlation between beverages pre-treatment and residue concentration was found.
Microbiological hazard was evaluated, also. The study included: determination of the total aerobic mesophilic bacteria count (TAMBC) (ISO 4833-1:2013); enumeration of coagulase-positive staphylococci (CPS) including Staphylococcus aureus and other species (ISO 6888-2:2021); detection and enumeration of total coliforms (ISO 4831:2006); detection and enumeration of Enterobacteriaceae (ISO 21528-1:2017); enumeration of sulphite-reducing anaerobic bacteria (ISO 15213:2003); detection, enumeration and confirmation through cereulid-toxin gene PCR-end point of Bacillus cereus (ISO 7932:2004/AMD 1:2020); enumeration of total yeast and mold (TYMC) (ISO 21527:2008); detection of Listeria monocytogenes (ISO 11290-1:2017) and Salmonella (UNI EN ISO 6579-1:2017).
Microbiological results showed that thermal processes improve microbiological safety. Pasteurization aims to reduce the microbial load by a 5 log.
Summing up plant-based beverages is a valid and healthy alternative of dairy milk. Due to their recent popularity cereals and vegetables beverages should be included in official control by competent authority like other commonly consumed food categories.

[1] Sethi, S., Tyagi, S. K., & Anurag, R. K. (2016). Plant-based milk alternatives an emerging segment of functional beverages: a review. Journal of Food Science and Technology 2016 53:9, 53(9), 3408–3423. https://doi.org/10.1007/S13197-016-2328-3
[2] Haas, R., Schnepps, A., Pichler, A., & Meixner, O. (2019a). Cow Milk versus Plant-Based Milk Substitutes: A Comparison of Product Image and Motivational Structure of Consumption. Sustainability 2019, Vol. 11, Page 5046, 11(18), 5046. https://doi.org/10.3390/SU11185046
[3] Bartula, K., Begley, M., Latour, N., & Callanan, M. (2023). Growth of food-borne pathogens Listeria and Salmonella and spore-forming Paenibacillus and Bacillus in commercial plant-based milk alternatives. Food Microbiology, 109. https://doi.org/10.1016/J.FM.2022.104143

APPLICATION AND IMPACT OF INFRARED RADIATION TECHNOLOGY ON MILK SAFETY AND QUALITY TRAITS

Luigi Danesi (1), Sara Panseri(1), Erica Tirloni (1), Giacomo Mosconi (1), Roberto Villa (1)

(1) Università degli Studi di Milano, Dipartimento di Medicina Veterinaria e Scienze Animali (DIVAS)

Corresponding Author: **Luigi Danesi** - luigi.danesi@unimi.it

The promotion of an environmentally friendly food system production is one of the greatest challenges of the next few years. The United Nations, through the Sustainable Development Goals, guide ways to achieve this result [1]. One of the priority pillars of the Green Deal is to develop new technologies able to reduce environmental impact while maintaining high safety and productive efficiency. In this scenario, the study is focalized on the dairy sector, characterized by strong energy consumption and a simultaneous rise in products request [2]. Among the innovations used, treating raw milk with infrared radiation (IR), as an alternative to pasteurization, is one of the most interesting applications. IR could be used due to its microorganisms' inactivation ability and offers different advantages: high heat transfer rate, low heating time, uniform heating, energy efficiency and water savage [3, 4]. This study aimed to evaluate the efficiency of IR based sanitation system, focalizing mainly on the safety and some quality traits of milk. In the first step of the work, the numeric reduction of Total Bacterial Count, Enterobacteriaceae, Coliforms and Lactic Acid Bacteria were evaluated in raw bovine milk samples before and after IR at different levels of energy radiation: 70, 80, 85 and 90. All bacterial species considered were reduced by the action of IR increasingly efficiently as the intensity of radiation raised. Volatile Organic Compounds (VOCs) profile by HS-SPME and GC/MS analyses was conducted to evaluate the most important compounds that have a strong impact on milk flavour (aldehydes, ketones and sulphurated compounds) to assess the capacity of IR to maintain the characteristics of the raw product. Considering the poorer performance recorded from the microbiological point of view, 70-energy milk was preliminarily discarded. The data were compared to raw, pasteurized, extended shelf-life (ESL) milk samples. Regarding the ketones content, IR milk at 80 and 85 showed similar values to raw milk: respectively 588.65, 580.45 and 564.67 ng g-1. Pasteurized milk and 80-energy milk showed a similar content to raw milk for aldehydes (values of 4.42 and 10.41 compared to 7.44 ng g-1). About sulphurate compounds, pasteurized milk profile was similar to raw milk, but 80-energy treated showed a similar content compared to ESL.

[1] Messerli, Peter, et al. "Global sustainable development report 2019: the future is now–science for achieving sustainable development." (2019).
[2] OECD/FAO (2019), OECD-FAO Agricultural Outlook 2019-2028, OECD Publishing, Paris, https://doi.org/10.1787/agr_outlook-2019-en
[3] Krishnamurthy, K. et al. (2008) 'Efficacy of infrared heat treatment for inactivation of staphylococcus aureus in milk', Journal of Food Process Engineering, 31(6), pp. 798–816. doi: 10.1111/j.1745-4530.2007.00191.x.
[4] Priyadarshini, A.; Rajauria, G.; O'Donnell, C.P.; & Tiwari, B.K. (2019) Emerging food processing technologies and factors impacting their industrial adoption, Critical Reviews in Food Science and Nutrition, 59:19, 3082-3101, DOI: 10.1080/10408398.2018.1483890.

AN EXPLORATORY STUDY ON THE USE OF ANTIBIOTICS IN THE PIG CHAIN AS AN ISSUE FOR PUBLIC HEALTH AND FOOD AUTHENTICITY

Maria Pia Fabrile (1), Sergio Ghidini (1), Augusta Caligiani (1), Federico Scali (2), Maria Olga Varrà (1), Giovanni Loris Alborali (2), Adriana Ianieri (1), Emanuela Zanardi (1)

(1) University of Parma, Department of Food and Drug
(2) Istituto Zooprofilattico Sperimentale della Lombardia e dell'Emilia-Romagna (IZSLER).

Corresponding Author: **Emanuela Zanardi** - emanuela.zanardi@unipr.it

Meat companies have introduced antibiotic-free lines of pork as part of good animal husbandry practices for a responsible use of antimicrobial agents in livestock. Antibiotic-free label claim is currently available on the market (Bradford et al, 2022) driven by the urgent need to reduce antimicrobial consumption - and thus antimicrobial resistance - and the growing customer demand. However, these claims lead to new challenges concerning authentication and traceability in the pigs' chain.

A metabolomics approach was employed to highlight putative metabolic differences in pigs due to antimicrobial administration; also, the feasibility of metabolomics as a valuable tool to investigate antimicrobial treatment was evaluated. Liver metabolome of 41 pigs reared in Northern Italy during 2020 was characterized by 1H Nuclear Magnetic Resonance (NMR) spectroscopy. Two groups of pigs, treatment (TX, n=19) and control (CTRL, n=22), were selected according to the Defined Daily Dose Animal for Italy for comparison. A biphasic extraction procedure was adopted to collect the two fractions of liver, polar and non-polar, differently analysed at the 600.17 MHz NMR.

A differentiation between TX and CTRL pigs' groups was observed by performing Orthogonal Partial Least Squared-Discriminant Analysis on polar and non-polar fractions. The most discriminant variables (Variable Importance in Projection > 1) driving the separation between TX and CTRL groups were selected and the statistical significance on relevant features was checked to identify the discriminant molecules and their regulation between the two groups. In liver polar fraction, discriminant molecules up-accumulated (Fold Change Ratio) in TX pigs' group were glucose, proline, tryptophan, glutathione, lactate, and choline. Higher levels of glucose and lactate in liver may suggest that the observed pigs were in a stressful condition requiring energy. In liver non-polar fraction, decreasing in fatty acids, cholesterol, and unsaturated fatty acids was observed in TX groups, probably due to the consumption of those substrates in case of body energy request.

The favourable results achieved so far suggest that the metabolomics approach might be seen as a promising tool for encouraging the intake of fewer drugs with antibacterial activity. Moreover, this technique might improve tracking and authentication systems along the chain of antibiotic-free pigs in confirming label claims and thwarting potential food fraud.

[1] Bradford H, McKernan C, Elliott C, & Dean M (2022) Consumers' perceptions and willingness to purchase pork labelled 'raised without antibiotics'. Appetite 171, 105900.

COMBINING LIGHT STABLE ISOTOPE RATIOS AND LANTHANIDE PROFILES FOR TRACEABILITY AND FRAUD PREVENTION IN THE SQUID INDUSTRY

Maria Olga Varrà (1), Emanuela Zanardi (1), Paola Iacumin (2), Mattia Rossi (2), Giovanni Tommaso Lanza (1), Adriana Ianieri (1), Sergio Ghidini (1).

(1) University of Parma, Department of Food and Drug
(2) University of Parma, Department of Chemistry, Life Sciences and Environmental Sustainability.

Corresponding Author: **Maria Olga Varrà** - mariaolga.varra@unipr.it

Regulators, industry participants, and consumers are very concerned about fraud in the fishery sector and ask for the provision of trustworthy and durable solutions to assist the traceability and safety of the products [1].
In this work, the efficacy of integrating light stable isotope ratios with the lanthanide profile of squids was investigated with the aim of preventing the fraudulent substitution by species and geographical origin.
A total of 80 specimens of European squids (L. vulgaris) and flying squids (T. sagittatus) were collected from the Mediterranean Sea (FAO fishing area 37.2.1) and the Atlantic Ocean (FAO fishing area 27.7). Stable isotope ratios of carbon and nitrogen (δ13C and δ15N) and 13 elements from the lanthanide block (La, Ce, Pr, Nd, Sm, Eu, Gd, Tb, Dy, Ho, Er, Yb, and Lu) were determined by isotope ratio mass spectrometry and inductively coupled plasma-mass spectrometry.
Following exploratory analysis, all the analytes were found to be statistically different between the two squid species, except for Eu, with δ13C, δ15N, La, Pr, and Nd values being higher in European squids ($p \leq 0.05$). In contrast, δ15N values did not significantly differ according to sample origin, while 13C and most of the lanthanides were more abundant in squids from the Mediterranean Sea ($p \leq 0.05$). A Classification and Regression Tree was then used as predictive machine learning algorithm to define decision rules based on specific cut-off values of the analytes to be used for both species and origin prediction. As a result, samples were classified by species and origin with 90% and 89% accuracy using only 2 out 15 discriminant analytes. Specifically, if Dy ≤ 0.52 µg/kg or Dy ≤ 0.52 µg/kg and δ15N > 14.87 ‰, the sample was classified as flying squid; if Dy > 0.52 µg/kg and δ15N ≤ 14.87 ‰, the sample was classified as European squid; if δ13C > –18.99 ‰ or δ13C ≤ –18.99 ‰ and Nd > 3.92 µg/kg the sample was classified as Mediterranean; if δ13C ≤ –18.99 ‰ and Nd ≤ 3.92 µg/kg, the sample was classified as Atlantic.
Considering that our previous findings suggested a strong impact of squid origin on food safety aspects related to the contamination by heavy metals as well as a major tendency of flying squid to accumulate Cd and Hg, tracking the provenance and monitoring species substitution is of utmost importance. Accordingly, this study suggests that the measurement of light stable isotopes and lanthanides can be regarded as an accurate tool for this purpose.

[1] Fox, M., Mitchell, M., Dean, M., Elliott, C., & Campbell, K. (2018). The seafood supply chain from a fraudulent perspective. Food Security, 10, 939–963.

EFFECT OF BAKERY BY-PRODUCTS INCLUSION IN BROILER DIET ON MICROBIOLOGICAL QUALITY AND PHYSICOCHEMICAL CHARACTERISTICS OF MEAT

Karthika Srikanthithasan (1), Marta Castrica (2), Claudia M. Balzaretti (3), Marta Gariglio (1), Edoardo Fiorilla (1), Valentina Bongiorno (1), Achille Schiavone (1), Claudio Forte (1), Dino Miraglia (4)

(1) Department of Veterinary Sciences, University of Turin, 10095 Grugliasco, Italy
(2) Department of Comparative Biomedicine and Food Science, University of Padova, Agripolis, Viale dell'Università 16, 35020 Legnaro, Italy
(3) Department of Veterinary Medicine and Animal Sciences, University of Milan, Via dell'Università 6, 26900 Lodi, Italy
(4) Department of Veterinary Medicine, University of Perugia, Via San Costanzo 4, 06126 Perugia, Italy

Corresponding Author: **Marta Castrica** - marta.castrica@unipd.it

This study evaluated the effect on microbiological and organoleptic meat quality of different dietary inclusions of bakery by-products (BBP) replacing corn and soybean meal in broilers' diet. In total, 200 one-day-old male ROSS 308 chicks were assigned to four dietary treatments according to their average live weight (5 replicates/group, 10 birds/pen). The 4 dietary groups were: Control (CTR: commercial feed), L-BBP (6.25% BBP), M-BBP (12.5% BBP), and H-BBP (25% BBP).
After slaughtering (34 days), 20 chicken breasts (n=5/group) were collected to assess the microbiological quality and physico-chemical characteristics of the meat at 6h post mortem (day 0), after 3, 5, 7, and 9 days of refrigerated storage (4°C±1).
The data showed that BBP dietary inclusions influenced the development of microbial counts during storage. In particular, total viable count and *B. thermosphacta* values at the end of the storage period were lower (P<0.01) in H-BBP and M-BBP groups compared to CTR group. These results are in line with the total volatile basic nitrogen values that resulted higher (P<0.001) in the CTR group compared to the BBP fed groups at the end of the observation period. Consequently, pH also varied; H-BBP and M-BBP groups showed lower values than the CTR group at each analyzed time point. Concerning colour coordinates, no differences in lightness (L*) redness (a*), and yellowness (b*) were recorded until the fifth observation day.
The meat from the treated groups maintained constant lightness (L*) and redness (a*) over time, and at the end of the storage period, the H-BBP and M-BBP samples were more redness than the CTR group (P<0.01). The results of shear force and thiobarbituric acid reactive substances assay (lipid oxidation) showed no difference between the groups. According to these preliminary results, dietary inclusion of BBP in broiler diet appears to exert positive effects on certain quality characteristics of broiler meat. Additionally, the inclusion of bakery by-products in livestock diets makes their use even more beneficial from a food waste reduction perspective [1]. Further research is ongoing to assess both the sensory attributes and nutritional profile of meat to propose a high-quality and environmentally friendly meat to the consumer.

[1] Pinotti, L., Luciano, A., Ottoboni, M., Manoni, M., Ferrari, L., Marchis, D., & Tretola, M. (2021). Recycling food leftovers in feed as opportunity to increase the sustainability of livestock production. Journal of Cleaner Production, 294, 126290.

76° CONVEGNO SISVET
BARI, 21-22-23 GIUGNO 2023

PERSISTENCE OF *SALMONELLA* SPP. IN FARMED INSECTS SPECIES, A SYSTEMATIC REVIEW OF EXPERIMENTAL STUDIES

Juliane Pinarelli Fazion (1), Filippo Marzoli (1), Alessandra Pezzuto (1), Michela Bertola (1), Pietro Antonelli (1), Beatrice Dolzan (1), Lisa Barco (1), Simone Belluco (1)

(1) Istituto Zooprofilattico Sperimentale delle Venezie (IZSVe), Legnaro (PD), 35020, Italy

Corresponding Author: Juliane Pinarelli Fazion - jpinarelli@izsvenezie.it

Due to their nutritional value, feed conversion index, and ecological potential, consumption of insects as food and feed has been suggested as an alternative to the rising global food need. The potential presence of food pathogens is one of the risks related to eating insects. *Salmonella* specific criteria for insect-based products are not provided by EU regulation on microbiological standards for food products. However, the commission implementing regulation that authorizes Novel Food establishes microbiological standards on a case-by-case basis and, in the case of edible insects, requires the absence of *Salmonella* in 25 g of product. Like with other food and feed items, it is crucial to monitor any potential safety hazards during the insect production process. The aims of this systematic review are to collect, select and evaluate, in the available scientific literature, studies investigating the persistence of *Salmonella* in insect species. Results were used to define survival ability and to suggest control measure for insect farms. In total, 36 papers investigating the persistence of *Salmonella* spp. in insects were included after screening. In detail, 27 and 14 studies were carried out on complete metamorphosis and on incomplete metamorphosis insects, respectively. Regarding complete metamorphosis insects, investigated in 27 studies, the longest survival of *Salmonella* was reported in *P. regina* in which survived for 29 days at 5°C. McAllister et al. (1994) reported a survival of *Salmonella* for 28 days in the feces of *A. diaperinus* using a high titer of inoculum for insect infection. Some papers investigated the persistence of *Salmonella* in complete metamorphosis insects continuously exposed to contaminated substrate. These data can represent a condition occurring in real farming environment. In this instance, *Salmonella* remained persistent in *C. vicina* for more than 16 days. Otherwise, among the 14 studies investigating the incomplete metamorphosis insect, the longest persistence of *Salmonella* (> 10 months) was observed in *B. germanica. P. americana* excreted *Salmonella* through feces until all the insects dead, which occurred at 44 days. The analysis of the persistence of *Salmonella* collect useful data for risk the assessors and decision makers involved in the safety of insect based food. This data can contribute to define the hygienic-sanitary requirements and risk mitigation measures along the supply chain. The *systematic review* protocol is registered in PROSPERO database (CRD42022329213).

[1] EFSA Scientific Committee, 2015. Scientific Opinion on a risk profile related to production and consumption of insects as food and feed. EFSA Journal 2015; 13 (10):4257, 60 pp. doi:10.2903/j.efsa.2015.4257
[2] European Commission (EC). Commission Regulation (EC) No. 2073/2005 of 15 November 2005 on microbiological criteria for foodstuffs. (2005).
[3] European Commission (EU). Commission Implementing Regulation (EU) 2017/2470 of 20 December 2017 establishing the Union list of novel foods in accordance with Regulation (EU) 2015/2283 of the European Parliament and of the Council on novel foods. (2017).
[4] McAllister, J. C., Steelman, C. D. & Skeeles, J. K. Reservoir Competence of the Lesser Mealworm (Coleoptera: Tenebrionidae) for *Salmonella typhimurium* (Eubacteriales: Enterobacteriaceae). Journal of Medical Entomology 31, 369–372 (1994).
Pinarelli Fazion, J., Marzoli, F., Belluco, S., Bertola, M., Antonelli, P., Dolzan, B., Barco, L., Pezzuto, A. Persistance of *Salmonella* spp. in farmed insects species, a systematic review of experimental studies. PROSPERO 2022 CRD42022329213 Available from: https://www.crd.york.ac.uk/prospero/display_record.php?ID=CRD42022329213

ACHETA DOMESTICUS AS A SUSTAINABLE AND SAFE FOOD SOURCE: STABILITY OF POWDER DURING ONE-YEAR STORAGE AND RISK OF SALMONELLA PRESENCE

Filippo Marzoli (1), Alessandra Tata (2), Juliane Pinarelli Fazion (1), Carmela Zacometti (2), Michela Bertola (1), Giulia Cento (1), Beatrice Dolzan (1), Pietro Antonelli (1), Roberto Piro (2), and Simone Belluco (1)

(1) Istituto Zooprofilattico Sperimentale Delle Venezie (IZSVe), PD, Legnaro, 35020, Italy
(2) Laboratorio di Chimica Sperimentale, Istituto Zooprofilattico Sperimentale delle Venezie, Vicenza, Italy

Corresponding Author: **Filippo Marzoli** - fmarzoli@izsvenezie.it

Insects represent a promising source of animal proteins for food and feed and, specifically, Acheta domesticus (AD) is a promising option from a sustainability and safety point of view [1, 2] and it was recently approved for market in Europe [3]. Concerning the hazards associated with the consumption of insects, food-borne pathogens may occur. EU legislation of microbiological criteria of food products does not provide specific criteria for Salmonella in insect-based food. However, the commission implementing regulation that authorizes Novel Food set microbiological criteria on a case-by-case basis and, regarding edible insects, requires the absence of Salmonella [4]. The present work addresses two main goals regarding AD as a food source: 1) microbiological and chemical evaluation of AD powders during one year of storage; 2) determine the occurrence of Salmonella in farmed AD or its derivative products through a systematic review (SR). The experimental study was conducted on cricket powders obtained by three different processes: drying at 80 °C and 120 °C, and lyophilization. The pathogens Listeria monocytogenes and Salmonella were not detected, while total viable count and lactic acid bacteria tended to decrease during the storage. For Bacillus cereus, only one replicate of dried powder at 80 °C exceeded the limit set by EU Regulation. The protein contents of the powders ranged between 60.6% and 64.3%. The peroxide values were higher than the limit set by the EU Regulation in all samples, while higher amounts of hexanal and pentanal were reported in oven-dried powders than in lyophilized ones. The SR was conducted by searching five scientific literature databases and showed that the proportion of AD based food positive to Salmonella is very low. Indeed, the presence of Salmonella was reported only in two studies that analyzed processed insects, while it was never detected in raw insects. In conclusion, a one-year shelf-life can be suitable for the analyzed cricket powder from a microbiological point of view, some concerns remain from a chemical point of view due to an early tendency towards rancidity. The processing stage represents a critical point to prevent the loss of quality of the product (oxidation) and consumer exposure to pathogens such as Salmonella. Such results suggest the need to implement insect species-specific post-rearing protocols and to better investigate the possibility of AD to be infected with Salmonella.

[1] Belluco, S., Kinyuru, J., Fisher, B.L., 2022. Editorial: Insects: A source of safe and sustainable food? Front. Sustain. Food Syst. 6.
[2] EFSA Panel on Nutrition, Novel Foods and Food Allergens (NDA): Dominique Turck, Torsten Bohn, Jacqueline Castenmiller, Stefaan De Henauw, Karen Ildico Hirsch-Ernst, Alexandre Maciuk, et al. 2021. Safety of frozen and dried formulations from whole house crickets (Acheta Domesticus) as a Novel Food pursuant to Regulation (EU) 2015/2283. EFSA Journal 19 (8): e06779.
[3] Commission Implementing Regulation (EU) 2022/188 of 10 February 2022 authorising the placing on the market of frozen, dried and powder forms of Acheta domesticus as a novel food under Regulation (EU) 2015/2283 of the European Parliament and of the Council, and amending Commission Implementing Regulation (EU) 2017/2470. Off. J. Eur. Union L 30/108.
[4] Commission Implementing Regulation (EU) 2017/2470 of 20 December 2017 establishing the Union list of novel foods in accordance with Regulation (EU) 2015/2283 of the European Parliament and of the Council on novel foods. Off. J. Eur. Union L 351.

AMV

DIFFERENTIAL GLYCOPATTERN IN THE HORSE PELVIC AND PENILE URETHRA EPITHELIUM

Desantis S. (1), Cinone M. (1), Albrizio M. (1)

(1) Department of Precision and Regenerative Medicine and Ionian Area (DiMePre-J), University of Bari Aldo Moro, Italy

Corresponding Author: **Salvatore Desantis** - salvatore.desantis@uniba.it

The urethra of the male has a dual function, acting as a route for urine and semen. Often the male urethra corresponds to the preferential entrance for pathogenic agents that can affect both the urinary and the reproductive systems. Thus, the physicochemical properties of the mucosal epithelium surface are of paramount importance. The luminal glycocalyx of human urethral cells contains acid mucopolysaccharides which are involved in the prevention of bacterial colonization [1]. Despite its considerable importance, scanty reports are available about the molecular composition of the urethral epithelium, particularly in non-human species. In this study, the glycan profile of pelvic and penile urethra was investigated. Tissue fragments from the pelvic and penile urethra of three adult horse stallions in good health status, were fixed in 4% (w/v) PBS-buffered paraformaldehyde, embedded in paraffin wax, and stained with a panel of twelve lectins. Surface cells of both the pelvic and penile urethra contained glycans terminating with galactose (Gal), fucose (Fuc), N-acetylglalactosamine (GlaNAc), as well as α2,6- and α2,3-linked sialic acids. Few surface cells of penile urethra also displayed Galβl,3GalNAc (T antigen). The luminal surface of both the pelvic and penile urethra expressed all the investigated sialo- and asialoglycans, although a high presence of GalNAc-terminating glycans was detected on the surface of the pelvic urethra. Interestingly, the urothelium showed secretory activity. The lumen of the pelvic urethra contained neutral glycans terminating with N-acetylglucosamine, Gal, Fuc, and GalNAc, as well as negative-charged glycans terminating with α2,3-linked sialic acids and sialic acid-linked GalNAc. In the lumen of the penile urethra only sialoglycans such as α2,3-linked sialic acids, sialic acid-linked GalNAc, and sialic acid-linked T antigen were detected. These findings suggest that the horse urethral epithelium is coated with a complex glycosylation pattern which in addition to protecting the urethra against pathogens, as in humans is capable of modifying the urethral milieu [2]. Since the seminal plasma (SP) also contains glycoproteins [3], the role of the urethra in the SP composition cannot be excluded.

[1] Alm P., Colleen S. Acta Path. Microbiol. Immunol. Scand. Sect. A, 90: 103-111, 1982
[2] Zecchi Orlandini S., Orlandini G. E. Arch. Androl. 23: 51-59,1989
[3] Pini T. et al. Reprod. Fertil. Dev. 30: 689-702, 2018

76° CONVEGNO SISVET
BARI, 21-22-23 GIUGNO 2023

MACROSCOPIC AND STEREOLOGICAL ASSESSMENT OF THE OVARIAN RESERVE IN CALVES BORN FROM NUTRIENT-RESTRICTED COWS

Lazzarini G. (1), Frau A. (2), Edache D. (3), Sadeghinezhad J. (4), Atzori A.S. (3), Gallo A. (5), Franciosi F. (6), Mossa F. (2), Miragliotta V. (1)

(1) Department of Veterinary Sciences, University of Pisa, Italy
(2) Department of Veterinary Medicine, University of Sassari, Sassari, Italy
(3) Department of Agriculture, University of Sassari, Sassari, Italy
(4) Department of Veterinary Anatomy, Faculty of veterinary medicine, University of Tehran, Tehran, Iran
(5) Department of Animal Science, University Cattolica del Sacro Cuore, Piacenza, Italy
(6) Department of Veterinary Medicine and Animal Sciences, University of Milan, Italy

Corresponding Author: **Vincenzo Miragliotta** - vincenzo.miragliotta@unipi.it

The Developmental Origins of Health and Disease (DOHaD) hypothesis postulates that prenatal undernutrition (among other factors) can affect an individual's long-term health. Previous data showed that offspring from nutritionally restricted cows display a diminished ovarian reserve, as assessed by a reduction in ultrasonographical antral follicle count, reduced serum concentration of anti-Mullerian hormone, and increased serum concentration of follicle-stimulating hormone[1].
Here, twenty-two female calves were born from three groups of differentially fed dams. Specifically, starting 10 days before artificial insemination, animals were individually fed at: 0.6 of their maintenance energy requirements (M) up to day 80 (Nutrient Restricted, NR80, N=8); 0.6 M up to day 120 of gestation (NR120, N=9); 1.8M until day 120 of pregnancy (Control, C, N=5). At slaughter (4.5 mo. of age), ovaries were measured, weighted, all visible antral follicles were counted before formalin fixation. Stereological observations were performed on 4 ovaries collected from NR120 and four from C. Systematic uniform random sections were obtained. Each ovary was embedded in 7% Agar and cut into parallel slabs with a fixed distance that were embedded in paraffin blocks. Two consecutive sections (dissector pairs) were cut from each block and stained with H&E. Each ovary's dissector pair was analyzed using the physical fractionator method to obtain the number of primordial and primary follicles. Total number of primordial and primary follicles was thus calculated by dividing the total counted follicles by the block section fraction (distance between pairs / distance between slabs). The student T test was used for statistical analysis.
Ovarian weight was lower ($p<0.05$) in NR120 compared to C and similar between NR120 and NR80 (C=10.4±1.3; NR80=7.4±0.9; NR120=6.7±0.5 g). NR120 heifers had less ($p<0.05$) visible antral follicles than C whereas no difference was detected between NR80 and C (C=197.2±36.5; NR80=150.1±20.9; NR120=104.2±10.7). Primordial follicles were 60525±17665 in C and 41275±23026 in NR120. Primary follicles were 25675±4242 in C and 13900±7970 in NR120 ($p<0.05$).
Maternal exposure to undernutrition from preconception to day 120 of gestation impaired the daughter's ovarian reserve. Despite contrasting data are reported on the number of ovarian follicles and their decrease with growth in cows[2-4], no attempts have to date been made to assess their number in a stereological way.

[1] Mossa, F., et al., Maternal undernutrition in cows impairs ovarian and cardiovascular systems in their offspring. Biol Reprod, 2013. 88(4): p. 92.
[2] Modina, S.C., et al., Reductions in the number of mid-sized antral follicles are associated with markers of premature ovarian senescence in dairy cows. Reprod Fertil Dev, 2014. 26(2): p. 235-44.
[3] Monniaux, D., Factors influencing establishment of the ovarian reserve and their effects on fertility. Anim Reprod, 2018. 15(Suppl 1): p. 635-647.
[4] Monniaux, D., et al., The ovarian reserve of primordial follicles and the dynamic reserve of antral growing follicles: what is the link? Biol Reprod, 2014. 90(4): p. 85.

MICROGLIA ANALYSIS: FROM THE MORPHOLOGICAL TO THE MULTIDIMENSIONAL PHENOTYPE

Lorenzini L. (1), Baldassarro V.A. (1), Sannia M. (2), Zanella L. (1), Moretti M. (1), Quadalti C. (3), DeGiorgio R. (4), Clavenzani P. (1), Giardino L. (1,2), Calzà L. (2,3)

(1) Department of Veterinary Medical Sciences, University of Bologna, Bologna, Italy
(2) IRET Foundation, Ozzano Emilia (BO), Italy
(3) Department of Pharmacy and Biotechnologies, University of Bologna, Bologna, Italy
(4) Department of Translational Medicine, University of Ferrara, Ferrara, Italy

Corresponding Author: **Luca Lorenzini** - luca.lorenzini8@unibo.it

Microglia is a self-renewing brain-resident immune cell population committed to maintain the microenvironmental homeostasis. The microglia-activated state is conventionally described as the morphological transition from a resting state ("immobile" highly branched, M0 type) into activated M1 pro- or M2 anti-inflammatory phenotype, by a variety of dystrophic morphological features. However, more complex and heterogeneous molecular signatures are emerging. Our laboratory is investigating if microglia physiopathological transition triggered by systemic inflammation worsen the age-dependent neuroinflammation observed in a mouse model of Alzheimer disease (Tg2576). We analyzed the morphological and molecular phenotype of microglia in Tg2576 vs WT age matching mice, in which colitis was induced as model of systemic inflammation. Mice were monitored for spatial learning and memory ability at baseline (2months of age) by Morris water maze test, then colitis was induced by Dextran Sodium Sulphate (DSS) in 3 months old mice, and animals tested again at 4 and 5 months. Systemic inflammation was monitored by cytokine plasma assay (IL6, IL10, IL17, TNF□) at different time-points after colitis induction. Neuroinflammation was monitored in the cerebral cortex and hippocampus by cytofluorimetric analysis of microglia surface markers (CD11, CCL2, CD88, CCR5, CD195, CD61, CD253, P2X7R) at the peak of colitis clinical score, by RT-PCR RNA expression analysis of neuroinflammation associated genes (Nfe2/2, Nlrp3, Cx3cr1, ApoE, P2ry12, Tmem119), by Iba1-IR microglia morphological analysis at long-term. The main results of the study are the followings: (i) TNFa plasma level are higher in young, asymptomatic Tg2576 mice compared to WT mice before colitis induction, and this correspond to microglia proliferation, as described by increased CD11-positive cells; (ii) peripheral inflammation related to DSS colitis worsen the age-dependent cognitive decline in Tg2576 mice; (iii) gene expression analysis reveals an impairment of microglia-related genes regulation by DSS in Tg2576 compared to WT. Finally, the simple morphological analysis of microglia branching, performed by artificial intelligence-assisted image analysis (Imaris 3D voxel-based software on confocal microscopy images) fails to detect the brain inflammatory state. We conclude that a multidimensional approach combining morphological and molecular data is needed for a proper description of microglia state.

[1] Bighinati A, Focarete ML, Gualandi C, Pannella M, Giuliani A, Beggiato S, Ferraro L, Lorenzini L, Giardino L, Calzà L. Improved functional recovery in rat spinal cord injury induced by a drug combination administered via an implantable polymeric delivery system. J. Neurotrauma, 2020, 37:1708-1719
[2] Giuliani A., Sivilia S., Baldassarro V.A., Gusciglio M., Lorenzini L., Sannia M., Calzà L., Giardino L., Age-related changes of the neurovascular unit in the cerebral cortex of Alzheimer's disease and age-matching wild-type mouse models: a neuroanatomical and molecular study J. Neuropathol. Exp. Neurol., 2019, 78:101-112
[3] Lorenzini L, Giuliani A, Sivilia S, Baldassarro VA, Fernandez M, Lotti Margotti M, Giardino L, Fontani V, Rinaldi S, Calzà L, REAC technology modifies pathological neuroinflammation and motor behaviour in an Alzheimer's disease mouse model. Scientific Reports, 2016, 6:35719-35728
[4] Borjini N, Fernandez M, Giardino L, Calza L Cytokines and chemokines alterations in tissue, CSF and plasma in early, presymptomatic phase of experimental allergic encephalomyelitis (EAE), a rat model of multiple sclerosis. J Neuroinflammation, 2016, 13:291-307.

MORPHOLOGICAL PHENOTYPING OF THE MOUSE AGING COCLEA: LINKING BASIC RESEARCH TO PRECLINICAL APPLICATIONS

Attanasio C. (1), Palladino A. (2), Mastrorilli V. (3,4), Matey A.T. (3,4), Varricchio E. (5), Scavizzi F. (3,4), Raspa F. (3,4), Scocco P. (6), de Girolamo P. (1), De Felice E. (6)

(1) Università degli Studi di Napoli - Dipartimento di Medicina Veterinaria e Produzioni Animali
(2) Università degli Studi di Napoli - Dipartimento di Agraria
(3) Consiglio Nazionale delle Ricerche (CNR) – Istituto di Biologia Cellulare e Neurobiologia
(4) Consiglio Nazionale delle Ricerche (CNR) - Istituto di Biochimica e Biologia Cellulare - International Campus of Development (EMMA-INFRAFRONTIER-IMPC)
(5) Università degli Studi del Sannio - Dipartimento di Scienze e Tecnologie.
(6) Università degli Studi di Camerino - Scuola di Bioscienze e Medicina Veterinaria

Corresponding Author: **Chiara Attanasio** - chiara.attanasio@unina.it

Morphological mouse phenotyping plays a pivotal role in the translational setting by providing a reliable framework for molecular and instrumental analysis and this is even more accurate in the arena of auditory research, where mouse is a central model organism due to a high degree of evolutionary genetic relationship and morpho-functional analogies with the human auditory system (1).
In this work, we approach the characterization of aging process in mouse cochlea by comparing significant morphological markers in mice belonging to two of the most common used inbred (C57BL/6) and outbred (CD1) strains in both male and females during aging, i.e. in animals ranging from 2 to 18 months of age (approved by Ministry of Health with protocol 1177/2020-PR). Further, we strive to integrate morphological data with those stemming from molecular and instrumental analyses (auditory brainstem response) to correlate morphological changes to transcriptional variation and functional discrepancies between both strains and sexes (2). At each timepoint, we stained the tissue by haematoxyilin-eosin to highlight morphological changes and identified by immunofluorescence key molecules in signal transduction to detect at which stage the sensory decline starts and, therefore, track the degenerative process. Specifically, to identify age-related neurodegeneration indicators, we featured the localization of TMC1, Gipc3, Myosin VIIa, and Cdh23 in the hair cells. Further to gain more insights on the correlation between synaptopathy and sensory hair cell loss we labeled NF200 which identifies neural cytoskeleton.
Over aging, we observed a clear drop in the selected marker occurrence and in the number of type 1 spiral ganglion neurons which are the first action potential generating neurons in the auditory pathway (3). Notably, our observations were performed in inbred and outbred mouse strains which may be an asset to investigate the physiological trigger of age-related hearing loss. In addition, this study is part of a broader project that includes the characterization of vision loss at the same timepoints to identify the potential onset of compensatory mechanisms between the two senses (4). Overall, our data may provide new insights in the translational setting as well as in comparing the phenotypic variation in the two most common used laboratory mouse strains (2).

[1] Bowl MR and Dawson S, The mouse as a model for age-related hearing loss. Gerontology 2015, doi:0.1159/000368399.
[2] Tuttle AH et al., between inbred and outbred mice. Nat Methods. 2018, 15(12): 994–996.
[3] Carricondo F and Romero-Gomez B, The Cochlear Spiral Ganglion Neurons: The Auditory Portion of the VIII Nerve. Anat Rec 2018, doi.org/10.1002/ar.23815.
[4] Mahmoudi E, Hearing, Vision, or Dual Sensory Impairment and Dementia Risk. JAMA Netw Open 2021;4(3):e211846.

AGE ALTERATIONS OF CEREBRAL CORTICAL FEATURES IN TURSIOPS TRUNCATES

Graic J.M. (1), Peruffo A. (1), Corain L. (1), Finos L. (1), Grisan E. (1), Orekhova K. (1), Gerussi T. (1), Centelleghe C. (1), Cozzi B. (1)

(1) Department of Comparative Biomedicine and Food Science, University of Padova, Italy

Corresponding Author: **Jean-Marie Graic** - jeanmarie.graic@unipd.it

The odontocete auditory cortex is a critical cortical area for their fitness and arguably their survival, given their heavy reliance on echolocation for foraging and hunting activity(1). There is partial evidence that dolphins are born with the capacity to produce and receive sounds, which they likely develop into adulthood and subsequently potentially lose in old age. There is relatively little data describing the cortical architecture in cetaceans and even less so comparing species or age classes (1,2). We compared the cortical neuron populations found in very young, adult and older age classes to try and find measurable differences pertaining putatively to cortical development and potential rearrangement. As expected, the magnitude of the changes were relatively minute since the cortex is mostly mature (myelinised) at birth in cetaceans as in many precocious mammals, but by analysing thousands of cells per specimen and per cortical layer, we were able to find discrete differences. Neurons became noticeably larger, with a larger area, diameter, perimeter and axes lengths, going from calves to older animals. Regarding surface density, layers 3, 5 and 6 showed a clear reduction from calves, to adults to older animals. Classifying the cells by their shape using morphometric thresholds, we were able to determine that there was a very significant change in the size of rounder cells from calves (smaller) to adult to old (larger). The shape of round and ellipsoid cells was most different across age classes in the first cortical layer, suggesting the involvement of interneurons and glia in these differences. Taken together, these changes suggest that mechanisms underlying neural aging could be unfolding differently from that of terrestrial artiodactyls (3).

[1] Cozzi, B., Huggenberger, S., & Oelschläger, H. (2016). Anatomy of dolphins: Insights into body structure and function. In Anatomy of Dolphins: Insights into Body Structure and Function. Academic Press.
[2] Fung, C., Schleicher, A., Kowalski, T., & Oelschläger, H. H. A. (2005). Mapping auditory cortex in the La Plata dolphin (Pontoporia blainvillei). Brain Research Bulletin, 66(4–6), 353–356. https://doi.org/10.1016/j.brainresbull.2005.02.019
[3] Hof, P. R., Glezer, I. I., Archin, N., Janssen, W. G., Morgane, P. J., & Morrison, J. H. (1992). The primary auditory cortex in cetacean and human brain: A comparative analysis of neurofilament protein-containing pyramidal neurons. Neuroscience Letters, 146(1), 91–95. https://doi.org/10.1016/0304-3940(92)90180-F

THE AGE-RELATED CHANGES IN VERTEBRATE RETINA: INSIGHTS FROM STUDIES IN INBRED AND OUTBRED MOUSE STRAINS

Palladino A. (1), Scavizzi F. (2,3), Raspa M. (2,3), Mastrorilli V. (2,3), Matey A.T. (2,3), Crasto A. (1), Lucini C. (4), P de Girolamo P. (5), De Felice E. (5), Attanasio C. (4)

(1) Università degli Studi di Napoli Federico II - Dipartimento di Agraria
(2) Consiglio Nazionale delle Ricerche - Istituto di Biologia Cellulare e Neurobiologia
(3) Consiglio Nazionale delle Ricerche - Istituto di Biochimica e Biologia Cellulare - International Campus EMMA-INFRAFRONTIER-IMPC
(4) Università degli Studi di Napoli Federico II - Dipartimento di Medicina Veterinaria e Produzioni Animali
(5) Università degli Studi di Camerino - Scuola di Bioscienze e Medicina Veterinaria

Corresponding Author: **Antonio Palladino** - antonio.palladino@unina.it

The vertebrate retina is a complex and highly organized tissue that plays a critical role in vision. As individuals age, the retina undergoes a variety of changes, including a decline in photoreceptor function, thinning of the layers, and alterations in the structure and function of glial retinal cells, that can impact visual function [1]. Such changes have been associated with a variety of visual disorders, including age-related macular degeneration and diabetic retinopathy [2,3]. Understanding the molecular mechanisms underlying these alterations is crucial for developing effective therapies to treat these disorders. Animal models provide valuable tools to study the aging of the retina and, based on their different genetic background, the combinatory use of both inbred and outbred mouse strains may lead to a more comprehensive understanding of the aging process. In this context, we propose a multimodal approach to identify and characterize stages of age-related retinal degeneration with high temporal resolution. To this end, we conducted in vivo, and ex vivo analyses using optical computed tomography (OCT), electroretinogram (ERG) and immunofluorescence (IF) experiments, respectively, to estimate the age-dependent decline of retina function. Mouse retinas from two different strains, an inbred (C57BL/6) and an outbred (CD-1), both male and female, were analyzed at four different time points (2, 6, 12, and 18 months). The research was approved by the Ministry of Health with protocol 1177/2020-PR. OCT provided structural information on the thickness of each retinal layer, documenting a decrease in retinal thickness associated with aging. ERG measures the light-induced electrical activity of the retina, and results indicate a progressive reduction of amplitudes of both a- and b- waves. Additionally, we conducted IF using retinal-specific markers to label key retinal structures, such as GNAT-2 for photoreceptor cells (PRCs), PKCα for rod bipolar cells (RBCs) and Calbindin for horizontal cells (HCs). We also investigated synaptophysin as a marker of cell synapses between photoreceptors and horizontal cells. Consistent with the in vivo data, IF analysis demonstrated a significant age-dependent reduction of PRCs, HCs, RBCs, as well as the area of synapses between PRCs and HCs. Taken together these findings demonstrate a decline in retinal layers associated with aging and suggest potential implications for age-related visual impairment in both mouse strains.

[1] Jackson GR, Owsley C. Visual dysfunction, neurodegenerative diseases, and aging. Neurol Clin. 2003;21(3):709-728. doi:10.1016/s0733-8619(02)00110-5
[2] Bourne, R. R. A., Stevens, G. A., White, R. A., Smith, J. L., Flaxman, S. R., Price, H., … Resnikoff, S. (2013). Causes of vision loss worldwide, 1990-2010: a systematic analysis. The Lancet Global Health, 1(6), e339–e349. https://doi.org/10.1016/S2214-109X(13)70113-7
[3] Wong, W. L., Su, X., Li, X., Cheung, C. M. G., Klein, R., Cheng, C.-Y., … Wong, T. Y. (2014). Global prevalence of age-related macular degeneration and disease burden projection for 2020 and 2040: a systematic review and meta-analysis. The Lancet Global Health, 2(2), e106–e116. https://doi.org/10.1016/S2214-109X(13)70145-1

DISTRIBUTION OF VASOACTIVE INTESTINAL PEPTIDE (VIP) IMMUNOREACTIVITY IN THE RAT PALLIAL AND SUBPALLIAL AMYGDALA AND COLOCALIZATION WITH γ-AMINOBUTYRIC ACID

Salamanca G. (2), Tagliavia C. (1), Graic J-M. (3), Grandis A. (2), Bombardi C. (2)

(1) Department of Veterinary Medicine, University of Teramo
(2) Department of Veterinary Medical Sciences, University of Bologna
(3) Department of Comparative Biomedicine and Food Science, University of Padua

Corresponding Author: **Giulia Salamanca** - giulia.salamanca2@unibo.it

The amygdaloid complex, also known as amygdala, is a heterogeneous group of distinct nuclear and cortical pallial and subpallial structures. The amygdala plays an important role in several complex functions including emotional behavior and learning. GABAergic neurons located in pallial and subpallial amygdala are immunohistochemically heterogeneous regarding the content of calcium binding proteins and peptides, some of which are expressed in separate groups of cells(McDonald, 2020). Vasoactive intestinal polypeptide (VIP) is present in specific subpopulations of GABAergic cells in the amygdala. Although VIP immunoreactivity has been observed in somatodendritic and axonal profiles of the rat basolateral and central amygdala, there is no currently available comprehensive analysis of the distribution of VIP immunoreactivity in the different amygdalar pallial and subpallial structures. The present study utilized immunohistochemical and morphometrical techniques to analyze the distribution and the neuronal localization of VIP immunoreactivity in the rat pallial and subpallial amygdala. In pallial amygdala VIP-IR neurons are local inhibitory interneurons that presumably can regulate directly and indirectly the activity of pyramidal excitatory neurons. In the subpallial amygdala VIP immunoreactivity is expressed in different inhibitory cell types that probably act as projection or local interneurons. The distribution of VIP immunoreactivity is nonhomogeneous throughout the different amygdalar areas, suggesting a distinct impact of this neuropeptide on neuronal amygdaloid circuits activities and, consequently, on the cognitive, emotional, behavioral and endocrine activities amygdala mediated.

[1] McDonald AJ. Functional neuroanatomy of the basolateral amygdala: Neurons, neurotransmitters, and circuits. Handb Behav Neurosci. 2020; 26:1-38.

THE EPITHELIAL-MESENCHYMAL-TENDON TRANSITION STATE OF AMNIOTIC EPITHELIAL STEM CELLS INFLUENCES TENDON REGENERATION: INSIGHTS FROM AN EXPERIMENTAL SHEEP ACHILLES TENDON INJURY MODEL

El Khatib M. (1), Barboni B. (1), Berardinelli P. (1), Mauro A. (1), Turriani M. (1), Peserico A. (1), Russo V. (1)

(1) Unit of Basic and Applied Biosciences, Department of Biosciences, and Agro-Food and Environmental Technologies, University of Teramo, 64100, Teramo, Italy

Corresponding Author: **Mohammad El Khatib** - melkhatib@unite.it

Tendinopathies remain a One Health unmet medical demand as conventional therapies, both in humans and animals, are unsatisfactory (1). In this context, the use of amniotic epithelial stem cells (AECs) has emerged as a promising strategy to address tendon pathology (2-4). In the present research, by exploiting the epithelial-mesenchymal-tendon transition state of AECs, it was possible to verify their role in tendon regeneration. To this aim, three different AEC subsets: epithelial (eAECs), mesenchymal (mAECs), and tendon-like (tdAECs) were allotransplanted in a validated experimental sheep Achilles tendon injury model. Tissue remodeling was compared at two endpoints, 14 and 28 days, and the results showed that all AEC subsets accelerated tendon regeneration compared to spontaneous healed tendon used as control. According to total histological scores, eAECs and tdAECs transplantations were more efficient than mAECs, with a significantly relevant effect shown 28 days post-transplantation. Furthermore, regardless of AEC state, allo-transplantations favored a pro-regenerative immune response characterized by increased IL-10/IL-12 levels compared to the control ($p<0.001$). The AECs' genotypic and phenotypic state influenced the immune response within the injury site where eAECs-transplanted tendons induced a significant upregulation of pro-regenerative M2 macrophages with respect to mAECs and tdAECs 14 days post-transplantation. An in-depth analysis allowed to evidence that eAECs and tdAECs exhibited two different underlying regenerative mechanisms within the tendons. eAECs positively influenced tendon regeneration by mainly shifting the pro-inflammatory response to a pro-regenerative one within the host tissue, followed by the deposition of organized extracellular matrix (ECM) and blood vessel remodeling. tdAECs chiefly acted during the proliferative phase, affecting the density of ECM and supporting a prompt recovery characterized by decreased cell density and improving cell alignment along the longitudinal axis of collagen fibers. These findings provide insights into how AECs with different phenotypes can orient tendon regeneration through a crosstalk with the host tissue, potentially leading to the development of new therapeutical approaches for tendon injuries. The evidence gathered indicates that eAECs can be a feasible and effective approach to managing acute tendinopathies, strengthening the rationale for advancing their utilization in clinical settings.

[1] V. Russo; M. El Khatib et al. Tendon Immune Regeneration: Insights on the Synergetic Role of Stem and Immune Cells during Tendon Regeneration. Cells. 2022. 11, 434.
[2] B. Barboni; V. Russo et al. Therapeutic Potential of HAECs for Early Achilles Tendon Defect Repair through Regeneration. J. Tissue Eng. Regen. Med. 2018, 12, e1594–e1608.
[3] B. Barboni; V. Russo et al. Achilles Tendon Regeneration Can Be Improved by Amniotic Epithelial Cell Allotransplantation. Cell Transpl. 2012, 21, 2377–2395.
[4] A. Mauro; V. Russo et al. M1 and M2 Macrophage Recruitment during Tendon Regeneration Induced by Amniotic Epithelial Cell Allotransplantation in Ovine. Res. Vet. Sci. 2016, 105, 92–102.

76° CONVEGNO SISVET
BARI, 21-22-23 GIUGNO 2023

THE DIFFERENTIATION POTENTIAL OF CANINE PERI-OVARIAN ADIPOSE TISSUE-DERIVED MESENCHYMAL STROMAL CELLS

Mirra G. (1), Herrera Millar V. (2), Lange Consiglio A. (3), Carnevale L. (3), Cremonesi F. (3), Veronesi M.C. (3), Bazzocchi C. (3), Martini V. (3), Comazzi S. (3), Modina S.C. (3), Di Giancamillo A. (2)

(1) Department of Comparative Biomedicine and Food Science, University of Padua, Viale dell'Università 16, Legnaro, 35020, Italy
(2) Department of Biomedical Sciences for Health, University of Milan, Via Mangiagalli 31, Milan, 20133, Italy
(3) Department of Veterinary Medicine and Animal Sciences (DIVAS), University of Milan, Via dell'Università 6, Lodi, 26900, Italy

Corresponding Author: **Giorgio Mirra** - giorgio.mirra@unipd.it

This study wanted to compare the proliferative and differentiative potential of mesenchymal stromal cells (MSCs) (1) isolated from peri-ovarian adipose tissue (POAT), which is an innovative source of MSCs, and from the commonly used subcutaneous adipose tissue (SAT). The adipose tissue was obtained as waste material following routine ovariectomy, from 5 healthy 30 Kg Labrador Retriever dogs (OPBA 137_2021).
As a preliminary analysis, a histological and histometrical comparison between SAT and POAT adipocytes was carried out. Results showed significant differences between cells (area and perimeter, POAT>SAT, $p<0.05$) from the two different sources, but no differences were observed in the stromal fraction. Subsequently, as the first step of our study, MSCs were isolated in vitro cultured and characterized. At passages P1, P3, and P5 the cell growth curve was realized, the colony-forming unit assay was performed, and the doubling time was calculated to assess MSCs' proliferative potential. MSCs were also screened for positive MSC markers (≥95%, CD44 and CD90) and negative for hematopoietic markers (≤2%, CD11b, CD31, CD34, CD45, and MHC-II), as described in literature (2). As expected, MSCs were positive for both mesenchymal markers, while they were negative for all hematopoietic markers with no significant differences between POAT and SAT-derived MSCs markers expression. The chondrogenic, osteogenic, and adipogenic differentiation potential was assessed and tested by specific histochemical stainings (Alcian blue, Alizarin Red, and Oil Red O staining, respectively). In both POAT and SAT, positive staining was observed thus confirming successful differentiation. The analysis carried out has established that, similarly to SAT, POAT is endowed with multipotentiality.
In the second phase of the study, the 3D chondrogenic differentiation potential of MSCs derived from the two sources was tested. Cells were also 3D-cultured in fibrin scaffolds and their differentiation was evaluated at different time points (1 day, 4 days, 7 days, and 10 days of culture) by Alcian Blue and Safranin-O stainings to assess, respectively, glycosaminoglycans and proteoglycans production. Results showed an increased production through the different time points.
In conclusion, the 3D differentiation is very promising, and it could be a starting point for MSCs transplantation for typical breed pathologies in Labradors.

Funding: Linea 2 UNIMI ADG 2021

[1] Caplan, A.I. Mesenchymal Stem Cells. Journal of Orthopaedic Research 1991, 9, 641–650, doi:10.1002/jor.1100090504.
[2] Hendawy, H.; Uemura, A.; Ma, D.; Namiki, R.; Samir, H.; Ahmed, M.F.; Elfadadny, A.;El-Husseiny, H.M.; Chieh-Jen, C.; Tanaka, R. Tissue Harvesting Site Effect on the Canine Adipose Stromal Vascular Fraction Quantity and Quality. Animals 2021, 11, 460, doi:10.3390/ani11020460

CATTLE CONGENITAL PSEUDOMYOTONIA AS ANIMAL MODEL: WHEN DIAPHRAGM MUSCLE CAN MAKE THE DIFFERENCE

Akyürek E.E. (1), Jacinto J.G.P. (2), Bolcato M. (2), Bianchini E. (3), Sandonà D. (3), Gentile A. (2), Sacchetto R. (1)

(1) Department of Comparative Biomedicine and Food Science, University of Padova;
(2) Department of Veterinary Medical Sciences, University of Bologna;
(3) Department of Biomedical Science, University of Padova

Corresponding Author: **Roberta Sacchetto** - roberta.sacchetto@unipd.it

Cattle "congenital pseudomyotonia" (PMT) is a muscular disorder described for the first time in Chianina cattle breed [1] and subsequently in Romagnola breed, characterized by an impairment of muscle relaxation induced by exercise. By DNA sequencing, we provided evidence of missense mutations in ATP2A1 gene, coding for SERCA1 isoform. Pathological muscles of PMT affected animals are characterized by a striking, selective reduction in the expression level of SERCA1 protein. Although present at low levels, the SERCA1 variants maintained the basic intrinsic properties of wild-type (WT) SERCA1, notably the Ca2+ dependent ATPase activity [2,3]. On the bases of clinical, genetic and biochemical analyses, our group has demonstrated that PMT is the true counterpart of human Brody myopathy, a rare and orphan disease for which an appropriate pharmacological treatment is now not available.
Our results showing that in bovine PMT, SERCA1 mutations do not abolish SERCA1 activity, prompted us to hypothesize that the retention of functional properties of mutated SERCA pump represents the requisite for the efficacy of a potential innovative therapy. We have recently designed a novel pharmacological approach based on the employment of protein folding CFTR correctors, specifically developed for rescuing type II Cystic Fibrosis Transmembrane Regulator (CFTR) mutants [4].
Our preliminary results show that CFTR correctors successfully rescued in vitro SERCA1 mutants, causing bovine PMT.
In vitro studies are fundamental for experimental settings and permit tight control of fundamental principles and the testing of therapeutic hypotheses. They represent the first step toward a cure, being the second step of preclinical research in drug development, represented by in vivo animal studies which are essential and necessary before clinical trial can be pursued. Mouse is undoubtedly the preferred model for preclinical therapeutic trials, nevertheless sometimes this model fails to mirror the disease phenotype and therefore is unsuitable to study certain human pathologies. This is the case of human Brody disease. Due to the wide difference in fiber type composition of diaphragm muscle between mice and large mammals, including humans, mouse is not appropriate as animal model for this pathology. To solve this problem, we treated locally bovine PMT affected muscle with CFTR correctors, resulted from the most promising in vitro studies.

[1] Sacchetto R., Testoni S., Gentile A., Damiani E., Rossi M., Liguori R., Drögemüller C., Mascarello F. (2009) A defective SERCA1 protein is responsible for congenital pseudomyotonia in Chianina cattle. Am. J. Pathol. 174:565-573.
[2] Bianchini E., Testoni S., Gentile A., Calì T., Ottolini D., Villa A., Brini M., Betto R., Mascarello F., Nissen P., Sandonà D., Sacchetto R. (2014) Inhibition of Ubiquitin Proteasome System Rescues the Defective Sarco(endo)plasmic Reticulum Ca2+-ATPase (SERCA1) Protein Causing Chianina Cattle Pseudomyotonia. J. Biol. Chem. 289:33073-33082.
[3] Akyürek E E, Busato F., Murgiano L., Bianchini E., Carotti M, Sandonà D., Drögemüller C., Gentile A., Sacchetto R. (2022) Differential analysis of Gly211Val and Gly286Val mutations affecting Sarco(endo)plasmic Reticulum Ca2+ATPase (SERCA1) in congenital pseudomyotonia Romagnola cattle. Int J Mol Sci. 23:12364.
[4] Scano, M., Benetollo, A., Nogara, L., Bondì, M., Dalla Barba, F., Soardi, M., Furlan, S., Akyürek E. E., Carotti, M., Sacchetto, R., Blaauw, B., Sandonà, D. (2021). CFTR corrector C17 is effective in muscular dystrophy, in vivo proof of concept in LGMDR3. Hum. Mol Genet.

SLAUGHTERED SOWS: CAN THEY REPRESENT A GOOD SPONTANEOUS MODEL OF OSTEOARTHRITIS?

Herrera Millar V.R. (1), Karjalainen V.P. (2), Finnila M.A.J. (2), Saarakkala S. (2,3) Mobasheri A. (2), Peretti G.M. (1,4) Pallaoro M. (5), Caniatti M. (5), Bassi J. (5), Di Giancamillo M. (5), Modina S. (5), Di Giancamillo A. (1)

(1) Department of Biomedical Sciences for Health, University of Milan, Milan, Italy
(2) Research Unit of Health Science and Technology, Faculty of Medicine, University of Oulu, Oulu, Finland
(3) Department of Diagnostic Radiology, Oulu University Hospital, Oulu, Finland
(4) IRCCS Istituto Ortopedico Galeazzi, Milan, Italy
(5) Department of Veterinary Medicine and Animal Sciences, University of Milan, Lodi, Italy

Corresponding Author: **Valentina Rafaela Herrera Millar** - valentina.herrera@unimi.it

Osteoarthritis (OA) is one of the most common chronic diseases affecting the joints and it is highly relevant in veterinary and human medicine [1]. To date, there are still no effective therapies except for relieving symptoms. This is most likely related to poorly known pathogenesis of the OA, although some risk factors, such as age and obesity, are known [2]. Intensive breeding of pigs for meat production exposes the animals to excessive weight gain in a few months. We further hypothesize that excess weight can spontaneously compromise the integrity of the knee. The aim of this study was to verify if the 250 kg-sow can represent a good model for the study of OA. Five knee joints of slaughter sows (250 kg) and five light pigs (110 kg) were collected. The knees were first scanned with clinical computed tomography, radiography, and magnetic resonance imaging. Synovial fluid was collected for cytological analysis, and medial menisci were divided into the anterior horn (AH), central body (CB), and posterior horn (PH): for each portion, two adjacent sections were collected for subsequent histology and micro-computed tomography (µCT). No degeneration or edema was detected, but in the synovial fluid of sows, proteinaceous material and increased cellularity were observed, consisting exclusively of large mononuclear cells, with central/paracentral round nucleus, and abundant weakly stained cytoplasm. Rare lymphocytes and neutrophils both without signs of nuclear degeneration were observed. The cytological outcomes were compatible with chronic degenerative arthropathy. The meniscus degeneration was also confirmed by histological findings. Picro-Sirius Red staining was used to evaluate the fibers trend, and Safranin-O staining for cell morphology and proteoglycan deposition. The criteria and scores of Pauli et al were used to make the histological evaluation [3]. Moderate and severe degeneration was observed in the menisci of 250 kg-sows (grades 3-4), while in light pigs the menisci appeared normal or in early stages of degeneration (grades 1-2). Fiber orientation in three-dimensional reconstruction, performed by µCT, confirmed that the 250 kg-sow exhibits the typical signs of meniscus degeneration. Preliminary data suggest that sow could be used as animal model for knee OA that is related to overloading.

[1] Szponder T, Latalski M, Danielewicz A, Krać K, Kozera A, Drzewiecka B, Nguyen Ngoc D, Dobko D, Wessely-Szponder J. Osteoarthritis: Pathogenesis, Animal Models, and New Regenerative Therapies. J Clin Med. 2022 Dec 20;12(1):5. doi: 10.3390/jcm12010005. PMID: 36614806; PMCID: PMC9821671.
[2] Grässel S, Muschter D. Recent advances in the treatment of osteoarthritis. F1000Res. 2020 May 4;9:F1000 Faculty Rev-325. doi: 10.12688/f1000research.22115.1. PMID: 32419923; PMCID: PMC7199286.
[3] Pauli C, Whiteside R, Heras FL, Nesic D, Koziol J, Grogan SP, Matyas J, Pritzker KP, D'Lima DD, Lotz MK. Comparison of cartilage histopathology assessment systems on human knee joints at all stages of osteoarthritis development. Osteoarthritis Cartilage. 2012 Jun;20(6):476-85. doi: 10.1016/j.joca.2011.12.018. Epub 2012 Feb 18. PMID: 22353747; PMCID: PMC3348372.

76° CONVEGNO SISVET
BARI, 21-22-23 GIUGNO 2023

ASSESSMENT OF NERO DI LOMELLINA PIG MEAT: PRELIMINARY RESULTS FROM THE MORPHOLOGICAL AND PHYSICAL CHARACTERIZATION OF THE SEMIMEMBRANOSUS MUSCLE

Pallaoro M. (1), Di Giancamillo A. (2), Rossi R. (1), Aidos L. (1), Herrera V. (2), Costa A. (1), Buoio E. (1), Mazzola S. (1), Di Giancamillo M. (1), Bassi J. (1), Modina S. (1)

(1) Università degli Studi di Milano, Dipartimento di Medicina Veterinaria e Scienze Animali, Via dell'Università 6, Lodi (LO), 26900
(2) Università degli Studi di Milano, Dipartimento di Scienze Biomediche per la Salute, Via Mangiagalli 31, Milano (MI), 20133

Corresponding Author: **Margherita Pallaoro** - margherita.pallaoro@unimi.it

Nero di Lomellina (NL) is a recently recognized Italian local pig breed of the area of Pavia. Being a rustic animal, it can easily resist adverse environmental conditions without altering its productive performance; additionally, its high-quality meat is already widely appreciated and identified with its geographical origin. Hence, NL represents a real richness for the territorial economy, but thus far very little is known about it [1].
This study aimed to characterize NL's Semimembranosus (SM), one of the main thigh muscles involved in cured meat production. NL data were compared with those obtained for Commercial Hybrid (CH) pigs, born and equally bred on the same farm: this provided us with a robust and reliable comparison.
Thighs from 160 kg pigs were collected at the slaughterhouse, and SM was sampled as a representative muscle of the whole thigh; characterization of muscle fibers morphology and typology was performed by histological, histochemical, and immunohistochemical analyses, followed by histometric quantification [2]. The pH and colour parameters were evaluated at 24 h *post mortem*. Instrumental tenderness, drip and cooking losses were also assessed [3]. Moreover, the muscle and the adipose masses were evaluated by Computed Tomography (CT) to verify NL thigh fat deposition [4].
The morphological evaluation of the fiber area and the cell count returned similar results for NL and CH. Fiber type composition was assessed by SDH histochemical and immunofluorescent analyses, revealing no differences between the two breeds. Similar results between NL and CH were found for meat physical parameters, except for the red index (a*) which resulted significantly higher in NL than CH. CT analyses found equal muscle and fat masses between the considered breeds.
It must be considered that heavy CH pigs are selected to develop thighs with boosted muscle and adipose depositions; on the other hand, NL is a rustic animal that never underwent such a selection. Even so, our preliminary results suggest strong similarities in muscle features, meat physical parameters, and thigh composition between the two breeds. NL may seem to be competitive towards CH to produce cured meat, although further data are still required to better define the chemical features and the consumer acceptance of its meat.

[1] O. Franci and C. Pugliese, "Italian autochthonous pigs: Progress report and research perspectives," Ital. J. Anim. Sci., vol. 6, no. SUPPL. 1, pp. 663–671, 2007, doi: 10.4081/ijas.2007.1s.663.
[2] S. Velotto, C. Vitale, and A. Crasto, "Relationship between morphometric characteristics of fibre types and adipocytes in Cinta Senese pig," Acta Agric. Scand. A Anim. Sci., vol. 60, no. 4, pp. 211–218, 2010, doi: 10.1080/09064702.2010.529937.
[3] R. Rossi, G. Pastorelli, S. Cannata, S. Tavaniello, G. Maiorano, and C. Corino, "Effect of long term dietary supplementation with plant extract on carcass characteristics meat quality and oxidative stability in pork," Meat Sci., vol. 95, no. 3, pp. 542–548, Nov. 2013, doi: 10.1016/j.meatsci.2013.05.037.
[4] M. Font-i-Furnols, A. Brun, and M. Gispert, "Intramuscular fat content in different muscles, locations, weights and genotype-sexes and its prediction in live pigs with computed tomography," Animal, vol. 13, no. 3, pp. 666–674, 2019, doi: 10.1017/S1751731118002021.

EFFECTS OF FUNCTIONAL FEED ON MORPHO-FUNCTIONAL CHARACTERISTICS AND ANTIOXIDANT CAPACITY IN FINISHING PIGS

Di Meo M.C. (1), Maruccio L. (2), Mandrone V. (1), Giaquinto D. (2), Palladino A. (3), De Felice E. (4), Pesce A. (5), Zarrelli A. (6), De Girolamo P. (2), Varricchio E. (1)

(1) Department of Science and Technology (DST), University of Sannio, Benevento, Italy
(2) Department of Veterinary Medicine and Animal Production, University of Naples Federico II, Naples, Italy
(3) Department of Agriculture, University of Naples Federico II, Naples, Italy
(4) School of Biosciences and Veterinary Medicine, University of Camerino, Italy
(5) Istituto Zooprofilattico Sperimentale del Mezzogiorno, Portici, Italy
(6) Department of Chemical Sciences, University of Naples Federico II, Naples, Italy

Corresponding Author: **Maria Chiara Di Meo** - mardimeo@unisannio.it

In recent years, the scientific community has focused on the effects on animal health [1] of antioxidant molecules present in the by-products and co-products of the agri-food supply chains, particularly used to supplement the feed ration of animals of veterinary interest [2].
The aim of this study was to formulate a feed functionalised with bioactive molecules of Olea europaea for adult 'commercial hybrid' Large White x Landrace x Duroc pigs fed with polyphenols during the finishing period for 90 days.
The study focuses on the functional quality of certain pig muscles (muscle fibres and intramuscular fat), specifically on the fatty acid composition and morpho-structural aspects of muscle fibres.
Pigs were divided into two groups: a control group fed a standard diet and a group fed a standard diet enriched with polyphenolic extract of Olea europaea L. (300 mg/head/day).
Qualitative-quantitative analyses of the bioactive compounds present in the diet were performed and after slaughtering, the psoas major and longissimus dorsi muscles of the two groups of pigs were sampled for histological and molecular evaluation in order to assess the effects of the diet supplemented with bioactive molecules compared to the standard diet.
In particular, the DPPH assay showed an increase in anti-radical activity in the muscle of the group fed with functionalised feed, while the fatty acid profile of the same group of pigs, obtained by gas-chromatographic analysis, showed a significant increase in total unsaturated fatty acids with a decrease in omega 6 and an increase in omega 3 (PUFA) and omega 9 (MUFA).
In conclusion, it can be hypothesised that feeding pigs with feed enriched with Olea europaea extracts modifies the intramuscular lipid composition and improves the oxidative stress response of the muscle. With a view to future perspectives, our results may contribute to improve breeding techniques aimed at animal welfare and production quality.

[1] Berbel, J., & Posadillo, A. (2018). Review and analysis of alternatives for the valorisation of agro-industrial olive oil by-products. Sustainability, 10(1), 237.
[2] Tsiplakou, E., Pitino, R., Manuelian, C. L., Simoni, M., Mitsiopoulou, C., De Marchi, M., & Righi, F. (2021). Plant feed additives as natural alternatives to the use of synthetic antioxidant vitamins in livestock animal products yield, quality, and oxidative status: A review. Antioxidants, 10(5), 780.

BLEOMYCIN-INDUCED LUNG FIBROSIS IN MICE: MORPHOLOGICAL DIFFERENCES BETWEEN MALE AND FEMALE

Maruccio L. (1), D'Avino D. (2), Cattaneo F. (3), Di Meo M.C. (4,2), Cerqua I. (2), Schädel P. (5), Granato E. (2), Ialenti A. (2), Werz O. (5), Roviezzo F. (2), de Girolamo P. (1), Rossi A. (2)

(1) Department of Veterinary Medicine and Animal Production, University of Naples Federico II, Naples, Italy
(2) Department of Pharmacy, School of Medicine and Surgery, University of Naples Federico II, Naples, Italy
(3) Department of Molecular Medicine and Medical Biotechnology, University of Naples Federico II, Naples, Italy
(4) Department of Sciences and Technologies (DST), University of Sannio, 82100 Benevento (BN), Italy
(5) Department of Pharmaceutical and Medicinal Chemistry, Institute of Pharmacy, Friedrich-Schiller-University Jena, Jena, Germany

Corresponding Author: **Lucianna Maruccio** - lucianna.maruccio@unina.it

Pulmonary fibrosis (PF) is considered a chronic and progressive lung disease, where the lung architecture is replaced by fibrotic tissue [1]. Recent studies have shown that PF and inflammation are two distinct pathways [2], with higher incidence in men than women [3]. Currently the cellular and molecular mechanisms underlying this sexual differentiation are not yet fully understood. Therefore, the aim of this study is to evaluate any sex-related differences both at the onset and during the progression of PF. PF was induced in adult, both male (N:6) and female (N:6), C57/Bl6 mice through subcutaneous injection of bleomycin (BLM) (2 mg/kg), 3 times/week for 4 weeks. While the control group (N:12) received saline injection. Animals were sacrificed at 1, 2, 3 and 4 weeks and lung samples were collected. We performed hematoxylin and eosin (H&E), Masson's Trichrome (MTC) and Picro Sirius Red (PSR) staining for morphological purpose. In addition, immunohistochemical analysis of neutrophils; measurement of leukotriene B4 (LTB4), interleukin-1β (IL-1β), prostaglandin E2 (PGE2) by ELISA; and levels of specialized proresolving lipid mediators (SPMs) by UPLC-MS/MS were carried out. H&E showed an onset of cellular infiltration at week 1 after BLM treatment along the subpleural area and between alveolar spaces, increasing up to weeks 2 from treatment, with an evident loss of lung architecture due to the presence of more fibrotic tissue in males than in females. This result was confirmed by the presence of higher neutrophilia in males compared to females up to the 2 weeks. The proinflammatory levels of LTB4 and IL-1β were significantly increased only in male mice. MTC and PSR showed a significant and progressive increase in collagen starting from 1 week in males, while in females a slight increase in collagen was observed up to 2 weeks, with a return to baseline at 4 weeks. The antifibrotic levels of PGE2 were therefore significantly increased only in female mice. Furthermore, a higher lung level of SPM was observed in females than in males. In addition, by evaluating expression levels of E-cadherin, N-cadherin, □-Smooth Muscle Actin and Vimentin through western blot analysis, we also observed an enhanced epithelial-mesenchymal transition in male mice compared to females. In conclusion, we can hypothesize that in the initial phase of PF, inflammation and fibrosis were equally induced in both sexes, although male mice expressed an exacerbation of the fibrotic process.

[1] T.E. King, et al.2011. Idiopathic pulmonary fibrosis. Lancet, 378: 1949–1961.
[2] Rydell-Törmänen K, et al. 2012. Extracellular matrix alterations and acute inflammation developing in parallel during early induction of pulmonary fibrosis. Lab Invest., 92(6):917-25.
[3] Gribbin J, et al. 2006. Incidence and mortality of idiopathic pulmonary fibrosis and sarcoidosis in the UK. Thorax, 61:980–985.

SUBSTANCE P EXPRESSION AND NEUROCHEMICAL CHARACTERIZATION IN THE ENTERIC NERVOUS SYSTEM OF THE PORCINE COLON

Mazzoni M. (1), Cabanillas L. (2), Clavenzani P. (1), Costanzini A. (3), Mulugeta M. (2), Larauche M. (2), De Giorgio R. (3), Sternini C. (2,4)

(1) Department of Veterinary Science, University of Bologna
(2) Division of Digestive Diseases, Department of Medicine, UCLA, Los Angeles, CA, USA
(3) Department of Translational Medicine, University of Ferrara.
(4) Department of Neurobiology, UCLA, Los Angeles, CA, USA

Corresponding Author: **Maurizio Mazzoni** - m.mazzoni@unibo.it

The pig is a good model for studying intestinal functions and diseases for its homologies with humans, e.g. microbiome composition, size, nutrition (both omnivores) and colon fermenters. The enteric nervous system (ENS) in pigs and humans has a multilayered submucosal plexus with an inner (ISP) and an outer submucous plexus (OSP) near the mucosa and the circular muscle, respectively, in addition to the myenteric plexus in the muscle layer. We showed differences in the density and distribution of functionally distinct neurons in different regions and plexuses of the porcine colon. This study focused on substance P (SP), a peptide with many modulatory effects in the gastrointestinal (GI) tract physiology. We tested whether there were differences in the density and neurochemical profile of SP neurons in the ISP, OSP and MP of the ascending (AC) and descending (DC) colon of 14 Yucatan minipigs. We processed colonic wholemounts for multiple labeling immunofluorescence with anti-HuCD (pan-neuronal marker), choline acetyltransferase (ChAT, marker for cholinergic excitatory neurons) and neuronal nitric oxide synthase (nNOS, maker for nitrergic inhibitory neurons) antibodies using confocal microscopy and Imaris software to quantify the number of neurons/mm2 and the % of total HuCD neurons. HuCD/SP-immunoreactive (IR) neurons were most abundant in the ISP vs. OSP and MP in both AC and DC ($p<0.01$-$p<0.0001$) with highest density in ISP of AC vs. DC ($p<0.0001$). SP-IR neurons represent 27-28% of HuCD-IR neurons in ISP followed by OSP (19-22%) and MP (13-17%, $p<0.05$- $p<0.0001$) in AC and DC. The majority of SP-IR neurons contain ChAT-IR (62-67% in the MP, 74-76% in the ISP, 70-80% in OSP). SP-/nNOS-IR neurons were much less abundant in both AC and DC with the highest density in the OSP 35% vs. 16% in the ISP, $p<0.05$, and 20% in the MP in AC, and in the ISP (31%) followed by OSP and MP (25% and 18%) in DC. There was a small population of SP-/ChAT-/nNOS-IR neurons in all plexuses in AC and DC. The different density and neurochemical profile likely reflect different functions of SP-IR neurons such as immune-modulation, regulation of mucosal function, control of vascular, myogenic and neurogenic activities. The ENS is key to maintain GI homeostasis via the release of different transmitters/modulators and mapping their sites of expression provides the basis for elucidating neuronal circuits controlling reflex pathways underlying GI functions and disorders.

THE CASE STUDY OF RESISTIN IN SHEEP: CAN DIET INFLUENCE THE MOLECULE'S LOCALIZATION IN THE ENDOCRINE PANCREAS?

Palmioli E. (1,2), Maranesi M. (2), Dall'Aglio C. (2), Scocco P. (3), Marini D. (2), De Felice E. (3), Anipchenko P. (2), Mercati F. (2)

(1) Department of Philosophy, Social Sciences, and Education, University of Perugia, 06123, Perugia, IT
(2) Department of Veterinary Medicine, University of Perugia, 06126, Perugia, IT
(3) School of Bioscience and Veterinary Medicine, University of Camerino, 62032, Camerino, IT

Corresponding Author: **Elisa Palmioli** - elisa.palmioli@studenti.unipg.it

Resistin (RETN), a recently discovered adipokine, is a cysteine-rich and secretory protein produced by adipocytes [1]. The RETN has been detected in several tissues, including human and lab animals' pancreas, wherein impairs glucose tolerance and insulin action and causes insulin resistance [2]. This study aimed to evaluate the presence and expression of RETN in the pancreas of 15 adult female sheep reared on Apennine pastures, which show a decrease in their nutritional value due to the increasing stress from summer drought [3]. The sheep were divided into 3 groups according to the diet they were subjected to maximum pasture flowering (MxF) group, maximum pasture dryness (MxD) group, and experimental (Exp) group which received a feed supplementation in addition to the MxD group feeding. As a preliminary analysis, immunohistochemistry (IHC) and immunofluorescence (IF) techniques were performed on formalin-fixed and paraffin-embedded sections of the pancreas to detect the RETN presence and to evaluate the co-expression of RETN with both glucagon (GCG)- and insulin (INS)-producing cells. Mouse monoclonal anti-RETN, rabbit polyclonal anti-GCG, and anti-INS, as primary antibodies, were used to carry out both IHC and IF. The RETN was observed in the endocrine cells and colocalized with both GCG-secreting alpha cells and INS-secreting beta cells, showing a wide distribution throughout the pancreatic islets. No differences in distribution and signal intensity of RETN, GCG, and INS were observed among the three groups of animals fed on different diets.
These data show that RETN protein is produced by the cells of pancreatic islets, including the alpha and beta cells. Nevertheless, further investigations are needed to determine the overall role of pancreatic RETN. The IHC investigation did not reveal any differences related to diet.
The RT-qPCR evaluation of RETN, GCG, and INS expression will further characterize the influence of different diets on pancreatic islet functionality.

[1] Minn et al. (2003). Resistin is expressed in pancreatic islets. Biochem Biophys Res Commun. 310: 641–645. doi.org/10.1016/j.bbrc.2003.09.061.
[2] Adeghate E. (2004). An update on the biology and physiology of resistin. Cell Mol Life Sci. 61:2485–2496. doi.org/10.1007/s00018-004-4083-2.
[3] Scocco et al. (2016). Effects of summer rainfall variations on sheep body state and farming sustainability in sub-mediterranean pastoral system. Span J Agric Res. 14:1-4. doi.org/10.5424/sjar/2016143-9230.

HISTOCHEMICAL AND IMMUNOHISTOCHEMICAL EVALUATION OF THE EFFECTS OF A LOW INPUT DIET ON DIFFERENT CHICKEN BREEDS

Fonsatti E. (1), Bortoletti M. (1), Bordignon F. (2), Ballarin C. (1), Trocino A. (2), Xiccato G. (2), Birolo M. (2), Bertotto D. (1), Radaelli G. (1)

(1) Dept. of Comparative Biomedicine and Food Science (BCA), University of Padua
(2) Dept. of Agronomy Food Natural Resources Animals and Environment (DAFNAE), University of Padua

Corresponding Author: **Giuseppe Radaelli** - giuseppe.radaelli@unipd.it

The reduction of the environmental impact in poultry farm, fitting in the goals of European Green Deal and in the concept of One Welfare, is possible considering different chicken breeds and diets. Local breeds are more resilient and their use enhances biodiversity [1]. These breeds are ideal for introducing low input diet based on national and local raw materials that are formulated for less demanding breeds [2].
This study aimed to compare the effects of the diet (standard vs low input in which soybean was partly replaced by fava bean (Vicia faba, var minor); 18.5% vs 17.5% CP; 3,050 vs 2921 kcal EM/kg) on the morphology response in the jejunum in a fast-growing genotype (Ross 308) compared with local breeds (Bionda Piemontese, BP, and Robusta Maculata, RM) and their crosses with a moderate-growth genotype (Sasso). Marker of jejunal inflammation were investigated with an immunohistochemical approach. Two days before the commercial slaughter, 6 animals per genotype per diet were used to sample jejunum mucosa. One sample was taken at the midpoint of the end of the duodenal loop and the Meckel's diverticulum, fixed in paraformaldehyde in PBS (0,1M; pH 7,4), dehydrated and embedded in paraffin. Serial sections of 4 µm were cut and stained with: haematoxylin/eosin for morphometric analysis, Alcian-PAS for counting goblet cells, and antibodies against CD3 intraepithelial T-cells and CD45 intraepithelial leukocytes. Villi length, crypts depth and goblet cells were measured with an image-analysis software (Aperio ImageScope, Leica GmbH)[3]. Significant differences among genotypes were recorded for jejunum morphology ($P<0.001$) with the highest value for villi height/crypt depth ratio in chickens belonging to RM and the lowest value in Ross chickens (14.7 vs 7.80). Ross showed the highest density for Goblet cells (21.6 cells/300 µm) followed by BP and its cross (19.2 and 19.4 cells/300 µm) and RM with its cross (17.7 and 17.9 cells/300 µm). Compared to the standard diet, the low input diet decreased villi height (1179 to 1049 µm; $P=0.05$) and the villi/crypt ratio (11.7 to 10.4; $P<0.05$) while tended to increase the density of CD3 cells (3092 to 3447 cell/µm2; $P<0.10$). In conclusions, relationships between performance and gut morphology should be considered in the comparison of the genotypes, but the effect of the low input diet on jejunum morphology could be ascribed to the replacement of soybean with faba bean and, possibly, its content in antinutritional factors.

[1] T. T. H. Nguyen, I. Bouvarel, P. Ponchant, and H. M. G. Van Der Werf, "Using environmental constraints to formulate low-impact poultry feeds," J. Clean. Prod., vol. 28, pp. 215–224, Jun. 2012, doi: 10.1016/J.JCLEPRO.2011.06.029.
[2] A. González Ariza, A. Arando Arbulu, F.J. Navas Gonzalez, S. Nogales Baena, J.V. Delgado Bermejo, M. E. Camacho Vallejo, "The Study of Growth and Performance in Local Chicken Breeds and Varieties: A Review of Methods and Scientific Transference," Anim. 2021, Vol. 11, no. 9, p. 2492, Aug. 2021, doi: 10.3390/ANI11092492.
[3] A. Pascual, M. Pauletto, M. Giantin, G. Radaelli, C. Ballarin, M. Birolo, C. Zomeño, M. Dacasto, M. Bortoletti, M. Vascellari, G. Xiccato & A. Trocino, "Effect of dietary supplementation with yeast cell wall extracts on performance and gut response in broiler chickens," J. Anim. Sci. Biotechnol., vol. 11, no. 40, May 2020, doi: 10.1186/S40104-020-00448-Z.

76° CONVEGNO SISVET
BARI, 21-22-23 GIUGNO 2023

THE CONTRIBUTION OF TOPOGRAPHICAL ANATOMY TO VETERINARY ACUPUNCTURE

Tagliavia C. (1), Salamanca G. (2), Canova M. (2), Bombardi C. (2), Grandis A. (2)

(1) Department of Veterinary Medicine, University of Teramo
(2) Department of Veterinary Medical Sciences, University of Bologna

Corresponding Author – **Claudio Tagliavia -** ctagliavia@unite.it

The growing interest in the West in Traditional Chinese Medicine (TCM) in veterinary medicine, and in acupuncture, has led to a proliferation of schools and texts dedicated to it (1-4). Currently, the literature provides students of the discipline with a representation of acupoints (called tsubo) on simple schematic or pictorial images of muscles and bones, accompanied by descriptions in varying degrees of detail. This seems insufficient for a good understanding of their location, especially for students at the beginning of their training.
In particular, the texts consulted (1-4) showed that both the descriptions and the illustrations contained several anatomical inaccuracies, few indications to simple repere points useful for correct needle insertion, and little or nothing about potentially dangerous structures should they be pierced by the needle.
Therefore, it was decided to investigate whether a detailed topographical and stratigraphic study of acupoints on dog cadavers could fill these gaps.
The investigation showed that: 1) there are discrepancies between the authors regarding the topography of some points, presumably due to whether or not they attempted to adapt the tsubo to human acupuncture; 2) it was possible to identify additional anatomical reference structures (palpable on the surface of the body) that would facilitate the needle placement; 3) particularly dangerous points were identified due to the possibility of perforating vessels, nerves, lymph nodes and glands, or the risk of penetrating one of the splanchnic cavities.
In conclusion, it is believed that a veterinary anatomist, specialist in gross anatomy, can also make an important contribution to this discipline by providing basic morphological data useful to his clinical colleague. It is desirable that new veterinary texts, with photographic atlases detailing the location of the acupuncture points and the risks associated with them, should be published in the near future.

[1] Demontoy A. (1986) - Manuel d'acupuncture canine. Ed. Le Point Vétérinaire (Parigi, Francia).
[2] Matern C. (2011) – Acupuncture for Dogs and Cats: A Pocket Atlas. Ed. Thieme Medical Publisher (New York).
[3] Molinier F. (1986) - Traitè d'Acupuncture Vétérinaire; Tome 1, Acupuncture fondamentale traditionelle et modern des carnivores; Fascicule 1: Notions élémentaires. Ed. Maisonneuve (Parigi, Francia).
[4] Smith D.S. (2011) – Animal Acupressure illustrated: Volume 1 Small Animals. An Acupressure guide for common ailments of the smaller family members. Ed. Julie D. Temple.

USING NEW PROTEIN SOURCES IN FEED FOR GILTHEAD SEABREAM (*S. AURATA*) BROODSTOCK: WHAT HAPPENS TO THE OFFSPRING?

Aidos L. (1), Mirra G. (2), Cialini C. (1), Bosi G. (1), Proietti L. (3), Foglio L. (3), Parati K. (3), Modina S. (1), Di Giancamillo A. (4)

(1) Department of Veterinary Medicine and Animal Science, University of Milan, Lodi, Italy
(2) Department of Comparative Biomedicine and Food Science, University of Padua, Padua, Italy
(3) Istituto Sperimentale Italiano "Lazzaro Spallanzani", Rivolta d'Adda, Italy
(4) Department of Biomedical Sciences for Health, University of Milan, Milan, Italy

Corresponding Author: **Lucia Aidos -** lucia.aidos@unimi.it

Constant intensification and growth of aquaculture production are dependent on the development of sustainable protein sources that can replace the most used protein sources in aquafeeds: fishmeal and soybean meal [1]. In this study, gilthead seabream (S. aurata) broodstock was given two diets where vegetable protein sources were replaced with alternative ones, such as algae, duckweed, insects, and microbiomes (bioflocs). Two inclusion levels (V2 and V3) were compared with a group receiving a control diet (V1). The effect of the diet was evaluated on larval development during endogenous feeding, which is a high vulnerability period, due to the small size of the larvae, incomplete development, and low energy reserves [2]. After hatching, larvae were reared at 19 °C and were sampled at hatch (T0) and the complete yolk sac absorption stage (T1). For both time points, larvae were evaluated in terms of 1) total length (TL); 2) gut development, by analyzing serial hematoxylin-eosin and AB-PAS stained sections; 3) muscle development, by whole-mount immune-fluorescence; 4) skeletogenesis, using a double staining whole-mount technique with Alcian Blue and Alizarin for cartilage and bone tissues respectively. The fish handling procedures and sampling methods used in the trial followed the E.U directive 2010/63/EU guidelines on the protection of animals used for scientific purposes.
TL was similar among groups at any time point. In all groups at T0, the gut appeared undifferentiated, consisting of an AB-PAS negative cubic cells monolayer, located dorsally to the yolk sac. At T1, in all groups, it was already possible to observe the gut folds and a PAS-positive layer. At the end of T1, the mouth was open and the yolk sac was absent. The lateral muscle was striated with 5 layers at T0 and 7 layers at T1 demonstrating a growth mechanism of hypertrophy and hyperplasia. Moreover, larvae from V1 showed a higher degree of ossification especially at the head level and at the distal portion of the notochord, at the future caudal fin position at T0, with no differences at T1.
The experimental diets tested in this study did not lead to any relevant development alterations of larvae from V2 or V3 when compared with V1. We can therefore affirm that the diet tested on the broodstock at both inclusion levels is compatible with regular larvae development, even if further studies are necessary to assess the impact of these diets on larvae after the onset of exogenous feeding.

Funding: AquaTech4Feed (B49C18000190005).

[1] Gatlin, D.M.; Barrows, F.T.; Brown, P.; Dabrowski, K.; Gaylord, T.G.; Hardy, R.W.; Herman, E.; Hu, G.; Krogdahl, Å.; Nelson, R.; et al. Expanding the Utilization of Sustainable Plant Products in Aquafeeds: A Review. *Aquac. Res.* 2007, *38*, 551–579, doi:10.1111/j.1365-2109.2007.01704.x.
[2] Fuiman, L.A.; Cowan, J.H. Behavior and Recruitment Success in Fish Larvae: Repeatability and Covariation of Survival Skills. *Ecology* 2003, *84*, 53–67.

IMMUNOHISTOCHEMICAL LOCALIZATION OF PIEZO PROTEIN, PIEZO1 AND PIEZO2 IN SENSORY ORGANS OF ZEBRAFISH (DANIO RERIO)

Aragona M. (1), Porcino C. (1), Briglia M. (1), Abbate F. (1), Gerrera M.C. (1), Laurà R. (1), Levanti M. (1), Germanà A. (1)

(1) Zebrafish Neuromorphology Lab, Department of Veterinary Sciences, University of Messina, 98168 Messina, Italy

Corresponding Author: **Marialuisa Aragona** - mlaragona@unime.it

Stretching-activated ion channels are a new class of mechanosensitive proteins. They convert the electrical signal into a biological response. Piezoelectric proteins, Piezo 1 and Piezo 2 located in the membrane cell, have recently been identified as mechanical ion channels activated by the strain. Piezomechanoensible ion channels are proteins evolutionarily conserved, essential for performing multiple normal physiological functions in development and maintenance [1]. These proteins have been linked to various pathophysiological conditions as hearing and deafness, and physiological and hypertrophic cardiac responses, such as dysfunction/kidney disease. Piezoelectric proteins are also involved in regulating mechanosensitive sensory neuron flows caused by inflammatory conditions [2] and play a role in maintaining homeostasis and cell turnover in zebrafish (D. rerio) epithelia. The role of piezo proteins in tension control is fundamental in aquatic vertebrates. In addition, the biophysical properties of the piezo protein in teleosts such as D. rerio resemble those of mammalian-dependent voltage channels [3]. The sensory system in fish consists of specialized sensory organs that contain differentiated cells able to detect the light, mechanical, and chemical environment stimuli and transforms them into electrical signals. Zebrafish is used as a model to study different human sensory disorders because they have 82% of orthologs genes associated with human disease [4]. Although studies on piezo proteins in zebrafish have begun, few is known about their localization and role in the sensory organs of adult zebrafish. In this study, we used paraffin embedded tissue of adult D. rerio to analyze the expression and localization of Piezo 1 and Piezo 2 proteins in the sensory patches of zebrafish sensory organs. Our results elucidate for the first time the localization of piezo proteins, Piezo 1 and Piezo 2, in different neuronal subpopulations of the sensory organs in adult zebrafish. These results taken together might be important to clarify the real role of Piezo proteins in the sensory epithelia of zebrafish adding new data for future translational studies of sensory organs disorders.

[1] Cobo, R.; García-Piqueras, J.; García-Mesa, Y.; Feito, J.; García-Suárez, O.; Vega, J.A. Peripheral Mechanobiology of Touch—Studies on Vertebrate Cutaneous Sensory Corpuscles. International Journal of Molecular Sciences 2020, 21, doi:10.3390/ijms21176221.
[2] Faucherre, A.; Nargeot, J.; Mangoni, M.E.; Jopling, C. piezo2b Regulates Vertebrate Light Touch Response. The Journal of Neuroscience 2013, 33, 17089, doi:10.1523/JNEUROSCI.0522-13.2013.
[3] Moroni, M.; Servin-Vences, M.R.; Fleischer, R.; Sánchez-Carranza, O.; Lewin, G.R. Voltage gating of mechanosensitive PIEZO channels. Nature Communications 2018, 9, 1096, doi:10.1038/s41467-018-03502-7.
[4] Germanà, A.; Guerrera, M.C.; Laurà, R.; Levanti, M.; Aragona, M.; Mhalhel, K.; Germanà, G.; Montalbano, G.; Abbate, F. Expression and Localization of BDNF/TrkB System in the Zebrafish Inner Ear. International Journal of Molecular Sciences 2020, 21, 5787, doi:https://doi.org/10.3390/ijms21165787.

ACUTE TOXICITY, TERATOGENETIC EFFECT AND BEHAVIORAL STUDIES OF SALVIA ROSMARINUS WATER EXTRACT IN DANIO RERIO LARVAE

Cometa M. (1), Briglia M. (1), Mhalhel K. (1), Aragona M. (1), Porcino C. (1), Abbate F. (1), Guerrera M.C. (1), Montalbano G. (1), Germanà A. (1)

(1) Zebrafish Neuromorphology Lab, Department of Veterinary Sciences, Univerisity of Messina 98168 Messina

Corresponding Author: **Marzio Cometa** - marzio.cometa@unime.it

Salvia rosmarinus, commonly known as rosemary, is an evergreen perennial shrub belonging to the *Lamiaceae* family [1]. Native to the Mediterranean region, rosemary is now cultivated around the world due to its use as a natural food preservative and flavouring agent. Recent studies have displayed that rosemary extracts have a large variety of medical properties like strong antibacterial, antifungal and antioxidant activity, but also hepatoprotective, antispasmodic, anticarcinogenic, anti-inflammatory, antidiabetic and neuroprotective activities [2]. These biological properties have made rosemary a potential new therapeutic agent in the treatment of many diseases. Teratogenesis is a process that disrupts the normal development of an embryo or *fetus* by causing growth retardation, permanent structural and functional abnormalities, or miscarriage in severe cases [3]. It can be caused by certain natural agents (teratogens), which interfere with embryonic development in multiple ways [4]. The present study aimed to investigate the acute toxicity and the possible teratogenic effects of Salvia rosmarinus water extract in Zebrafish embryos, an important animal model used in toxicology/teratogenic studies due to their special characteristics such as external fertilization, rapid embryonic development and optical transparency. After exposure to different concentrations of Salvia rosmarinus extract in 96 well plates, we examined the larvae using VAST BioImager system (Vertebrate Automated Screening Technology), a set of tools developed for zebrafish researchers. This system requires the collection of images from a large number of 2-5 dpf zebrafish larvae, in dorsal, lateral and ventral view to improve the analysis of the teratogenic effects of the extract used. Our results demonstrated that different concentration of Salvia rosmarinus water extract negatively affects survival. The embryos that survived at 0.78 mg/ml concentration showed numerous defects, including pericardial and yolk sac edema, body curvature, necrosis and craniofacial malformation like snoutjaw defect.

[1] Rosmarinus officinalis (rosemary)". Centre for Agriculture and Bioscience International. 3 January 2018. Retrieved 13 July 2018.
[2] Al Sereitia MR, Abu Amerb KM, Sena P (1999) Pharmacology of rosemary (Rosmarinus officinalis Linn.) and its therapeutic potentials. Indian Journal of Experimental Biology 37(2): 124-131.
[3] Gilbert-Barness E. Teratogenic causes of malformations. Ann Clin Lab Sci. 2010;40(2):99–114. [PubMed] [Google Scholar]
[4] Brent RL. The cause and prevention of human birth defects: What have we learned in the past 50 years. Con Anom. 2001;41(1):3–21. [Google Scholar]

THE IMPACT OF INTERMITTENT FASTING (IF) IN CELL PROLIFERATION: NEW INSIGHTS IN ADULT NEUROGENESIS

D'Angelo L. (1), Giaquinto D. (1), Raggio M. (2), Fonsatti E. (3), Bortoletti M. (3), Ballarin C. (3), Bertotto D. (3), Lucini C. (1), Radaelli G. (3), de Girolamo P. (1)

(1) Dept of Veterinary Medicine and Animal Production, University of Naples Federico II
(2) Dept. of Sciences and Technologies, University of Sannio
(3) Dept. of Comparative Biomedicine and Food Science (BCA), University of Padua

Corresponding Author: **Livia D'Angelo** - livia.dangelo@unina.it

Dietary interventions, *i.e.* intermittent fasting, are known to enhance cognition and adult cell proliferation turnover in murine models [1]. We decided to explore this effect in zebrafish, a model with high cell proliferation plasticity, which enables to completely repair the brain parenchyma upon traumatic injury [2]. At this aim, we investigated the effect of three months of intermittent fasting (feeding animals each other day) on adult zebrafish and compared with the effect determined by the short-term fasting (96 hours) and control-matched animals. The experimental protocol was approved by the Ministry of Health [291/2022-PR]. Upon 96 hours of fasting, levels of ribosome phosphorylation (pS6), a marker of neural activity in mammals and fish [3,4], were unchanged in the whole brain compared to IF and control animals in comparison to ribosome protein S6. Comparable expression levels of pS6 in IF and control animals suggest a metabolic adaptation, while unchanged levels in 96 hours of fasting suggest that this species needs a prolonged exposure to fasting stimulus. Remarkably, the levels of NPY, a well-known orexigenic neuropeptide [4], seemed increased in the whole brain of animals upon 96 hours of fasting and, to a less extent, in IF compared to control animals. We then analysed the mitotic activity of cells in response to the two different metabolic conditions by employing the marker Ki-67, expressed at high levels only in proliferating cells, to assess i) the increase of proliferative activity in response to IF; ii) the brain region with higher Ki-67 immunodensity; iii) the ratio between proliferating/activated cells. Finally, we questioned whether the increase of NPY occurring earlier than the neuronal activation can be involved in modulating the zebrafish cell proliferation. Overall, these data shed light on the potential beneficial effect of dietary interventions on brain cell proliferation and open the avenue for new research strategies on adult neurogenesis.

[1] Pereira Dias G. et al. (2021). Intermittent fasting enhances long-term memory consolidation, adult hippocampal neurogenesis, and expression of longevity gene Klotho. Molecular Psychiatry 26,6365–6379 https://doi.org/10.1038/s41380-021-01102-4.
[2] Cacialli P. et al. (2018). Neuronal expression of brain derived neurotrophic factor in the injured telencephalon of adult zebrafish. J Comp Neurol. 526(4):569-582 doi: 10.1002/cne.24352.
[3] Knight et al. (2012). Molecular Profiling of Activated Neurons by Phosphorylated Ribosome Capture. Cell 151, 5, 1126-1137. https://doi.org/10.1016/j.cell.2012.10.039.
[4] Montesano A. et al. (2019). Age-related central regulation of orexin and NPY in the short-lived African killifish Nothobranchius furzeri. J Comp Neurol. 527(9):1508-1526. doi: 10.1002/cne.24638.

CHARACTERIZATION OF A NEW NOTHOBRANCHIUS FURZERI MODEL OF A DIET-INDUCED OBESITY

Giaquinto D. (1), Maruccio L. (1), Palladino A. (2), Attanasio C. (1), Scocco P. (3), Varricchio E. (4), de Girolamo P. (1), D'Angelo L. (1), De Felice E. (3)

(1) Department of Veterinary Medicine and Animal Production, University of Naples Federico II, 80137 Naples, IT
(2) Department of Agricultural Sciences, University of Naples Federico II, 80055 Portici, IT
(3) School of Biosciences and Veterinary Medicine, University of Camerino, 62032, Camerino, IT
(4'+) University of Studies of Sannio, Department of Sciences and Technologies, 82100, Benvento, IT

Corresponding Author: **Elena De Felice** - elena.defelice@unicam.it

The global epidemic of obesity is a major public health problem today. Due to augmented life expectancy, obesity is growing in prevalence among older people. Increasing efforts therefore need to be made to identify effective strategies able to promote healthy aging and curb the obesity pandemic [1]. The use of animal models to study the phenomena underlying obesity (genetic, physiological, epigenetic and environmental), as well as research on potential treatments, offers enormous potential. However, to understand the impact of ageing on obesity, it is primary to conduct studies on old animals, in order to phenocopy the systemic ageing context [2]. In the last few years, the African killifish Nothobranchius furzeri has emerged as an important model system for the study of vertebrate biology. N. furzeri is an annual fish that inhabits seasonal freshwater ponds in the southeast of Africa and is characterized by rapid growth and early sexual maturation. This fish, with a median lifespan of 3 and 7 months, is currently considered the shortest-lived vertebrate that can be bred in captivity. Importantly, despite its short lifespan, N. furzeri recapitulates typical age-dependent phenotypes and pathologies making this fish a suitable model for aging research [3]. The goal of our work is to establish and characterize a new model of diet-induced obesity (DIO). The research was approved with protocol 1141/2020-PR by the Ministry of Health. First objective is to define the dietary protocol to determine the obese phenotype. To this aim, 77 fishes belonging to MZM 04/10 strain, both male and female, at 6 weeks of age were divided into different group diets and fed for 14 weeks with increasing doses of Chironomus spp. as follows: a) 300mg/die (ctrl); b) 540mg/die; c) 660mg/die; d) 780mg/die. Every two weeks morphometric measurements, i.e. body weight and length, were individually taken to assess growth curves and define the body mass index. Morphological studies on whole trunk histological sections allowed us to a) analyze liver structure, b) measure the thickness of visceral and subcutaneous adipose tissue, c) characterize the complex carbohydrates. Two master-key lipogenic factors, namely PPARγ and SREBP-1, have also been tested on liver homogenates by means of Western blot. Our preliminary results indicate that the diet administered to group d is the most obesogenic. Our pilot study is the first step toward the analysis of obese phenotype during vertebrate ageing.

[1] Colleluori G. and Villareal D.T. (2021). Aging, obesity, sarcopenia and the effect of diet and exercise intervention. Exp Gerontol. 155: 111561. doi: 10.1016/j.exger.2021.111561.
[2] Suleiman J.B. et al. (2020). A systematic review on different models of inducing obesity in animals: advantages and limitations. J Adv Vet Anim Res. 7(1): 103-114. doi: 10.5455/javar.2020.g399.
[3] Terzibasi Tozzini E. and Cellerino A. (2020). Nohobranchius annual killifishes. EvoDevo 11: 25. doi: 10.1186/s13227-020-00170-x.

AGING AND OBESITY CHANGES THE CENTRAL AND PERIPHERAL CHEMICAL SENSORY PERCEPTION IN AFRICAN TURQUOISE KILLIFISH

Giaquinto D. (1), Attanasio C. (1), De Felice E. (2), Palladino A. (3), de Girolamo P. (1), D'Angelo L. (1)

(1) Department of Veterinary Medicine and Animal Production, University of Naples Federico II, 80137 Naples, IT
(2) School of Bioscience and Veterinary Medicine, University of Camerino, 62032, Camerino, IT
(3) Department of Agricultural Sciences, University of Naples Federico II, 80055 Portici, IT

Corresponding Author: **Daniela Giaquinto** - daniela.giaquinto@unina.it

Morphophysiological changes in the chemical senses are well investigated in research in human elderly, as well as considered key factors in the development of obesity1.Key biological mechanisms underlying these changes are largely hypothesized and not completely understood yet. The aims of our research are to broaden knowledge on the interplay of chemical senses in the metabolic control via nutrient-sensing mechanism under two different physiologically altered metabolic conditions, aging and overfeeding.At this aim, we carried out our study in the model Nothobranchi us furzeri, the shortest-lived vertebrate that can be bred in captivity2, coupling behavioral observations, western blot and immunohistochemical analyses. A total of 10animals/conditions was used. In vivo observations confirmed a remarkable difference in the food selection: old and obese fish did not discriminate among different types of food, while young and normal fed subjects demonstrated a clear selectivity. For ex vivo analyses, we mainly focused on the brain and taste buds in oral cavity, respectively involved in the central and peripheral chemical sensory perception, and we collected samples of animals at 16 weeks old (normal fed and overfed) and 52 weeks (old animals). We investigated key chemoattractant-mediated signal transduction (PLCB2, TRPM5) and NPY, an appetite-related neuropeptide with functional role in aging3 and obesity. At central level, mainly in the hypothalamus, we observed occurrence of a) TRPM5 in all experimental groups; b) PLCB2 in adults and obese, and drastic reduction in old fish; c) NPY in all conditions with a sharp increase in the old. In the taste buds, we observed a homogeneous distribution of TRPM5 and PLCB2 without evident differences among groups, and a decrease of NPY in old animals.Our results suggest i) a physiological age-related decrease of taste receptors at central level, in line with the behavioral observations; ii) an increase of PLCB2 at central level supporting the hypothesis that overfeeding continuously activates receptors, while we suppose that the peripheral perception of taste is reduced likely due to a lack of satiety feedback, which leads animals to feed continuously; iii) the age-related different central and peripheral regulation of NPY which prompts for a clear role of NPY in the peripheral sensorineural decline. Altogether, these preliminary data may have many implications for translational research into nutrition, aging and obesity.

[1] Tam BT, Morais JA, Santosa S. Obesity and ageing: Two sides of the same coin. Obesity Reviews. 2020;1–21. https://doi.org/10.1111/obr.12991
[2] Terzibasi E. et al. The short-lived fish Nothobranchius furzeri as a new model system for aging studies. Experimental Gerontology 42 (2007) 81–89
[3] Giaquinto D, et al. Central and Peripheral NPY Age-Related Regulation: A Comparative Analysis in Fish Translational Models. International Journal of Molecular Sciences. 2022; 23(7):3839. https://doi.org/10.3390/ijms23073839 1. Montesano A, et al. Age-related central regulation of orexin and NPY in the short-lived Africa

URTICA DIOICA EXTRACT COUNTERACT THE TOXIC EFFECT OF AN ORGANOPHOSPHATE PESTICIDE (CHLORPYRIFOS) ON ZEBRAFISH LARVA

Mhalhel K. (1), Ben C.A. (2), Cometa M. (1), Germanà G. P. (1), Laurà R. (1), Levanti M. (1), Saoudi M. (2), Montalbano G. (1)

(1) Zebrafish Neuromorphology Lab, Department of Veterinary Sciences, University of Messina
(2) Animal Ecophysiology Laboratory, Faculty of Scinece of Sfax, University of Sfax, Tunisia

Corresponding Author: **Kamel Mhalhel** - kamel.mhalhel@unime.it

The increase in the world's population in the 21st century could not have been possible without a parallel expansion in food production, enhanced by technological advances and the use of pesticides. Although around one-third of crop production depends on applying pesticides, the extensive use of these chemicals results in soil, water, and turf contamination. Indeed, those chemical compounds remain in the yields and enter the food chain, posing a hazard to human beings by affecting the physiological pathways and organs, resulting in acute or chronic disorders [1].
Poisoning by acute exposure to certain pesticides has well-known neurotoxic effects, including increased prevalence of neurologic symptoms and changes in neurobehavioral performance, reflecting cognitive and psychomotor dysfunction [2].
Moreover, many studies have shown that natural compounds possess protective activity against many neurological disorders and have neuro-pharmacological effects by increasing levels of specific cell survival proteins [3,4].
Considering all the data mentioned above, we thought to investigate the effects of Urtica dioica ethanolic extract on counteracting the toxic effects of chlorpyrifos, a broad-spectrum organophosphate insecticide, on the development and neurobehavioral performance of zebrafish larvae.
We have performed morphological, histological, and immunohistochemical approaches on control, pesticide, Urtica dioica extract, preventive and curative larval groups to fulfill our goal.
Our finding includes the teratogenicity of chlorpyrifos exposure in zebrafish embryos and its capacity to delay normal hatching time and alter neurobehavioral responses. All the toxic effects of the pesticide were counteracted by the ethanolic extract of Urtica dioica exposition performed prior (preventive) or subsequent (curative) to the pesticide exposure.

[1] Sharma, A.; Kumar, V.; Shahzad, B.; Tanveer, M.; Sidhu, G.P.S.; Handa, N.; Kohli, S.K.; Yadav, P.; Bali, A.S.; Parihar, R.D.; et al. Worldwide Pesticide Usage and Its Impacts on Ecosystem. SN Applied Sciences 2019, 1, 1446, doi:10.1007/s42452-019-1485-1.
[2] Kamel, F.; Hoppin, J.A. Association of Pesticide Exposure with Neurologic Dysfunction and Disease. Environ Health Perspect 2004, 112, 950–958, doi:10.1289/ehp.7135.
[3] Rauf, A.; Rahman, M.M. Potential Therapeutics against Neurological Disorders: Natural Products-Based Drugs. Front Pharmacol 2022, 13, 950457, doi:10.3389/fphar.2022.950457.
[4] Mhalhel, K.; Sicari, M.; Pansera, L.; Chen, J.; Levanti, M.; Diotel, N.; Rastegar, S.; Germanà, A.; Montalbano, G. Zebrafish: A Model Deciphering the Impact of Flavonoids on Neurodegenerative Disorders. Cells 2023, 12, doi:10.3390/cells12020252.

IMMUNOHISTOCHEMICAL EVALUATION OF DNA OXIDATIVE DAMAGE AND HEAT SHOCK PROTEIN (HSP70) IN ADULT ZEBRAFISH (D. rerio) EXPOSED TO FASTING

Fonsatti E. (1), Giaquinto D. (2), D'Angelo L. (2), Bortoletti M. (1), Ballarin C. (1), Bertotto D. (1), Lucini C. (2), de Girolamo P. (2), Radaelli G. (1)

(1) Dept. of Comparative Biomedicine and Food Science (BCA), University of Padua
(2) Dept of Veterinary Medicine and Animal Production, University of Naples Federico II

Corresponding Author: **Giuseppe Radaelli** - giuseppe.radaelli@unipd.it

The improvement of animal welfare is an important issue, and increasingly relevant for fishes. Knowledge on stress and its manifestations is essential to guarantee animal welfare, minimize the adverse effects, and improve the management of fish farming and fish manipulation. Changes in the natural environment and variation in the abiotic factors in a facility or in a fish farm could cause stress in fish, inducing oxidative stress thus promoting the onset of potential alterations. Oxidative stress could be investigated using stress biomarkers. 8-hydroxy-2'-deoxyguanosine (8-OHdG), a product of deoxyguanosine oxidation, is an example of DNA damage due to bases' modification. 8-OHdG analysis has been widely applied in assessing the DNA damage being the major product of oxidative DNA damage with clear mutagenic potential for G to T transversions [1]. Another biomarker at the cellular levels, to measure the stress response includes special stress proteins, namely heat shock proteins (HSPs), which represent a class of highly conserved cellular proteins. Among HSPs, HSP70 represents the most widely studied implicated in numerous important chaperoning functions [2].
In the present study, an immunohistochemical analysis on the cellular localization of 8-OHdG and HSP70 has been performed in adult zebrafish subjected to two different fasting conditions: i) 96 hours of fasting (acute stress) and ii) 12 weeks of intermittent fasting (chronic stress). The experimental plan was approved by the Ministry of Health [291/2022-PR]. Six animals for each experimental conditions were fixed in Bouin's solution, dehydrated, and embedded in paraffin. Sections of 4 μm were obtained using a microtome. Immunohistochemical staining was performed using the VECTASTAIN® Universal Quick HRP Kit (Vector Laboratories, Inc., California). Primary antibodies anti-8-OHdG and anti-HSP70 (Abcam, UK) were used for the analysis.
The anti-8-OHdG antibody exhibited a nuclear positivity in: i) epithelial cells of skin; ii) enterocytes of the intestinal mucosa; iii) hepatocytes of liver parenchyma and iv) skeletal muscles cells. Positive immunostaining to HSP70 antibody was mainly detected in the epithelial cells of intestine and in the cytoplasm of hepatocytes. From a preliminary analysis, animals subjected to chronic stress exhibited the highest immunopositivity to both antibodies, thus indicating the potential use of these stress markers to assess the effect of intermittent fasting in zebrafish.

[1] B. Halliwell and J. M. C. Gutteridge, Free radicals in biology and medicine, 4th ed. Oxford: Oxford University Press, 2001.
[2] M. Bortoletti, L. Maccatrozzo, G. Radaelli, S. Caberlotto, and D. Bertotto, "Muscle cortisol levels, expression of glucocorticoid receptor and oxidative stress markers in the teleost fish argyrosomus regius exposed to transport stress," Animals, vol. 11, no. 4, 2021, doi: 10.3390/ANI11041160.

ANIV

BIOMOLECULAR APPROACH TO THE DIAGNOSIS AND GENETIC CHARACTERIZATION OF FELINE LEUKAEMIA VIRUS (FELV)

Veronica Facile (1), Laura Gallina (1), Nicola Roda (1), Maria Chiara Sabetti (2), Alessia Terrusi (1), Lorenza Urbani (1), Martina Magliocca (1), Francesco Dondi (1), Andrea Balboni (1), Mara Battilani (1)

(1) Dipartimento di Scienze Mediche Veterinarie, Alma Mater Studiorum – Università di Bologna
(2) Dipartimento di Scienze Veterinarie, Università di Parma

Corresponding Author: **Andrea Balboni** - a.balboni@unibo.it

Feline leukaemia virus (FeLV), genus Gammaretrovirus, is responsible for feline leukaemia syndrome in domestic cats (1). The prevention and control of disease caused by FeLV are primarily based on direct measures, in particular on the identification and isolation of infected subjects. Antigen diagnostic methods, which are the most widely used in clinical practices, showed limitations in the latent and regressive infections: in these cases, the use of molecular tests is recommended. In this study, a SYBR Green Real Time qPCR assay (2) was validated and used to detect FeLV proviral DNA in blood and serum samples from antigenic positive cats (SNAP Combo Plus FeLV Ag/FIV Ab, IDEXX) referred to the University Veterinary Hospital (University of Bologna) in 2018-2021. To genetically characterise the identified viruses, a portion of the viral env gene was amplified from all cats using four different end-point PCR (3) and sequences were analysed. The env gene encodes for the surface glycoprotein and transmembrane protein that constitute the viral envelope and subtype differences arise from mutations in this gene often acquired in vivo during FeLV replication. Twenty-six FeLV seropositive domestic short-haired cats, 15 males and 11 females, with median age of 6 years and 1 month (6 months-15 years) were included in the study. Twenty-two of 26 (84%) cats included in the study tested positive by qPCR assay. By sequencing the env gene, all the four qPCR negative cats had endogenous viral sequences, physiologically present in the genome of cats and not involved in the disease, suggesting a high specificity of the qPCR. Further studies are required to investigate why the antigen test showed positive results in cats that had only endogenous FeLV sequences. From env gene analysis, 15/22 qPCR positive cats tested positive for subtype A and 5/15 shown coinfection with subtype B. No env sequence was obtained for 7/22 qPCR positive cats. The variability of circulating viruses emerged in this study, confirms the need to an integrated approach, adopting different tests, to accurately diagnose the infection status of cats. Furthermore, the use of a specific and sensitive method like Real Time qPCR could improve the blood donors screening (4).

[1] Jarrett WFH, Crawford EM, Martin WB, et al. (1964). A virus-like particle associated with leukemia (lymphosarcoma). Nature 202:567-568. Feline leukaemia virus: Half a century since its discovery
[2] Tandon R, Cattori V, Gomes-Keller MA, Meli ML, Golder MC, Lutz H, Hofmann-Lehmann R. (2005). Quantitation of feline leukaemia virus viral and proviral loads by TaqMan real-time polymerase chain reaction. J Virol Methods;130(1-2):124-32.)
[3] Watanabe S, Kawamura M, Odahara Y, Anai Y, Ochi H, Nakagawa S, Endo Y, Tsujimoto H, Nishigaki K. (2013). Phylogenetic and structural diversity in the feline leukemia virus env gene. PLoS One;8(4)
[4] Nesina S, Katrin Helfer-Hungerbuehler A, Riond B, et al. (2015). Retroviral DNA–the silent winner: blood transfusion containing latent feline leukemia provirus causes infection and disease in naive recipient cats. Retrovirology;12:105

DEVELOPMENT OF A PASSIVE SURVEILLANCE SYSTEM FOR THE MONITORING AND THE MANAGEMENT OF POTENTIAL HOSPITAL-ACQUIRED INFECTIONS (HAIS) IN A VETERINARY UNIVERSITY HOSPITAL (VUH)

Raffaele Scarpellini (1), Giammarco Assirelli (1), Erika Esposito (1), Elisabetta Mondo (1), Silvia Piva (1)

(1) University of Bologna - Department of Veterinary Medical Sciences

Corresponding Author: **Raffaele Scarpellini** - raffaele.scarpellini@studio.unibo.it

Bacterial hospital-acquired infections (HAIs) are considered an emerging threat in small animal practice, especially in relation with the onset of antimicrobial resistant (AMR) infection-causing bacteria. In particular, veterinary university hospitals (VUHs) have been highlighted as high-risk settings due to the presence of students and the high frequency of referred patients [1]. The development of internal surveillance systems able to detect and analyze HAIs prevalence, involved bacterial species, antimicrobial resistance patterns and related risk factors is an effective tool to collect data useful to fight the onset of HAIs [2]. This study describes the application of a passive surveillance system that analyzed samples submitted for diagnosis (without an active selection by the investigators) in the Bologna VUH – Small Animal section. It was focused on specimens often related with HAIs such as blood cultures, nosocomial urinary tract infections (UTIs), and surgical site infections (SSIs). From 2020 to 2022, 138 strains from specimens of potential HAIs were collected and analyzed, representing 14.7% of the total number of isolated strains in the same timeframe. AMR and multi-drug resistance (MDR) rates ranged from 81.6 to 93.9% and from 61.2% to 73.5%, respectively. In all the three specimen typologies, the most commonly isolated species was Escherichia coli (overall 27%), followed by Klebsiella pneumoniae in nosocomial UTIs (19%), Enterobacter spp. in blood cultures (10%) and SSIs (18%). Compared with the average non-susceptibility rates for the single drug, large differences in terms of non-susceptibility were registered for cefazolin/cephalothin (51.3-61.3% versus 30.73%), ceftiofur (46.7-59.5% versus 25.51%) and piperacillin-tazobactam (24.5-34.7% versus 15.8%). Risk factors related with the onset of potential HAIs were identified in the previous use of invasive devices and the previous/current antimicrobial use (all with p=0.00001). This study confirms that HAIs are an important threat in small animal healthcare setting, especially in relation with AMR and MDR rate. A surveillance system can be a cost-effective and tailored instrument not only to monitor HAIs, but also to improve their management by addressing specific policies (eg., antimicrobial stewardship, preventive measures).

[1] Benedict K.M. et al. Characteristics of biosecurity and infection control programs at veterinary teaching hospitals, Journal of the American Veterinary Medical Association, 233(5):767-773, 2008.
[2] Harbarth S. Et al. The preventable proportion of nosocomial infections: an overview of published reports, Journal of Hospital infection, 54(4): 258-266, 2003.

FIRST CHARACTERIZATION OF STREPTOCOCCUS EQUI SUBSP. ZOOEPIDEMICUS SEQUENCE TYPE 525 IN DONKEYS OF ABRUZZO REGION, ITALY

Maria Chiara Cantelmi (1), (2), Michele Martino (1), Antonio Cocco (1), Giovanni Di Teodoro (1), Daniela Averaimo (1), Alexandra Chiaverini (1), Lucilla Ricci (1), Antonio Petrini (1), Francesca Cito (1), Pietro Badagliacca (1), Marco Rulli (1), Carmine Merola (1), (2)

(1) Istituto Zooprofilattico Sperimentale dell'Abruzzo e Molise, Teramo, Italy;
(2) Department of Biosciences and Technology for Food, Agriculture and Environment, University of Teramo, Teramo, Italy

Corresponding Author: **Maria Chiara Cantelmi -** m.cantelmi@izs.it

Streptococcus equi subsp. zooepidemicus (SEZ) is a Lancefield group C β-haemolytic Streptococcus that is usually considered a commensal bacterium of the oral cavity, pharynx, and respiratory tract of horses (1). In the present case study series, we described the diagnostic process applied to characterize the streptococcal infection caused by a novel SEZ sequence type (ST525) in donkeys located on a farm in Abruzzi, Italy. A total of 4 donkeys died between March and April 2022 after showing respiratory signs. The diagnostic process began with history taking and with anatomopathological investigations which showed hemorrhagic pneumonia and septicemia. Then, SEZ infection was confirmed by applying an innovative diagnostic strategy that put together standard bacterial isolation techniques, analytical tools for bacteria identification (MALDI-TOF MS), and molecular techniques (qPCR). Furthermore, the application of the DNA sequencing approach helps us to characterize the bacterial strains and the virulence factors involved in animal diseases. The novel SEZ-ST525 was identified in all the cases of the disease. This new sequence type was isolated from the lung, liver, and spleen in Case 1, and from retropharyngeal lymph nodes in Case 2. Moreover, the presence of the virulence gene mf2, a virulence factor carried by prophages in Streptococcus pyogenes, was also found for the first time in an SEZ strain. Our results demonstrated the need to apply new diagnostic tools, such as genomic sequencing technologies, for the identification and tracking of pathogenic strains of SEZ, shedding new light on the revaluation of this bacteria as a causative agent of disease in animals and humans.

[1] Preziuso, S., Moriconi, M., & Cuteri, V. (2019). Genetic diversity of Streptococcus equi subsp. zooepidemicus isolated from horses. Comparative Immunology, Microbiology and Infectious Diseases, 65, 7-13.

RISK FACTORS AND IMPACT ON FERTILITY OF THREE *EQUUS CABALLUS* PAPILLOMAVIRUS STRAINS ON HORSES IN ITALY

Filippo Dell'Anno (1), Livia De Paolis (1), Katia Cappelli (2,3), Chiara Grazia De Ciucis (1), Samanta Mecocci (2,3), Floriana Fruscione (1), Maria Ines Crescio (1), Marco Pepe (2,3), Gian Guido Donato (4), Rodolfo Gialletti (2,3), Tiziana Nervo (4), Luca Mechelli (2,3), Alessandro Ghelardi (5), Elisabetta Razzuoli (1)

(1) Istituto Zooprofilattico Sperimentale del Piemonte, Liguria e Valle d'Aosta, National Reference Center of Veterinary and comparative Oncology (CEROVEC), Piazza Borgo Pila 29/34, 16129 Genova, Italy
(2) Dipartimento di Medicina Veterinaria, Università degli Studi di Perugia, Via San Costanzo 4, 06126 Perugia, Italy
(3) Centro di Ricerca Sul Cavallo Sportivo, Università degli Studi di Perugia, Via San Costanzo 4, 06126 Perugia, Italy
(4) Dipartimento di Scienze Veterinarie, Università degli Studi di Torino, Largo Paolo Braccini 2, 10095 Grugliasco (TO), Italy
(5) Azienda Usl Toscana Nord-Ovest, UOC Ostetricia e Ginecologia, Nuovo Ospedale Apuane, Via Enrico Mattei 21, 54100 Massa, Italy

Corresponding Author: **Filippo Dell'Anno** - filippo.dellanno@izsto.it

Papillomaviruses (PVs) are small non-enveloped viruses, ubiquitous across the animal kingdom. PVs induce different kind of infection, such as cutaneous papillomas, genital papillomatosis, and carcinomas [1]. To date, a novel published *Equus caballus* PV (EcPV10) [2] (not yet deposited in https://pave.niaid.nih.gov/search/search_database) along with 14 species of PVs have been reported to infect equines such as horses and donkeys: three *Bos taurus* PVs (BPV1, BPV2 and BPV13), two *Equus asinus PVs* (EaPV1 and EaPV2), and nine *Equus caballus* PVs (EcPV1–9) [3]. As in humans, PV infections in horses can lead to the development or progression of different kinds of lesions, such as aural plaques, genital masses, and verrucous lesions [3]. With the aim to evaluate the impact of these viruses on horse health and fertility, we performed a study on Italian equine population to quantify the risk of an increased susceptibility to EcPV2, EcPV9, and EcPV10 infection associated with age and breed. Then we focused on mares to assess risk factors associated with the reproduction, such as type of mating (natural covering vs artificial), and the status (pluriparous or maiden). Finally, as EcPV10 was recently characterized in an infertile mare [2], we evaluated the impact of each PV strain on fertility. The statistical analysis was performed fitting logistic models to estimate the odds ratios (OR) of being positive to each PV strain and risk factors.
This study focused on 171 horses (largely sampled from the Pedimont Italian region), mostly females, with a mean age of 10.3 (± 4.4). Most of the analysed animals belonged to the Standardbred (49.7%) and Thoroughbred (38%). The OR did not show any increase in susceptibility for EcPV9 and EcPV10 by age classes. Conversely, Thoroughbred seems to have a higher risk of infection from EcPV9, if compared to the other breeds.
Similarly, the susceptibility to EcPV2 infection increased with age and was higher in Thoroughbred. Neither the type of mating nor the status were associated with the increased risk of PVs infection. Finally, our results suggest that the presence/absence of the virus did not appear to influence the fertility in the analysed mares. However, future studies involving a larger number of subjects, should be carried on validating our observations.

[1] Doorbar, J. Host control of human papillomavirus infection and disease. Best Pract. Res. Clin. Obstet. Gynaecol. 2018, 47 , 27–41
[2] Turco, S.; Gabbianelli, F.; Mavian, C.N.; Pietrucci, D.; De Paolis, L.; Gialletti, R.; Mechelli, L.; De Ciucis, C.G.; Cappelli, K.; Dell'Anno, F.; Mecocci, S.; Donato, G.G.; Nervo, T.; Fruscione, F.; Crescio, M.I.; Ghelardi, A.; Chillemi, G.; Razzuoli, E. Genetic Characterization of a Novel Equus caballus Papillomavirus Isolated from a Thoroughbred Mare. Viruses 2023, 15, 650.
[3] Li, C.-X.; Chang, W.-S.; Mitsakos, K.; Rodger, J.; Holmes, E.C.; Hudson, B.J. Identification of a Novel Equine Papillomavirus in Semen from a Thoroughbred Stallion with a Penile Lesion. Viruses 2019, 11, 713.

MOLECULAR SURVEY FOR SELECTED FELINE VIRAL PATHOGENS IN SICILY, SOUTHERN ITALY

Mira F. (1,2), Schirò G. (1,2), Giudice E. (2), Guercio A. (1), Vicari D. (1), Di Pietro S. (2), Antoci F. (1), Gucciardi F. (1), Lastra A. (1), Purpari G. (1)

(1) Istituto Zooprofilattico Sperimentale della Sicilia "A. Mirri" - Italy
(2) Department of Veterinary Science, University of Messina, Polo Universitario dell'Annunziata, Messina, Italy

Corresponding Author: **Simona Di Pietro** - simona.dipietro@unime.it

To date, information on the viral infections in cat (Felis catus) in Sicily region (southern Italy) is limited. A retrospective study, based on samples of domestic cats collected from 2020 to 2022 in Sicily, was conducted to determine the prevalence of selected viral athogens. A total number of 64 dead or alive cats, suspected of a viral infectious disease, were analysed for the presence of carnivore protoparvovirus type 1 (CPPV-1) species, including feline panleukopenia virus (FPV) and canine parvovirus type 2 (CPV-2), feline coronavirus (FCoV), feline calicivirus (FCV), feline herpesvirus type 1 (FHV-1), norovirus (NoV), and rotavirus (RoV). Tissue amples or rectal swab/faeces were collected from each cat from different Sicilian provinces. A set of classical PCR/RT-PCR or Real-Time RT-PCR assays and sequence analyses were performed. Single (45%) or mixed (38%) viral infections were detected in 83% (53/64) tested cats: 66% (42/64) and 3% (2/64) of the cats tested positive to FPV and CPV-2c, respectively, 30% (19/64) to FCoV, 19% (12/64) to FCV, 5% (3/64) to RoV and to NoV, and 3% (2/64) to FHV-1. FPV remains the main viral cause of infection, alone or in co-infection with other viruses, and sequence analysis showed high rates of nucleotide identity between the virus identified. A CPV-2c genomic variant, to date described in domestic dogs (1), was detected in the tested cats. FCoV enteric (8%) or systemic (20%) infections were detected in dead cats, mainly associated with FPV. Systemic FCV infection was observed in 7/8 positive cats. Other less commonly reported viruses (GIV.2 and GVI.2 NoVs, RoV), potentially related to the animal/human interface, were also detected with lower rates. Mixed infections were observed in 45% of the tested positive animals. The evidence of a heterogenous viral infectious agents in cats suggests the need to use of a wide diagnostic panel and to improve the prevention, immunization, and biosecurity levels, particularly in critical environments. Moreover, further studies are required to better elucidate the epidemiological and pathogenetic role for noroviruses and rotaviruses (2,3) in feline species.

[1] Mira F, Purpari G, Di Bella S, Colaianni ML, Schirò G, Chiaramonte G, Gucciardi F, Pisano P, Lastra A, Decaro N, Guercio A. Spreading of canine parvovirus type 2c mutants of Asian origin in southern Italy. Transbound Emerg Dis. 2019 Nov;66(6):2297-2304. doi: 10.1111/tbed.13283.
[2] Di Martino B, Di Profio F, Melegari I, Marsilio F. Feline Virome-A Review of Novel Enteric Viruses Detected in Cats. Viruses. 2019 Sep 30;11(10):908. doi: 10.3390/v11100908.
[3] Amoroso MG, Serra F, Miletti G, Cardillo L, de Martinis C, Marati L, Alfano F, Ferrara G, Pagnini U, De Carlo E, Fusco G, Montagnaro S. A Retrospective Study of Viral Molecular Prevalences in Cats in Southern Italy (Campania Region). Viruses. 2022 Nov 21;14(11):2583. doi: 10.3390/v14112583.

76° CONVEGNO SISVET
BARI, 21-22-23 GIUGNO 2023

APOPTOSIS AND ITS ANTIVIRAL ROLE DURING FELINE HERPESVIRUS TYPE 1 INFECTION

Gianmarco Ferrara (1), Consiglia Longobardi (1), Francesca Sgadari (1), Sara Damiano (1), Roberto Ciarcia (1), Giuseppe Iovane (1), Ugo Pagnini (1), Serena Montagnaro (1)

(1) Department of Veterinary Medicine and Animal Production, Federico II, Naples

Corresponding Author: **Gianmarco Ferrara** - gianmarco.ferrara@unina.it

Apoptosis is an important component of the host's defensive systems against viral infection since it limits viral multiplication and transmission (1). Many intrinsic and extrinsic signaling pathways build a complicated network to modulate apoptosis, despite viruses having diverse strategies for promoting or suppressing apoptosis to avoid this process (2,3). Nothing is known about the relationship between Feline Herpesvirus type 1 (FeHV-1), the etiological agent of feline rhinotracheitis, and apoptosis. The purpose of this work was to understand more about this interaction by investigating the impact of FeHV-1 infection of permissive cells on apoptosis. Monolayers of Crandell Rees Feline Kidney Cell (CRFK) were infected at different multiplicity of infection (MOI) and time points and were evaluated by flow cytometry analysis using an Annexin V-FITC detection kit and by Western blot targeting specific markers of apoptosis (Cas 8, Cas 9, Cas 3, Bcl-XL, and Bcl-2). Our results suggested that FeHV-1 triggers the apoptosis process starting with 12 hours post infection. In fact, a large percentage of early apoptotic cells were detected by the flow cytometry analysis at this time point, while late apoptotic cells increased at 24 hours after infection. The western blot analysis showed the cleavage of caspase 3 after 48 hours post-infection and the cleavage of caspase 9 after 24 hours. The results, combined with the reduction of the expression of the anti-apoptotic proteins Bcl-XL and Bcl-2, suggested the induction of apoptosis as a response to FeHV-1 through the intrinsic (mitochondrial) pathway. The mitochondrial damage induced by the virus was further investigated in immunofluorescence assay using Mytotracker™ and a specific monoclonal antibody against FeHV-1. Subsequently, we assessed the role of apoptosis during FeHV-1 by the evaluation of the effects caused by the pharmacological inhibition and induction of apoptosis (in terms of cell viability and viral titer). The use of Z-VAD-FMK, a pan-caspase inhibitor, was associated with an increase in viral titer and a reduction in cell viability, on the other hand, when Ionomycin (an apoptosis inducer) was used we observed a reduction in viral titers and an increase in cell viability. In this work, we have described for the first time the relationship between FeHV-1 and apoptosis, the mechanisms behind its induction, and its antiviral role, thereby improving the knowledge related to the pathogenesis of this virus.

[1] Sun M, Hou L, Song H, Lyu C, Tang YD, Qin L, Liu Y, Wang S, Meng F, Cai X. The relationship between autophagy and apoptosis during pseudorabies virus infection. Front Vet Sci. 2022 Dec 20;9:1064433. doi: 10.3389/fvets.2022.1064433.
[2] Prasad A, Remick J, Zeichner SL. Activation of human herpesvirus replication by apoptosis. J Virol. 2013 Oct;87(19):10641-50. doi: 10.1128/JVI.01178-13. Epub 2013 Jul 24.
[3] Kvansakul M. Viral Infection and Apoptosis. Viruses. 2017; 9(12):356. https://doi.org/10.3390/v9120356

FUNICONE-LIKE COMPOUNDS TOWARDS CANINE CORONAVIRUS INFECTION IN A72 CELL LINE

Claudia Cerracchio (1), Francesco Serra (2), Maria Michela Salvatore (3,4), Maria Grazia Amoroso (2), Rosario Nicoletti (5,6), Anna Andolfi (3,7), Filomena Fiorito (1,7)

(1) Department of Veterinary Medicine and Animal Production, University of Naples Federico II, 80137 Naples
(2) Istituto Zooprofilattico Sperimentale del Mezzogiorno, Unit of Virology, Department of Animal Health 80055 Portici (NA)
(3) Department of Chemical Sciences, University of Naples Federico II, 80126 Naples
(4) Institute for Sustainable Plant Protection, National Research Council, 80055 Portici, Naples
(5) Council for Agricultural Research and Economics, Research Centre for Olive, Fruit and Citrus Crops,81100 Caserta
(6) Department of Agricultural Sciences, University of Naples Federico II, 80055 Portici (NA)
(7) BAT Center-Interuniversity Center for Studies on Bioinspired Agro-Environmental Technology, University of Naples Federico II, 80055, Portici (NA)

Corresponding Authors: **Filomena Fiorito** - filomena.fiorito@unina.it; **Anna Andolfi** - andolfi@unina.it

Coronaviruses (CoVs), like canine coronavirus (CCoV), can infect different hosts like mammals and birds. Due to the tendency of CoVs genome to mutate and recombine, they can develop new strains able to cross the species barriers. CCoV generally provokes enteric symptoms and self-limiting diseases in dogs; however, extremely virulent CCoV-II strains were identified from pups with lethal infection. Recently, novel canine-feline recombinant strains were identified from humans in different geographic locations [Vlasova et al., 2022], suggesting that these viruses emerge independently. Occurrence of emerging and potentially more dangerous coronaviruses is intensifying the research activity based on discovering novel antiviral drugs. Interestingly, natural products, such as fungal secondary metabolites have revealed antiviral properties. Indeed, 3-O-methylfunicone (OMF), a benzo-γ-pyrone produced by Talaromyces pinophilus LT6, can decrease the infectivity of CCoV (Cerracchio et al., 2022a), through an original mechanism of action. Hence, in this study the potential efficacy of vermistatin (VER) and penisimplicissin (PS) derived from the same strain against CCoV was evaluated on A72, a canine fibrosarcoma cell line.

Vermistatin is the most representative compound of the subgroup of funicone phthalide type [Salvatore et al., 2022]. It is frequently extracted along with some derivatives, such as penisimplicissin. Vermistatin showed antiproliferative activity against different tumor lines, probably through the inhibition of RNA synthesis. Recently, OMF, VER and PS were isolated for T. pinophilus along with a new macrolide, talarodiolide (Salvatore et al., 2022).

During infection with the reference CCoV strain S/378 in a canine fibrosarcoma (A-72) cell line, in vitro bio-screen, immunofluorescence staining, cytomorphological and virus yield analyses were carried out.

Following infection, the non-toxic doses of PS and VER were identified and used during CCoV infection. They remarkably diminished virus yield and downregulated the expression of viral nucleocapsid protein. These findings were accompanied by a noticeable downregulation of aryl hydrocarbon receptor (AhR). Overall, these results, not only confirm the implication of AhR in CCoV infection, as recently demonstrated (Cerracchio et al., 2022b), but also suggest targeting AhR for developing new antivirals towards CCoV infections (Cerracchio et al., 2022a).

[1] Vlasova, A.N.; Toh, T.H.; Lee, J.S.Y.; Poovorawan, Y.; Davis, P.; Azevedo, M.S.P.; Lednicky, J.A.; Saif, L.J.; Gray, G.C. Animal alphacoronaviruses found in human patients with acute respiratory illness in different countries. Emerg. Microbes Infect. 2022, 11, 699–702.
[2] Salvatore, M. M., DellaGreca, M., Andolfi, A., & Nicoletti, R. (2022). New insights into chemical and biological properties of funicone-like compounds. Toxins, 14(7), 466.
[3] Cerracchio C, Iovane V, Salvatore MM, Amoroso MG, Dakroub H, DellaGreca M, Nicoletti R, Andolfi A, Fiorito F. Effecti-veness of the Fungal Metabolite 3-O-Methylfunicone towards Canine Coronavirus in a Canine Fibrosarcoma Cell Line (A72). Antibiotics (Basel). 2022a, 11;11(11):1594.
[4] Cerracchio, C.; Serra, F.; Amoroso, M.G.; Fiorito, F. Canine coronavirus activates aryl hydrocarbon receptor during in vitro infection. Viruses 2022b, 14, 2437.

EQUINE HEPACIVIRUS (EgHV): BIOMOLECULAR PREVALENCE AND PHYLOGENETIC ANALYSIS IN ITALIAN HORSES

Giulia Pacchiarotti (1), Roberto Nardini (1); Giuseppe Manna (1); Raffaella Conti (1); Marcello Sala (1); Romina Salpini (2); Maria Teresa Scicluna (1)

(1) National Reference Center for Equine Diseases, Istituto Zooprofilattico Sperimentale del Lazio e della Toscana M. Aleandri, Via Appia Nuova, 1411 - 00178 Roma
(2) Dipartimento di Medicina Sperimentale, Università degli Studi di Roma Tor Vergata Via Montpellier, 1 - 00133 Roma

Corresponding Author: **Maria Teresa Scicluna** - teresa.scicluna@izslt.it

Equine hepacivirus (EqHV) is a small, enveloped, RNA virus belonging to the Flaviviridae family, genus Hepacivirus, and is the closest known homologue of human HCV. EqHV infection is usually cleared within a few weeks and infected horses can show from no symptoms to those of sub-clinical hepatitis, with modifications in liver enzyme levels, especially GGT, SDH and GLDH. Fatigue, jaundice and lethargy are related to a chronic infection, where also substantial liver enzymes level modifications can be detected, along with liver failure, which can lead to death, occasionally described.
EqHV is reported worldwide since 2012 [1], with an the estimated biomolecular and serological prevalence in equids respectively of 6.95% and 47.11% [2]. Data for Italy is limited to few regions with a prevalence of 4.7% [3].
The present study includes 1801 horse sera, collected during 2019-22, at national level by the animal health laboratories network from Equine Infectious Anemia surveillance activities. The sampling was designed for an expected prevalence of 50%, a Confidence Level of 95% and a Standard Error of 5%; and stratified in four activity categories: equestrian (recreational or pet horses), competition (competitive sport horses), work/eeat and reproduction. The total number of expected samples was then distributed according to premises number in each Region and screened by RT Real-Time PCR [1]. NS3 fragment of positive samples was sequenced and phylogenetic analysis was performed.
In total, 77 on 1801 samples were positive (4.27%). Prevalence for each category as listed above was respectively 3.42%, 4.96%, 3.85% and 5.02%. No statistical differences among categories were detected by Fisher Exact Test. The phylogenetic analysis confirmed that all the samples cluster within the three described EqHV subtypes (1-2-3).
The prevalence data is coherent with what previously reported in Italy [3] and demonstrate that the virus is widely distributed within equid population regardless of their activity. This study supports also the need to consider this virus in the differential diagnosis of hepatitis and in the screening protocols for subjects employed in the production of haemoderivates, together with other emerging hepatotropic viruses as Equine Parvovirus.

[1] Burbelo, P.D.; Dubovi, E.J.; Simmonds, P.; Medina, J.L.; Henriquez, J.A.; Mishra, N.; Wagner, J.; Tokarz, R.; Cullen, J.M.; Iadarola, M.J.; et al. Serology-Enabled Discovery of Genetically Diverse Hepaciviruses in a New Host. J. Virol. 2012, 86, 6171–6178.
[2] Pacchiarotti, G.; Nardini, R.; Scicluna, M.T. Equine Hepacivirus: A Systematic Review and a Meta-Analysis of Serological and Biomolecular Prevalence and a Phylogenetic Update. Animals 2022, 12, 2486. https://doi.org/10.3390/ani12192486.
[3] Elia, G.; Lanave, G.; Lorusso, E.; Parisi, A.; Cavaliere, N.; Patruno, G.; Terregino, C.; Decaro, N.; Martella, V.; Buona voglia, C. Identification and genetic characterization of equine hepaciviruses in Italy. Vet. Microbiol. 2017, 207, 239–247.
[4] Wu, L.; Ou, J.; Cai, S.; Ji, J.; Ren, Z.; Shao, R.; Li, S. First identification and genomic characterization of equine hepacivirus sub-type 3 strain in China. Virus Genes 2020, 56, 777–780.

CANID ALPHAHERPESVIRUS 1 AND ENTEROCOCCUS FAECIUM MIXED INFECTION IN A LABRADOR RETRIEVER BREED LITTER: A CASE REPORT

Schirò G., Mira F., Vicari D., Giudice E., Purpari G., Liotta L., Giarratana F., Di Pietro S., Di Bella S., Puleio R., Castronovo C., Carrozzo A., Guercio A.

Istituto Zooprofilattico Sperimentale della Sicilia "A. Mirri" - Italy (Mira F., Vicari D., Purpari G., Di Bella S., Puleio R., Castronovo C., Carrozzo A., Guercio A.)
Department of Veterinary Science, University of Messina, Polo Universitario dell'Annunziata, Messina, Italy (Schirò G., Mira F., Giudice E., Liotta L., Giarratana F., Di Pietro S.)

Corresponding Author: **Francesco Mira** - dottoremira@gmail.com

Canine stillbirth and neonatal mortality are considered relatively high in domestic dogs and can be related to different factors or causes (1). Infectious agents, such as Canid alphaherpesvirus 1 (CaHV-1) and bacteria causing sepsis, are thought to be among the most common causes of perinatal death in dogs (2). However, clinical and diagnostic information on microbiological causes of canine neonatal mortality is still limited. Here, we report a mixed viral and bacteriological infection in a Labrador retriever breed litter of 7 puppies in Italy. Five 2- to 4-week-old puppies dead two days after onset of clinical signs (including weakness, vocalization, respiratory distress, and pulmonary oedema) and were submitted for necropsy. Other two puppies showed no clinical signs. Pulmonary oedema and multifocal areas of hemorrhage in lungs, enlarged and haemorragic lymph nodes, full bladder with urinary sediment were observed at necropsy, and tissue samples were then collected for virological and bacteriological assays. Milk, vaginal, and rectal swab samples from the mother were also collected. For the CaHV-1 screening, a nested PCR assay (3)
was performed. Other viral pathogens (canine parvovirus type 2, canine distemper virus, canine adenovirus type 1 and 2, canine coronavirus) were also screened. Bacterial isolation was performed on selective and differential agar media, and the identification was carried out with the biochemical API 20 Strep System and confirmed by MALDI-TOF MS with SARAMIS software. The antimicrobial susceptibility was also evaluated. All samples from puppies tested positive for CaHV-1. Enterococcus faecium strains were isolated from almost all tested samples from puppies, and from milk breast and rectal swab samples collected from the mother. Resistances to antimicrobials were detected. A mixed infection has been then considered to be directly transmitted by the mother and the leading cause of the fatal outcome of pups. This study contributes to update the current knowledges on clinical and anatomopathological natural CaHV-1 infection, and the potential different outcome in littermates. As little is information on Enterococcus faecium systemic infection in dogs is available, this study highlighted the opportunity to include this bacterial species as cause of neonatal sepsis. Moreover, as Enterococcus faecium is included within the faction of the ESKAPE pathogens (4), its evidence should be carefully considered in companion animals.

[1] R. Tønnessen, K. Sverdrup Borge, A. Nødtvedt, A. Indrebø. Canine perinatal mortality: A cohort study of 224 breeds. Theriogenology, 2012, 77:9, 1788-1801, ISSN 0093-691X, doi.org/10.1016/j.theriogenology.2011.12.023.
[2] E.W. Fisher, Neonatal Diseases of Dogs and Cats. British Veterinary Journal, 1982, 138:4, 277-284, ISSN 0007-1935, doi.org/10.1016/S0007-1935(17)31031-X.
[3] C Schulze, W. Baumgartner, Nested polymerase chain reaction and in situ hybridization for diagnosis of canine herpesvirus infection in puppies. Veterinary Pathology, 1998; 35, 209 217, doi.org/10.1177/030098589803500306
[4] Pendleton J.N., Gorman S.P., Gilmore B.F. Clinical relevance of the ESKAPE pathogens. Expert Review of Anti-infective Therapy, 2013, 11:3, 297-308, doi.org/10.1586/eri.13.12

76° CONVEGNO SISVET
BARI, 21-22-23 GIUGNO 2023

EVALUATION OF DRUG EFFLUX ACTIVITY IN ANTIMICROBIAL RESISTANT STAPHYLOCOCCUS PSEUDINTERMEDIUS CLINICAL ISOLATES

Elisa Rampacci (1), Tommaso Felicetti (2) Francesco Birettoni (1), Rolando Arcelli (1), Riccardo Zelli (1), Stefano Sabatini (2), Fabrizio Passamonti (1)

(1) Dipartimento di Medicina Veterinaria, Università degli Studi di Perugia
(2) Dipartimento di Scienze Farmaceutiche, Università degli Studi di Perugia

Corresponding Author: **Elisa Rampacci** - elisa.rampacci@gmail.com

Staphylococcus pseudintermedius is responsible for a large variety of opportunistic infections in dogs and cats and it has the potential to be virulent in human hosts [1]. The treatment of diseases caused by this bacterium is considered a clinical challenge nowadays, as the resistance of S. pseudintermedius to the majority of antimicrobials has been increasing significantly over time [2]. Knowledge of the resistance mechanisms of S. pseudintermedius remains limited, particularly the overexpression of drug efflux pumps (EPs). The aim of this study was to examine the contribution of efflux systems in the resistance of S. pseudintermedius clinical isolates to common antibiotics, and the capability of different chemotypes to reverse drug resistance. A collection of 108 canine antibiotic-resistant S. pseudintermedius was examined. The efflux phenotypes were screened by the determination of the MIC of ethidium bromide (EtBr), a known substrate of bacterial EPs, and compared to the basal efflux expression of a wild-type strain (ATCC 49444). Selected strains were then advanced toward fluorometry-based experiments to test their capability to accumulate and extrude EtBr in the presence/absence of the EP inhibitors (EPIs) CCCP, thioridazine and an in-house molecule. The ability of these EPIs to synergize with failing antibiotics was evaluated. The presence of plasmid-encoded EPs was investigated for strains exerting high efflux activity. Ten out of 108 antibiotic-resistant S. pseudintermedius (9.3%) had EtBr MICs higher than that for the wild-type strain, suggesting active efflux. Of these, six showed efflux 2.5-fold higher than that of S. pseudintermedius ATCC 49444, considered significant and representative of an efflux phenotype. Although the use of EPIs had variable inhibiting effect on EtBr efflux, these did not fully restore the susceptibility of the isolates to failing antibiotics; however, synergism was observed for combinations EPI/ciprofloxacin and EPI/clindamycin. The following plasmid-encoded EPs were detected in the six high-efflux strains: smr (2/6), qacG (6/6), qacJ (1/6), qacH (3/6), msrA/B (1/6), tetK (3/6).
This study underlines the contribution of EPs in the emergence of high-level resistance to ciprofloxacin and clindamycin. However, high-efflux strains were supposed to harbour mutations in antibiotic targets that limited the therapeutic usefulness of EPIs. The efficacy of EPIs to block resistance evolutionary pathways should be investigated.

[1] Lynch, S.A.; Helbig, K.J. The Complex diseases of Staphylococcus Pseudintermedius in canines: Where to Next? Vet. Sci. 2021, 8, 11.
[2] EFSA Panel on Animal Health and Welfare (AHAW); Nielsen, S.S.; Bicout, D.J.; Calistri, P.; Canali, E.; Drewe, J.A.; Garin-Bastuji, B.; Gonzales Rojas, J.L.; Gortazar Schmidt, C.; Herskin, M.; Michel, V.; Miranda Chueca, M.A.; Padalino, B.; Pasquali, P.; Roberts, H.C.; Sihvonen, L.H.; Spoolder, H.; Stahl, K.; Velarde, A.; Viltrop, A.; Winckler, C.; Guardabassi, L.; Hilbert, F.; Mader, R.; Aznar, I.; Baldinelli, F.; Alvarez, J. Assessment of animal diseases caused by bacteria resistant to antimicrobials: dogs and cats. EFSA J. 2021, 19, e06680.

PRESENCE OF PASTEURELLA MULTOCIDA IN DOGS TRAINED FOR ANIMAL-ASSISTED THERAPY

Antonio Santaniello (1), Susanne Garzillo (1), Alessia Amato (1), Luca Borrelli (1), Tamara Pasqualina Russo (1), Adriano Minichino (1), Lorena Varriale (1), Ludovico Dipineto (1), Alessandro Fioretti (1)

(1) Department of Veterinary Medicine and Animal Productions, Federico II University of Naples, 80134 Naples, Italy

Corresponding Author: **Antonio Santaniello** - antonio.santaniello2@unina.it

As is known, dogs also play a central role in animal-assisted therapy (AAT) as co-therapists or as a support for people with physical or mental health problems. AAT is a non-pharmacological therapy aimed at people with physical and/or mental disabilities. Therefore, it is necessary to carry out interventions that guarantee its benefits for patients while also avoiding the risk of zoonoses due to contact with the animals or their mucous membranes. Considering that bodily contact is the main way to ensure the efficacy of AAT, with the high prevalence of P. multocida in the mouth microbiome of dogs and the possibility of transmission during the interaction time in the setting, the present study was carried out to detect the occurrence of Pasteurella multocida in the oral cavity of dogs attending a "dog educational centre" and training for future AAT interventions. In addition, some of the potential predictable factors of infection (i.e., age, sex, breed, and living conditions) were analyzed. The present study was performed from May to November 2018 on 200 dogs attending a dog educational centre in Southern Italy. As a preliminary step, the owners were interviewed using a pretested, standardized questionnaire to obtain a full history for each dog. Each dog was individually sampled using sterile, cotton-tipped swabs in the oral cavity (palate, internal gums and teeth, and tongue). All sampled dogs showed an absence of a periodontal procedure. In total, 25/200 dogs examined (12.5%; 95% confidence interval = 8.4–18.1%) were positive for P. multocida, as confirmed by PCR. Sex, breed, and living conditions were risk factors associated with P. multocida as revealed by the logistic regression analysis. Specifically, cross-bred female dogs living prevalently outdoors were significantly associated with the presence of P. multocida ($p < 0.05$). This study represents the first epidemiological survey of the prevalence of P. multocida in the oral cavity of dogs involved subsequently in AAT interventions, highlighting the potential risk of P. multocida infection in patients, often belonging to risk categories (e.g., children, the elderly, and immunocompromised individuals). Therefore, healthcare guidelines could be suggested to integrate the current literature related to the health check of dogs involved in AAT. In this way, it could be ensured that, even with bodily contact during AAT, the risk of pathogen transmission by the co-therapist dog can be avoided.

[1] Menna, L.F.; Santaniello, A.; Todisco, M.; Amato, A.; Borrelli, L.; Scandurra, C.; Fioretti, A. The Human-Animal Relationship as the Focus of Animal-Assisted Interventions: A One Health Approach. Int. J. Environ. Res. Public Health 2019, 16, 3660
[2] Santaniello, A.; Sansone, M.; Fioretti, A.; Menna, L.F. Systematic Review and Meta-Analysis of the Occurrence of ESKAPE Bacteria Group in Dogs, and the Related Zoonotic Risk in Animal-Assisted Therapy, and in Animal-Assisted Activity in the Health Context. Int. J. Environ. Res. Public Health 2020, 17, 3278
[3] Overgaauw, P.A.M.; Vinke, C.M.; Hagen, M.A.E.V.; Lipman, L.J.A. A One Health Perspective on the Human-Companion Animal Relationship with Emphasis on Zoonotic Aspects. Int. J. Environ. Res. Public Health 2020, 17, 3789
[4] Ghasemzadeh, I.; Namazi, S.H. Review of bacterial and viral zoonotic infections transmitted by dogs. J. Med. Life 2015, 8, 1–5.

EMERGING DISEASES IN EQUIDS: BIOMOLECULAR INVESTIGATIONS AND PREVALENCE OF EQUINE PEGIVIRUSES, CORONAVIRUS AND PARVOVIRUS IN HORSE POPULATIONS IN ITALY

Ida Ricci (1), Giulia Pacchiarotti (1), Francesca Rosone (1), Antonella Cersini (1), Andrea Carvelli (1), Carlo Alberto Minniti (2), Reno Caforio (3), Silvia Tofani (1), Raffaella Conti (1), Roberto Nardini (1), Erica Tilesi (1), Maria Rita Viola (1), Maria Teresa Scicluna (1)

(1) Istituto Zooprofilattico Sperimentale del Lazio e della Toscana 'M. Aleandri', Via Appia Nuova 1411, 00178 Rome, Italy
(2) Dipartimento per l'Organizzazione Sanitaria e Veterinaria - Comando Generale dell'Arma dei Carabinieri (Head of the Veterinary Service - Department for Health and Veterinary Organization - Carabinieri Corps General Head Quarters)
(3) Esercito Italiano (Italian Army)

Corresponding Author: **Teresa Scicluna -** teresa.scicluna@izslt.it

Sport and breeding horses represent a solid business worldwide: highly valuable individuals often travel and may be screened for several diseases to prevent infection risk and spread. In this scenario, new and re-emerging infections are often overlooked, especially when transmission routes are still elusive. To provide further knowledge on the distribution of these diseases in equids, four viruses were chosen on their relative importance in the literature, to assess their prevalence in selected Italian horse populations: Pegivirus D and E (previously known as EPgV-2 and EPgV-1), Equine Coronavirus (EcoV) and Equine Parvovirus (EqPV-H). Pegiviruses were initially considered responsible of equine hepatic disease, but recent data ascribes them as bone marrow tropic viruses with limited pathogenicity [1]; EcoV was detected in symptomatic horses, but also in clinically healthy subjects [2]; EqPV-H was recently ascribed as the aetiological agent of the Theiler's disease (fulminant serum hepatitis), previously associated to Pegivirus D [3].
The sampling focused on the equine population of the Italian Armed Forces (Army and Carabinieri Corps). Of a total of 1,068 subjects, including 24 donkeys, 443 equids (195 from the Italian Army and 248 from the Carabinieri Corps) were randomly selected (P:unknown; CL:95%; desired precision:5%) and their sera and rectal swabs, collected between 2021 and 2022, were tested by Real Time PCR protocols selected from the literature.
Pegivirus E had the highest prevalence (8.35%; 95%CI: 6.12-11.3), while Pegivirus D was detected only in nine horses (2.03%; 95%CI: 1.07-3.82). EqPV-H was detected in 2.26% individuals (95%CI: 6.12-11.3), of which one was a donkey.
Lastly, only one rectal swab was positive for EcoV (0.22%; 95%CI: 1.23-4.10).
Amplicons suitable for phylogenetic analysis are undergoing sequencing for genetic characterization. To our knowledge, this is the first study that provides data on the presence of Pegiviruses in specific equine Italian populations. Results demonstrate that the studied infections are present within the monitored populations, and to be considered in case of the observation of clinically related signs. Due to presence of asymptomatic cases during which viremia can be persistent for some time [3], it is crucial to also contemplate Equine Parvovirus and Pegiviruses as blood borne viruses when using hemoderivatives; for this, monitoring of these infections should be implemented.

[1] Tomlinson JE, Wolfisberg R, Fahnøe U, Sharma H, Renshaw RW, Nielsen L, Nishiuchi E, Holm C, Dubovi E, Rosenberg BR, Tennant BC, Bukh J, Kapoor A, Divers TJ, Rice CM, Van de Walle GR, Scheel TKH. Equine pegiviruses cause persistent infection of bone marrow and are not associated with hepatitis. PLoS Pathog. 2020 Jul 10;16(7):e1008677. doi: 10.1371/journal.ppat.1008677. PMID: 32649726; PMCID: PMC7375656.
[2] Pusterla N, Mapes S, Wademan C, White A, Ball R, Sapp K, Burns P, Ormond C, Butterworth K, Bartol J, Magdesian KG. Emerging outbreaks associated with equine coronavirus in adult horses. Vet Microbiol. 2013 Feb 22;162(1):228-31. doi: 10.1016/j.vetmic.2012.10.014. Epub 2012 Oct 16. PMID: 23123176; PMCID: PMC7117461.
[3] Ramsauer AS, Badenhorst M, Cavalleri JV. Equine parvovirus hepatitis. Equine Vet J. 2021 Sep;53(5):886-894. doi: 10.1111/evj.13477. Epub 2021 Jul 4. PMID: 34101906; PMCID: PMC8457058.

ANTIMICROBIAL ACTIVITY OF HEMP EXTRACT SEED OIL AGAINST STAPHYLOCOCCUS PSEUDINTERMEDIUS AND PSEUDOMONAS AERUGINOSA STRAINS ISOLATED FROM PYODERMA AND EXTERNAL OTITIS IN DOGS

Valeria Toppi (1), Elisa Rampacci (1), Laura Musa (1), Giorgia della Rocca (1) , Alessandra Di Salvo (1), Marina De Benedetti (2), Giacomo Di Giacinto (1), Maria Pia Franciosini (1), Patrizia Casagrande Proietti (1)

(1) Department of Veterinary Medicine, University of Perugia, Perugia, Italy
(2) BioAgrigea Compary, Padova, Italy

Corresponding Author: **Valeria Toppi** - valeria.toppi@studenti.unipg.it

Several scientific studies report that Hemp (Cannabis sativa) and its extracts (essential oil, seed oil and cannabinoids) possess antimicrobial properties against both Gram-positive and Gram-negative multi-resistant pathogenic bacteria and fungi. These findings make Hemp, and especially its derivatives, promising candidates for the future development of new, unconventional and innovative antimicrobial therapies against pathogenic bacteria of zoonotic and veterinary clinical interest. Among these, Staphylococcus pseudintermedius and Pseudomonas aeruginosa are responsible for serious infections in dogs and, according to EFSA, they have been identified among the most relevant antimicrobial-resistant bacteria in Europe for companion animals. For this, the aim of this study was to in vitro evaluate the antimicrobial activity of Hemp extract seed oil against Staphylococcus pseudintermedius strains and Pseudomonas aeruginosa strains isolated from pyoderma and external otitis in dogs. Twenty bacterial strains (respectively 10 methicillin-resistant S. pseudintermedius and 10 P. aeruginosa) were tested in this work. The assessment of the antimicrobial activity of Hemp extract seed oil was carried out by broth-microdilution method to determine the Minimum Inhibitory Concentration (MIC). The Hemp extract seed oil used in this study was characterized by the absence of tetrahydrocannabinol (THC) and a very low value of cannabidiol (CBD). We tested scalar dilutions of the Hemp seed oil in Dimethyl sulfoxide (DMSO) starting from a maximum value of 0.2%. The results of the work showed that the Hemp seed oil had an higher activity against S. pseudintermedius than against P. aeruginosa. The MIC value for all S. pseudintermedius strains and P. aeruginosa strains was indeed 0.05% and >0.2%, respectively. In conclusion, despite the fact that CBD and THC are widely reported to be responsible for the antimicrobial capacity of Hemp extracts, our results highlight that even Hemp seed oil without THC and with very low CBD has antimicrobial properties against the S. pseudintermedius strains. Further studies will be necessary to confirm these preliminary data and to expand the number of bacterial strains tested. Moreover, subsequent investigations will be needed to unveil the mechanisms underlying the antibacterial activity of low CBD and THC free-hemp seed oil and to establish its potential as topical treatment for skin infections.

[1] Mikulcová V, Kašpárková V, Humpolíček P, Buňková L. Formulation, Characterization and Properties of Hemp Seed Oil and Its Emulsions. Molecules. 2017;22(5):700. Published 2017 Apr 27. doi:10.3390/molecules22050700;
[2] Iseppi R, Brighenti V, Licata M, Lambertini A, Sabia C, Messi P, Pellati F, Benvenuti S. Chemical Characterization and Evaluation of the Antibacterial Activity of Essential Oils from Fibre-Type Cannabis sativa L. (Hemp). Molecules. 2019 Jun 21;24(12):2302. doi: 10.3390/molecules24122302. PMID: 31234360; PMCID: PMC6631254;
[3] Blaskovich MAT, Kavanagh AM, Elliott AG, Zhang B, Ramu S, Amado M, Lowe GJ, Hinton AO, Pham DMT, Zuegg J, Beare N, Quach D, Sharp MD, Pogliano J, Rogers AP, Lyras D, Tan L, West NP, Crawford DW, Peterson ML, Callahan M, Thurn M. The antimicrobial potential of cannabidiol. Commun Biol. 2021 Jan 19;4(1):7. doi: 10.1038/s42003-020-01530-y. PMID: 33469147; PMCID: PMC7815910;

76° CONVEGNO SISVET
BARI, 21-22-23 GIUGNO 2023

HEALTH INDICATORS AS SCREENING METHOD FOR WELFARE ASSESSMENT IN DAIRY HERDS

Alessandro Bellato (1), Alessandro Mannelli (1), Francesca Fusi (2,3), Valentina Lorenzi (2), Stefania Bergagna (4)

(1) Università degli Studi di Torino, Dipartimento di Scienze Veterinarie, Grugliasco, Italy
(2) Italian Reference Centre for Animal Welfare, Istituto Zooprofilattico Sperimentale della Lombardia e dell'Emilia-Romagna, Brescia, Italy
(3) Università degli Studi di Parma, Dipartimento di Scienze degli Alimenti e del Farmaco, Parma, Italy
(4) Istituto Zooprofilattico Sperimentale del Piemonte, Liguria e Valle d'Aosta, Torino, Italy

Corresponding Author: **Alessandro Bellato** - alessandro.bellato@unito.it

Various parameters serve as health indicators (HI) to describe the health status of a herd. In dairy cattle inter-calving interval (ICI), fresh-cow replacement rate, ketosis and mastitis incidence rates are evaluated [1]. Also, in Italy, thanks to ClassyFarm platform, a voluntary assessment of animal welfare is performed regularly [2]. The evaluation is made on-site about 5 assessment areas (AA) scored as percentages based on the meeting of criteria.
The aim of this exploratory study was to assess the potentiality of such HI as early predictor of poor welfare in dairy cattle herds.
A cohort of 1241 dairy herds in Piedmont were followed for 5 years. Data were collected by the Italian Breeders Association for dairy herd improvement program (DHI) and used to estimate HI adjusting by individual-cow risk factors using random-effect regression models [1]. Latent profile analysis (LPA) was performed on the model-derived random intercepts to detect herd profiles. The use of HI to identify herds with low welfare scores was explored by discriminant analysis (DA) on 277 herds that underwent the ClassyFarm welfare evaluation.
The herds which underwent welfare evaluation and were also involved in DHI had better scores for all AA.
LPA results showed the existence of 4 herd profiles (A, B, C, D), with 0.9, 9.1, 79.5, and 10.5% probability, respectively. Compared with C, the most frequent profile, A had higher mastitis and fresh-cow replacement rate, and longer ICI; B had shorter ICI but higher fresh-cow replacement rate; and D had higher ketosis rate but shorter ICI.
DA could discriminate herds with overall welfare score in the lowest quartile (poor welfare) with only 12% sensitivity but 96% specificity. It had a positive predictive value equal to the toss of a coin (50%), but a 78% negative predictive value.
LPA results displayed the existence of few herds that performed significantly worse than the rest on all HI. Unfortunately, for none of them welfare evaluation was available. Among the others three different profiles were identified with slightly different AA scores.
The better welfare scores observed for herds involved in DHI confirmed the importance of constant monitoring and breeder awareness. The findings of this preliminary analysis prevent from the use of HI as early predictors of poor welfare in dairy cattle herds. Yet, based on the high specificity, they could be used to identify low-risk herds thus reducing the workload for the public health system.

[1] A. Bellato, A. Tondo, L. Dellepiane, A. Dondo, A. Mannelli, S. Bergagna. Estimates of dairy herd health indicators of mastitis, ketosis, inter-calving interval, and fresh cow replacement in the Piedmont region, Italy. Prev Vet Med, 212, 10583, 2023.
[2] L. Bertocchi, F. Fusi, A. Angelucci, L. Bolzoni, S. Pongolini, R.M. Strano, J. Ginestreti, G. Riuzzi, P. Moroni, V. Lorenzi; "Characterization of hazards, welfare promoters and animal-based measures for the welfare assessment of dairy cows: elicitation of expert opinion. Prev Vet Med, 150, 8-18, 2018.

PREVALENCE OF BOVINE MASTITIS PATHOGENS AND BETA LACTAMASE RESISTANCE IN APULIA REGION

Bianco Angelica (1), Del Sambro Laura (1), Schino Giulia (1), Milano Angelica (1), Catalano Elisabetta (1), Caffò Annamaria (1), Castellana Antonella (1), Trisolini Carmelinda (1), Parisi Antonio (1), Capozzi Loredana (1)

(1) Experimental Zooprophylactic Institute of Apulia and Basilicata, Via Manfredonia, 20 - 71121 Foggia

Corresponding Author: **Angelica Bianco** - biancoange87@libero.it

Bovine mastitis is the most common disease affecting the mammary glands of cows. It is caused by an inflammation triggered mainly by pathogenic bacteria, which poses serious problems for the health of dairy cows and, in turn, for the productivity of livestock [1]. The use of antibiotics represents the only available therapeutic option for bacterial mastitis. Nevertheless, the use and misuse of antibiotics has significantly increased antibiotic resistance globally, representing a risk for public health. Generally, milk somatic cell count (SCC) is the method to make diagnose. However, several non-infectious factors, such as lactation stage, season, milking frequency can influence the SCC, leading to false positive results [2]. In this study we screened milk samples for the presence of 14 pathogens and the blaZ gene, associated to beta-lactamase resistance. We collected a total of 545 milk samples; DNA was purified using MagMAX™ CORE Mastitis & Panbacteria Module. The detection of 15 targets was performed by RealTime PCR using VetMAX™ MastiType Multi Kit.

Of the samples tested, 432 were positive for at least one of the pathogen targets tested. Sthaphylococcus spp was identified in 79% of the samples. In particular, S. aureus was identified in 28% of them. With a similar frequency (27%) was identified S. uberis. Other pathogens were identified less frequently: E. Coli and C. bovis (20%); Enteroccocus spp (17%); S. dysgalactiae (16%); S.agalactiae (14%); Klebsiella oxytoca/Klebsiella pneumoniae, T. pyogenes/ P. indolicus and S. marcescens (5%); Mycoplasma spp (3%); M. bovis (2%); Prototheca spp (1%).

The beta lactamase gene was identified in 35% of the samples tested. Interestingly, in 90% of the samples that tested positive for Staphylococcus spp., was found this gene that confers resistance to penicillin, which is the antibiotic of choice for the treatment of mastitis sustained by gram-positive bacteria.

Based on the preliminary results, it is clear that the most commonly encountered pathogens are S. aureus, S. uberis, and E. coli, as previously described [3, 4]. The evidence for the penicillin resistance gene, although we do not know which strains carry this resistance, highlights a potential risk towards effectiveness of the beta-lactam antibiotics in treating the bacterial infections. The project will continue trying to precisely deepen this aspect.

[1] Ezzat Alnakip M, Quintela-Baluja M, Böhme K, Fernández-No I, Caamaño-Antelo S, Calo-Mata P, et al. The Immunology of Mammary Gland of Dairy Ruminants between Healthy and Inflammatory Conditions. J Vet Med. 2014;2014:659801.
[2] Knežević K, Dobranić V, Đuričić D, Samardžija M, Benić M, Getz I, et al. Use of somatic cell count in the diagnosis of mastitis and its impacts on milk quality. Veterinarska stanica. 2021;52:751–64.
[3] Khan MZ, Wang J, Ma Y, Chen T, Ma M, Ullah Q, et al. Genetic polymorphisms in immune- and inflammation-associated genes and their association with bovine mastitis resistance/susceptibility. Front Immunol. 2023;14:1082144.
[4] Kabelitz T, Aubry E, van Vorst K, Amon T, Fulde M. The Role of Streptococcus spp. in Bovine Mastitis. Microorganisms. 2021;9:1497.

STUDY OF A NEW IMMUNIZATION PROTOCOL AGAINST BUBALINE ALPHAHERPESVIRUS 1 IN WATER BUFFALOES

Anna Donniacuo (1), Alessandra Martucciello (1), Anna Balestrieri (1), Cecilia Righi (2), Giovanna Cappelli (1), Eleonora Scoccia (2), Carlo Grassi (1), Loredana Cozzzolino (1), Giorgio Galiero (1), Giovanna Fusco (1), Francesco Feliziani (2), Esterina De Carlo (1)and Stefano Petrini (2)

(1) National Reference Centre for Hygiene and Technology of Breeding and Buffalo Production, Istituto Zooprofilattico Sperimentale del Mezzogiorno, 84131, Salerno (SA), Italy;
(2) National Reference Centre for Infectious Bovine Rhinotracheitis (IBR), Istituto Zooprofilattico Sperimentale Umbria-Marche "Togo Rosati", 06126 Perugia, (PG) Italy;
(3) Azienda Sanitaria Locale Salerno, 84014 Nocera Inferiore (SA), Italy;

Corresponding Author: **Giovanna Cappelli** - giovanna.cappelli@izsmportici.it

The water buffaloes (Bubalus bubalis) is a specie susceptible to Bovine alphaherpesvirus 1 (BoHV-1) and Bubaline alphaherpesvirus 1 (BuHV-1) (1,2). These viruses are comprised to the Herpesviridae family (3). As several studies have shown that the IBR gE-marker vaccine can induce cross-protection between BoHV-1 and BuHV-1 (2,5), in the present study, we hypothesized that a new immunization protocol using two IBR gE-marker vaccines could protect buffaloes against challenge infection against BuHV. Ten water buffaloes, divided into two groups (A, B) of five each, were selected. Group A was first vaccinated with two doses of live gE marker vaccine administered intranasally at an interval of 30 days, starting at the age of three months. Third and fourth doses of inactivated gE marker vaccine were injected intramuscularly at ten and eleven months of age. Group B represented negative control. Two hundred seventy post-vaccination days (PVD), all buffaloes were challenge infected with a wild-type (wt) BuHV-1 strain and the animals were observed for 63 post-challenge days (PCD). Rectal temperatures, nasal swabs and serum samples were taken during the entire experimental period. The samples were used for virus isolation and neutralization test (VNT) against BuHV-1 and gE-ELISA. The protocols used were in accordance with the OIE Manual. No clinical signs were observed during the experimental period. The virus was isolated from 2 to 7 PCD. An increase in the BuHV-1 NA titer was detected in group A from 1:8 on PVD 30 to 1:43 on PVD 240. In group B, no NAs, to BuHV-1 were detected. The NA titre against BuHV-1 of the vaccinated animals increased after challenge infection, reaching a value of 1:1024 and 1:1800 on PCD 10 and PCD 63, respectively. In group B, NAs against BuHV-1 were detected on PCD 15 with an average titre of 1:56. These titres increased to 1:550 PCD 63. No seroconversion was detected in the unvaccinated control. In all water buffaloes, a positive signal for gE was detected on PCD 30 until the end of the challenge. The results obtained in this study differ from those published previously (1,2). To conclude, the present study shows that in water buffaloes, the immunized protocol using BoHV-1 gE-deleted marker vaccines against BoHV-1 did not protect them against challenge infection with wt-BuHV-1.

This research was funded by the Italian Ministry of Health (grant number: Research Pro-ject IZSM RC 004/2017).

[1] Petrini, S.; Martucciello, A.; Grandoni, F.; De Matteis, G.; Cappelli, G.; Giammarioli, M.; Scoccia, E.; Grassi, C.; Righi, C.; Fusco, G.; et al. Evaluation of Safety and Efficacy of an Inactivated Marker Vaccine against Bovine alphaherpesvirus 1 (BoHV-1) in Water Buffalo (Bubalus bubalis). Vaccines 2021
[2] Montagnaro, S.; De Martinis, C.; Iovane, V.; Ciarcia, R.; Damiano, S.; Nizza, S.; De Martino, L.; Iovane, G.; Pagnini, U. Bovine herpesvirus type 1 marker vaccine induce cross-protection against bubaline herpesvirus type 1 in water buffalo. Prev. Vet. Med. 2014 1;116(1-2):56-62.
[3] International Committee on Taxonomy of Viruses: ICTV. Available online: https://ictv.global/taxonomy/taxondetails?taxnode_id=202101442 (access on 05/01/23)
[5] Petrini, S.; Konig, P.; Righi, C.; Iscaro, C.; Pierini, I.; Casciari, C.; Pellegrini, C.; Gobbi, P.; Giammarioli, M.; De Mia, G.M. Serological Cross-Reactivity Between Bovine alphaherpesvirus 2 and Bovine alphaherpesvirus 1 in a gB-ELISA: A Case Report in Italy. Front. Vet. Sci. 2020, 28;7:587885.

SEROLOGICAL AND MOLECULAR CHARACTERIZATION OF SMALL RUMINANT LENTIVIRUSES IN MOROCCO AND GREECE

Colitti Barbara (1), Daif Soukaina (2), Scalas Daniela (1), Ilias Bouzalas (3), Sergio Rosati (1)

(1) Department of Veterinary Science, University of Turin, Turin, Italy
(2) Institut Agronomique et Veterinaire Hassan II, Rabat, Morocco
(3) Veterinary Research Institute, Campus of Thermi, Thessaloniki, Greece

Corresponding Author: **Barbara Colitti** - barbara.colitti@unito.it

Genomic data show that small ruminant lentiviruses (SRLVs) have circulated in mammals for millions of years. Recent studies investigated the origins of SRLV strains and their distribution among domestic ruminants (1,2). Aim of the study was to investigate SRLV circulation in Greece and Morocco, two Countries in which the small ruminant population has an important economic and social role.
In Morocco, a total of 724 serum samples (578 sheep and 146 goats), were selected from 51 farms in different geographical locations. In Greece, 60 defatted bulk milk samples (9 sheep, 46 goats and 6 mix) collected from different geographical municipalities were examined. All samples were tested by screening ELISA followed by genotyping (Eradikit SRLV, In3diagnostic srl). Whole blood and cell pellet samples from Morocco and Greece respectively were used for DNA extraction and nested gag PCR (3). Prevalence of antibody-positive sheep and goats in Morocco was 5,3% and 0,7% respectively. Sample size allows an estimation of prevalence lower than 20% (CI 95%) in 17 sheep and 10 goat flocks. Surprisingly, a large proportion of screening-positive samples was not correctly serotyped. Serological prevalence of bulk milk in Greece was 59% and 31 samples (6 sheep, 22 goats and 3 mix) were successfully PCR amplified. Sanger and NGS amplicon sequencing approaches were used for DNA samples. To date, 14 new sequences from Morocco and 19 from Greece were generated. Serological data in Morocco support the evidence of a low prevalence of infection in the population, with an intrinsic difficulty of SRLV to spread, due to management practices. The rate of success by genotyping ELISA in this study did not reach 40%, leading to suppose that divergent strains might have escaped from diagnostic tools, partially confirmed by the evidence of a genotype A subtype carrying a mismatch in serotyping epitope. Sequence analysis reveals the circulation of a novel B and a recombinant A/B subtype. In Greece the prevalence of SRLV infection is more similar to that in Mediterranean milk breeds. Circulation of B1 and B2 subtypes were identified and new viral clusters referred to as genotype A were detected suggesting that heterogeneity of MVV group may be even larger than expected. This study highlighted the importance of monitoring viral sequences to develop specific diagnostic tests.

[1] Molace V. et al. (2020) 'Phylogenetic Analysis of Small Ruminant Lentiviruses in Germany and Iran Suggests Their Expansion with Domestic Sheep', Scientific Reports, 10: 2243.
[2] Carrozza ML et al. (2023) Emergence and pandemic spread of small ruminant lentiviruses. Virus Evol. 18;9(1):vead005
[3] Grego E et al. (2005) Genetic and antigenic characterization of the matrix protein of two genetically distinct ovine lentiviruses. Veter. Microbiol. 106:179–185

POSSIBLE ETIOLOGICAL ASSOCIATION OF OVINE PAPILLOMAVIRUSES WITH BLADDER TUMORS IN CATTLE

Bianca Cuccaro (1), Francesca De Falco (1), Anna Cutarelli (2), Roberta De Tullio (3), Alberto Alberti (4), Esterina De Carlo (2), Sante Roperto (1)

(1) Dipartimento di Medicina Veterinaria e delle Produzioni Animali, Università degli Studi di Napoli Federico II, Napoli
(2) Istituto Zooprofilattico Sperimentale del Mezzogiorno, Portici (NA)
(3) Dipartimento di Medicina Sperimentale (DIMES), Università degli Studi di Genova, Genova
(4) Dipartimento di Medicina Veterinaria, Università degli Studi di Sassari, Sassari

Bladder tumors of cattle are very uncommon accounting from 0.1% to 0.01% of all bovine malignancies. However, disease is very frequently seen in cattle and water buffaloes reared on pasturelands, where bracken fern
(Pteridium spp.) grows abundantly. In these endemic areas, up to 90% of adult animals may be affected. A synergism
between chronic ingestion of Pteridium spp. and bovine papillomavirus (BPV) infection can result in chronic inflammation and urinary bladder tumors, which are responsible for bovine enzootic hematuria (BEH), a clinical syndrome characterized by bloody urine, anemia, progressive emaciation, and death of affected animals. Currently, bovine deltapapillomaviruses, namely, bovine papillomavirus types 1, 2, 13, and 14 are the only viral agents known to be associated with bladder neoplasia in cattle. Ovine papillomaviruses (OaPVs) are oncogenic viruses comprising four genotypes. OaPV1, OaPV2, and OaPV4 belong to the genus Deltapapillomavirus, whereas OaPV3 is a Dyokappapapillomavirus. To date, all benign and malignant OaPV-associated tumors have been recorded only in sheep. Aim of the study: To investigate the potential association of ovine papillomavirus (OaPV) infection with bladder carcinogenesis of cattle. Methods: Droplet digital PCR was used to detect and quantify the nucleic acids of OaPVs in bladder tumors of cattle that were collected at public and private slaughterhouses. Results: OaPV DNA and RNA were detected and quantified in 10 bladder tumors of cattle that were tested negative for bovine papillomaviruses. The most prevalent genotypes were OaPV1 and OaPV2. OaPV4 was rarely observed. Furthermore, we detected a significant overexpression and hyperphosphorylation of pRb and a significant overexpression and activation of the calpain-1 as well as a significant overexpression of E2F3 and of phosphorylated (activated) PDGFβR in neoplastic bladders in comparison with healthy bladders, which suggests that E2F3 and PDGFβR may play an important role in OaPV-mediated molecular pathways that lead to bladder carcinogenesis.
Conclusion: In all tumors, OaPV RNA could explain the causality of the disease of the urinary bladder.
Therefore, persistent infections by OaPVs could be involved in bladder carcinogenesis. Our data showed that there is
a possible etiologic association of OaPVs with bladder tumors of cattle.

EVALUATING THE ANTIMICROBIAL RESISTANCE IN THE ENVIRONMENT: A MODEL BASED ON THE USE OF APIS MELLIFERA COLONIES

Giovanni Cilia (1), Ilaria Resci (1,2), Laura Zavatta (1), Silvia Piva (2), Elisabetta Mondo (2), Sergio Albertazzi (1), Antonio Nanetti (1), Laura Bortolotti (1)

(1) Research Centre for Agriculture and Environment (CREA-AA), Council for Agricultural Research and Agricultural Economics Analysis, Via di Corticella 133, 40128 Bologna, Italy
(2) Department of Veterinary Sciences, University of Bologna, Via Tolara di Sopra, 43, 40064 Ozzano dell'Emilia (BO), Italy

Corresponding Author: **Giovanni Cilia** - giovanni.cilia@crea.gov.it

Antibiotic resistance is increasingly relevant to public health [1]. Assessing the environmental occurrence of this phenomenon is essential for human, animal and environmental health. Honey bees (Apis mellifera L.) are widely used as bioindicators due to their morphological and behavioural characteristics like high mobility and hairs capturing environmental pollutants and microorganisms [2,3].
This study aimed to evaluate the use of honeybee colonies as bioindicators for antimicrobial resistance in the anthropogenic environment of the apiary.
Within the BeeNet project, from November 2021 to September 2022, 33 apiaries in the Emilia-Romagna region were used to evaluate the environmental spread of antimicrobial resistance. Ten forager bees were collected from each colony. Microbiological cultures from the body surface of the bees and their gut microbiota were performed to isolate bacterial strains. After strain identification at the genus level, antibiotic resistance was assessed using the Kirby-Bauer method by testing 18 of the most used antibiotics in Italy. A predictive statistical model was used to correlate the identified bacteria and the antimicrobial resistance with the different environmental and anthropic elements present within a 1.5 km radius around the apiaries.
High heterogeneity of plant, animal, and human-associated bacteria was detected, including several emerging opportunistic pathogens of epidemiological relevance. The highest resistance values were found for amoxicillin (63%), penicillin (62.3%), aztreonam (40%), and vancomycin (30.9%). Apiaries near wetlands showed a higher probability of strains resistant to aztreonam, cefotaxime, cefoxitin, cephalothin, nalidixic acid, and streptomycin. Aztreonam-, cefotaxime-, cefoxitin-, and cephalothin-resistant strains were significantly related to apiaries near agricultural areas. Gentamycin, erythromycin, cefotaxime and cefoxitin were related to water treatment plants. Aztreonam-resistant strains were significantly related to hospitals, while erythromycin and trimethoprim/sulfamethoxazole were related to livestock and urban fabric.
The results of this study indicate that A. mellifera colonies are useful bioindicators to monitor the antimicrobial resistance in environmental bacteria. The anthropogenic impact promotes the spread of resistance. Further studies are needed to clarify to what extent wild and managed bees may be used for sanitary monitoring in a One Health approach.

[1] Larsson DGJ, Flach CF. (2022). Antibiotic resistance in the environment. Nature Reviews Microbiology 20:257-269. doi: 10.1038/s41579-021-00649-x.
[2] Cilia G, Bortolotti L, Albertazzi S, Ghini S, Nanetti A. (2022). Honey bee (Apis mellifera L.) colonies as bioindicators of environmental SARS-CoV-2 occurrence. Science of the Total Environment, 805:150327. doi: 10.1016/j.scitotenv.2021.150327.
[3] Piva S, Giacometti F, Marti E, Massella E, Cabbri R, Galuppi R, Serraino A. (2020). Could honey bees signal the spread of antimicrobial resistance in the environment? Letters in Applied Microbiology 70:349-355. doi: 10.1111/lam.13288.

IDENTIFICATION OF SWINEPOX VIRUS FROM NATURAL OUTBREAKS IN SWINE FREE-RANGE FARMS IN SICILY (ITALY)

Vincenzo Di Marco Lo Presti (1), Dorotea Ippolito (1), Flavia Pruiti Ciarello (1), Luigi Bertolotti (2), Michele Fiasconaro (1), Barbara Colitti (2), Antonella Cersini (3), Maria Teresa Scicluna (3), Chiara Giudice (4), Giusy Cardeti (3), Maria Teresa Capucchio (2)

(1) Istituto Zooprofilattico Sperimentale della Sicilia, Area Territoriale Barcellona Pozzo di Gotto, Viale S. Andrea 96, 98051, Barcellona Pozzo di Gotto (ME)
(2) Department of Veterinary Science, University of Turin, Largo P. Braccini 2, 10095, Grugliasco, TO
(3) Istituto Zooprofilattico del Lazio e Toscana, Virology Unit, Via Appia Nuova, 1411 – 00178 Roma
(4) Department of Veterinary Medicine and Animal Sciences, University of Milan, Via Dell'Universita, 6 26900 LODI (LO)

Corresponding Author: **Vincenzo Di Marco Lo Presti** - dimarco.vince@gmail.com

Swinepox (SWP) is a disease caused by a species-specific Poxvirus affecting pigs, that is nowadays largely neglected due to the overall self-limiting progression, but still causing economic losses and animal welfare impairment. The study describes two outbreaks which occurred in Sicily (Italy) in 2019 and 2021 (Farm A and B). The cases were referred due to abnormal mortality rates in 2-4 months old pigs, showing SWP-like lesions. After a clinical examination, nasal, ocular and cutaneous swabs were collected and submitted for PCR, sequencing [1] and negative staining electron microscopy (nsEM). Carcasses underwent post-mortem-examination (PME), during which altered tissues were sampled for histology. Severe overcrowding and poor sanitary conditions were observed in both farms as also a severe infestation by pig louse (Haematopinus suis), a vector of SwPV. Affected animals (25/200, 12.5% in Farm A and 150/895, 16.75% in Farm B) showed aspecific symptoms and multifocal, pustular dermatitis in the abdomen, inner surface of the legs, the groin and periocular/labial areas. Histologically, disseminated ulcerative dermatitis with epithelial hyperkeratosis, acanthosis, ballooning degeneration, spongiosis and focally extensive necrosis were present. Haemorrhages and non-suppurative inflammation with granulation tissue were also observed. In the epidermis, eosinophilic, rounded inclusion bodies were detected. Moreover, 70% of the affected animals from Farm B showed ocular lesions, ranging from mild conjunctivitis to severe conjunctivitis/blepharitis. The lesions were confirmed histologically, but no inclusion bodies were seen. No other organ alterations were observed during the PME. PCR detected SWPV in skin and an ocular swab samples. Sequencing of the PCR amplicon showed an identity of 99.79% compared to an isolate detected in a German outbreak [2]. The nsEM detected brick shaped viral particles in one skin sample, approximately 220nm in length and 160nm in width, with irregularly arranged surface tubules. SWPV was previously reported in Italy[3], but the presented outbreaks are the first described in Sicily. Although SWPV is species-specific, spill-over events by other Poxvirus are possible [4]. The recent zoonotic emergence of the Monkeypox virus has revived interest in this viral family as a cause of a potential new pandemic. Thus, SWPV should be monitored, especially in areas where pigs are raised outdoors, lacking biosecurity and proper husbandry practices.

[1] Medaglia, M.L.G.; Pereira, A. de C.; Freitas, T.R.P.; Damaso, C.R. Swinepox Virus Outbreak, Brazil, 2011. Emerg Infect Dis 2011, 17, 1976–1978, doi:10.3201/eid1710.110549.
[2] Kaiser, F.K.; Wiedemann, A.; Kühl, B.; Menke, L.; Beineke, A.; Baumgärtner, W.; Wohlsein, P.; Rigbers, K.; Becher, P.; Peters, M.; et al. Swinepox Virus Strains Isolated from Domestic Pigs and Wild Boar in Germany Display Altered Coding Capacity in the Terminal Genome Region Encoding for Species-Specific Genes. Viruses 2021, 13, 2038, doi:10.3390/v13102038.
[3] Mariano, V.; Nardi, A.; Vergari, E.; Carletti, F.; Barbieri, L.; Cardeti, G. Poxvirus in a Swine Farm in Italy: A Sporadic Outbreak? Large Animal Review 2015, 21, 219–220.
[4] Essbauer, S.; Pfeffer, M.; Meyer, H. Zoonotic Poxviruses. Veterinary Microbiology 2010, 140, 229–236, doi:10.1016/j.vetmic.2009.08.026.

CHARACTERIZATION OF BOVINE HERPESVIRUS -4 (BOHV-4) ORF45

Luca Russo (1), Emanuele Capra (2), Valentina Franceschi (1), Davide Cavazzini (3), Roberto Sala (4), Barbara Lazzari (2), Sandro Cavirani (1), Gaetano Donofrio (1)

(1) Dipartimento di Scienze Medico Veterinarie, Università di Parma, Parma, Italy
(2) Istituto di Biologia e Biotecnologia Agraria, Consiglio Nazionale delle Ricerche IBBA CNR, Lodi, Italy
(3) Dipartimento di Scienze Chimiche, della Vita e della Sostenibilità Ambientale, Università di Parma, Parma, Italy. 4.Dipartimento di Medicina e Chirurgia, Università di Parma, Parma, Italy

Corresponding Author: **Gaetano Donofrio** - gaetano.donofrio@unipr.it

Bovine herpesvirus 4 (BoHV-4) is a Gammaherpesvirus of the genus Rhadinovirus, his natural host is the bovine whereas the African Buffalo the natural reservoir. Anyhow, BoHV-4 infection is not associated to a specific disease. Genome structure and genes are well conserved in Gammaherpesvirus, and orf45 gene and its product, ORF45, is one of those (1). BoHV-4 ORF45 has been suggested to be a tegument protein, however, BoHV-4 ORF45 structure and function have not yet been experimentally characterized. In the present study, it is shown that BoHV-4 ORF45, despite its poor homology with other characterized Rhadinovirus ORF45s, is structurally related to Kaposi's sarcoma-associated herpesvirus (KSHV) (2), is a phosphoprotein and localizes in the host cell nuclei. Through the generation of an ORF45-null mutant BoHV-4 and its pararevertant, it was possible to demonstrate that ORF45 is essential for BoHV-4 lytic replication and is associated to the viral particles, as for the other characterized Rhadinovirus ORF45s. Finally, the impact of BoHV-4 ORF45 on cellular transcriptome was investigated, an aspect poorly explored or not at all for other Gammaherpesvirus. Many cellular transcriptional pathways were found to be alterate, mainly those involving p90 ribosomal S6 kinase (RSK) and signal-regulated kinase (ERK) complex (RSK/ERK). It was concluded that BoHV-4 ORF45 has similar characteristics of those of KSHV ORF45 and its unique and incisive impact on cell transcriptome pave the way to further investigations.

[1] Palmeira, L., Machiels, B., Lete, C., Vanderplasschen, A., and Gillet, L. (2011). Sequencing of bovine herpesvirus 4 v.test strain reveals important genome features. Virol J 8, 406. doi: 10.1186/1743-422X-8-406.
[2] Atyeo, N., and Papp, B. (2022). The ORF45 Protein of Kaposi's Sarcoma-Associated Herpesvirus and Its Critical Role in the Viral Life Cycle. Viruses 14(9). doi: 10.3390/v14092010.

76° CONVEGNO SISVET
BARI, 21-22-23 GIUGNO 2023

ASSESSMENT OF BOVINE HERPESVIRUS-4 (BOHV-4) BASED VECTOR VACCINE INTRANASALLY ADMINISTERED IN A HAMSTER CHALLENGE MODEL OF LUNG DISEASE

Seok-Chan Park (3), Laura Conti (2), Valentina Franceschi (1), Byungkwan Oh (3), Myeon-Sik Yang (3), Gaeul Ham (3), Antonino Di Lorenzo (2), Elisabetta Bolli (2), Federica Cavallo (2), Bumseok Kim (3), Gaetano Donofrio (2)

(1) Department of Medical Veterinary Sciences, University of Parma, Strada del Taglio 10, 43126 Parma, Italy.
(2) Department of Molecular Biotechnology and Health Sciences, University of Torino, via Nizza 52, 10126 Torino, Italy.
(3) Biosafety Research Institute and Laboratory of Veterinary Pathology, College of Veterinary Medicine, Jeonbuk National University, Iksan 54596, Korea.

Corresponding Author: **Gaetano Donofrio** - gaetano.donofrio@unipr.it

Bovine Herpesvirus 4 (BoHV-4) is a bovine Rhadinovirus not associated to a specific pathological lesion or disease and experimentally employed as viral vector vaccine. BoHV-4-Based Vector (BoHV-4-BV) has been shown to be effective in immunizing and protecting several animal species when systemically administrated through the intramuscular, subcutaneous, intravenous, or intraperitoneal rout (1,2,3). However, BoHV-4-BV has never been intranasally tested for respiratory disease protection. In the present study, a recombinant BoHV-4, BoHV-4-A-S-ΔRS-HA-ΔTK, delivering an expression cassette for SARS-CoV-2 Spike glycoprotein, was constructed and its immunogenicity, as well as its capability to transduce cells of the respiratory tract, was tested in mice. Next, to test the intranasal administration of BoHV-4-A-S-ΔRS-HA-ΔTK efficacy in terms of protection toward a SARS-CoV-2 challenge, the well-established COVID-19/Syrian Hamster model was adopted. Intranasal administration of BoHV-4-A-S-ΔRS-HA-ΔTK elicited protection against SARS-CoV-2, with improved clinical signs, including significant reduction of body weight loss, significant reduction of viral load in trachea or lung and significant reduction of histopathologic lung lesions if compared with intramuscular administered BoHV-4-A-S-ΔRS-HA-ΔTK. These results suggested that intranasal immunization with BoHV-4-BV induced a protective immunity and BoHV-4-BV could be a vaccine platform potentially appliable for the protection of other animal species toward respiratory diseases.

[1] Donofrio, G., Cavirani, S., Simone, T., and van Santen, V.L. (2002). Potential of bovine herpesvirus 4 as a gene delivery vector. J Virol Methods 101(1-2), 49-61. doi: 10.1016/s0166-0934(01)00419-0
[2] Conti, L., Bolli, E., Di Lorenzo, A., Franceschi, V., Macchi, F., Riccardo, F., et al. (2020a). Immunotargeting of the xCT Cystine/Glutamate Antiporter Potentiates the Efficacy of HER2-Targeted Immunotherapies in Breast Cancer. Cancer Immunol Res 8(8), 1039-1053. doi: 10.1158/2326-6066.CIR-20-0082.
[3] Pedrera, M., Macchi, F., McLean, R.K., Franceschi, V., Thakur, N., Russo, L., et al. (2020). Bovine Herpesvirus-4-Vectored Delivery of Nipah Virus Glycoproteins Enhances T Cell Immunogenicity in Pigs. Vaccines (Basel) 8(1). doi: 10.3390/vaccines8010115.

PREVALENCE AND ANTIBIOTIC SUSCEPTIBILITY OF ENTEROPATHOGENS FROM CASES OF NEONATAL CALF DIARRHEA IN CALVES FROM REFERRAL HOSPITAL

Laura Filippone Pavesi (1), Guido Grilli (1), Tiziana Vitiello (1), Maria Filippa Addis (1), Martina Penati (1), Giulia Sala (2) Antonio Boccardo (1), Vincenzo Ferrulli (1), Davide Pravettoni (1), Valerio Bronzo (1)

(1) Dipartimento di Medicina Veterinaria e Scienze Animali, Università degli Studi di Milano, Milan, Italy
(2) Dipartimento di Scienze Veterinarie, Università degli Studi di Pisa, Pisa, Italy

Corresponding Author: **Laura Filippone Pavesi** - laura.filippone@unimi.it

This study aimed to identify bacteria and their antimicrobial susceptibility test (AST) in 210 calves with neonatal calf diarrhoea (NCD) admitted to the Veterinary Teaching Hospital (VTH) of the University of Milan from 2020 to 2023. Fecal samples were collected during the clinical examination, stored at 4 °C, and processed in the Microbiology Lab. of the VTH. All feces were inoculated onto 5% sheep blood agar plates and MacConkey agar plates and incubated at 37°C for 18-24 h under aerobic conditions. Using the direct transfer method, the species identification was accomplished via matrix-assisted laser desorption-ionization mass spectrometry (MALDI-TOF; Bruker Daltonics, Fremont, CA). The isolates were subjected to AST by the disc diffusion method according to the Clinical and Laboratory Standards Institute (CLSI) guidelines [1], testing the following antimicrobials: amoxicillin/clavulanic acid (30µg), ampicillin (10µg), ceftiofur (30µg), enrofloxacin (5µg); oxytetracycline (30µg), penicillin (10iu) marbofloxacin (5µg), florfenicol (30µg), trimethoprim/sulfamethoxazole (TMP/SMX)(25µg). Antibiotic susceptibility of isolates was categorized by measuring the inhibition zone according to the CLSI guidelines [2]. Thirty-seven fecal samples were collected upon request of bacteriological assays by the farmer. Five samples had mixed bacteria flora and no AST was performed. A fecal sample showed a mixed infection by Escherichia coli and Enterococcus spp. E. coli was detected in 27 other samples. Enterococcus spp. was detected in another sample. Klebsiella pneumoniae, already described as an NCD pathogen [3], was detected in 3 samples. All isolates of K. pneumoniae were resistant to ampicillin, oxytetracycline, penicillin, and TMP/SMX. All isolates of Enterococcus were resistant to marbofloxacin, oxytetracycline, and TMP/SMX. The 73.8% of E. coli were resistant to the antibiotics tested. As for ampicillin and oxytetracycline, 92.9% of the isolates were resistant. In contrast, the lowest percentage of E. coli resistance to an antibiotic tested was 43% to ceftiofur. Except for the ampicillin resistance of K. pneumoniae, which is intrinsic and due to the chromosomal beta-lactamase gene SHV-1 [4], the other antibiotic resistance rates show an increased acquired antibiotic resistance not only to those classified as D and C by the EMA but also to those in category B. The reported results are preliminary and further tests will be carried out.

[1] Clinical and Laboratory Standards Institute. Performance Standards for Antimicrobial Disk and Dilution Susceptibility Tests for Bacteria Isolated From Animals—Fourth Edition: Approved Standard VET01-A4. CLSI, Wayne, PA, USA, 2013.
[2] CLSI (Clinical and Laboratory Standards Institute) (3rd ed.), Performance standards for antimicrobial disk and dilution susceptibility tests for bacteria collected from animals; Approved standard, 28, Clinical and Laboratory Standards Institute, Wayne, PA (2008) No. 8.
[3] Haggag Y.N., Samaha H.A., Nossair M.A., Awad W.K., Epidemiological studies on diarrhea in some dairy cattle farms. Alexandria Journal of Veterinary Sciences, 51(2): 282-289, 2016.
[4] Wyres KL, Holt KE. Klebsiella pneumoniae as a key trafficker of drug resistance genes from environmental to clinically important bacteria. Curr Opin Microbiol. 2018, 45: 131-139. doi: 10.1016/j.mib.2018.04.004. Epub 2018 May 1. PMID: 29723841.

EARLY ABORTION RELATED TO UREAPLASMA DIVERSUM INFECTION IN CATTLE.

Milano A. (1), Pugliese D. (1), Sposato A. (1), Schino G. (1), Catalano E. (1), Bianco A. (1), Capozzi L. (1), Del Sambro L. (1), Trisolini C. (1), Parisi A. (1)

(1) Istituto Zooprofilattico Sperimentale di Puglia e Basilicata, Sezione di Putignano (BA), Contrada San Pietro Piturno, 70017, Putignano, BA, Italy

Corresponding Author: **Angelica Milano** - angelica.milano@izspb.it

The study investigated the infectious causes responsible for early abortions in cattle in the province of Bari, using microbiological and molecular methods, adding useful information to the bibliography and epidemiology already existent. The investigation involved a dairy cattle farm of 42 animals located in Gioia del Colle reporting respiratory problems, due to Ureaplasma diversum, and reproductive problems investigated with 11 vaginal swabs, having 4 positives for the same microorganism. The aim of this report is the identification of the infectious causes of an early abortion (about 70 days). The fetus was examined at the Experimental Zooprophylactic Institute of Apulia and Basilicata laboratories. A careful inspection and necropsy did not reveal macroscopic lesions, at the same time samples were taken from the placenta, amniotic fluid, umbilical cord, lung, gastrointestinal tract and brain. The samples obtained were cultured on selective media for the detection of Salmonella spp. (ISO6579-1:2017), the genetic material was extracted with MagMAX™ CORE Nucleic Acid Purification kit and a Real-Time PCR was set up to detect the main abortigenic pathogens: Chlamydia spp., Toxoplasma gondii, Coxiella burnetti, Mycoplasma bovis, Ureaplasma diversum, Bovine herpesvirus-1 (BoHV-1), Bovine viral diarrhea virus (BVDV) (1). The bacteriological examination for Salmonella spp. gave a negative result. Molecular analyzes showed positive results for Ureaplasma diversum in the placenta, amniotic fluid, umbilical cord and fetal lung, while all samples analyzed were negative for the other pathogens. PCR technique and the sequencing confirmed the presence of U. diversum in the same samples. The BLAST results showed an identity >98% with U. diversum (2). The vertical transmission was confirmed detecting U. diversum in the fetal organs, in line with the existing bibliography (3). The association of the pathogen with early abortions, compared to late abortion or stillbirth cases reported in the literature (3) could be an interesting point for further investigations. The results obtained suggest improving the attention in the role of the pathogen U. diversum as a cause of early abortion in cattle.

[1] Tramuta C., Lacerenza D., Zoppi S., Goria M., Dondo A., Ferroglio E., Nebbia P., & Rosati S. (2011). Development of a set of multiplex standard polymerase chain reaction assays for the identification of infectious agents from aborted bovine clinical samples. Journal of veterinary diagnostic investigation : official publication of the American Association of Veterinary Laboratory Diagnosticians, Inc, 23(4), 657–664. https://doi.org/10.1177/1040638711407880

[2] Camacho C., Coulouris G., Avagyan V., Ma N., Papadopoulos J., Bealer K., & Madden T. L. (2009). BLAST+: architecture and applications. BMC bioinformatics, 10, 421. https://doi.org/10.1186/1471-2105-10-421

[3] Santos Junior M. N., de Macêdo Neres N. S., Campos G. B., Bastos B. L., Timenetsky J., & Marques L. M. (2021). A Review of Ureaplasma diversum: A Representative of the Mollicute Class Associated with Reproductive and Respiratory Disorders in Cattle. Frontiers in veterinary science, 8, 572171. https://doi.org/10.3389/fvets.2021.572171

HIGH CAPACITY OF BIOFILM FORMATION IN MULTIDRUG-RESISTANT SALMONELLA INFANTIS STRAINS

Laura Musa (1), Valentina Stefanetti (1), Valeria Toppi (1), Maria Pia Franciosini (1), Noah Spata (1), Giacomo Di Giacinto (1), Patrizia Casagrande Proietti (1)

(1) Department of Veterinary Medicine, University of Perugia

Corresponding Author: **Laura Musa** - laura.musa@studenti.unipg.it

Salmonellosis is considered the second most common foodborne and zoonotic disease after campylobacteriosis. Poultry farms, in particular broiler chicken production chain, are an important source of Salmonella spp., and the contamination of broiler farms has been increasingly associated with persistent serovars, such as S. Infantis. Meat/egg contamination can occur at multiple stages in food chain production [1]. The ability of Salmonella spp. to adhere to surfaces and form biofilms is a direct link between environmental contamination and food processing/contamination [2]. The emergence of multidrug-resistant (MDR) phenotypes has been attributed to the structural properties of biofilms [3]. Since MDR S. Infantis isolates are highly prevalent in the broiler meat industry and may represent a Public Health concern, the purpose of this study was to investigate the ability of biofilm-formation of 80 MDR and Extended Spectrum β-lactamase S. Infantis strains isolated from broiler food chain production and to characterize biofilm production through Whole Genome Sequencing. Biofilm forming ability has been evaluated with the method described by Pate et al. (2019) [4] and the experiments were performed in triplicate for three independent assays. Our results demonstrated that all tested strains were biofilm producers, although with varying degrees of production. Six/80 strains resulted moderated biofilm producers and 74/80 strong biofilm producers. The WGS analysis was performed on 6 S. Infantis strains resulted strong biofilm-producers. This assay showed the presence of the fim cluster (fimADF) encoded for fimbrial production, also described in S. Typhimurium. Fimbriae are considered major players in the initial steps of biofilm formation. Biofilm presence has an impact also on antimicrobial therapy. The population of bacterial cells harnessed in the biofilm is in a state defined as "dormant," or metabolically inactive, and this may contribute to the mechanism of antibiotic resistance, especially for those antibiotics that target cells in active replication. It is important to emphasize that within the broiler farm, the environmental temperature is between 18-22°C which is the optimum temperature for in vitro biofilm formation by Salmonella spp. S. Infantis persistence in broiler flocks may be related precisely to its ability to form biofilms, making disinfection protocols on the farm and in the production chain more difficult, posing serious Public Health concerns.

[1] Mughini-Gras, L.; van Hoek, A.H.A.M.; Cuperus, T.; Dam-Deisz, C.; van Overbeek, W.; van den Beld, M.; Wit, B.; Rapallini, M.; Wullings, B.; Franz, E.; et al. Prevalence, Risk Factors and Genetic Traits of Salmonella Infantis in Dutch Broiler Flocks. Vet. Microbiol. 2021, 258, 109120, doi:10.1016/j.vetmic.2021.109120.
[2] Merino, L.; Procura, F.; Trejo, F.M.; Bueno, D.J.; Golowczyc, M.A. Biofilm Formation by Salmonella Sp. in the Poultry Industry: Detection, Control and Eradication Strategies. Food Res. Int. Ott. Ont 2019, 119, 530–540, doi:10.1016/j.foodres.2017.11.024.
[3] Bhardwaj, D.K.; Taneja, N.K.; Taneja, P.; Patel, P. Phenotypic and Genotypic Characterization of Multi-Drug Resistant, Biofilm Forming, Human Invasive Strain of Salmonella Typhimurium SMC25 Isolated from Poultry Meat in India. Microb. Pathog. 2022, 173, 105830, doi:10.1016/j.micpath.2022.105830.
[4] Pate, M.; Mičunovič, J.; Golob, M.; Vestby, L.K.; Ocepek, M. Salmonella Infantis in Broiler Flocks in Slovenia: The Prevalence of Multidrug Resistant Strains with High Genetic Homogeneity and Low Biofilm-Forming Ability. BioMed Res. Int. 2019, 2019, 4981463, doi:10.1155/2019/4981463.

INDUCTION OF SERONEGATIVE LATENT CARRIERS BY ADMINISTRATION OF TWO LIVE IBR MARKER VACCINES IN PASSIVELY IMMUNIZED CALVES

Petrini S., Righi C., Pirani S., Costantino G., Gobbi P., Scoccia E., Iscaro C., Giammarioli M., Viola G., Feliziani F.

(1) National Reference Centre for Infectious Bovine Rhinotracheitis (IBR), Istituto Zooprofilattico Sperimentale Umbria-Marche "Togo Rosati", 06126 Perugia (PG), Italy;
(2) Veterinary Practitioner, 62026 San Ginesio (MC), Italy

Corresponding Author: **Stefano Petrini** - s.petrini@izsum.it

Bovine alphaherpesvirus 1 (BoHV-1) is associated with several clinical manifestations, including infectious bovine rhinotracheitis (IBR). Different papers have shown that it is possible to induce so-called Seronegative Latent Carrier (SNLC) calves after traditional modified live vaccines (1) administration or after infection (2) in calves with passive immunity (p.i.). These animals are very important in genetic centres, control plans and BoHV-1-free herds, as the virus may be present in calves without showing any seropositivity. This study aimed to evaluate whether two live IBR marker vaccines administered to calves with and without p.i. can generate SNLC. Twelve calves were divided into four groups of three animals each. Groups A and C were injected with a live gE/tk-deleted marker vaccine. Groups B and D were inoculated with a live gE-deleted marker vaccine. Groups A and B were fed with colostrum and milk with p.i. The other groups were fed with IBR-free colostrum and milk. After vaccination, clinical, virological and serological investigations were carried out. When the animals became BoHV-1 seronegative, each calf was treated with dexamethasone (DMS). Further, nasal swabs and blood samples were collected for virological and serological investigations. All surveys and the DMS treatments were performed by Petrini et al. (3). No viral vaccine shedding and clinical form of IBR was observed during the experiments. The calves became BoHV-1 seronegative at 270 (Group A), 180 (Group B), and 336 (Group C and D) post-vaccination days. Only the calves in Group A showed seropositivity on gB-ELISA 74 days post-treatment (DPT) with DMS. Also, an increase in neutralizing antibodies (NAs) of 1.38 log10 120 DPT with DMS was observed in the same group. In addition, viral circulation was excluded in all groups as the gE-ELISA always gave negative results. The results obtained in this study differ from those published previously (4). To conclude, it is possible to induce SNLC calves by administering a live gE/tk-deleted marker vaccine in the presence of p.i. The increase of NAs can be considered an indicator of viral vaccine reactivation in the absence of viral shedding.

This research was funded by the Italian Ministry for Health (Ricerca Corrente IZSUM 09/2019)

[1] Menanteau-Horta A.M., Ames T.R., Johnson D.W., Meiske J.C. (1985). Effect of maternal antibody upon vaccination with infectious bovine rhinotracheitis and bovine virus diarrhea vaccines. Canadian Journal of Comparative Medicine, 49: 10-14.
[2] Bradshaw B.J., Edwards S. (1996). Antibody isotype responses to experimental infection with bovine herpesvirus 1 in calves with colostrally derived antibody. Veterinary Microbiology, 53, 143-151.
[3] Petrini S., Martucciello A., Righi C., cappelli G., Torresi C., Grassi C., Scoccia E., Costantino G., Casciari c., Sabato R., Giammarioli M., De Carlo E., Feliziani F. Assessment of Different Infectious Bovine Rhinotracheitis Marker Vaccines in Calves. Vaccines 2022, 10(8), 1204.
[4] Lemaire M., Mayer G., Baranowski E., Schynts F., Wellemans G., Kerkhofs P., Thiry E. Production of Bovine Herpesvirus type 1-seronegative latent carriers by administration of a live-attenuated vaccine in passively Immunized calves.

STUDY OF CROSS-REACTIVITY ANTIBODY RESPONSE BETWEEN BOVINE GAMMAHERPESVIRUS 4 AND BOVINE ALPHAHERPESVIRUS 1: PRELIMINARY RESULTS

Righi C.(1), Martucciello A. (2), Costantino G. (1) , Cappelli G. (2) , Rossi E. (1), Grassi C. (2) , Marenzoni M.L. (3), Pellegrini C. (1), De Carlo E. (2), Feliziani F.(1), Petrini S. (1)

(1) National Reference Centre for Infectious Bovine Rhinotracheitis (IBR), Istituto Zooprofilattico Sperimentale Umbria-Marche "Togo Rosati", 06126 Perugia (PG), Italy;
(2) National Reference Centre for Hygiene and Technology of Breeding and Buffalo Production Istituto Zooprofilattico Sperimentale del Mezzogiorno, 84131 Salerno, Italy;
(3) Department of Veterinary Medicine, University of Perugia, via San Costanzo 4, 06124 Perugia, Italy.

Corresponding Author: **Cecilia Righi** - c.righi@izsum.it

Bovine alphaherpesvirus 1 (BoHV-1) causes a variety of clinical signs, including a respiratory disease called infectious bovine rhinotracheitis (IBR) [1]. A serological cross-reactivity has been observed between BoHV-1 and other herpesviruses [2]. This phenomenon could lead to severe consequences in BoHV-1 serology, resulting in an incorrect diagnosis of BoHV-1, both in areas where there are active control/eradication plans for IBR and in performance test stations. This study aimed to evaluate the occurrence of serological cross-reactivity between BoHV-1 and Bovine gammaherpesvirus 4 (BoHV-4). Five calves, devoid of BoHV-1 and BoHV-4 neutralizing antibodies (NAs), were selected for this study. According to the European legislation on the protection of animals used for scientific purposes, four calves were subjected to challenge infection with BoHV-4 85/16 TV strain, and a calf served as control. Serum samples were collected on 0, 14 and 21 post-challenge days (PCDs) and tested for BoHV-1 and BoHV-4 antibodies, using, via manufacturer's instruction, the: competitive IBR gE-ELISA (A); competitive IBR gB-ELISA (B); and three indirect BoHV-4 ELISA tests (C, D, E). Virus neutralization (VN) tests were performed according to the protocols previously published [3-4] and were used in parallel to examine the collected sera. No seropositivity for BoHV-1 in any ELISA and VN tests was observed during the experiments. In all calves, antibodies for BoHV-4 were not detected on PCDs 0 by ELISA and VN tests. Otherwise, BoHV-4 ELISA tests (C, D, E) showed discordant results. In particular, on PCDs 14 and 21, by ELISA tests (C, E), only 1 out of 4 samples was detected as positive to BoHV-4. Differently, on PCD 14, the ELISA test (D) discovered 2 out of 4 samples as positive and on PCD 21, 4 out of 4 samples. In VN, the mean titer of the BoHV-4 recovered from all infected animals, already on PCD 14 was 1:8, to stay like this on PCD 21. To conclude, no serological cross-reactivity between BoHV-4 and BoHV-1 with different commercial IBR ELISA tests and BoHV-1 VN was highlighted. Moreover, indirect BoHV-4 ELISA tested have shown different diagnostic sensitivity.
This research was funded by the Italian Ministry for Health (Ricerca Corrente IZSUM 10/2021).

[1] Nandi, S.; Kumar, M.; Manohar, M.; Chauhan, S. Bovine herpes virus infections in cattle. Anim. Health Res.Rev. 2009, 19, 85–98.
[2] Petrini, S.; Righi, C.; Iscaro C.; Viola, G.; Gobbi P.; Scoccia E.; Rossi E.; Pellegrini C.; De Mia G.M. Serological Cross-Reactivity Between Bovine alphaherpesvirus 2 and Bovine alphaherpesvirus 1 in a gB-ELISA: A Case Report in Italy; Front. Vet. Sci. (2020). 7:587885; doi.org/10.3389/fvets.2020.587885.
[3] Petrini, S.; Martucciello, A.; Grandoni, F.; De Matteis, G.; Cappelli,G.; Giammarioli, M.; Scoccia, E.; Grassi, C.; Righi, C.; Fusco , G.; Galiero, G., Pela, M., De Mia, G.M. and De Carlo. Evaluation of Safety and Efficacy of an Inactivated Marker Vaccine against Bovine alphaherpesvirus 1 (BoHV-1) in Water Buffalo (Bubalus bubalis). Vaccines 2021, 9, 355. https://doi.org/10.3390/vaccines9040355 int/fileadmin/Home/eng/Health_standards/tahm/3.04.11_IBR_IPV.pdf (accessed on 26 September 2019).
[4] Martin, W.B.; Castrucci, G.; Frigeri, F.; Ferrari, M. A serological comparison of some animal herpesviruses. Comp. lmmun. Microbiol. infect. Dis. Vol. 13, No. 2, pp. 75-84, 1990.

BOVINE DERMATOPHILOSIS IN AN APULIAN DAIRY CATTLE

Sebastiano Tinelli (1), Giovanni D'Onghia (1), Adriana Trotta (2), Claudia Cafarchia (2), Marialaura Corrente (2)

(1) Veterinary Practitioner
(2) Department of Veterinary Medicine, University of Bari Aldo Moro, Italy

Corresponding Author: **Sebastiano Tinelli** - tinelli.s@tiscali.it

Dermatophilosis is a chronic and contagious exudative-proliferative dermatitis that mainly affects wild and domestic ruminants. It is caused by Dermatophilus congolensis, a Gram positive bacterium. The disease is currently considered endemic in Africa, North America, Australia and New Zealand. The few Italian reports are not related to domestic cattle in which the infection is characterized by high morbidity and low mortality with distinctive epidermal lesion like "paintbrush". In domestic and wild ruminants, it is a multifocal inflammatory disease of superficial layers of the skin of the back, carpus and hock. The aggravated forms can result in high fever and in a consistent reduction in dairy production. The diagnosis is usually made by culture isolation of the crusts on Columbia Blood Agar (CBA) incubated in microaerophilic condition at 37°C for 5 days. Aim of this work is to describe a case of dermatophilosis occurred in an Apulian dairy cattle. The characteristic skin lesions involved 30% of the animals and some animals showed anorexia and high fever accompanied by drastic reduction of dairy production. Two samples (recent crusts from two different animals) were subjected to culture isolation for bacteriological and mycological tests, as well as molecular identification by PCR with specific primers for *D. congolensis*. The amplicons were sequenced by MiSeq NGS technology and the sequence, aligned with the reference sequences present in the BLAST database. The two sequences perfectly matched with reference sequences of *D. congolensis*. Diagnosis based on culture isolation often reveals skin bacteria and fungi contaminants, as occurred in this case, that can interfere with *D. congolensis* growth. For this reason this method is often not useful as diagnostic test and it is necessary to use reliable diagnostic tests such as PCR in order to obtain more precise information on the real diffusion of this pathogen.

[1] Amor A., Enriquez A., Corcuera M.T., Toro C., Herrero D., Baquero M. (2011) Is Infection by Dermatophilus congolensis Underdiagnosed? J. Clin. Microbiol. 49 (1): 449-451.
[2] Loria G.R., La barbera E., Monteverde V., Piraino C., Caracappa S. (2004) Dermatophilus congolensis: la malattia in Italia. Large Animals Review. 4: 1-4.
[3] Larrasa J., Garcia A., Ambrose N.C., Alonso M.J., Parra A., Hermoso De Mendoza M., Salazar J., Rey J., Hermoso De Mendoza J. (2002) A single random amplified polymorphic DNA genotyping method for field isolates of Dermatophilus congolensis. J. Vet. Med. B 49: 135-141.

CHARACTERIZATION OF STREPTOCOCCUS UBERIS ASSOCIATED WITH SHEEP MASTITIS: ANTIBIOTIC RESISTANCE AND GENOMIC ANALYSIS.

Rosa M.N. (1), Canu A. (1), Longheu C.M. (1), Azara E. (1), Cillara G. (1), Puddu G. (1), Broccias S. (1), Molotzu M. (1), Pisanu M. (1), Tola S. (1)

(1) Istituto Zooprofilattico Sperimentale della Sardegna, 07100 Sassari

Corresponding Author: **Sebastiana Tola** - sebastiana.tola@izs-sardegna.it

Streptococcus uberis is one of the main causative agents of ovine mastitis [1]; however, little is known about this global, environmental pathogen and its genomic mechanisms of disease. A bank of 124 S. uberis isolates (one per farm), collected from mastitis-infected sheep in Sardinia, were analyzed for this study. Bacterial identification at the species level was determined by PCR-RFLP and MALDI-TOF MS, as described in a previous study [2]. Isolates were analyzed by multilocus-sequence typing (MLST) and pulsed field gel electrophoresis (PFGE) for genetic relatedness. All isolates were also subjected to antimicrobial susceptibility analysis by the disk diffusion test using a panel of 14 antimicrobials. Among the 124 S. uberis isolates, 46 were randomly selected for whole genomic sequencing with an IonTorrent Personal Genome Machine (PMG). Almost all (96.8%) isolates were resistant to at least one antimicrobial. The highest resistance rate was found against streptomycin (93.5%), kanamycin (79.8%) and gentamicin (64.5%), followed by novobiocin (25%) and tetracycline TE (19.3%). Seventy-four (59.7%) isolates were simultaneously resistant to all aminoglycosides tested. Seventeen isolates (13.7%) exhibited multidrug resistance. Of the three erythromycin-resistant isolates, two harboured ermB gene while one carried ermC gene. The ermA, ermTR and mefA genes were not detected among the analyzed isolates. Among the twenty-four S. uberis isolates resistant to TE, ten were positive for tetM, six for tetO, two for tetK and one for tetO. Of the six penicillin-resistant isolates, five harbored the blaZ gene. All aminoglycosides-resistant isolates were PCR negative for aad-6 and aphA-3′ genes. MLST analysis identified 86 different allelic combinations; among these, 13 were STs present in the database while the remaining 73 (84.9%) STs were found for the first time in this study. The most frequently detected STs were ST808, ST1177, ST1192 and ST1247 with 4 isolates each, while the major lineage found was the clonal complex (CC)143. High level of polymorphism was observed among the SmaI profiles; in fact, a total of 121 pulsotypes (PTs) were unique. Pangenomic analysis identified 1445 core genes. This study indicates the epidemiological complexity of S. uberis isolated from ovine milk. The lack of a predominant strain suggests that most of the S. uberis IMI cases are due to contamination of the mammary gland from the environment.

[1] Marogna G. et al. Clinical findings in sheep farms affected by recurrent bacterial mastitis. Small Ruminant Research. 2010;88(2):119–25. [2] Rosa NM et al. Comparison of PCR-RFLP, API® 20 Strep and MALDI-TOF MS for identification of Streptococcus spp. collected from sheep and goat milk samples. Small Ruminant Research 2019;180:35–40.

DETECTION OF INFECTIOUS AGENTS IN RED FOXES (VULPES VULPES) IN EMILIA ROMAGNA REGION (ITALY)

Martina Magliocca (1), Roberta Taddei (2), Lorenza Urbani (1), Giulia Maioli (2), Gianluca Rugna (2), Silva Rubini (2), Maria Sampieri (2), Alessia Terrusi (1), Veronica Facile (1), Mara Battilani (1), Andrea Balboni (1)

(1) Dipartimento di Scienze Mediche Veterinarie (DIMEVET), Alma Mater Studiorum – Università di Bologna
(2) Istituto Zooprofilattico Sperimentale della Lombardia e dell'Emilia-Romagna (IZSLER)

Corresponding Author: **Andrea Balboni** - a.balboni@unibo.it

Wildlife is subject to surveillance or monitoring to assess the circulation of infectious agents that also affect domestic animals and humans. Especially, red fox (Vulpes vulpes), having the ability to adapt to local environmental conditions, can live in urban, suburban and rural areas. Foxes can be infected by pathogens responsible for important diseases for domestic dogs and humans, such as Canine distemper virus (CDV), Carnivore protoparvovirus 1 (CPPV-1), Canine adenovirus type 1 and 2 (CAdV-1 and CAdV-2), Canine circovirus (CanineCV), and Leptospira spp [1-4]. In this study, red foxes died in provinces of Bologna, Modena and Ferrara (Emilia Romagna region, Italy), from January to May 2022, were screened for CDV, CPPV-1, CAdV, CanineCV, and Leptospira spp. using specific real-time PCR or real-time RT-PCR methods and the identified pathogens were genetically characterised analysing the whole genome or informative genes. For this purpose, 25 red foxes found dead in study area were subjected to necroscopic examination at the Istituto Zooprofilattico Sperimentale della Lombardia e dell'Emilia Romagna (IZSLER) and spleen and intestine were sampled for molecular screening and stored at -20°C until analysis. For each animal included in the study, signalment data (area and data of collection/culling, sex and presumed age) and necroscopic findings were recorded. Of the 25 red foxes, nine were positive at least to one tested pathogen and two of them were coinfected. Five out of 25 red foxes tested positive to CPPV-1 DNA and six to CanineCV DNA. No foxes tested positive to CDV, CAdV or Leptospira spp. For CPPV-1, VP2 gene analysis revealed the circulation of two canine parvovirus type 2 variants (CPV-2a and CPV-2b) widespreads in domestic dogs, indicating that the main source of infection for foxes may be direct or indirect contact with domestic dogs. The complete genome analysis of the detected CanineCVs showed a close correlation with a group of viruses identified in Europe, mostly in foxes, indicating a host predilection or a limited transmission between foxes and other wild carnivores or domestic dogs.

[1] Akerstedt et al., Serosurvey for Canine Distemper Virus, Canine Adenovirus, Leptospira interrogans and Toxoplasma gondii in free-ranging canids in Scandinavia and Svalbard. Journal of Wildlife Diseases, 46(2), 474-480, 2010.
[2] Plumer et al.,Rapid urbanization of red foxes in estonia: distribution, behaviour, attacks on domestic animals, and health-risks related to zoonotic diseases. PLoS ONE 9(12): e115124, 2014.
[3] Pluemer et al., Red foxes (Vulpes vulpes) and coyotes (Canis latrans) in an urban landscape: prevalence and risk factors for disease. Journal of Urban Ecology, 5(1), 1-9, 2019.
[4] Costanzi et al., Beware of dogs! Domestic animals as a threat for wildlife conservation in Alpine protected areas. European Journal of Wildlife Research, 67(4), 70, 2021.

PILOT INVESTIGATION ON THE PRESENCE OF FISH PATHOGENIC VIRUSES IN WILD FISH-FEEDING BIRDS

Sara Ciulli (1), Francesca Errani (1), Caterina Lupini (1), Giulia Graziosi (1), Elena Catelli (1), Enrico Volpe (1)

(1) Dipartimento di Scienze Mediche Veterinarie, Alma Mater Studiorum, Università di Bologna, Ozzano Emilia, BO, Italia

Corresponding Author: **Sara Ciulli** - sara.ciulli@unibo.it

Aquaculture is a continuously growing sector to support global animal protein food demand. However, infectious diseases limit aquaculture development significantly, causing huge economic losses. Infectious agents can be transmitted by several routes, including mechanical vectors such as animals other than fish. In this regard, wild birds are considered potential vectors of several fish pathogenic viruses; however, only few studies have investigated the presence of fish pathogenic viruses in wild fish-feeding birds. Birds can spread the viruses by carrying them on their feathers, feet and the bill. They can also move infected fish or parts of them from one farm to another. Furthermore, viruses with high resistance to acid conditions can also be spread by regurgitated feed and faeces. Specifically, infectious particles of the infectious pancreatic necrosis virus (IPNV, family Birnaviridae) were isolated from faeces of birds experimentally fed with infected fish. On the other hand, infectious particles of the haemorrhagic septicaemia virus (VHSV, family Rhabdoviruses) were isolated from regurgitated feed [1]. However, to the best of our knowledge, so far only one study has investigated the presence of fish pathogenic viruses in faeces of wild fish-feeding birds, pointing out the presence of the nervous necrosis virus (NNV, family Nodaviridae) in bird faeces collected from a fish farming area in South East Asia [2]. In this study, the presence of fish pathogenic viruses, including NNV, IPNV and VHSV, was investigated in faeces and cloacal swab samples of wild fish-feeding birds (n=43), collected in aquatic habitats in north-east Italy. Bird samples included the grey heron (Ardea cinerea), the sandwich tern (Thalasseus sandvicensis) and the great cormorant (Phalacrocorax carbo). The viral genome presence was investigated through validated molecular methods (real time PCR) which pointed out the presence of NNV in great cormorant samples; this bird species covers a wide geographical area and feeds on several fish species, thus being able to represent a potential vector of the virus among different geographical areas.

[1] Peters, F., & Neukirch, M. (1986). Transmission of some fish pathogenic viruses by the heron, Ardea cinerea. Journal of Fish Diseases 9, 539-544.

[2] Kuo, H. C., Wang, T. Y., Hsu, H. H., Lee, S. H., Chen, Y. M., Tsai, T. J., Ou, M. C., Ku, H. T., Lee, G. B., & Chen, T. Y. (2012). An automated microfluidic chip system for detection of piscine nodavirus and characterization of its potential carrier in grouper farms. PloS one, 7(8), e42203. https://doi.org/10.1371/journal.pone.0042203

VIRAL ENCEPHALOPATHY AND RETINOPATHY IN DUSKY GROUPERS (*EPINEPHELUS MARGINATUS*) FROM TWO MARINE PROTECTED AREAS OF NORTHERN MEDITERRANEAN SEA

Volpe E. (1), Mandrioli L. (1), Errani F. (1), Garcia Hartmann M. (2), Merotto L. (3), Girons A. (4), Ciulli S. (1)

(1) Dipartimento di Scienze Mediche Veterinarie, Alma Mater Studiorum, Università di Bologna, Cesenatico, FC, Italia
(2) MarLab, Mougins, France
(3) Area Marina Protetta di Portofino, Santa Margherita Ligure, Italy
(4) ICTIOVET S.L., Barcelona, Spain

Corresponding Author: **Sara Ciulli** - sara.ciulli@unibo.it

Betanodavirus infection is responsible for viral encephalopathy and retinopathy (VER), a worldwide infectious disease affecting several fish species and clinically associated with nervous signs and high mortality. In the Mediterranean Basin, VER affects especially the European seabass (Dicentrarchus labrax), causing severe losses in the aquaculture sector. Betanodavirus has also been detected in several wild fish species, often in association with an asymptomatic infection. Groupers (family Serranidae, subfamily Epinephelinae) are highly susceptible to betanodavirus infection and VER has long been reported in farmed Asian groupers. Less frequently, betanodavirus infection has been reported in Mediterranean wild groupers in association with mortality outbreaks, particularly in Southern Italy (Apulia and Sicily regions), Tunisia, Algeria and the Balearic Islands in Spain, most of which have been associated with extraordinary climatic conditions characterized by unusually high water temperatures [1-4].
In this study, mortality outbreaks in Northern Mediterranean wild groupers were investigated. Affected groupers were collected from two marine protected areas (MPAs): the Monaco Principality MPA in 2018 and 2019 and the Portofino MPA in Italy in 2019. Four dusky groupers (Epinephelus marginatus) presenting swimming behaviour changes, floating and blindness were examined. Histopathological findings of the brain were highly consistent with viral infection. Betanodavirus was isolated from the nervous tissues of all examined groupers. The isolated virus was genetically characterised through amplification and sequencing of both RNA1 and RNA 2 viral genome fragments. Phylogenetic analysis showed a high similarity among viruses isolated from groupers collected in the two MPAs in different years; all the isolated viruses belong to the Redspotted grouper nervous necrosis virus (RGNNV) species. On the other hand, a wider analysis including betanodaviruses detected in both farmed and wild fish showed a lack of species specificity for betanodaviruses isolated from wild grouper. These viruses, in fact, clustered together with betanodaviruses detected in other fish species, including both wild and farmed fish.

[1] Vendramin, N., Patarnello, P., Toffan, A., Panzarin, V., Cappellozza, E., Tedesco, P., Terlizzi, A., Terregino, C., & Cattoli, G. (2013). Viral Encephalopathy and Retinopathy in groupers (Epinephelus spp.) in southern Italy: a threat for wild endangered species? BMC veterinary research, 9, 20. https://doi.org/10.1186/1746-6148-9-20
[2] Haddad-Boubaker S., Boughdir W., Sghaier S., Ben Souissi J., Megdich A., Dhaouadi R., Amara A., Panzarin V., & Fakhfakh E. (2014). Outbreak of Viral Nervous Necrosis in Endangered Fish species Epinephelus costae and E. marginatus in Northern Tunisian Coasts. Fish Pathology, 49 (2), 53–56.
[3] Valencia, J. M., Grau, A., Pretto, T., Pons, J., Jurado-Rivera, J. A., Castro, J. A., Toffan A., Catanese, G. (2019). Viral encephalopathy and retinopathy (VER) disease in Epinephelus marginatus from the Balearic Islands marine protected areas. Diseases of Aquatic Organisms, 135(1), 49–58. https://doi.org/10.3354/dao03378
[4] Boukedjouta, R., Pretto, T., Abbadi, M., Biasini, L., Toffan, A., & Mezali, K. (2020). Viral encephalopathy and retinopathy is endemic in wild groupers (genus Epinephelus spp.) of the Algerian coast. Journal of fish diseases, 43(7), 801–812. https://doi.org/10.1111/jfd.13181

A ROADMAP FOR DOMESTIC CAT HEPADNAVIRUS (DCH) SEROLOGICAL MARKERS TO PREDICT THE STAGE OF INFECTION

Paola Fruci (1), Andrea Palombieri (1), Vittorio Sarchese (1), Giovanni Aste (1), Federica Di Profio (1), Barbara Di Martino (1)

(1) Università degli studi di Teramo, Dipartimento di Medicina Veterinaria

Corresponding Author: **Paola Fruci** - pfruci@unite.it

In Australia in 2016, a novel orthohepadnavirus (Domestic Cat Hepadnavirus, DCH) similar to hepatitis B virus (HBV), was identified[1]. The epidemiology and pathobiology of this novel hepadnavirus in cats is still uncertain, but several evidence seem suggest a possible association with chronic liver diseases, mirroring clinical and histopathologic findings observed in HBV-associated hepatopathies. For detection of HBV and for predicting the stage of infection, antigens, antibodies, and viral genome are profiled/quantified and this information is coupled with haematological tests [2]. Similarly, investigating patterns and phases of hepadnavirus infection in cats cannot rely solely on molecular diagnostics. Recently, antibodies specific to the recombinant DCH core antigen (DCHcAg) have been found in feline sera, proving that exposure to infection is 2.5 times higher than that obtained by using molecular diagnostic tools [3]. Herein, we report the development of an antibodies detection ELISA based on the recombinant DCH surface (DCHs) protein expressed in baculovirus system. The in-house DCHs antibodies (DCHsAbs) ELISA was employed to evaluate seroconversion in a collection of 94 cat sera that included 27 samples positive only for anti-DCHcAg, 17 contained also DCH DNA and 50 (control group) resulted serologically or molecularly negative. IgG anti-DCHsAg was found in a total of 16 samples with an overall prevalence of 17.0%. All the seropositive sera were molecularly negative but possessed IgG anti-DCHcAg. When re-testing the subset of 50 seronegative specimens for DCHcAbs, none of the tested sera showed seroconversion against DCHsAg. Among the HBV serological markers, the presence of antibodies raised to the core antigen is compatible with acute, resolved, chronic and occult HBV infection, while a concomitant seroconversion to HBV surface (HBs) is observed in the blood of human patients who recover from infection. In addition, it is the only detectable serological marker in those who successfully respond to hepatitis B immunization. In this survey, we found that more than half DCHcAbs positive samples (59.3%) contained also IgG anti-DCHsAg, a condition that may be compatible with the possible recovery from the infection. By converse, anti-DCHsAg was not found when assessing sera concomitantly positive for viral DNA, suggesting that molecular tools combined with serological markers, may be useful to discriminate between an active, occult or resolved infection.

[1] Aghazadeh M, et al. A Novel Hepadnavirus Identified in an Immunocompromised Domestic Cat in Australia. Viruses. 2018;10(5):269.
[2] Lee JH, Kim HS. Current laboratory tests for diagnosis of hepatitis B virus infection. Int J Clin Pract. 2021;75(12):e14812.
[3] Fruci P, et al. Detection of antibodies against domestic cat hepadnavirus using baculovirus-expressed core protein. Transbound Emerg Dis. 2022;69(5):2980-2986.

MOLECULAR CHARACTERIZATION OF RESISTANT STRAINS OF SALMONELLA ENTERICA SUBSP. ENTERICA ISOLATED FROM MIGRATORY BIRDS

Capozzi L., Bianco A., Del Sambro L., Caldarola G., Pugliese D., Sposato A., Palazzo L., Pietragalla I., Romano A. C., Parisi A.
Istituto Zooprofilattico Sperimentale della Puglia e della Basiliata, Foggia, Italia

Corresponding Author: **Parisi A.**

Antibiotic resistance represents one of the major global health challenges of our century. Understanding the mechanisms of environmental spread of resistance factors, whose pathways are often interconnected1, remains a key issue in this emergency. Some studies highlight the potential role of migratory birds as sentinels for the surveillance of multi-resistant pathogens2, while others emphasise their role in the dissemination and spread of genetic factors that confer antibiotic resistance3,4,. Increasingly, wildlife finds a source of sustenance in urban dumps, and this leads to the intake of potentially harmful substances such as drug residues along with waste materials. The contact of these wild animals with antimicrobial drugs, now ubiquitously present in water and the environment, stimulates the acquisition of new resistance mechanisms by the bacterial strains for which birds are a reservoir. This phenomenon is important from a scientific point of view, since bacterial strains with zoonotic potential are carried with high frequency.
In this study, two strains of Salmonella enterica isolated from the faeces of migratory wild birds, a buzzard and a kestrel respectively, detected in March 2022 in the province of Potenza (Apulia, Italy) were genotyped and molecularly characterised. DNA was purified from bacterial colonies isolated on TSI agar using the DNeasy Blood & Tissue kit (Qiagen). Genomic libraries were prepared with Illumina DNA Prep kit (Illumina), following the manufacturer's instructions. Whole genome sequencing (WGS) was conducted on the Illumina MiSeq platform. The genome sequences obtained were submitted to Enterobase for species identification, serotype definition and Sequence Type (ST). The use of the Abricate tool allowed the analysis of the resistome and virulence factors.
In silico analysis enabled the strains sampled from the buzzard and kestrel to be identified as belonging to the S. enterica species and to the serotypes and ST Derby (ST682) and Kentucky (ST152), respectively.
The study of the resistome revealed in both strains the presence, respectively, of about 50 genes involved in the mechanisms of resistance to 19 antibiotic classes and different disinfectants. Plasmid analysis detected in both strains the presence of IncX1, a plasmid carrier of genes coding for resistance factors against β-lactams, aminoglycosides, trimetroprim, tetracyclines and aminoglycosides.
The periodic transfer of these animals gives them an important role in the dissemination of resistant pathogens along migratory routes.
Since genetic elements that are so crucial to human and animal health are acquired and spread through migratory activities by wildlife, it will be increasingly unlikely to be able to maintain control over them. Investigating this critical aspect for the transmission of antibiotic resistance is a key step in reconstructing the pathways and identifying the protagonists of this phenomenon of environmental evolution, with the ultimate aim of contributing to the understanding and management of the resistance crisis as a whole.

[1] Fletcher S. Understanding the contribution of environmental factors in the spread of antimicrobial resistance. Environ Health Prev Med. 2015;20(4):243-252. doi:10.1007/s12199-015-0468-0 [2] Martín-Maldonado, B.; Rodríguez-Alcázar, P.; Fernández-Novo, A.; González, F.; Pastor, N.; López, I.; Suárez, L.; Moraleda, V.; Aranaz, A. Urban Birds as Antimicrobial Resistance Sentinels: White Storks Showed Higher Multidrug-Resistant Escherichia coli Levels Than Seagulls in Central Spain. Animals 2022, 12, 2714. https://doi.org/10.3390/ani12192714
[3] Yuan, Y., Liang, B., Jiang, B.W., Zhu, L.W., Wang, T.C., et al. Migratory wild birds carrying multidrug-resistant Escherichia coli as potential transmitters of antimicrobial resistance in China. 2018. PLOS ONE 16(12): e0261444. https://doi.org/10.1371/journal.pone.0261444
[4] Elsohaby, I., Samy, A., Elmoslemany, A., Alorabi, M., Alkafafy, M., Aldoweriej, A., Al-Marri, T., Elbehiry, A., & Fayez, M. (2021). Migratory Wild Birds as a Potential Disseminator of Antimicrobial-Resistant Bacteria around Al-Asfar Lake, Eastern Saudi Arabia. Antibiotics (Basel, Switzerland), 10(3), 260. https://doi.org/10.3390/antibiotics10030260

INFLUENZA A VIRUS SURVEILLANCE IN DOMESTIC AND WILD SUIDAE IN EMILIA-ROMAGNA REGION (NORTHERN ITALY)

Alice Prosperi (1), Laura Soliani (1), Laura Baioni (1), Camilla Torreggiani (1), Simona Perulli (1), Laura Fiorentini (1), Giovanni Pupillo (1), Annalisa Santi (1), Ana Moreno (1), Andrea Luppi (1), Chiara Chiapponi (1,2)

(1) Istituto Zooprofilattico Sperimentale della Lombardia e dell'Emilia-Romagna "Bruno Ubertini", Via Bianchi, 9 - 25124 Brescia, Italy - WOAH (OIE) Reference Laboratory for Swine Influenza
(2) Department of Life Sciences, Biochemistry and Molecular Biology Unit, University of Parma, Italy

Corresponding Author: **Alice Prosperi** - alice.prosperi@izsler.it

Influenza A viruses – IAVs are the most widespread agents of respiratory infections in humans and swine. In this study, IAV viral circulation was monitored between 2017-2023 in wild boars (active surveillance) and domestic swine (passive surveillance) in Emilia-Romagna region. Nasal swabs, oral fluids or lung samples were screened in pools for IAV using real-time RT-PCR, with a maximum of five individuals per pool. Positive samples were molecularly subtyped by PCR and viral isolation was attempted. A selection of viral isolates was subjected to whole genome sequencing by NGS and phylogenetic analysis was performed. From 1 January 2017 to 23 March 2023, 5722 wild boar lung samples were analysed in 2985 SwIAV real-time RT-PCR tests. Out of those, 17 (0.57%) were positive and 7 (41.18%) SwIAVs were subtyped at the H-N level (3 H1pdm09N1, 2 H1avN1, 1 H1avNx, 1 H1N2). Finally, 3 viruses were isolated and WGS-analysed: (i)HA-1C-N1av (1C.2.1) in genotype U; (ii)HA-1C-N1av (1C.2.1) in genotype A; (iii)H1pdm09N1-derived subtype (HA-1A-N1av—1A.3.3.2) in genotype 31. At the same time, 7926 samples collected in swine farms affected by PRDC were delivered for diagnostic purposes to IZSLER and analysed in 2119 tests; 382 (18%) were SwIAV positive. Within the positives, 221 (57%) were subtyped and classified preliminarily as H1N1, H1N2, H3N2 and H1N1pdm09. A selection of isolated strains was fully sequenced (106 viruses). An increasing genotypic diversity was detected within the analysed strains: H1N2 was the subtype with the highest variability (with 18 different gene combinations and 10 genotypes), followed by H1N1 (with 5 genotypes), whilst the H3N2 strains showed a stable genetic pattern (1 genotype). The SwIAV subtypes detected showed an evolving situation and the strains detected in wild boars highlighted the simultaneous circulation of the same genotypes in domestic and wild Suidae, which suggests SwIAV spillover events at the wildlife–livestock interface. The importance of monitoring SwIAV genetic variability is strengthened by the economic importance of swine influenza as a PRDC etiological agent but also the swine role as an IAVs mixing vessel. Suidae are able to favour viral hypervariability, creating new viral variants with reassortment events, which might lead to pandemic events. In the post-SARS-CoV2-pandemic scenario, the surveillance of potentially pandemic viruses within a One-Health approach is more desirable than ever.

This research was partially supported by EU funding within the NextGenerationEU-MUR PNRR Extended Partnership initiative on Emerging Infectious Diseases (Project no. PE00000007, INF-ACT) and by Italian Ministry of Health (grant numbers RC IZ LER 2018009 and RC IZ LER 2020005).

[1] Henritzi, D.; Petric, P.P.; Lewis, N.S.; Graaf, A.; Pessia, A.; Starick, E.; Breithaupt, A.; Strebelow, G.; Luttermann, C.; Parker, L.M.K.; et al. Surveillance of European Domestic Pig Populations Identifies an Emerging Reservoir of Potentially Zoonotic Swine Influenza A Viruses. Cell Host Microbe 2020, 28, 614–627.e6. https://doi.org/10.1016/j.chom.2020.07.006.
[2] OIE. OIE (2018): Influenza A virus of Swine (NB: Version Adopted in May 2015). Chapter 3.9.7 in Manual of Diagnostic Tests and Vaccines for Terrestrial Animals 2021. Available online: https://www.oie.int/fileadmin/Home/eng/Health_standards/tahm/3.09.07_INF_A_SWINE.pdf
[3] Chiapponi, C.; Prosperi, A.; Moreno, A.; Baioni, L.; Faccini, S.; Manfredi, R.; Zanni, I.; Gabbi, V.; Calanchi, I.; Fusaro, A.; et al. Genetic Variability among Swine Influenza Viruses in Italy: Data Analysis of the Period 2017–2020. Viruses 2021, 14, 47. https://doi.org/10.3390/v14010047.
[4] Prosperi A, Soliani L, Canelli E, Baioni L, Gabbi V, Torreggiani C, Manfredi R; et al. Influenza A in Wild Boars: Viral Circulation in the Emilia-Romagna Region (Northern Italy) between 2017 and 2022. Animals (Basel). 2022 Jun 20;12(12):1593. doi: 10.3390/ani12121593. PMID: 35739929; PMCID: PMC9220169.

DETECTION OF HELICOBACTER SPP. IN CAPTIVE NON-DOMESTIC FELIDS FROM ITALY

Gabriele Ratti (1), Giuliano Ravasio (1), Paola Roccabianca (1), Alessia Giordano (1), Maurizio Oltolina (2), Luca Bertola (1, 3), Manuel Morici (4), William Magnone (5), Stefania Lauzi (1)

(1) Università degli Studi di Milano, Dipartimento di Medicina Veterinaria e Scienze Animali
(2) Parco Faunistico Le Cornelle
(3) Mouse and Animal Pathology Lab (MapLab)
(4) Pombia Park
(5) Parco Natura Viva Garda Zoological Park

Corresponding Author: **Gabriele Ratti** - gabriele.ratti@unimi.it

Colonization by Helicobacter spp. has been reported in several animal species, including non-domestic felids. Helicobacter spp. infections often remain unrecognized and along with other factors may contribute to progressive gastritis [1-2]. In May 2022, Helicobacter spp. was detected by nested-PCR [3] in the gastric biopsy of a lion admitted to the Veterinary Teaching Hospital (VTH) of Lodi with a history of chronic gastritis. Following this finding, aim of this study was to investigate the presence of Helicobacter spp. in captive non-domestic felids from Italy. Fecal samples were selected from the caseload of the VTH laboratory diagnostic routine between 2019 and 2023. DNA from feces was analyzed using a nested-PCR targeting the 16S gene of Helicobacter spp. PCR positive fecal and gastric biopsy samples were investigated by partial 16S gene sequencing for Helicobacter species identification by BLAST and phylogenetic analyses. Samples from a total of 40 non-domestic felids (family Felidae) were included in this study. Animals came from 3 zoological facilities and one circus. Overall, Helicobacter spp. DNA was detected in 3/40 (7.5%) animals belonging to two different zoological facilities. More precisely, positive samples were obtained from the lion's gastric biopsy and the fecal samples from two snow leopards. Phylogeny based on the partial 16S gene showed that Helicobacter spp. from snow leopards grouped within the cluster including other Helicobacter canis sequences, whereas Helicobacter spp. from the lion grouped with other Helicobacter acinonychis stains previously identified in other non-domestic felids belonging to the Panthera genus, confirming BLAST analysis results. Our preliminary results showing the presence of Helicobacter spp. in captive lion and snow leopards in Italy confirm Helicobacter infection previously reported in non-domestic felids [2]. A lower presence of Helicobacter species was detected in our study compared to previous reports. This result may likely reflect different management practices in the zoological facilities analyzed or the use of fecal samples, that may have underestimated the presence of Helicobacter spp. compared to gastric biopsies, even if it represents a non-invasive sampling strategy to detect bacterial circulation [4]. Given the presence of severe gastritis in one infected lion, further studies are recommended to investigate the pathogenic role of Helicobacter infection in captive non-domestic felids in Italy.

[1] Tegtmeyer, N., Rivas Traverso, F., Rohde, M., Oyarzabal, O. A., Lehn, N., Schneider-Brachert, W., Ferrero, R. L., Fox, J. G., Berg, D. E., & Backert, S. (2013). Electron microscopic, genetic and protein expression analyses of Helicobacter acinonychis strains from a Bengal tiger. PloS one, 8(8), e71220. https://doi.org/10.1371/journal.pone.0071220
[2] Mangiaterra, S., Marker, L., Cerquetella, M., Galosi, L., Marchegiani, A., Gavazza, A., & Rossi, G. (2022). Chronic Stress-Related Gastroenteric Pathology in Cheetah: Relation between Intrinsic and Extrinsic Factors. Biology, 11(4), 606. https://doi.org/10.3390/biology11040606
[3] Fox, J. G., Dewhirst, F. E., Shen, Z., Feng, Y., Taylor, N. S., Paster, B. J., Ericson, R. L., Lau, C. N., Correa, P., Araya, J. C., & Roa, I. (1998). Hepatic Helicobacter species identified in bile and gallbladder tissue from Chileans with chronic cholecystitis. Gastroenterology, 114(4), 755–763. https://doi.org/10.1016/s0016-5085(98)70589-x
[4] De Witte, C., Lemmens, C., Flahou, B., De Laender, P., Bouts, T., Vercammen, F., Ducatelle, R., Smet, A., & Haesebrouck, F. (2018). Presence of Helicobacter and Campylobacter species in faecal samples from zoo mammals. Veterinary microbiology, 219, 49–52. https://doi.org/10.1016/j.vetmic.2018.04.014

MULTI-DRUG RESISTANT KLEBSIELLA PNEUMONIAE STRAINS ISOLATED FROM LESSER KESTREL (FALCO NAUMANNI)

Samarelli R. (1), Pugliese N. (1), Pazzani C. (2), Oliva M. (2), Lombardi R. (1), Galante D. (3), Zizzadoro C. (1), Crescenzo G. (1), Circella E. (1), Camarda A. (1)

(1) Dipartimento di Medicina Veterinaria, Università degli Studi di Bari Aldo Moro
(2) Dipartimento di Bioscienze, Biotecnologie e Ambiente, Università degli Studi di Bari Aldo Moro
(3) Istituto Zooprofilattico Sperimentale della Puglia e Basilicata

Corresponding Author: **Rossella Samarelli** - rossella.samarelli@uniba.it

Antimicrobial resistance (AMR) is a primary challenge both in human and veterinary medicine. This is one of the basic assets of the OneHealth approach, which aspires to optimize the health of people, animals, and the environment (1). With that premise, this study was aimed at characterizing 17 multi-drug resistant (MDR) K. pneumoniae strains, isolated during a larger survey that identified enrofloxacin-resistant strains from lesser kestrels (Falco naumanni), between 2021 and 2022. After isolation and identification by MALDI-TOF, K. pneumoniae strains were tested for their susceptibility to ampicillin (AMP), cefotaxime (CTX), cefepime (FEP), imipenem (IMP), chloramphenicol (CHL), co-trimoxazole (SXT), streptomycin (STR), gentamycin (GEN), apramycin (APR), tetracycline (TET), and colistin (COL) by the disc diffusion method. Eight strains (A-MDR) were resistant to 8 antibiotics (ENR-AMP-CTX-FEP-STR-GEN-SXT-TET), 8 (B-MDR) to 7 antibiotics (ENR-AMP-CTX-STR-GEN-SXT-TET), and 1 to 6 antibiotics (ENR-AMP-CTX-STR-GEN-SXT). The strains of the B-MDR group had an intermediate susceptibility to FEP. The MIC to ENR of all of them was 32 ug/mL. The whole genome sequence of two strains, representative of the A-MDR and B-MDR groups, respectively, was determined by the Nanopore system. Both strains harbored a conjugative plasmid, which hosted most of the genetic determinants of resistances, specifically blaTEM-1 (AMP resistance), blaOXA-1 (an extended-spectrum beta-lactamase, ESBL), blaCTX-M-15 (cephalosporin resistance), qnrB1 (ENR resistance), strA and strB (STR resistance), aac(6')-Ib-cr and aac(3)-II (aminoglycoside resistance), dfrA14 (trimethoprim resistance), and sul2 (sulfamethoxazole resistance).No gene was associated with TET resistance was detected, but the gene kpnF was found, which encodes a small antibiotic efflux pump. Considering the MDR profiles and the resistance genes, which include ESBL, cephalosporin beta-lactamases, and fluoroquinolone target protectors, it is likely that the MDR strains were not selected in the lesser kestrels, which did not come in contact with typically human antibiotics. Rather, they were selected in humans and spread in the environment. The mobility traits increase the transfer potential and the circulation of resistance determinants in bacterial species and strains that may be selected in humans, colonize the gut of wild birds, be disseminated in the environment and return to humans with newer and more concerning features (2,3).

[1] Aslam, B., Khurshid, M., Arshad, M. I., Muzammil, S., Rasool, M., Yasmeen, N., Shah, T., Chaudhry, T. H., Rasool, M. H., Shahid, A., Xueshan, X., & Baloch, Z. (2021). Antibiotic Resistance: One Health One World Outlook. Frontiers in cellular and infection microbiology, 11, 771510.
[2] Wang, X., Zhao, J., Ji, F., Wang, M., Wu, B., Qin, J., Dong, G., Zhao, R., & Wang, C. (2023). Genomic Characteristics and Molecular Epidemiology of Multidrug-Resistant Klebsiella pneumoniae Strains Carried by Wild Birds. Microbiology spectrum, e0269122. Advance online publication.
[3] Wyres, K. L., & Holt, K. E. (2018). Klebsiella pneumoniae as a key trafficker of drug resistance genes from environmental to clinically important bacteria. Current opinion in microbiology, 45, 131–139

MOLECULAR INVESTIGATION FOR CRESS DNA VIRUSES IN CAPTIVE NON-HUMAN PRIMATES, ITALY

Vittorio Sarchese (1), Andrea Palombieri (1), Paola Fruci (1), Federica Di Profio (1), Klaus Gunther Friedrich (2), Barbara Di Martino (1)

(1) Università degli Studi di Teramo, Dipartimento di Medicina Veterinaria
(2) Fondazione Bioparco di Roma, Viale del Giardino Zoologico, Roma

Corresponding Author: **Vittorio Sarchese** - vsarchese@unite.it

Replication-associated protein (Rep)-encoding single-stranded (CRESS) DNA viruses have been discovered in a diversity of prokaryotes and eukaryotes. CRESS DNA viruses encompass several viral families including Circoviridae. Circoviruses are non-enveloped, spherical viruses with a circular DNA genome of approximately 2 kb length. Members of the family Circoviridae are classified into two genera, Circovirus (CV) and Cyclovirus (CyV) [1] that have been identified in human stools, blood, and cerebrospinal fluid (CSF), as well as in various domestic and wild vertebrates including non-human primates (NHPs). In NHPs, CVs and CyVs were firstly discovered in 2010 in a molecular survey conducted from faecal samples of healthy chimpanzees (Pan troglodytes) [2]. In addition, CyVs were found using a metagenomic approach in a stool collected from a drill monkey (Mandrillus leucophaeus) housed in a sanctuary in Nigeria [3]. In this study, to better understand the ecology of these viruses in NHPs we investigated the presence of CVs and CyVs in animals housed at the Zoological Garden (Bioparco) of Rome, Italy. A total of 48 samples (24 sera and 24 stools paired samples), collected during 2021-2022, were molecularly screened by a nested PCR protocol using broadly reactive primers designed to amplify a conserved region of Rep gene among members of the family Circoviridae [2]. Overall, viral DNA was detected in 5 (20.8%) out of 24 stools samples collected from a macaque (Macaca fuscata) and four mandrills (Mandrillus sphinx), respectively. By BLAST and FASTA analyses, amplicons displayed the highest nucleotide (nt) identity with two CyVs strains identified in fecal samples collected in Italy from a maltese lizard (Podarcis filfolensis) (98.4-99.3% nt) and in Tunisia from a patient with acute flaccid paralysis (99.3-99.7%), respectively. Furthermore, nt identity (78.4-78.7%) was also found to CyVs identified in children with respiratory infections in Chile. Upon phylogenetic analyses based on the partial Rep gene, all strains segregated into the genus Cyclovirus, along with other CyVs strains previously identified in animals and humans. The finding of this study provides further evidence for the circulation of CyVs in NHPs. The identification of closely related CyVs in human and animal hosts suggests a potential cross-species and zoonotic transmissions. Further studies are required to elucidate the role of animals in CyVs ecology.

[1] Rosario K. et al. 2017. Revisiting the taxonomy of the family Circoviridae: establishment of the genus Cyclovirus and removal of the genus Gyrovirus. Arch Virol 162:1447–1463.
[2] Li L. et al. 2010. Multiple diverse circoviruses infect farm animals and are commonly found in human and chimpanzee feces. J Virol 84:1674-82.
[3] George U. et al. Genome Sequences of Novel Members of Previously Described DNA and RNA Virus Families, Isolated from Feces of a Drill Monkey in Nigeria. Microbiol Resour Announc 9(17):e00092-20.

STUDENT COMPETITION "MICROBIOLOGY CAN BE…2022", SECOND EDITION

Team 1- Gerli Caterina1, Lupo Cecilia1, Mongiat Marta1, Passerini Chiara1, Pianorini Matteo1, Quartesan Allegra Benedetta1, Team 2- Bartoccini Virginia1, Boutiznar Rokia1, Cavallo Valentina1, Garelli Virginia1, Massei Fiamma Lucia1, Team 3- Bertes Margherita1, Borchi Teresa1, Chiriatti Valerio1, Genco Davide1, Ippodimonte Francesca1, Pallini Francesco1, Team 5- Antonelli Annalisa1, Chiatti Maria Francesca1, Del Pizzo Emanuela1, Filugelli Alessia1, Fosconi Agnese1, Nerti Lara1, Team 6- Fabian Sara1, Guglielmi Giulia1, Orselli Sara1, Panit Catherine Arguelles1, Santilli Marta1, Schiavon Elena1, Team 7- Arrabi Nur1, Cohen Shirinoam1, Chermaz Caterina1, Pirgher Francesca1, Romano Chiara1, Team 9- Barbarulo Sara1, Ciurli Costanza1, Dusan Markovic1, Gianino Nadia1, Grilli Gabriele1, Leandri Michele1, Ciappelloni Raoul2, Marenzoni Maria Luisa1

(1) Università degli Studi di Perugia, Dipartimento di Medicina Veterinaria
(2) Istituto Zooprofilattico Sperimentale Umbria e Marche "Togo Rosati"

Corresponding Author: **Maria Luisa Marenzoni** - marialuisa.marenzoni@unipg.it

Based on the experience of the previous edition, results of the second edition of the student contest "Microbiology can be..." aa. 2021/2022, performed during the third year of the course of the veterinary medicine, are presented. The ability to effectively communicate knowledge about infectious diseases is a critical skill for society, as demonstrated during the COVID-19 pandemic and in the ongoing fight against antibiotic resistance. The public must be informed to access and understand scientific research, particularly related to health (i.e. health literacy), and trust in science to make informed decisions and take appropriate actions. Students must be trained to provide competent and straightforward scientific information through effective communication.
"Microbiology can be..." activities were organized in groups of 5-6 students who learned to utilize and combine different skills to perform various types of communication on microbiology and infectious diseases. In this way, they learned to recognize their potential, especially in group settings, and develop communication skills useful for their future profession. In this edition, the students produced new informative materials such as: drawings, comics, games, and videos, that will be useful in disseminating knowledge about infectious diseases to other students and society.

[1] Scavone et al. Microbiology can be comic. FEMS Microbiol Lett. 1;366(14):fnz171, 2019.
[2] Marenzoni et al. "Microbiology can be ..." An educational communication project in the field of microbiology and infectious diseases. SPVet.it 133/2022.
[3] Timmis et al. The urgent need for microbiology literacy in society. Environ Microbiol. 2019 May;21(5):1513-1528.

USE OF ARTIFICIAL INTELLIGENCE FOR WRITING A FLASH FICTION FOR THE MICROEPIDEMIC PROJECT

Maria Luisa Marenzoni (1), Silva Costarelli (2), Raoul Ciappelloni (2)

(1) Dipartimento di Medicina Veterinaria, Università degli Studi di Perugia
(2) Istituto Zooprofilattico Sperimentale dell'Umbria e delle Marche "Togo Rosati"

Corresponding Author: **Maria Luisa Marenzoni** - marialuisa.marenzoni@unipg.it

This study describes how ChatGPT®, an Artificial Intelligence (AI) application, was used to generate a short story about the zoonotic disease Leptospirosis as part of the "Micro Epidemic One Health" Italian Health System Project, which focuses on storytelling and collaborative writing activities. The AI application was engaged to develop a story about a group of teenagers who contracted Leptospirosis after swimming in a river contaminated with the Leptospira bacteria, and what people can learn about zoonoses from this experience. The story was generated within seconds and was deemed plausible. It was then validated by veterinarians and linguistic experts to assess the medical assumptions and quality of English translation. The use of AI in writing and storytelling is becoming increasingly feasible, with the platform capable of transforming data into true stories. The quality of responses generated by AI applications can be considered good, and the reader may not even notice the text's artificial origin. Advantages of this process include speed of execution and suggestions provided by the AI. However, its use cannot take place independently, and experts must provide more technical information for implementation.

[1] Ciappelloni R. (2016). Macchine parlanti e scriventi per una editoria automatica - Speaking and writing machines, for an automatic publishing. Sanità Pubblica Veterinaria, n. 98, Ottobre. . https://spvet.it/archivio/numero-98/edi98.html.
[2] Lund, B.D., Wang, T. (2023), Chatting about ChatGPT: how may AI and GPT impact academia and libraries?, Library Hi Tech News, Vol. ahead-of-print No. ahead-of-print. https://doi.org/10.1108/LHTN-01-2023-0009
[3] Studart Galdino G., Veras de Sandes-Freitas T., Modelli de Andrade L. G., Caetano Adamian C. M., Cavalcante Meneses G., Bezerra da Silva Junior G., De Francesco Daher E. (2022). Development and validation of a simple machine learning tool to predict mortality in leptospirosis. Scientific Reports - Nature, Mar 18;13(1):4506. doi: 10.1038/s41598-023-31707-4.

VIROLOGICAL SURVEILLANCE IN THE INFECTIOUS ISOLATION AREA OF THE VETERINARY UNIVERSITY HOSPITAL OF THE UNIVERSITY OF BOLOGNA: MOLECULAR DETECTION OF CANINE AND FELINE PARVOVIRUS CONTAMINATION

Alessia Terrusi (1), Ilaria Magnagnoli (1), Simona Vilardi (1), Roberta Troia (1), Massimo Giunti (1), Martina Magliocca (1), Laura Gallina (1), Lorenza Urbani (1), Mara Battilani (1), Andrea Balboni (1)

(1) Dipartimento di Scienze Mediche Veterinarie (DIMEVET), Alma Mater Studiorum-Università di Bologna

Corresponding Author: **Andrea Balboni** - a.balboni@unibo.it

Healthcare-associated infections (HAI) are nosocomial acquired infections that are not present or incubating at the time of admission to a hospital. Their frequency is likely to increase in human and veterinary medicine, and prevention and control programs are essential to limit their impact [1,2]. This study focused on virological surveillance in the infectious isolation area of the Veterinary University Hospital (VUH) of Bologna, between October 2021 and June 2022. During the study period, canine parvovirus (CPV-2) and feline panleukopenia virus (FPV) DNA was detected and quantified by real-time PCR in dogs and cats with a confirmed diagnosis of parvovirosis admitted in the isolation area of VUH. A standardised protocol was applied to assess faecal viral elimination, viral contamination of hair, environment and operators, and the effectiveness of disinfection. The identified viruses were characterized by VP2 gene sequencing and deduced protein analysis. Seven patients were included in the study: 5 dogs and 2 cats. All subjects had high faecal elimination rate during hospitalization (10^4-10^9 copies of viral DNA/µl extract) and at hospital discharge (10^4-10^6 copies/µl), as well as high hair contamination (10^3-10^8 copies/µl). These data suggest that animals continue to eliminate viruses and carry them with the hair at the hospital discharge, representing a risk for other animals. Environmental monitoring revealed high contamination at the time of hospital discharge, potentially related to the magnitude of faecal excretion but not to the number of days of hospitalization. The disinfection protocol adopted was mostly effective, with significant reduction of viral DNA quantity in almost all sampled areas and equipment. Criticalities were revealed in some sampled surfaces and probably related to an underestimation of the risk of dissemination associated with the operator activities. The adoption of a filter zone resulted effective to prevent viral contamination outside the isolation area. High genetic variability emerged for the viruses identified (CPV-2a, CPV-2b, CPV-2c and FPV). Circulation of different parvoviruses in the VUH could predispose to the occurrence of multiple infections with potential implication on the outcome of disease. Further studies are needed to correlate the molecular data to the viability of the viruses detected in the environment with the aim to applied efficacious hospital infection-control programs.

[1] Milton, Arockiasamy Prince A. Nosocomial Infections and Their Surveillance in Veterinary Hospitals. Adv Anim Vet Sci. 2015;3(2s):1–24.
[2] Marenzoni M L, Conti M B, Rossi E, Rinoldo R, Franceschini R, Tesei E, Porciello F, Mancini S, Favi B, Gamboni M, Boni P, Raffaele O, Desario C, Franciosini M P, Buonavoglia C, Decaro N. Detection of Environmental Contamination with Feline and Canine Parvoviruses: New Perspectives and Challenges». J Appl Microbiol. 2021;131(1):499–512.

76° CONVEGNO SISVET
BARI, 21-22-23 GIUGNO 2023

EXPLORING THE BEE GUT MICROBIOTA AS THE VECTOR FOR ZOONOSES IN A ONE HEALTH PERSPECTIVE

Bruno Tilocca (1), Mariachiara Paonessa (1), Viviana Greco (2,3), Roberto Bava (1), Fabio Castagna (1), Vincenzo Musella (1), Andrea Urbani (2,3), Domenico Britti (1), Paola Roncada (1)

(1) University "Magna Graecia" of Catanzaro- Department of Health Science
(2) Catholic University of the Sacred Heart- Department of Basic Biotechnological Sciences, Intensivological and Perioperative Clinics
(3) Fondazione Policlinico Universitario A. Gemelli IRCCS- Department of Diagnostic and Laboratory Medicine, Unity of Chemistry, Biochemistry and Clinical Molecular Biology

Corresponding Author: **Bruno Tilocca**- tilocca@unicz.it

Bees research is gaining increasing attention acknowledged their pivotal ecological role as pollinators and biological spies of the environmental health status (e.g. pollution, climate change, anthropization). Besides, covering short-to-mid distances by flying, bees held the potential to "move" microorganisms (including pathogens) among diverse environments and across the three spheres of life (i.e. human, animals and environment), in line with the One-Health approach. Exploring the bee-associated microbiota would represent an effective strategy for monitoring microbial routes and forecasting animal infection outbreaks, along with tracking the diffusion of the antimicrobial resistance traits each microorganism carries over its moving. As of today, only a handful of studies are available in the literature, mostly aimed at the description of the functional bee microbiota concerning honeybee susceptibility to infections and the quality of the produced honey (1-3). To the best of our knowledge, no studies have dealt with bee gut metaproteomics to highlight the microbiological potential of bees.

Here, the functional microbiota of four adult bee workers representatives is investigated by mass spectrometry-based metaproteomics and bioinformatics data analysis to explore the structure and activities of bacteria, fungi and protists, meant as the major gut colonizers. The outcomes of our explorative research underline the massive presence of bacteria in relation to the other investigated taxa. Interestingly, pathogenic or potentially pathogenic specimens have been identified in all the surveyed taxa, supporting the hypothesized role of bees while bridging the microbial routes across the pillars of life. Functional featuring of the bee gut microbiota is also detailed for the bacteria, fungi, and protists members providing evidence of the metabolic strength of the microbiota and its involvement in conditioning microbial phenotypic behaviours, which unavoidably condition the onset and progression of infective outbreaks in the animal, human and environmental field.

Altogether, our study contributes to the pioneering knowledge on the functional bee microbiota and provides a first glimpse into the future modulation of the structure and function of such microbial communities towards a reduced diffusion of pathogens and antimicrobial resistance traits via this yet underestimated route (4).

Work supported by Dept. of Health Sciences- UniCZ. Project BE-ONE (BT).

[1] Boncristiani et.al, 2021.World Honey Bee Health: The Global Distribution of Western Honey Bee (Apis mellifera L.) Pests and Pathogens. DOI:10.1080/0005772X.2020.1800330
[2] Müller et.al, 2015.Involvement of secondary metabolites in the pathogenesis of the American foulbrood of honey bees caused by Paenibacillus larvae. DOI:10.1039/c4np00158c
[3] Wang et.al, 2023. Atrazine exposure can dysregulate the immune system and increase the susceptibility against pathogens in honeybees in a dose-dependent manner. DOI: 10.1016/j.jhazmat.2023.131179
[4] Cui et.al, 2022. Community composition, bacterial symbionts, antibacterial and antioxidant activities of honeybee-associated fungi. DOI: 10.1186/s12866-022-02580-4

DETECTION OF VIRAL PATHOGENS IN ITALIAN RED FOXES USING PCR AND METAGENOMIC ANALYSIS

Maria Irene Pacini (1), Micaela Sgorbini (1), Valentina Virginia Ebani (1), Maurizio Mazzei (1)

(1) Department of Veterinary Science of University of Pisa, Pisa, Italy

Corresponding Author: **Maria Irene Pacini** - mariairene.pacini@phd.unipi.it

In recent years, the incidence of emerging infectious diseases has increased dramatically worldwide, threatening human and animal health and biodiversity [1]. Wildlife plays an important role in the spread of pathogens and represents a valuable resource for the early detection [2]. Unfortunately, disease surveillance in wildlife tends to be challenging, with some practical difficulties, and consequently the literature is still scarce [3]. Foxes are widespread and feed on a wide range of food resources, including carcasses and waste. They have therefore often been proposed as a sentinel species for the health status of ecosystems [4]. Our work investigated the presence of viral pathogens in foxes from two study areas in central Italy: the province of Pisa and the Foreste Casentinesi National Park (Arezzo). These areas differ in the degree of interaction between humans, domestic animals and wildlife. The proposed methodology involves the use of faeces as non-invasive samples and the synergistic application of conventional molecular techniques and metagenomic analysis. A total of 27 faecal samples were collected and tested by PCR for a panel of viral targets, including the major viral species detected in European fox populations. They were also analysed by metagenomic techniques using a shotgun approach. Molecular analysis identified Canine adenovirus-1, Canine parvovirus, Bocavirus spp, Kobuvirus spp, Coronavirus spp, Fox astrovirus, Avian astrovirus, Avian adenovirus, Rodent circovirus, Pigeon circovirus, Porcine circo-like virus, Torque teno felis virus, Unclassified Anelloviridae, Fox adeno-associated virus -1, Tod virus, Porcine picobirnavirus and Unclassified Picornavirales. The results revealed the presence of numerous viruses, including some not previously reported in Italian foxes and some common to other species. These data confirm the possible role of foxes as sentinels for the spread of infectious diseases in the environment and suggest the potential of the proposed methodology in wildlife disease monitoring.

[1] Daszak, P.; Cunningham, A.A.; Hyatt, A.D. Emerging Infectious Diseases of Wildlife - Threats to Biodiversity and Human Health. Science (80-.). 2000, 287, 443–449
[2] Cunningham, andrew A. A Walk on the Wild Side—Emerging Wildlife Diseases. Bmj 2005, 331, 1214– 1215, doi:10.1136/bmj.331.7527.1214.
[3] Garcês, A.; Pires, I. Secrets of the Astute Red Fox (Vulpes Vulpes, Linnaeus, 1758): An InsideEcosystem Secret Agent Serving One Health. Environ. 2021, Vol. 8, Page 103 2021, 8, 103, doi:10.3390/ENVIRONMENTS8100103
[4] Ryser-Degiorgis, M.P. Wildlife Health Investigations: Needs, Challenges and Recommendations. BMC Vet. Res. 2013, 9, 1–17.

DEVELOPMENT OF NEUTRALIZATION TEST AGAINST BACILLUS ANTHRACIS TOXINS TO EVALUATE THE ANTIBODY IN RESPONSE TO STERNE 34F2 VACCINATION

Lorenzo Pace (1), Valeria Rondinone (1), Iolanda Padalino (1), Viviana Manzulli (1), Dora Cipolletta (1), Furio Ferringo (1), Antonio Lopolito (1), Marcella Regina (1), Valerio Vetritto (1), Luigi Giangrossi (1), Antonio Fasanella(1), Domenico Galante (1)

(1) Istituto Zooprofilattico Sperimentale della Puglia e della Basilicata - Anthrax Reference Institute of Italy, Foggia, Italy

Corresponding Author: **Lorenzo Pace** - lorenzo.pace@izspb.it

Anthrax is a zoonotic disease caused by Bacillus anthracis, a Gram-positive, aerobic, spore-forming bacterium. Most anthrax vaccines for animals utilize the toxigenic and uncapsulated B.anthracis strain 34F2, responsible for the induction of protective antibodies (1).
The aim of this study was to set up and demonstrate the efficacy of a B.anthracis toxin neutralization assay (TNA) for the in vitro evaluation of the neutralizing antibody response induced following immunization with Sterne 34F2 vaccine. This test would allow to evaluate the efficacy of the Sterne vaccine without used of experimental infection with pathogenic strains of B.anthracis.
We used 10 New Zealand White rabbit and 10 sheep of a southern Italy farm vaccinated with two doses of Sterne vaccine. Five animals for species were used as a control. Sera were collected before the first vaccination (T0), before the booster dose (T1) and 15 days after the second dose (T2). The TNA was conducted using the RAW 264.7, a murine macrophage cell line. The 1:160 diluition of serum samples were tested to evaluate antibodies that neutralized the infectivity of B.anthracis toxins in cell monolayers. At each dilution of serum samples, 200 ng/ml of Recombinant Anthrax Protective Antigen and 640 ng/ml of Anthrax Lethal Factor were added (2,3).
To assess cell viability, we used the Vybrant® MTT Cell Proliferation Assay Kit, following the protocol indicated by the company.
The concentration of recombinant toxins used resulted in 80% mortality of cells in the absence of neutralizing antibodies. All serum samples analyzed from vaccinated animals showed production of neutralizing antibodies at both T1 and T2, while no neutralizing antibody response was found at T0 and in all control samples.
In vaccinated sheep, a significant booster effect of the vaccine dose was noted, rising from a cell viability of 32% at T1 to about 80% at T2. In contrast in rabbits there was no booster effect, in fact at both T1 and T2 the cell viability was about 80%.
In this study, the test developed and standardized showed that animals vaccinated with the Sterne 34F2 strain produced a good amount of antibodies neutralizing B.anthracis toxins, demonstrating how important this test is for determining post-vaccinal serology. Finally, the toxins neutralization test can offer some advantages such as (i) doesn't require the use of the challenge test with live pathogen strains, (ii) shows the real neutralizing effect of the antibodies produced.

[1] Adone R, Sali M, Francia M, Iatarola M, Donatiello A, Fasanella A. Development of a Sterne-Based Complement Fixation Test to Monitor the Humoral Response Induced by Anthrax Vaccines. Front Microbiol. 2016 Jan 28;7:19.
[2] Lu H, Catania J, Baranji K, Feng J, Gu M, Lathey J, et al. Characterization of the native form of anthrax lethal factor for use in the toxin neutralization assay. Clin Vaccine Immunol. 2013 Jul;20(7):986-97
[3] Ngundi MM, Meade BD, Lin TL, Tang WJ, Burns DL. Comparison of three anthrax toxin neutralization assays. Clin Vaccine Immunol. 2010 Jun;17(6):895-903.

ARNA

76° CONVEGNO SISVET
BARI, 21-22-23 GIUGNO 2023

METABOLIC PROFILE FOR THE EVALUATION OF ADAPTATION TO EXERCIZE IN YOUNG HORSES AT CONDITIONING

Senes A. (1), Podda A. (1), Carboni E. (1), Biggio G.P. (2), Cherchi R. (2), Cossu I. (2), Cappai M.G. (1)

(1) Department of Veterinary Medicine, University of Sassari, Via Vienna No. 2, 07100 Sassari, Italy
(2) Department of Equine Breeding and Reproduction Research, Autonomous Region of Sardinia, 4th of Lucrezia Borgia Square, 07040 Ozieri

Corresponding Author: **Maria Grazia Cappai** - mgcappai@uniss.it

Young horses undergoing conditioning adapt their metabolic response differently according to activity level and this is believed to be individual dependent. The aim of this research was to monitor the metabolic adaptation to exercise in young horses during conditioning, in comparison with adult horse undergoing same activity level to monitor the metabolic recovery from exercise required to bring selected parameters back to baseline values of pre-exercise.
A total of eight horses from a same stable (five females and three males, age: 2 years, n. 5; 3 years, n. 2; 7 years, n.1; Body weight, BW; 375 - 548 kg; body condition score 1-9: 4.5-5 points) were included in the study. Horses were housed in individual boxes within the same stable and were subjected to the same conditioning programme in covered paddock with sandy flooring, according to Sigler (2019) [1]. Animals were fed a diet consisting of high-quality hay and mixed compound feed, following the feeding practice of the stable to meet requirements [2], with *ad libitum* water. BW and heart rate (HR) were monitored: horses were sampled for whole blood before and within 0' (T1), 30' (T2), 60' (T3), 120' (T4) of exercise. Hemogram, biochemical profile and serum protein fractions were determined. All data were analyzed with a mixed procedure model with sampling time and time in training as fixed factors and individual horse as random effect.
Explored metabolic parameters varied in relation to the exercise and turned back to pre-exercise values differently for individual horse. However, the hematocrit (HCT) displayed to vary significantly ($p=0.03$; mean HCT: T0, 0.38; T1, 0.43; T2, 0.35; T3, 0.36; T4, 0.36, pooled-SD: 0.06) from values at rest and after exercise in all the horses involved, over time. Globulines (alfa2 and beta1) also displayed to vary significantly ($p=0.018$ and $p=0.001$, respectively).
Given the importance of HCT and ALB in body fluid distribution in individual horse over time for the establishment of recovery from exercise, those metabolic parameters may be good indicators to adapt specific nutrient provision accordingly and also for the transfer of experimentally acquired skills to the sport horse sector, through appropriate animal management.

[1] Sigler, D.H. (2019). Conditioning and Retraining the Equine Athlete
[2] NRC (2007), Nutrient Requirements of Horses. Ed. By National Research Council, Washington (USA)

76° CONVEGNO SISVET
BARI, 21-22-23 GIUGNO 2023

NUTRITIONAL MANAGEMENT OF HYPERLIPAEMIA AND NON ALCOHOLIC FATTY LIVER (NAFL) IN A SARDINIAN DONKEY

Sarah Morrone (1), Alice Senes (1), Sara Sechi (1), Eraldo Sanna Passino (1), Carlo Carta (1), Maria Grazia Cappai (1)

(1) Department of Veterinary Medicine, University of Sassari, Via Vienna No. 2, Sassari, 07100, Italy

Corresponding Author: **Maria Grazia Cappai** - mgcappai@uniss.it

This clinical case reports the nutritional approach in a Sardinian adult jenny suffering from severe malnutrition along with hyperlipaemia (common in donkeys with low feed intake [1,2,3]), likely following a traumatic event. The jenny came from a regional park in Sardinia (Italy) and showed to be underweight (body weight, BW: 88 kg; body condition score, BCS (1-5): 1.75), reluctant to eat, with poor faecal score (FS (1-8 point):2, watery/loose feces) and wounds complicated by ulcerations on the elbow, limbs and sternum. The results of analyses showed systemic inflammatory status and increased liver enzymes (ALB[g/l] 21,5-31,6: 17,6; AST[u/l] 279-430: 657; ALT[u/l] 5,0-14,0: 60,0) and hyperlipaemia (TG [mmol/l] 0,6-2,8: 8,7). On ultrasound examination, enlarged and hyperechogenic liver was also observed. The nutritional approach considered a two-step treatment, with subsequent feeding practices: at first, the animal was stimulated to consume feed voluntarily by using highly palatable hay and ad hoc feeding technique (free access to pasture and water, hay 1.80 kg/d and 50 g/d of mash compound feed). The jenny was weighed after one week (BW: 96 kg) and showed increased BCS (reaching 2.25 and FS: 4). The second step was intended to improve the body conditions and restore blood parameters and lasted 4 weeks. The feeding practice consisted of hay (1500g/d), mash compound (300g/d) and free access to pasture and water. After four weeks, the parameters improved markedly and got closer to the physiological range with apparent recovery. Appetite was improved along with overall nutritional conditions. The patient was then moved to graze in a plot with other donkeys to improve animal social and feeding behaviour. No changes in BCS, faecal score, or clinical condition were observed. After 30 weeks, blood parameters were monitored at the follow up complete blood cell count (CBC) along with nutritional biochemistry fell within physiological ranges. The jenny finally weighed 118 kg, with a normal BCS of 3 out of 5 and a faecal score of 5 out of 8. The two-step nutritional approach had remarkable results, with complete clinical and nutritional recovery of the patient in four weeks, beginning from a severe condition and poor health.

[1] Burden, F. A., Du Toit, N., Hazell-Smith, E., & Trawford, A. F. (2011). Hyperlipemia in a population of aged donkeys: description, prevalence, and potential risk factors. Journal of Veterinary Internal Medicine, 25(6), 1420–1425;
[2] Mendoza, F. J., Toribio, R. E., & Perez-Ecija, A. (2018). Donkey internal medicine—Part I: Metabolic, endocrine, and alimentary tract disturbances. Journal of Equine Veterinary Science, 65, 66–74;
[3] Thiemann, A. K., Buil, J., Rickards, K., & Sullivan, R. J. (2022). A review of laminitis in the donkey. Equine Veterinary Education, 34(10), 553–560.

EFFECTS OF SOYBEAN MEAL REPLACEMENT WITH 00-RAPESEED MEAL ON PARMIGIANO REGGIANO TYPE CHEESE CHARACTERISTICS

D. Cavallini (1), L. Mammi (1), A. Palmonari (1), G. Canestrari (1), M. Fustini (1), E. Bortolazzo (2), L. Grazia (1), A. Formigoni (1)

(1) University of Bologna
(2) CRPA

Corresponding Author: **Damiano Cavallini** - damiano.cavallini@unibo.it

Parmigiano Reggiano (PR) is one of the best-known and most exported Italian PDO cheese all over the world. A PDO product must be entirely manufactured within a delimited area, follow specific processing and feeding rules [1]. The objectives of this study were to investigate the effects of substituting soybean meal with rapeseed meal on PR type cheese quality and characteristics. The study was composed by a long-term farm trial with 40 free stall Holstein cows divided in two groups (TRT and CTR). Rations were formulated to mimic common TMRs used in the PR cheese production area of Italy. Treatments differed in protein source: CTR (9.3% DM basis of soybean meal) and TRT (13.2% 00-rapeseed meal DM basis). Other dietary components, as well as chemical composition of the diets, were the same among treatments, with different proportions to maintain isoenergetic and isoproteic level. The milk was sampled weekly and experimental cheese-making trials were conducted. Milk and cheese samples were analysed for composition, fatty acid profile, sensorial characteristics and goitrin content. Data were analyzed using a mixed model procedure. Results shown that no significant differences were reported in milk composition, coagulation time, nor on cheese yield. Main milk and cheese sensory characteristics were similar between treatments, with the exception of milk butter/cream and lactic acid odors, which resulted higher in TRT (+0.3 and +0.2 points). A more intense color and less hotness in TRT treatment cheese were observed. The inclusion of rapeseed meal in the ratio did not compromise the cheese-making characteristics, the quality of the milk and the PR type cheese produced. The cheese fatty acid profile resulted similar between treatments with higher long chain and polyunsaturated fatty acids (mainly n6) in TRT group. Goitrin resulted undetectable in CTR milk, while it was 1.43 µmol/g in TRT treatment. Overall, all the cheese samples resulted in no goitrin. In conclusion, our results show that rapeseed meal could be included in Italian PDO cheese regulation.

[1] Cavallini, D., Mammi, L. M. E., Biagi, G., Fusaro, I., Giammarco, M., Formigoni, A., & Palmonari, A. (2021). Effects of 00-rapeseed meal inclusion in Parmigiano Reggiano hay-based ration on dairy cows' production, reticular pH and fibre digestibility. Italian Journal of Animal Science, 20 (1). https://doi.org/10.1080/1828051X.2021.1884005

INTRODUCTION OF LIVE YEASTS, FERMENTATION EXTRACTS FROM ASPERGYLLUS ORYZAE AND SUCROSE IN FEED TO BE USED IN THE TRANSITION PERIOD OF THE DAIRY COW

Difonzo Michele Vito, Guareschi Andrea, De Carlo Domenico

Corresponding Author: **Vito Michele Difonzo** - m.v.difonzo60@gmail.com

The transition period also coincides with a higher incidence of two metabolic disorders: hypocalcemia (HC) and negative energy balance (NEB). Four Holstein farms were selected in the province of Bari, of which: two form control group A (CON) without STEAMING-UP, and two form sample B (SP) with " ICIM STEAMING-UP PL (sucrose, *Saccharomyces Cerevisiae* ncyc sc 47, fermentation extracts from *Aspergillus Oryzae*, MgCl2, NaCl)" feed administered to starting 20 days before delivery. Blood samples were taken from the animals' tail vein in sterile EDTA-containing tubes. A VetScan VS2 portable biochemistry analyzer was used for the analyses, on 40 cows (20 CON and 20 SP) at 7 days both before and after calving. To 7 days before calving, Ca values in SP are 2.47 mmol/L and in CON are 2,42 mmol/L, show no significant differences, the P levels in SP are 1.855 mmol/L and in CON are 2.54 mmol/L, so with a difference than 0.69 mmol/L (p=0.01), the of Mg in SP are 1.06 mmol/L and in CON are 0.87 mmol/L with a difference than 0.19 mmol/L, CK values in SP are 180 U/L and in CON are 311 U/L with a difference than 131 U/L (p=0.01), ALB in SP are 37 g/L and in CON are 32 g/L with an increase than 5 g/L (p=0.01); ALP in SP are 148 and in CON are 58 U/L with an increase than 90 U/L; AST in SP are 68 U/L and in CON are 145 with a difference than 77 U/L. To 7 days after calving, the SP group compared with the CON group had Ca levels in SP are 2.45 mmol/L and in CON are 2.28 mmol/L with an increase than 0.15 mmol/L, P in SP are 1.82 mmol/L and in CON are 2.32 mmol/L with a difference than 0.5 mmol/L (p=0.01), the of Mg in SP are 0.91 mmol/L and in CON are 0.94 mmol/L show no significant differences, ALB in SP are 36 g/L and in CON are 30 g/L with a difference than 6 g/L (p=0.01), AST in SP are 83 U/L and in CON are 162 g/L with a difference than 79 U/L (p=0.01), CK in SP are 159 U/L and in CON are 313 U/L with a difference than 154 U/L (p=0.01).At 7 postpartum, the SP group compared with the CON group had Ca levels greater than 0.15 mmol/L, P less than 0.5 mmol/L, ALB greater than 5.35 U/L, AST less than 79.3 U/L and CK less than 153.6 U/L. Analyzing the first functional check relating to multiparous cows, the average milk production in kg was: 34.5 kg in the CON group at about 20 DIM and 40.4 kg in the SP group at about 23 DIM, therefore production increased by 5.9 kg of milk. The use of STEAMING-UP ICIM PL, from 20 days before the birth, can improve milk production.

[1] R. C. Neves,B.M. Leno,T. Stokol,T. R. Overton,and J. A. A. McArt:Risk factors associated with postpartum subclinical hypocalcemia in dairy cows,J. Dairy Sci. 100:3796–3804

[2] Bouda, J., Doubek, J., Muzik, J., Toth, J: Parturition induction in cows and its effects in the development of biochemical and hematological values in the bloodofcalves.VetarinarniMedicina 39 223-230

[3] J.H. Silva, L.V.Q. Contreras,C.F. Ponath,C. Bondan,M.C. Machado, F. González:Blood concentrations of calcium, phosphorus and magnesium and dietary cation-anion difference in dairy cows Arq. Bras. Med. Vet. Zootec., v.75, n.1, p.127-136, 2023

[4] Wei Yang, Bingbing Zhang, Chuang Xu, Hongyou Zhang, Cheng Xia:Effects of ketosis in dairy cows on blood biochemical parameters, milk yield and composition, and digestive capacity J Vet Res 63, 555-560, 2019 DOI:10.2478/jvetres-2019-0059

EFFECT OF HYDROLYSABLE TANNINS' METABOLITES ON FEED FERMENTATION, GAS PRODUCTION AND RUMINAL ECOSYSTEM USING A LONG-TERM IN VITRO RUMEN FERMENTATION APPROACH (RUSITEC)

Michele Manoni (1), Sergej Amelchanka (2), Melissa Terranova (2), Luciano Pinotti (1,3), Paolo Silacci (4), Marco Tretola (1,4)

(1) University of Milan, Department of Veterinary Medicine and Animal Science, Via dell'Università 6, Lodi, 26900, Italy
(2) ETH Zurich, AgroVet-Strickhof, Eschikon 27, Lindau, 8315, Switzerland
(3) CRC I-WE, University of Milan, Milan, 20134, Italy
(4) Agroscope, Posieux, 1725, Switzerland

Corresponding Author: **Michele Manoni** - michele.manoni@unimi.it

Livestock production is currently facing the growing demand for animal-source food and the need to reduce the impact on the environment. Ruminants are among the largest contributors to greenhouse gas (GHG) emissions, in particular methane (CH4). Different strategies to lower greenhouse gas (GHG) emissions in ruminants are being investigated, and one of them relies on the dietary tannin supplementation. The dietary supplementation with tannins in ruminants can affect the rumen microbial community and thus the enteric fermentation, leading to reduced methane emissions. Following a previous *in vitro* screening, we investigated the effect of two hydrolysable tannin metabolites, named ellagic acid (EA) and gallic acid (GA), in a long-term in vitro rumen fermentation approach. EA and GA were supplemented to a control diet (CTR: ryegrass hay and barley concentrate (7.5:2.5), 10 g DM/day) in an 8-fermenter rumen simulation technique (Rusitec), running for 10 days. Three experimental conditions were investigated: i) EA 75 mg/g DM, ii) GA 75 mg/g DM, iii) EA 75 mg/g DM + GA 75 mg/g DM. The measurements were performed in the last 5 days of the experimental period. Total gas production was not significantly altered over the last 5 days, whereas daily
methane (CH4) production was significantly decreased by EA (-45%) and EA+GA (-60%), compared to control. CH4 production per unit of dietary organic matter (OM) and short-chain fatty acids (SCFA) was also reduced by EA (-48% and -32%) and EA+GA (-65% and -58%), and less by GA (-19% and -22%). Ammonia formation was significantly reduced by EA (-46%), GA (-19%) and EA+GA (-86%). Total SCFA production was decreased by EA and EA+GA (-26%, -16%), but not by GA. Similarly, EA and EA+GA, but not GA, reduced rumen degradability of OM, crude fibre (CF) and crude protein (CP). All the treatments increased the bacterial count and decreased the protozoal count (except for GA). Furthermore, EA and EA+GA modulated the relative abundance of selected fibrolytic and cellulolytic rumen bacterial taxa. The results showed that both EA and GA decreased average daily CH4 production and ammonia formation, with EA being most effective than GA for almost all the parameters observed. Nevertheless, GA showed a lower impairing effect on the degradability of nutrients in rumen and on SCFA production. Further research on the dynamics of rumen microbiota and the metabolism of hydrolysable tannins in rumen will strengthen the outcome of this study.

[1] Soliva CR, Amelchanka SL, Duval SM, Kreuzer M. Ruminal methane inhibition potential of various pure compounds in comparison with garlic oil as determined with a rumen simulation technique (Rusitec). Br. J. Nutr. 2011, 106:114-22.
[2] Soliva CR, Amelchanka SL and Kreuzer M. The requirements for rumen-degradable protein per unit of fermentable organic matter differ between fibrous feed sources. Front. Microbiol. 2015, 6:715.
[3] Li Y, Kreuzer M, Clayssen Q, Ebert MO, Ruscheweyh HJ, Sunagawa S, Kunz C, Attwood G, Amelchanka S, Terranova M. The rumen microbiome inhibits methane formation through dietary choline supplementation. Sci. Rep. 2021, 11:21761.

76° CONVEGNO SISVET
BARI, 21-22-23 GIUGNO 2023

PRECISE MEASUREMENT OF RADIOSTRONTIUM AND URANIUM ISOTOPES IN ANIMAL FEEDS BY LIQUID SCINTILLATION COUNTING AND ALPHA SPECTROMETRY

Gabriele Trotta (1), Nicola Bortone (1), Rita Damiano (1), Michele Nicolini (1), Valeria Nardelli (1), Marco Iammarino(1)

(1) Istituto Zooprofilattico Sperimentale della Puglia e della Basilicata - Centro di Referenza Nazionale per la Ricerca della Radioattività nel Settore Zootecnico – Veterinario

Corresponding Author: **Marco Iammarino** - marco.iammarino@izspb.it

Radiostrontium (Sr-90) and Uranium (U) are two chemical elements affecting the health of livestock if present in feed. Possible health effects can range from reduced growth and reproduction to death. Therefore, measuring Sr-90 and U levels in animal feed is crucial for ensuring both animal wellness and the safety of derived products such as meat, milk, cheese and eggs. Recent studies demonstrated that the levels of Sr and U in pet food can exceed the safety limits set by National authorities [1]. Furthermore, consumption of contaminated feed can lead to accumulation of Sr-90 and U in animal tissues, increasing the overall human exposure and associated risk. More in depth, high levels of these elements in feed can influence the chemical composition of milk and meat, compromising their quality and safety for human consumption [3]. Measuring Sr-90 and U levels in animal feeds is therefore essential for ensuring animal wellness and the safety of food of animal origin. Given the possible negative effects of these radionuclides on animal health and food safety, National authorities and livestock producers have to adopt preventive measures to minimize Sr-90 and U contamination in animal feed.

In this work, the results obtained from the development of a rapid and innovative radiochemical method for determining Sr-90 and U isotopes concentration in animal feed are described.

This new method is based on the use of a characteristic scintillation cocktail obtained by organic solvents and "doped" in such a way as to selectively chelate both U and Y-90, the short-lived daughter of Sr-90, then emitting ultraviolet light with a specific spectrum, also capable of alpha/beta discrimination. Intralaboratory comparisons with accredited methods of the National Radioactivity Reference Center [4], evaluated as correlation between detected levels (R2), also demonstrated the robustness and reliability of this method, suitable as both screening and confirmatory tool.

Moreover, the results of a national monitoring developed to evaluate the overall contamination level of different types of feed are reported and commented. Low levels of Sr-90 (ranging from 0.07 to 8.67 Bq/kg) and uranium isotopes (ranging from 0.05 to 20 Bq/kg) were found. Considering the typical feed consumption, the toxicological reference data available from IAEA and related mathematical models, the average annual dose of Sr-90 and U was calculated for different animals, confirming safe levels, below 1.0 mSv/y.

[1] Chen, C., Wang, J., Zhang, J., Zhou, Y., & Zhang, Z. (2018). Radioactive strontium (90Sr) and uranium (U) in pet food: a potential health threat to pets and their owners. Environmental Science and Pollution Research, 25(29), 29193-29201. DOI: 10.1007/s11356-018-2952-4

[2] Wang, J., Zhang, J., Chen, C., Zhou, Y., & Zhang, Z. (2017). Levels of radioactive strontium (90Sr) and uranium (U) in swine feed in China. Environmental Pollution, 222, 74-82. DOI: 10.1016/j.envpol.2016.12.021

[3] Li, S., Zhang, Y., Wang, J., Zhang, J., & Zhou, Y. (2021). Effect of dietary strontium and uranium on milk quality and heavy metal accumulation in lactating cows. Environmental Science and Pollution Research, 28(13), 16602-16611. DOI: 10.1007/s11356-020-12146-0

[4] Marchesani G., Trotta G., De Felice P., Bortone N., Damiano R., Nicolini M., Accettulli R., Chiaravalle A.E., Iammarino M. (2022). Fast and Sensitive Radiochemical Method for 90Sr Determination in Food and Feed by Chromatographic Extraction and Liquid Scintillation Counting Food Analytical Methods, 15 (6), pp. 1521-1534.

RNIV

76° CONVEGNO SISVET
BARI, 21-22-23 GIUGNO 2023

EFFECT OF REARING SYSTEMS ON IMMUNE STATUS, STRESS PARAMETERS, INTESTINAL MORPHOLOGY AND MORTALITY IN CONVENTIONAL AND LOCAL CHICKEN BREEDS

Valentina Stefanetti (1), Alice Cartoni Mancinelli (2), Luisa Pascucci (1), Laura Menchetti (3), Cesare Castellini (2), Cecilia Mugnai (4), Edoardo Fiorilla (4), Barbara Miniscalco (4), Diletta Chiattelli (2), Maria Pia Franciosini (1), and Patrizia Casagrande Proietti (1)

(1) Department of Veterinary Medicine, University of Perugia, Via S. Costanzo 4, 06124 Perugia
(2) Department of Agricultural, Environment and Food Science, University of Perugia, Borgo XX Giugno 74, 06124, Perugia
(3) Department of Agricultural and Agri-Food Sciences and Technologies, University of Bologna, Viale Fanin 46, 40138 Bologna
(4) Department of Veterinary Sciences, University of Turin, Largo Braccini 2, 10095, Grugliasco, Torino

Corresponding Author: **Valentina Stefanetti** - valentina.stefanetti@unipg.it

Most poultry meat derived from intensively-housed birds (1). However, in recent years, consumer preference has demanded poultry producers to develop more sustainable farming systems. Although free-range system is considered beneficial for animal welfare, it is less standardizable than intensive one, thus the choice of bird genotype appears to be crucial (2). In the present study, we aimed to evaluate the effect of conventional and free-range rearing systems on immune status, stress parameters, intestinal morphology, and mortality in commercial hybrid (Ross-308) and local poultry strains, Bionda Piemontese (BP), Robusta Maculata (RM), BP x Sasso (BPxS), RM x Sasso (RMxS). Three hundred chicks of five different genotypes were randomly divided into two rearing systems. At the slaughterhouse, blood samples were collected for heterophil/lymphocyte ratio determination; a segment of jejunum was fixed in formalin and RNA Later for morphological and biomolecular analyses, respectively. It has been evaluated the relative expression of interleukin (IL)-2, IL-6, IL-10, IL-18, IL-1β, inducible nitric oxide synthase (iNOS), toll-like receptor (TLR)-4 and interferon gamma (IFN-γ) (3). Data were statistically analyzed by two-way ANOVA. We found that the rearing system significantly affected the expression of IL-10, iNOS, IL-2 and IL-6, where these genes were up-regulated in free-range system. A significant interaction between the rearing system and the genotype was also showed. Within the free-range rearing system, the BPxS, BP, RM showed significantly higher expression values of IL-6 and iNOS compared to the broiler line. This could be due to allocation resource theory which postulates that in fast-growing animals, the body energy is mainly allocated toward growth and maintenance and thus the amount required to mount the immune response could be insufficient. In accordance with this hypothesis, we found significant increased H/L ratio, mortality rates and worse jejunum morphology in Ross hybrids, compared to the local breeds in free-range system. Overall, our results confirm that the intense selection for growth in broiler chickens may have reduced their ability to react to the environmental stimuli related to free-range system, resulting in lower adaptability, making them inappropriate for alternative farming system (4). On the contrary, local chicken breeds are able to adapt and survive to the free-range system, thus representing an important genetic resource.

[1] Scheuermann, G. N., Bilgili, S. F., Hess, J. B. & Mulvaney, D. R. Breast muscle development in commercial broiler chickens. Poult Sci 82, 1648–1658 (2003).
[2] Dal Bosco, A., Mattioli, S., Cartoni Mancinelli, A., Cotozzolo, E. & Castellini, C. Extensive Rearing Systems in Poultry Production: The Right Chicken for the Right Farming System. A Review of Twenty Years of Scientific Research in Perugia University, Italy. Animals (Basel) 11, 1281 (2021).
[3] L Jarosz, A Marek, Z Gradzki, M Kwiecien, B Zylinska, B Kaczmarek.Effect of feed supplementation with zinc glycine chelate and zinc sulfate on cytokine and immunoglobulin gene expression profiles in chicken intestinal tissue. Poult Sci. 2017 Dec 1;96(12):4224-4235.
[4] Castellini, C., Bosco, A. D., Mugnai, C. & Bernardini, M. Performance and behaviour of chickens with different growing rate reared according to the organic system. Italian Journal of Animal Science 1, 290–300 (2002).

NITRIC OXIDE-RELEASING SOLUTION: NEW COMPOUNDS TO COMBAT ANTIBIOTIC RESISTANCE IN LIVESTOCK

Chiara Grazia De Ciucis (1), Chiara Guglielmetti (1), Angela Pontei (1), Veronica Planta (1), Elena Berrone (1), Cristiano Corona (1), Valeria Listorti (1), Floriana Fruscione (1), Lisa Guardone (1), Alessandro Ricci (2), Claudio Bellino (2), Alessandro Dondo (1), Livia De Paolis (1), Simona Zoppi (1), Elisabetta Razzuoli (1)

(1) Istituto Zooprofilattico Sperimentale del Piemonte Liguria e Valle d'Aosta, Italy
(2) Dipartimento di Scienze Veterinarie, Italy

Corresponding Author: **Elisabetta Razzuoli** - elisabetta.razzuoli@izsto.it

Bacterial diseases represent one of the major economic losses in cattle breading. To date antibiotics represent the gold standard therapy for uterine (UP) and respiratory pathologies (RP). The incorrect use or the repetition of treatments can cause selection of antimicrobial resistant bacteria (1). In the last years, Nitric Oxide-Releasing Solution (NORS), a liquid formulation that releases nitric oxide, a broad-spectrum antimicrobial substance, was demonstrated to be a potential antimicrobial treatment for bovine mastitis. Two experiments were performed, as previously described (2), to evaluate: a) cytotoxicity and immunomodulatory properties of NORS' on continuous (IPEC-J2) and primary (FLECA) cell lines in terms of gene expression; b) NORS' effect on different pathogens. The in vitro experiments were repeated thrice and the following concentrations of NORS were tested: 75, 45, 22.5, 9, 3 and 1 mM. Our results showed a significant cytotoxic effect ($p<0.05$) both on FLECA and IPEC-J2 by 75 mM. IPEC-J2 were also sensitive to 45 mM, so we decided to use a concentration ≤22.5 mM for other tests.

Treatments with NORS caused a down regulation of MYD88 and an up-regulation of TLR4 at 22.5 mM, after 24 h of exposure in FLECA cell lines. Moreover 3 mM caused a down-regulation of inflammation (decreased gene expression of NFKB1, MYD88, IL18 and TGFB). In IPEC-J2 we observed at 22.5 mM, after 24 h of exposure, up regulation of CXCL8, NOS2, TLR5 and IFNB.

Concerning antibacterial activity, the experiments were performed, initially, with NORS solutions at 75, 45 and 22.5 mM for 2, 15 and 30 minutes on different bacterial strains (*E. coli, C. perfringens, P. multocida, Mycoplasma mycoides subsp. capricolum, T. pyogenes, S. Typhimurium, H. somni*). With NORS solutions at 75 and 45 mM, after 15 minutes, there is a total inhibition of bacterial growth for all strains. Further experiments are still ongoing at lower mM concentrations in order to identify the lowest concentration able to guarantee a bacteriostatic action, while limiting the potential inflammatory risk.

Our preliminary data would suggest to use NORS in vivo test at 22.5 mM for 24 h. Indeed, this combination of concentration and time does not determine alteration of innate immunity in terms of inflammation in uterine and intestinal cell line.

[1] Trevisi, E.; Zecconi, A.; Cogrossi, S.; Razzuoli, E.; Grossi, P.; Amadori, M. Strategies for Reduced Antibiotic Usage in Dairy Cattle Farms. Res. Vet. Sci. 2014, 96, 229–233, doi:10.1016/j.rvsc.2014.01.001.
[2] Capellini, F.M.; Vencia, W.; Amadori, M.; Mignone, G.; Parisi, E.; Masiello, L.; Vivaldi, B.; Ferrari, A.; Razzuoli, E. Characterization of MDCK Cells and Evaluation of Their Ability to Respond to Infectious and Non-Infectious Stressors. Cytotechnology 2020, 72, 97–109, doi:10.1007/s10616-019-00360-z.

UNVEILING THE TUMOR IMMUNE MICROENVIRONMENT (TIME) OF ECPV2-ASSOCIATED EQUINE VULVO-VAGINAL SQUAMOUS CELL CARCINOMAS (EVSCCS)

Livia De Paolis (1), Ilaria Porcellato (2), Samanta Mecocci (2), Luca Mechelli (2), Benedetta Passeri (3), Malgorzata Ciurkiewicz (4), Chiara Grazia De Ciucis (1), Katia Varello (5), Vittoria Montemurro (5), Floriana Fruscione (1), Katia Cappelli (1), Wolfgang Baumgärtner (4), Christina Puff (4), Alessandro Ghelardi (6), Federico Armando (4) and Elisabetta Razzuoli (1)

(1) Istituto Zooprofilattico Sperimentale del Piemonte Liguria e Valle d'Aosta, Centro di Referenza Nazionale Oncologia Veterinaria e Comparata, S.S Genova, Piazza Borgo Pila 24-39, 16129-Genova, Italy
(2) Università degli Studi di Perugia, Dipartimento di Medicina Veterinaria, Via San Costanzo 4, 06126, Perugia, Italy
(3) Università degli Studi di Parma, Dipartimento di Medicina Veterinaria, Via Taglio, 8, 43100 Parma, Italy
(4) Department of Pathology, University of Veterinary Medicine Hannover, Bünteweg 17, 30559 Hannover, Germany
(5) Istituto Zooprofilattico Sperimentale del Piemonte Liguria e Valle d'Aosta, Via Bologna, 148, 10154 Torino, Italy
(6) Azienda Usl Toscana Nord-Ovest, UOC Ostetricia e Ginecologia, Ospedale Apuane, Massa, Italy

Corresponding Author: **Livia De Paolis** - livia.depaolis@izsto.it

Squamous cell carcinoma (SCC) represents the most common malignant cutaneous tumor in horses, accounting for 7-37% of all skin lesions. It can develop at any site on the skin and mucosa, although non-pigmented skin and muco-cutaneous junctions, such as external genitalia of both male and female horses, are most likely the preferential sites of SCCs [1]. Growing evidence has been indicating *Equus Caballus papillomavirus* type 2 (EcPV2) as the causative agent of equine penile and vulvar SCCs [2]. Among these tumors, equine vulvo-vaginal SCC (evSCC) represents a malignant neoplasia of unknown prevalence in mares, generally occurring as a de novo onset or arising from precursor lesions such as plaques and papillomas [1]. Recently, tumor immune microenvironment (TIME) of EcPV2-induced equine penile SCC (epSCC) has been investigated, highlighting histological and immunephenotypical similarities to human papillomavirus (HPV)-induced SCC [3]. Thus far, little is known about the role of host's immune response in EcPV-induced evSCC. The aim of the study was to investigate the TIME of evSCC, trying to unveil the interplay between EcPV2 and the host's immune system. To this purpose, 11 malignant lesions (M), 8 preneoplastic/benign lesions (PB) and 5 healthy controls (C) were retrospectively selected. Real Time PCR was performed for assessing the presence of EcPV2 DNA, while E6/E7 oncogene expression was evaluated by RNAscope. All PB and M samples were positive for EcPV2-L1 viral DNA and showed the expression of E6/E7 oncogenes. In order to investigate the expression of immune-related markers, immunohistochemistry (IHC) and RT-qPCR were carried out. Comparing the intratumoral and extratumoral area of samples at IHC, CD3+, MPO+ and MUM1+ cells were higher within the intratumoral tissue, while CD20+ cells were higher in the extratumoral compartment. When comparing the PB and M lesions, the expression of intratumoral CD204+ cells was significantly higher within M group (Mann-Whitney test). RT-qPCR results highlighted a significant upregulation of interleukin 8 gene (CXCL8), interferon gamma (IFNG), and interleukins IL12A and IL23 in M with respect to C (Kruskal Wallis test). Moreover, IL17A was significantly increased in M when compared to both PB and C. Finally, our results suggest the presence of a protumoral state within the TIME, characterized by a M2-polarization of macrophages CD204+ [4]. However, further studies are needed to confirm these preliminary results.

[1] Sykora, S.; Brandt, S. Papillomavirus Infection and Squamous Cell Carcinoma in Horses. Vet. J. Lond. Engl. 1997 2017, 223, 48–54, doi:10.1016/j.tvjl.2017.05.007.
[2] Ramsauer, A.S.; Kubacki, J.; Favrot, C.; Ackermann, M.; Fraefel, C.; Tobler, K. RNA-Seq Analysis in Equine Papillomavirus Type 2-Positive Carcinomas Identifies Affected Pathways and Potential Cancer Markers as Well as Viral Gene Expression and Splicing Events. J. Gen. Virol. 2019, 100, 985–998, doi:10.1099/jgv.0.001267.
[3] Porcellato, I.; Mecocci, S.; Mechelli, L.; Cappelli, K.; Brachelente, C.; Pepe, M.; Orlandi, M.; Gialletti, R.; Passeri, B.; Ferrari, A.; et al. Equine Penile Squamous Cell Carcinomas as a Model for Human Disease: A Preliminary Investigation on Tumor Immune Microenvironment. Cells 2020, 9, 2364, doi:10.3390/cells9112364.
[4.] Seung, B.-J.; Lim, H.-Y.; Shin, J.-I.; Kim, H.-W.; Cho, S.-H.; Kim, S.-H.; Sur, J.-H. CD204-Expressing Tumor-Associated Macrophages Are Associated With Malignant, High-Grade, and Hormone Receptor–Negative Canine Mammary Gland Tumors. Vet. Pathol. 2018, 55, 417–424, doi:10.1177/0300985817750457.

76° CONVEGNO SISVET
BARI, 21-22-23 GIUGNO 2023

IN VITRO EVALUATION OF ANTICANCER ACTIVITY OF ATTENUATED *SALMONELLA TYPHIMURIUM* (STM6ZNUABC) ON CANINE MAMMARY TUMOR

Livia De Paolis (1), Barbara Chirullo (2), Genny Del Zotto (3), Filippo Dell'Anno (1), Michela Tarantino (2), Ilaria Porcellato (4), Paola Petrucci (2), Chiara Grazia De Ciucis (1), Antonello Bufalari (4), Katia Cappelli (4), Giulia Moretti (4), Samanta Mecocci (4), Eleonora Monti (4), Floriana Fruscione (1) and Elisabetta Razzuoli (1)

(1) National Reference Center of Veterinary and Comparative Oncology (CEROVEC), Istituto Zooprofilattico Sperimentale del Piemonte, Liguria e Valle D'Aosta, Piazza Borgo Pila 39/24, 16129 Genoa, Italy
(2) Unit of Emerging Zoonoses, Department of Food Safety, Nutrition and Veterinary Public Health Istituto Superiore di Sanità, Viale Regina Elena 299, 00161 Rome, Italy
(3) IRCCS Istituto Giannina Gaslini, Genoa, Italy
(4) Department of Veterinary Medicine, University of Perugia, Via San Costanzo 4, 06126 Perugia, Italy

Corresponding Author: **Livia De Paolis** - livia.depaolis@izsto.it

Cancer represents a leading cause of death worldwide and remains one of the most critical challenges, constantly driving scientists' attention. Although conventional antineoplastic treatments have undergone significant improvement, the increasing prevalence of drug-resistant tumors prompted the need for alternative strategies against cancer [1]. In the last few decades, bacteria-based cancer therapies have been proposed as alternative strategies in oncology, due to their anti-inflammatory and immunogenic properties [2]. Most recently, several studies highlighted tumor-targeting and antitumor activities of an attenuated mutant strain of Salmonella enterica ser. Typhimurium (STMΔznuABC) [3]. However, it is unclear whether this activity is tumor-induced or species-specific nor the mechanism of the pathogen-host-tumor interaction is known. Thus far, the anticancer activity of STM6znuABC has been tested on several cell lines, including murine and human ones, but no data are present in literature regarding STM6znuABC effects on canine mammary tumors (CMTs). The aim of the study was to evaluate in vitro the antineoplastic and immunogenic properties of STM6znuABC on CMTs. To this purpose, four CMT cell lines (CF33, TM51, TM52, TM53) were treated with STM6znuABC for 1 h. Antiproliferative activity (MTT assay), bacterial invasion and gene expression analysis (RT-qPCR) of innate immune-related markers were evaluated. According to the results obtained, STMΔznuABC was able to penetrate in TM51 (Log 2 33.4±10.3), TM52 (Log 2 32.2±7.1), TM53 (Log 2 29.8±08.5) and CF33 (Log 2 10.6±2.7), with significant reduction of cell viability. Indeed, STM6znuABC infection led to a significant upregulation of interleukins 8 (CXCL8), IL18, IL10, Toll-like Receptor 4 (TLR4) and DNA repair protein homolog 1 (RAD51) in TM53, while cluster of differentiation 14 (CD14), interleukins IL4, IL6, Tumor Protein 53 (TP53), Phosphatase and Tensin homolog (PTEN) , Signal Transducer and Activation of Transcription 5 (STAT5), Toll-like Receptor 5 (TLR5) and Transforming Growth Factor B (TGFB) were downregulated. Similar results were highlighted in CF33 and TM52 cell lines. Concerning TM51, a significant downregulation of CXCL8, cluster of differentiation 44 (CD44), erb-b2 receptor tyrosine kinase (ERRB2) and TP53 was highlighted. This study demonstrated the anti-proliferative and immunomodulatory effects of STM6znuABC on CMTs cell lines, paving the way for a new anticancer treatment.

[1] Pucci, C.; Martinelli, C.; Ciofani, G. Innovative Approaches for Cancer Treatment: Current Perspectives and New Challenges. ecancermedicalscience 2019, 13, 961, doi:10.3332/ecancer.2019.961.
[2] Hoffman, R.M. The Preclinical Discovery of Bacterial Therapy for the Treatment of Metastatic Cancer with Unique Advantages. Expert Opin. Drug Discov. 2012, 7, 73–83, doi:10.1517/17460441.2012.644534.
[3] Chirullo, B.; Ammendola, S.; Leonardi, L.; Falcini, R.; Petrucci, P.; Pistoia, C.; Vendetti, S.; Battistoni, A.; Pasquali, P. Attenuated Mutant Strain of Salmonella Typhimurium Lacking the ZnuABC Transporter Contrasts Tumor Growth Promoting Anti-Cancer Immune Response. Oncotarget 2015, 6, 17648–17660, doi:10.18632/oncotarget.3893.

EVALUATION OF IMMUNE GENE EXPRESSION IN *MARCHIGIANA* BREED CATTLE NATURALLY INFECTED WITH *MYCOBACTERIUM AVIUM* SUBSP. *PARATUBERCULOSIS* TO CHARACTERIZE PROFILES OF PARATUBERCULOSIS SUSCEPTIBLE OR RESISTANT ANIMALS

Katia Cappelli (1), Samanta Mecocci (1), Linda Petrucci (2), Martina Torricelli (2), Roberta Ratto (1), Federica Riva (3), Marco Ermini (4), Carla Sebastiani (2), Massimo Biagetti (2), Anna Fratto (2), Piera Mazzone (2)

(1) Dipartimento di Medicina Veterinaria - Università degli Studi di Perugia
(2) Istituto Zooprofilattico Sperimentale dell'Umbria e delle Marche "Togo Rosati"
(3) Dipartimento di Medicina Veterinaria - Università degli Studi di Milano
(4) Azienda Sanitaria Territoriale Macerata – Servizio Veterinario di Sanità Animale

Corresponding Author: **Katia Cappelli** - katia.cappelli@unipg.it

Bovine paratuberculosis (PTB) is a chronic enteritis caused by *Mycobacterium avium* ssp. *paratuberculosis* (MAP). Infection of calves occurs early via fecal-oral route but the clinical signs of PTB do not appear always and anyway not before 2-3 years of age. In the early stages of infection, the host innate and cell-mediated immunity are essential to contrast MAP infection or to contain PTB progression, and thus it is related to PTB susceptibility/resistance (S/R). After MAP ingestion, its interaction with the intestinal immune cells induces the expression of proinflammatory cytokines, differentiation of T helper1 (Th1) cells, secretion of interferon-gamma (IFN-γ) and activation of macrophages. Transition from latent to clinical PTB occurs later, when Th2 cells secrete anti-inflammatory cytokines that reduce macrophage activation and Th1 cytokine secretion, while promoting humoral response (1). Thus, peripheral blood cells show different cytokines' gene expression profiles depending on the different stage of PTB progression. Several studies investigated immune regulatory genes variability, as a quote of genetic heritability exists in determining the different response to MAP infection (2, 3). Through a longitudinal study, in a MAP-infected herd of *Marchigiana* breed cattle, IFN-γ tests, ELISA, qPCR, and cultures were performed to evaluate MAP infection progression. Fifteen subjects were categorized in three phenotypic groups of five each: healthy uninfected; healthy but MAP-infected (IFN-γ test positive); PTB affected cattle (ELISA, qPCR, culture positive). Gene expression levels of interleukins (*IL1α*, *IL17A*, *IL12B*, *IL18*, *IL36*), IL receptors (*IL10RA*, *IL36RN*), tumor necrosis factor alpha (*TNFα)* and *IFNγ* were evaluated by RT-qPCR to characterize the profiles of PTB S/R animals starting from whole blood post-stimulation with specific antigens Avian (A)PPD and Johnin (J)PPD. Four out of eight tested genes resulted modulated among experimental conditions. A significant up-regulation ($p < 0.05$) of *IL18* and *IL1α* was observed in infected animals compared to healthy ones after stimulation with APPD and JPPD, while *IFNγ* resulted up-regulated in both infected and affected animals compared to the healthy subjects. Considering the overall infected animal group, an up-regulation of *IL36RN* was found compared to healthy animal group.
These data, defining immunogenetic pathways, allow us to better characterize cattle breeds carriers of potential PTB genetic resistance traits.

[1] Stabel JR, Bannantine JP. (2020) Divergent antigen-specific cellular immune responses during asymptomatic subclinical and clinical states of disease in cows naturally infected with *Mycobacterium avium subsp. paratuberculosis*. Infect Immun 88:e00650-19. https://doi.org/10.1371/journal.pone.0196502
[2] Park H-E, Park H-T, Jung YH, Yoo HS (2018) Gene expression profiles of immuneregulatory genes in whole blood of cattle with a subclinical infection of *Mycobacterium avium subsp. paratuberculosis*. PLoS ONE 13(4): e0196502. https://doi.org/10.1371/journal. pone.0196502
[3] Mazzone P, Di Paolo A, Petrucci L, Torricelli M, Corneli S, Sebastiani C, Ciullo M, Sebastianelli M, Costarelli S, Scoccia E, Sbarra F, Gabbianelli F, Chillemi G, Valentini A, Pezzotti G and Biagetti M. (2023) Evaluation of Single Nucleotide Polymorphisms (SNPs) Associated with Genetic Resistance to Bovine Paratuberculosis in Marchigiana Beef Cattle, an Italian Native Breed. Animals 2023, 13, 587. https://doi.org/10.3390/ani13040587

76° CONVEGNO SISVET
BARI, 21-22-23 GIUGNO 2023

DECIPHERING THE TRANSCRIPTION REGULATION OF HORSE BOVINE PAPILLOMAVIRUS-RELATED SARCOIDS THROUGH OMIC INTEGRATED APPROACH

Samanta Mecocci (1,2), Livia De Paolis(3), Luca Mechelli(1), Roberta Ratto(1), Rodolfo Gialletti(1), Benedetta Passeri(4), Porcellato Ilaria(1), Capomaccio Stefano(1,2), Alessandro Ghelardi(5), Elisabetta Razzuoli(1), Katia Cappelli(1,2)

(1) Department of Veterinary Medicine, University of Perugia, 06123 Perugia, Italy
(2) Sports Horse Research Center (CRCS), University of Perugia, 06123 Perugia, Italy

Corresponding Author: **Samanta Mecocci** - samantamecocci@gmail.com

Sarcoids are reported to be the most common skin tumors in horses and approximately represents up to 90% (35-90%) of skin neoplasms in this species, often present (14-84% of cases) as multiple tumors [1]. This is a highly impacting pathology for the veterinary field due to the high incidence, resistance to therapy and the presence of recurrences. Although the etiology is multifactorial, the association between genetic factors and bovine papillomaviruses (PVs) types 1, 2 and 13 infections seem to underlie the tumor development [2]. PVs are DNA viruses with tropism for skin and mucous membrane cells; the infection occurs through microlesions that allow the virus to penetrate and reach the basal layer of the epithelium where the viral genome can persist in episomal form or be integrated into the cellular genome [3]. In sarcoids related to BPVs, the tumor progression is often associated with an altered immune response, and studies are needed to identify a suitable therapy. The aim of this work was to better understand the host-pathogen interaction by implementing knowledge on the tumor microenvironment in terms of sarcoid lesion gene expression. To this purpose, sarcoid lesions from 12 affected animals, resulted positive to BPV infection, and 12 portion of healthy skin were selected. In order to evaluate the whole content of mRNAs and small RNAs through an high-throughput NGS approach, total RNA was extracted and two separated Illumina® sequencing libraries were constructed. Two dedicated conventional pipelines were applied for the analysis of the two RNA types, obtaining two sets of differentiallly expressed genes (DEGs): 6036 for mRNA (3620 down-regulated and 2415 up) and 106 micro-RNAs (miRNAs - 40 down and 66 up) in sarcoid lesions compared to healthy skin. For miRNAs all target mRNAs were retrieved and subjected to a functional analysis together with DEGs. The most enriched biological processes for miRNA targets were related to transcriptional modulations, cytoskeleton organization processes, DNA and RNA packaging and transport and host-viral interaction. While for DEGs, cellular pathways implicated in cytokine and chemokine production, growth factors and collage binding were found. Moreover, cell adesion and development, tissue morphogenesis, inflammatory and immune response were found as enriched biological processes. Overall all these processes could be related to infection and cellular transformation.

[1] Bergvall KE. Sarcoids. Vet Clin North Am Equine Pract. 2013 Dec;29(3):657-71. doi: 10.1016/j.cveq.2013.09.002. PMID: 24267682.
[2] Chambers G, Ellsmore VA, O'Brien PM, Reid SWJ, Love S, Campo MS, Nasir L. Association of bovine papillomavirus with the equine sarcoid. J Gen Virol. 2003 May;84(Pt 5):1055-1062. doi: 10.1099/vir.0.18947-0. PMID: 12692268.
[3] Munday JS. Bovine and human papillomaviruses: a comparative review. Vet Pathol. 2014 Nov;51(6):1063-75. doi: 10.1177/0300985814537837. Epub 2014 Jun 30. PMID: 24981715.

IMMUNOMODULATORY POTENTIAL OF SMALL RNAS ENCLOSED IN WATER BUFFALO'S EXTRACELLULAR VESICLES FROM COLOSTRUM AND MILK

Samanta Mecocci (1), Daniele Pietrucci (2), Marco Milanesi (3), Stefano Capomaccio (1), Chiara Evangelista (3), Loredana Basiricò (4), Umberto Bernabucci (4), Giovanni Chillemi (3), Katia Cappelli (1)

(1) Department of Veterinary Medicine, University of Perugia, Perugia, Italy
(2) Institute of Biomembranes, Bioenergetics and Molecular Biotechnologies, IBIOM, CNR, Bari, Italy
(3) Department for Innovation in Biological, Agro-food and Forest systems (DIBAF), University of Tuscia, Viterbo, Italy
(4) Department of Agriculture and Forest Sciences (DAFNE), University of Tuscia, Viterbo, Italy

Corresponding Author: **Katia Cappelli** - katia.cappelli@unipg.it

Extracellular vesicles (EVs) are small signaling structures that recently gain lively interest for their capability to cross biological barriers, resist acidification in the gastric environment and, most importantly, to exert modulation of the immune system, mainly through their cargo (mostly miRNA) [1]. Since milk is particularly enriched in EV (mEVs) we tried to characterize the small-RNA cargo of colostrum EVs (colosEVs) and mEVs of Mediterranean buffalo through a next generation sequencing approach [2,3]. Colostrum (immediately after parturition) and milk (50 days after) were collected from 7 subjects reared in central. ColosEVs and mEVs were isolated through differential centrifugations, an EDTA treatment and ultracentrifugations were performed to recover vesicles in the pellets [4]. Transmission electron microscopy for shape and contamination assessment, and Exoview technology for concentration, dimension and positivity to EV-markers were applied and confirmed the EV isolation. Total RNA was extracted and the small-RNA libraries were sequenced through the Illumina® technology. After the bioinformatic pipeline, in both sample sources, most of the small-RNAs referred to miRNAs (95% for colosEVs and 96% for mEVs). Of these, 350 were shared, 17 were colosEV-specific while 73 mEV-specific. The differential gene expression analysis showed 1504 differentially expressed genes (DEGs, log2 Fold Change - log2FC > |1| and adjusted p < 0.05), 961 up-regulated and 543 down-regulated, in colosEVs compared to mEVs. The RNA types with a highest number of DEGs were protein coding (918 up-regulated and 281 down-regulated) and miRNAs (28 up-regulated and 193 down-regulated). Target genes for DE miRNAs, were retrieved through the investigation of a protein-protein interaction (PPI) network. Gene ontology (GO) enrichment analysis was carried out highlighting different groups of molecules: for targets of up-regulated miRNAs, enriched terms related to the innate immune response, miRNAs, transmembrane receptor protein kinase activity, mitochondrion, DNA methylation or demethylation, smooth muscle cell proliferation and nitric oxide metabolic process; down-regulated miRNAs revealed terms such as cellular response to cytokine stimulus and innate immune response, I-kappaB kinase/NF-kappaB signaling, DNA methylation and organization, signal transduction, stress-activated MAPK cascade, posttranscriptional regulation of gene expression and RNA splicing emerged as enriched terms.

[1] Mecocci, S.; Trabalza-Marinucci, M.; Cappelli, K. Extracellular Vesicles from Animal Milk: Great Potentialities and Critical Issues. Animals 2022, 12, 3231, doi:10.3390/ani12233231.
[2] Mecocci, S.; Pietrucci, D.; Milanesi, M.; Pascucci, L.; Filippi, S.; Rosato, V.; Chillemi, G.; Capomaccio, S.; Cappelli, K. Transcriptomic Characterization of Cow, Donkey and Goat Milk Extracellular Vesicles Reveals Their Anti-Inflammatory and Immunomodulatory Potential. IJMS 2021, 22, 12759, doi:10.3390/ijms222312759.
[3] Capomaccio, S.; Cappelli, K.; Bazzucchi, C.; Coletti, M.; Gialletti, R.; Moriconi, F.; Passamonti, F.; Pepe, M.; Petrini, S.; Mecocci, S.; et al. Equine Adipose-Derived Mesenchymal Stromal Cells Release Extracellular Vesicles Enclosing Different Subsets of Small RNAs. Stem Cells International 2019, 2019, doi:10.1155/2019/4957806.
[4] Mecocci, S.; De Paolis, L.; Zoccola, R.; Fruscione, F.; De Ciucis, C.G.; Chiaradia, E.; Moccia, V.; Tognoloni, A.; Pascucci, L.; Zoppi, S.; et al. Antimicrobial and Immunomodulatory Potential of Cow Colostrum Extracellular Vesicles (ColosEVs) in an Intestinal In Vitro Model. Biomedicines 2022, 10, 3264, doi:10.3390/biomedicines10123264.

76° CONVEGNO SISVET
BARI, 21-22-23 GIUGNO 2023

HETEROGENEITY OF PHENOTYPIC AND FUNCTIONAL CHANGES TO PORCINE MONOCYTE-DERIVED MACROPHAGES TRIGGERED BY DIVERSE POLARIZING FACTORS IN VITRO

Giulia Franzoni (1), Lorena Mura (1), Chiara Grazia De Ciucis (2), Floriana Fruscione (2), Filippo Dell'Anno (2), Susanna Zinellu (1), Tania Carta (1,3), Antonio G. Anfossi (3), Silvia Dei Giudici (1), Elisabetta Razzuoli (2), Annalisa Oggiano (1)

(1) Department of Animal Health, Istituto Zooprofilattico Sperimentale della Sardegna, 07100 Sassari, Italy
(2) National Reference Center of Veterinary and Comparative Oncology (CEROVEC), Istituto Zooprofilattico Sperimentale del Piemonte, Liguria e Valle d'Aosta, 16129 Genova, Italy
(3) Department of Veterinary Medicine, University of Sassari, 07100 Sassari, Italy

Corresponding Author: **Giulia Franzoni** - giulia.franzoni@izs-sardegna.it

Macrophages are innate immune cells characterized by remarkable plasticity, and they can quickly change their function and phenotype in response to external stimuli [1,2]. Swine are attracting increasing attention as a biomedical model, due to many immunological similarities with humans. Several studies have suggested that pigs are a better model than mice to understand human innate immunity; and pigs likely present higher predictive values than rodents in preclinical studies [3,4]. However, porcine macrophage polarization has not been extensively analyzed in this species. We therefore investigated porcine monocyte-derived macrophages (moMΦ) triggered by either IFN-γ and LPS (classical activation), and by diverse 'M2 related' polarizing factors: IL-4, IL-10, TGF-β, and dexamethasone, through microscopy, flow cytometry, multiplex ELISA, gene expression assays (PCR array and RT-PCR). IFN-γ and LPS polarized moMΦ toward a pro-inflammatory phenotype, although a significant IL-1Ra response was observed. Exposure to IL-4, IL-10, TGF-β, and dexamethasone gave rise to four distinct phenotypes, all antithetic to IFN-γ and LPS. Some peculiarities were observed: both IL-4 and IL-10 enhanced expression of IL18, and none of the 'M2 related' stimuli induced IL10 expression. Exposure to TGF-β and dexamethasone were characterized by enhanced levels of TGFβ2, whereas stimulation with dexamethasone, but not TGF-β2, triggered CD163 up-regulation and induction of CCL23. Macrophages stimulated with IL-10, TGF-β, or dexamethasone presented a decreased ability to release pro-inflammatory cytokines in response to TLR2 or TLR3 ligands: IL-10 showed a powerful inhibitory activity for CXCL8 and TNF release, whereas TGF-β provided a strong inhibitory signal for IL-6 production. Whilst our results emphasized porcine macrophage plasticity broadly comparable to human and murine macrophages, it also highlighted some peculiarities in this species.

[1] Gordon, S. Alternative activation of macrophages. Nat Rev Immunol 2003, 3, 23-35.
[2] Hume, D.A. The Many Alternative Faces of Macrophage Activation. Front Immunol 2015, 6, 370.
[3] Pabst, R. The pig as a model for immunology research. Cell Tissue Res 2020, 380, 287–304.
[4] Fairbairn, L.; Kapetanovic, R.; Sester, D.P.; Hume, D.A. The mononuclear phagocyte system of the pig as a model for understanding human innate immunity and disease. J Leukoc Biol 2011, 8, 855–871.

GOAT MILK EXTRACELLULAR VESICLES: IMMUNO-MODULATION EFFECTS ON PORCINE MONOCYTE-DERIVED MACROPHAGES IN VITRO

Giulia Franzoni (1) Samanta Mecocci (2), Chiara Grazia De Ciucis (3), Lorena Mura (1), Filippo Dell'Anno (3), Susanna Zinellu (1), Carta Tania (1,4), Floriana Fruscione (3), Livia De Paolis (3), Antonio G. Anfossi (4), Silvia Dei Giudici (1), Elisabetta Chiaradia (2), Luisa Pascucci (2), Annalisa Oggiano (1), Katia Cappelli (2), Elisabetta Razzuoli (3)

(1) Department of Animal Health, Istituto Zooprofilattico Sperimentale della Sardegna, 07100 Sassari, Italy
(2) Department of Veterinary Medicine, University of Perugia, 06123 Perugia, Italy
(3) National Reference Center of Veterinary and Comparative Oncology (CEROVEC), Istituto Zooprofilattico Sperimentale del Piemonte, Liguria e Valle d'Aosta, 16129 Genova, Italy
(4) Department of Veterinary Medicine, University of Sassari, 07100 Sassari, Italy

Corresponding Author: **Giulia Franzoni** - giulia.franzoni@izs-sardegna.it

Extracellular vesicles (EVs) are nanometric spherical structures, enclosed in a lipid bilayer membrane, which can be recovered by any biological fluids, including milk [1]. Milk extracellular vesicles (mEVs) have increasingly drawn interest for their theranostic potential [1]. Before any biomedical application, it is important to investigate the impact of these nanosized molecules on immune cells, such as macrophages. Macrophages are professional phagocytes, involved in the defense to both infective and not infective stressors [2]. Indeed, the impact of foreign molecules on these professional phagocytes may trigger unwanted immunotoxicity. In this study, swine was used as a biomedical model, due to many immunological similarities with humans [3, 4]. The immunomodulating effects of goat mEVs on porcine untreated (moMΦ) and classically activated (moM1) monocyte-derived macrophages isolated from healthy pigs was investigated through flow cytometry, multiplex ELISA, microscopy and gene expression assays. Two non- cytotoxic concentrations of mEVs (60 and 0.6 µg protein weight) were used for immunological tests. We observed that these particles were efficiently internalized by macrophages and the high dose triggered the up-regulation of MHC II DR and MHC I on moMΦ, whereas no effect was observed on moM1. Goat mEVs enhanced the expression of several cytokines in moMΦ, although with differences between concentrations: exposure to the low dose of mEVs increased IL10, EBI3, IFNB gene expression, whereas the high dose treatment of mEVs up-regulated several cytokines (IL1B, IL6, CXCL8, IL1, IL12B, EBI3, IFNB). These milk-derived nanosized structures modestly modulated the cytokine expression on moM1, reducing the gene expression of few toll like receptors (TLRs) on both moMΦ and moM1. In moMΦ, the exposure to the low dose of goat mEVs did not affect cytokine contents in culture supernatants (with the exception IL-1α, IL-1β, CXCL8 levels in mild manner), whereas the high dose promoted the sustained release of several cytokines (IL-1α, IL-1β, IL-1Ra, IL-6, CXCL8, IL-10, IL-12, TNF). In agreement with gene expression data, the exposure of moM1 to mEVs did not alter cytokine content in culture supernatants. Overall, our in vitro data hint a potential application of goat mEVs in the biomedical field: these nanosized structures polarized macrophages toward a M1-like phenotype, but then other mechanisms seem to occur to control this activation, avoiding the development of an exacerbated immune response.

[1] Mecocci, S.; Trabalza-Marinucci, M.; Cappelli, K. Extracellular Vesicles from Animal Milk: Great Potentialities and Critical Issues. Animals 2022, 12, 3231.
[2] Hume, D.A. The Many Alternative Faces of Macrophage Activation. Front Immunol 2015, 6, 370.
[3] Pabst, R. The pig as a model for immunology research. Cell Tissue Res 2020, 380, 287–304.
[4]Fairbairn, L.; Kapetanovic, R.; Sester, D.P.; Hume, D.A. The mononuclear phagocyte system of the pig as a model for understanding human innate immunity and disease. J Leukoc Biol 2011, 8, 855–871.

SICLIMVET

TECHNOLOGIES FOR A NOVEL CLINICAL APPROACH OF A CASE OF COMPLEX VASCULAR ANOMALY: THE "EXTENDED REALITY"

S. Cupido (1), S. Nicoli (3), P. Bargellini (2), D. Caivano (1), F. Birettoni (1), A. Bortolotti (3), M. Rishniw (4), F. Porciello (1)

(1) Department of Veterinary Medicine – University of Perugia, Italy
(2) AniCura Tyrus Veterinary Clinic – Terni, Italy
(3) Touchlabs 3Ddistrict – Bologna
(4) Department of Clinical Sciences – Cornell University, Ithaca, NY, USA

Corresponding Author: **Simone Cupido** - simone.cupido@icloud.com

A 12-month-old, 11 Kg, intact female French Bulldog was referred to the Perugia University Teaching Hospital for exercise intolerance and dyspnea. Physical and biochemical examination yielded normal findings, except for a soft machinery murmur best heard on the left second intercostal space area and a moderate grade plateau murmur best heard on the mitral focus. Two-dimensional and Doppler echocardiography demonstrated severe left atrio-ventricular enlargement with decreased fractional shortening, a mild functional mitral regurgitation, and signs of atrial hypertension. Continuous, turbulent blood flow was detected in the main pulmonary artery, arising in a more distal position than is typical for a patent ductus arteriosus and flowing perpendicular to the pulmonary artery flow. Thoracic ultrasonography showed B-lines consistent with pulmonary edema and furosemide and pimobendan were started. Once clinically stable, an ECG-gated computed tomographic angiographic study with a 128-row Multi Detector Computed Tomography (CT) unit was performed. The CT scan demonstrated an anomalous vessel compatible with the broncho-esophageal artery with numerous, complex branches arising and connecting with a complex network comprising the aortic arch and main pulmonary artery. Specific software permitted the creation of a high-resolution tridimensional model of the vascular anomalies. The model was displayed in an extended reality (XR) environment using Surgeree software (Brain Storm Dubai, U.A.E.). In this XR environment surgeons and cardiologists were rendered as avatars and could manipulate anatomical structures to determine the safest and most effective surgical approach. The visualization allowed surgeons to pinpoint the exact ligation site of the abnormal vessel. The surgical procedure was performed as planned by left thoracotomy. Immediately after the surgical procedure a new CT scan was performed, confirming the absence of residual flows. No surgical or clinical complications occurred during or after the procedure. Furosemide was discontinued. The three-days follow-up echocardiography showed an increase of fractional shortening to normal values. The one-month follow-up echocardiography demonstrated absence of residual flow, normal fractional shortening and so the pimobendan was discontinued. This report documents the first use of extended reality for the visualization, planning and execution of a surgical correction of a complex vascular defect in a dog.

[1] Yamane T, Awazu T et al. Aberrant Branch of the Bronchoesophageal Artery Resembling Patent Ductus Arteriosus in a Dog. J. Vet. Med. Sci. 2001; 63(7): 819-822
[2] Markovic LE, Kellihan HB et al. Advanced multimodality imaging of an anomalous vessel between the ascending aorta and main pulmonary artery in a dog. J. Vet. Cardol. 2014; 16, 59-65
[3] Fujii Y, Aoki T et al. Arteriovenous shunts resembling patent ductus arteriosus in dogs: 3 cases. J. Vet. Cardiol. 2009; 11, 147-151

76° CONVEGNO SISVET
BARI, 21-22-23 GIUGNO 2023

GENE EXPRESSION PROFILING OF ENDOMYOCARDIAL BIOPSIES FROM DOGS WITH DILATED CARDIOMYOPATHY

A. Di Loria (1), C. Ferravante (2), Y. D'Agostino (2,3), G. Giurato (2,4), M. Tursi (5), E. Grego (5), M. Perego (6), A. Weisz (2,4), P. Ciaramella (1), R. Santilli (6,7)

(1) Department of Veterinary Medicine and Animal Productions, University Federico II, Napoli, 80130, Italy
(2) Department of Medicine, Surgery and Dentistry 'Scuola Medica Salernitana', University of Salerno, 84081, Baronissi, SA, Italy
(3) Medical Genomics Program, AOU 'SS. Giovanni di Dio e Ruggi d'Aragona', University of Salerno, 84131 Salerno, Italy
(4) Genome Research Center for Health, Campus of Medicine, University of Salerno, 84081 Baronissi, Italy
(5) Department of Veterinary Sciences, University of Turin, 10095 Turin, Italy
(6) Clinica Veterinaria Malpensa, Viale Marconi 27, Samarate, 21017 Varese, Italy
(7) Department of Clinical Sciences, Cornell University, 930, Campus Road, 14853, Ithaca, New York, USA

Corresponding Author: **Antonio Di Loria** - adiloria@unina.it

In the past decade, the Next-Generation Sequencing (NGS) technology has become a very attractive toll to improve the diagnostic yield and clinical utility of endomyocardial biopsy. Nine privately-owned dogs previously diagnosed with dilated cardiomyopathy (DCM) related and not related to myocarditis pattern as well as three healthy control dogs were enrolled in this study.
Endomyocardial biopsies (EMB) were collected and NGS, histologic examination and PCR performed. The study protocol was approved by the Ethical Animal Care and Use Committee of the University of Naples Federico II (Protocol No. 67990/2015). An informed consent was obtained from all owners. Nine dogs with a diagnosis of DCM were enrolled in the study. Data from healthy control dogs (n=3) were retrieved from a previous approved study (NCBI BioProject PRJNA78827). Endocardial fibrosis was observed in 6/9 dogs, while a mild form of myocarditis was present in 3/9. Two dogs were positive for canine parvovirus 2 and one dog for canine distemper virus. The three control samples consisted of 61,373,812 reads in total, with an average of 20,457,937.33 reads per samples (range 20,429,448 - 20,484,644 reads). Gene cardiac expression pathways were differently observed in DCM dogs, showing an altered cellular energy metabolism and involvement of structural functional cardiac protein. Moreover, in dogs with myocarditis the NGS analysis confirmed the role of genes related to inflammation and pathogen infection.
The NGS performed on EMB allows to highlight molecular and genetic alterations related to DCM in dogs advancing the knowledge on the pathogenesis of this type of cardiomyopathy.

[1] Santilli, R.A., Battaia, S., Perego, M., Tursi, M., Grego, E., Marzufero, C., Gianella, P., 2017. Bartonella-associated inflammatory cardiomyopathy in a dog. Journal of Veterinary Cardiology 19, 74–81
[2] Santilli, R.A., Grego, E., Battaia, S., Gianella, P., Tursi, M., di Girolamo, N., Biasato, I., Perego, M., 2019. Prevalence of selected cardiotropic pathogens in the myocardium of adult dogs with unexplained myocardial and rhythm disorders or with congenital heart disease. Journal of the American Veterinary Medical Association 255, 1150–1160.
[3] Friedenberg, S.G., Chdid, L., Keene, B., Sherry, B., Motsinger-Reif, A., Meurs, K.M., 2016. Use of RNA-seq to identify cardiac genes and gene pathways differentially expressed between dogs with and without dilated cardiomyopathy. American Journal of Veterinary Research 77, 693–9.

76° CONVEGNO SISVET
BARI, 21-22-23 GIUGNO 2023

CLINICAL, CARDIOVASCULAR AND PROGNOSTIC FINDINGS IN DOGS WITH MYOCARDIAL INJURY: A RETROSPECTIVE ANALYSIS ON 102 CASES

Giovanni Romito, DVM, SMIPPV, MSc, PhD, Dipl. ECVIM-CA (Cardiology),1 Maria Chiara Sabetti, DVM,2 Mario Cipone, DVM1

(1) Department of Veterinary Medical Sciences, Alma Mater Studiorum-University of Bologna, Ozzano dell'Emilia, Italy
(2) Department of Veterinary Sciences, University of Parma, Parma, Italy

Corresponding Author: **Giovanni Romito** - giovanni.romito2@unibo.it

Introduction. Data on canine myocardial injury (MI) are currently sparse.1-3 Therefore, the aim of this study was to describe this clinical entity in dogs.
Materials and Methods. For this retrospective study, dogs diagnosed with MI were searched in the databases of one veterinary centre. MI was diagnosed in the case of increased cardiac troponin I (cTnI) associated with one/more disease(s) capable to induce MI in dogs and concomitant documentation of ≥1 echocardiographic and/or electrocardiographic (ECG) change typical of MI.1-3 Signalment, history, clinical, laboratory, radiographic, ECG, echocardiographic, treatment and outcome data were retrieved. Based on the cause of MI, dogs were classified in 9 etiological categories: unknown (Unk), infective (Inf), inflammatory (Infl), neoplastic (Npl), metabolic (Met), toxic (Tox), nutritional (Nut), immune-mediated (I-M) and mechanical (Mec). Based on cardiovascular findings, 4 groups were created: dogs with (Echo) and without echocardiographic changes (No-Echo) and dogs with (ECG) and without ECG changes (No-ECG). Echo dogs were further classified in 5 subgroups: ventricular dilation and systolic dysfunction (DCM), ventricular wall thickening (HCM), DCM+HCM, isolated echogenicity changes (IEC) and endocarditis (Endo). ECG dogs were further classified in 3 subgroups: abnormal impulse generation (AIG), abnormal impulse conduction (AIC) and abnormal ventricular repolarization (AVR). Statistical analysis included comparison of selected variables between dogs of different categories/groups/subgroups (Mann-Whitney, Kruskall-Wallis, Pearson $\chi 2$) and evaluation of potential effects of selected variables on outcome (logistic regression).
Results. 102 dogs were enrolled (Unk=34, Inf=20, Infl=18, Npl=8, Met=8, Tox=6, Nut=3, I-M=3, Mec=2), including 84 Echo (DCM=52, HCM=22, DCM+HCM=4, Endo=4, IEC=2) and 76 ECG (AIG=54, AIC=14, AVR=8). Outcome was known in 88 dogs (alive=42, death=46). Concentration of cTnI not differed significantly between dogs of the 9 etiological categories, between Echo vs No-Echo dogs and between ECG vs No-ECG dogs. Nor cTnI concentration neither presence/absence of echocardiographic/ECG changes had a statistically relevant effect on outcome.
Conclusion. This study provides, for the first time, a detailed description of clinical features of MI in dogs.

[1] Romito G et al. J Vet Cardiol 2021; 36:131-40.
[2] Romito G et al. Animals (Basel) 2021; 11:3506.
[2] Romito G et al. J Vet Med Sci 2022.

76° CONVEGNO SISVET
BARI, 21-22-23 GIUGNO 2023

PRELIMINARY DATA ON ENDOSCOPIC EVALUATION OF THE CLOACA IN *OPISTOGLYPH COLUBRIDS*

Marino Matteo (1), Knotek Zdenek (2), Di Giuseppe Marco (3), Morici Manuel (4), Oliveri Matteo (2), Spadola Filippo (1)

(1) University of Messina, Department of Veterinary Science, Messina, Italy
(2) Avian and Exotic Animal Clinic, Faculty of Veterinary Medicine, University of Veterinary and Pharmaceutical Sciences Brno, Brno, Czech Republic
(3) Medicina del Coniglio, www.medicinadelconiglio.it, Palermo, Italy
(4) Veterinary Department, Pombia Safari Park, Pombia, Italy

Corresponding Author: **Matteo Marino** - matteomarino1999@gmail.com

The aim of this work is to describe for the first time the cloacal anatomy, pathological findings and sex determination in males and females of *opistoglyph colubrids* using the cloacal endoscopic tecnique. The study was conducted on a group of captive breed snakes composed of: a female of *Toxicodryas blandigi*, a male of Boiga cyanea and a female of Boiga nigriceps, of different age and size, from a private owner using a rigid endoscope 4 mm diameter, 0°, 8.5 cm length, (Olympus medical, Japan) with a working sheath connected via one port to a syringe (60 mL, Pic solutions/Artsana, Italy) camera (Telecam DX-II, Karl Storz, Germany) and a documentation system (TELE PACK, Karl Storz, Germany). Animals were contained in dorsal decubitus without using anesthetics while endoscope was inserted into the cloaca in a caudal-cranial direction and to have a clear view warm sterile saline solution supplemented with lidocaine was infused in cloaca. These procedures allowed direct observation of the *proctodeum*, *urodeum*, and *coprodeum*; the visualization of *urodeum* can be used to distinguish differences between the sexes. In the females *urodeum*, a urogenital papilla and two hosts, separated by a septum, representing the entrance to the oviducts; in the male instead there is only the urogenital papilla which ends blindly as in the saurians. In this work the cloacoscopy was/is proposed like an observation tool for the study of compared anatomy, sex determination and opens new horizons as a diagnostic technique for the obstructive pathologies of the cloaca most frequently observed in captivity such as dystocia, faecal impaction, foreign bodies which could be removed or aspirated immediately after their identification. the study of the organs annexed to the cloaca, their comparison with the species of snakes whose anatomy is best known, the techniques of semen collection and artificial insemination will be the object of future work.

[1] Oliveri Matteo, Bartoskova Alena, Spadola Filippo, Morici Manuel, Di Giuseppe Marco and Knotek Zdenek. (2018). Method of semen collection and artificial insemination in snakes. Journal of Exotic Pet Medicine 27, pp 75–80.
[2] Spadola F., Sgroi P., Lubian E. & Morici M. (2021). Cloacal anatomy and sex determination in Tiliqua sp. Herpetological Bulletin 156, pp 11-13.
[3] Spadola Filippo, Morici Manuel, Di Giuseppe Marco, Lubian Emanuele, Oliveri Matteo, Bartoskova Alena & Knotek Zdenek. (2022). Evaluation of cloacoscopy for sex determination in the Argentine black and white tegu Salvator merianae. Herpetological Bulletin 161, pp 1-4.
[4] Spadola Filippo, Insacco Gianni. (2009). Endoscopy of cloaca in 51 Emys trinacris (Fritz et al., 2005): morphological and diagnostic study. Acta Herpetologica 4(1): 73-81.

76° CONVEGNO SISVET
BARI, 21-22-23 GIUGNO 2023

AN AI-BASED ALGORITHM FOR THE AUTOMATIC EVALUATION OF IMAGE QUALITY IN CANINE THORACIC RADIOGRAPHS

Silvia Burti (1), Marek Wodzinski (2,3), Eleonora Vettore (1), Henning Muller (3), Alessandro Zotti (1), Tommaso Banzato (1)

(1) Department of Animal Medicine, Production and Health, University of Padua, Viale dell'Università 16, 35020 Legnaro, Padua, Italy
(2) AGH University of Science and Technology, Department of Measurement and Electronics, Krakow, PL32059, Poland
(3) Information Systems Institute, University of Applied Sciences - Western Switzerland (HES-SO Valais), 3960 Sierre, Switzerland

Corresponding Author: **Silvia Burti** - silvia.burti@phd.unipd.it

Radiography is the most widely used imaging technique for the evaluation of the canine thorax. Obtaining high-quality images is paramount for a correct interpretation of thoracic radiographs (1). In human medicine specific guidelines on diagnostic image quality were established (2). To date, no specific standards for diagnostic image quality were established in veterinary medicine.
The aim of this study was to develop and test an artificial intelligence (AI)-based algorithm, based on ResNet-50 pre-trained on ImageNet, for detecting common technical errors in canine thoracic radiography. The algorithm was trained using a database of 7975 thoracic radiographs (4776 latero-lateral and 3199 sagittal projections) from three different veterinary clinics in Italy. The radiographs were evaluated for image quality by three experienced veterinary diagnostic imagers. The algorithm was designed to classify the images as correct or having one or more of the following errors: rotation (superimposition of opposite ribs), underexposure (evident quantum mottle or lack of detail of pulmonary structures), overexposure, incorrect limb positioning, incorrect neck positioning, blurriness (evident motion artefacts), cut, or presence of foreign objects or medical devices. All the tags, except for "correct", were not mutually exclusive and therefore a multi-label deep-learning approach was used. The algorithm correctly identified errors in thoracic radiographs with an overall accuracy of 81.5% in latero-lateral and 75.7% in sagittal images. The most accurately identified errors were limb mispositioning and underexposure both in latero-lateral (AUC 0.93 and 0.88, respectively) and sagittal images (AUC 0.88 and 0.92, respectively). The accuracy of the developed model in the classification of technically correct radiographs was fair in latero-lateral (AUC=0.77) and good in sagittal (AUC=0.81) images. The authors conclude that their AI-based algorithm is a promising tool for improving the accuracy of radiographic interpretation by identifying technical errors in canine thoracic radiographs.

[1] Thrall, D. E. Principles of Radiographic Interpretation of the Thorax. Textbook of Veterinary Diagnostic Radiology (Elsevier Inc., 2018). doi:10.1016/b978-0-323-48247-9.00040-1.
[2] Wilson, D. U., Bailey, M. Q. & Craig, J. The role of artificial intelligence in clinical imaging and workflows. 63, 897–902 (2022).

APPLICATION OF THE ARTIFICIAL INTELLIGENCE-BASED METHOD TO DIAGNOSE THE SEVERITY OF MYXOMATOUS MITRAL VALVE DISEASE FROM CANINE CHEST RADIOGRAPHS

Carlotta Valente (1), Tommaso Banzato (1), Alessandro Zotti (1), Helen Poser (1), Marek Wodzinski (2)(3), Henning Müller (3), Carlo Guglielmini (1)

(1) Department of Animal Medicine, Production and Health, University of Padua, Legnaro, Italy
(2) Department of Measurement and Electronics, AGH University of Science and Technology, Krakow, Poland
(3) Information Systems Institute, University of Applied Sciences – Western Switzerland (HES-SO Valais), Sierre, Switzerland

Corresponding Author: **Carlotta Valente** - carlotta.valente@unipd.it

Myxomatous mitral valve disease (MMVD) is the most common acquired cardiac disease in the dog and can eventually lead to congestive heart failure. The American College of Veterinary Internal Medicine (ACVIM) has classified MMVD into four stages according to clinical, radiographic and echocardiographic parameters [1]. Even if transthoracic echocardiography is the gold standard imaging technique, it requires trained and specialised operators and may not always be performed immediately. Nevertheless, it is usually suggested once signalment, history and clinical findings support suspicion of cardiac disease. In this scenario, the use of artificial intelligence (AI) - based tools for early identification of dogs affected by MMVD could assist the clinician during clinical routine [2]. The aim of this study was to develop an AI-based algorithm capable of identifying dogs affected by MMVD from chest radiographs. Subsequently, we also tried to implement the algorithm to stage MMVD according to the ACVIM classification. In this retrospective study, thoracic radiographs performed between 2012 and 2022 in dogs referred to the Cardiology and Diagnostic Imaging Units of the Padua Veterinary Teaching Hospital were selected. Dogs with thoracic radiographs and echocardiographic examinations were included. Animals were classified according to the ACVIM guidelines as healthy, B1, B2 and C+D [1]. In the first part of the study, a binary classification of patients was used, namely dogs with compensated and decompensated MMVD (that is, B1+B2 vs. C+D). In the second part, all stages of ACVIM were considered. The algorithm was trained only on the right and left lateral radiographic views. A total of 425 lateral radiographic views were included and, based on the ACVIM guidelines, 31 (7.3%), 147 (34.6%), 93 (21.9%) and 154 (36.2%) were classified as healthy, and stage B1, B2, and C+D, respectively. The algorithm showed a good performance in the binary classification task with a precision of 84% and 82% and a f1-score of 87% and 78% for compensated and decompensated dogs, respectively. On the other hand, a markedly lower accuracy (63%) was obtained in the prediction of the different ACVIM classes. In this preliminary study, the developed AI-based method was a useful tool to distinguish compensated from decompensated dogs with MMVD. However, further studies with a larger sample size are necessary to develop an AI-method for the correct identification of MMVD stages.

[1] Keene BW et al. ACVIM consensus guidelines for the diagnosis and treatment of myxomatous mitral valve disease in dogs, J Vet Intern Med, 33(3):1127-1140, 2019.
[2] Li S et al. Pilot study: Application of artificial intelligence for detecting left atrial enlargement on canine thoracic radiographs, Vet Radiol Ultrasound, 61(6):611-618, 2020.

76° CONVEGNO SISVET
BARI, 21-22-23 GIUGNO 2023

INTESTINAL MICROBIOME AND METABOLOME IN SEPTIC DOGS AFFECTED BY PARVOVIRAL ENTERITIS

R. Di Prinzio (1), C. De Rose (1), M.V. Giordano (1), G. Aste (1)

(1) Università Degli Studi di Teramo, Dipartimento Medicina Veterinaria. SP 18, Loc Piano D'accio, 64100 Teramo, Italia

Corresponding Author: **Cristina De Rose** - cderose@unite.it

Canine *Parvovirus* (CPV-2) enteritis is a predisposing factor for sepsis, thus making infected dogs a suitable study population as far as sepsis is concerned. Few studies investigated the potential impact of this infection on the canine gut microbiome.
The aim of this study is to evaluate the gut microbiome and metabolome in septic dogs with CPV-2 infection. Septic CPV-2 infected puppies ("P group") and healthy puppies ("H" group), admitted to the Veterinary Teaching Hospital of the University of Teramo (2020-2022) were prospectively enrolled in this study. Patients with suspected microbiome alteration of any other nature were excluded. Fecal samples were collected at presentation (P1) for both groups, and on the third (P2) and seventh day (P3) of hospitalization for the P group. All samples were frozen and shipped to the GI Lab of Texas A&M University to estimate the fecal abundance of target bacterial taxa and assess the Dysbiosis Index (DI) (2). A targeted metabolomic approach was utilized to measure the concentrations of unconjugated fecal bile acids, ursodeoxycholic acid, fatty acids, and sterols. All datasets were tested for normal distribution. One-way ANOVA were used for statistical purposes (GraphPad Prism 9.0). Metaboanalyst was used to assess heatmaps for metabolomics data. Twelve dogs were included in the H group: 9 male and 3 females. Fourteen dogs were included in the P group: 8 male and 6 females. The median age was 5.50 months (range 3-9) in H dogs and 4.5 months in P dogs (range 2-11). The median weight was 16 kg (range 7-25) in H dogs and 6 kg (range 2-23) in P dogs .A significant change in abundance was registered between group H and P1, for Blautia and Faecalibacterium taxa, and between group H and all P1, P2 and P3 for the Streptococcus taxon. Statistically significant changes in the DI (p 0.04) were observed between T1(-0.6643) and T3 (-4.050) of the P group. The Streptococcus taxa progressively decreased, with statistically significant changes between the H group e all P groups. Regarding the metabolomic analysis, fatty acids increased in the P group, while the H group presented an increased expression of sterols and bile acids, with an inverted pattern. Despite the acute onset of the disease, results suggested a lipid imbalance in dogs with parvovirus that matches those of previous studies in dogs with chronic enteropathy. The restoration of fecal microbiota and metabolites follow the clinical improvement of the dogs.

[1] Alves F, Prata S, Nunes T, Gomes J, Aguiar S, Aires da Silva F, Tavares L, Almeida V, Gil S. Canine parvovirus: a predicting canine model for sepsis. BMC Vet Res. 2020 Jun 15;16(1):199. doi: 10.1186/s12917-020-02417-0. PMID: 32539830; PMCID: PMC7294767.
[2] AlShawaqfeh MK, Wajid B, Minamoto Y, Markel M, Lidbury JA, Steiner JM, Serpedin E, Suchodolski JS. A dysbiosis index to assess microbial changes in fecal samples of dogs with chronic inflammatory enteropathy. FEMS Microbiol Ecol. 2017 Nov 1;93(11). doi: 10.1093/femsec/fix136. PMID: 29040443.
[3] Honneffer, Julia Beth (2017). Microbiota and Metabolomic Changes Across Various Canine Gastrointestinal Diseases. Doctoral dissertation, Texas A & M University. Available electronically from https://hdl.handle.net /1969.1/165742.

76° CONVEGNO SISVET
BARI, 21-22-23 GIUGNO 2023

MODULATION OF INTESTINAL MICROBIOTA IN CANINE CHRONIC LIVER DISEASE: PRELIMINARY RESULTS

Verena Habermaass (1), Eleonora Gori (1), Daniela Olivero (2), Alessio Pierini (1), Chiara Mariti Ilaria Lippi (1), Veronica Marchetti (1)

(1) Department of Veterinary Sciences, University of Pisa, viale delle Piagge 2, 56124 Pisa (PI), Italy
(2) Veterinary Analysis Lab, BiEsseA Scilvet, via A. D'Aosta 7, 20133 Milano (MI), Italy

Corresponding Author: **Verena Habermaass** - verena.habermaass@phd.unipi.it

Human chronic liver disease (CLD) is associated with intestinal dysbiosis and administration of pre-probiotics is part of its therapeutical management [1]. The aim of the present study was to evaluate intestinal microbiota in dogs with CLD and any potential benefits in symbiotic administration. Client-owned dogs with a diagnosis of CLD were included. Dogs were randomized into groups: one treated with symbiotic Florentero® Candioli (Group A; n=15) and the other non-treated as controls (Group B; n=16). No modifications of diet and ongoing therapies were done. Microbial taxa, clinical signs, and biochemical parameters were compared between groups at T0 and T1 (5-6 weeks recheck). Resolution of clinical signs and improvement of ALT (≥30% reduction of T0) was investigated at T1. All DNA fecal samples were subjected to maximum parallel sequencing using next generation technique (ION TORRENT NGS) with analysis of 16SrRNA hypervariable regions. No significant differences were observed at T0 and T1 between Groups A and B in canine fecal microbiota phyla (Firmicutes, Bacteroidetes, Fusobacteria, Proteobacteria, Actinobacteria). Even without statistical significance, T1 Actinobacteria and Firmicutes were higher in Group A (2.3% and 58.9%) than Group B (0.65% and 53.45%). Contrarily, Fusobacteria and Proteobacteria were lower in Group A (4.7% and 13.1%) than Group B (7.4% and 15.5%). Six dogs (40%) of Group A were symptomatic at T0, and at T1 all but one became asymptomatic. In Group B, 4 dogs (25%) were symptomatic at T0 and, at T1 one dog had persisting clinical signs, 3 dogs had clinical resolution, whereas 3 dogs became symptomatic. At T0, 8 (53%) and 9 (56%) dogs in A and B groups respectively, presented increased ALT. At T1, 7/8 (87.5%) patients of Group A presented ALT improvement, and in Group B, only 2 dogs (25%) had an ALT improvement, whereas 2 dogs presented increasing ALT. The tendence to increase of Firmicutes and decrease of Proteobacteria could be positive changes according to a general concept of intestinal dysbiosis in chronic enteropathies [2]. Even if no significance were detected, some positive modifications were observed after symbiotic administration. Further studies are needed to investigate if a longer administration or a larger cohort could provide significative modification in fecal microbiota of dogs with CLD. Since it seems to provide clinical and biochemical improvement, a symbiotic administration should be considered in dogs with CLD.

[1] Liu S, Yang X. Intestinal flora plays a role in the progression of hepatitis-cirrhosis-liver cancer. Front Cell Infect Microbiol. 2023 Mar 9; 13:1140126. doi: 10.3389/fcimb.2023.1140126. PMID: 36968098; PMCID: PMC10034054.
[2] Ziese AL, Suchodolski JS. Impact of Changes in Gastrointestinal Microbiota in Canine and Feline Digestive Diseases. Vet Clin North Am Small Anim Pract. 2021 Jan;51(1):155-169. doi: 10.1016/j.cvsm.2020.09.004. Epub 2020 Oct 29. PMID: 33131916.

THE WELFARE OF DECAPOD CRUSTACEANS AT SLAUGHTER: A PROPOSAL FOR FUTURE LEGAL FRAMEWORK

Landi A. (1), Pugliese M. (2), Conte F. (2) Fruscella L. (3,4), Vitale D. (3), Biondi V. (2), Ricciuti A. (3), Passantino A. (2)

(1) Veterinario libero professionista, Torino
(2) Dipartimento di Scienze Veterinarie, Università degli Studi di Messina
(3) ALI, Animal Law Italia ETS
(4) Department of Landscape, University of Greenwich, Londra, Regno Unito

Corresponding Author: **Michela Pugliese** - michela.pugliese@unime.it

The production of decapod crustaceans (e.g., lobsters, shrimp, and crabs) for human consumption is a growing sector, and as such deserves particular attention, also considering that these animals are increasingly being recognised as sentient beings in the scientific community, as well as in some countries. In fact, several studies have shown that some species of decapod crustaceans possess a relatively complex cognitive system and may have the capacity to suffer. These discoveries render the health and welfare of these animals' key issues, not only for the scientific community, but also for aquaculturists, food business operators, control and inspection authorities, and policy makers. Although now recognised as sentient beings, decapod crustaceans are at present not protected in terms of their welfare during slaughter; this is reflected in Council Regulation (EC) No 1099/2009, which does not include specific provisions for decapod crustaceans at the time of killing in the EU. Based on these considerations, the authors propose a list of 'best practice' guidelines for the slaughtering of these animals that, according to the latest literature, aims at minimising pain and suffering. It is thereby argued that a precautionary approach should be adopted to protect these animals from possible pain and suffering. Finally, it is recommended that standardised welfare methods for stunning and slaughtering crustaceans be included in the Italian and EU legislations. The adoption of welfare recommendations is also likely to result in a high level of consumer acceptance, in turn enhancing the commercial production of these animals.

[1] Birch, J. Animal sentience and the precautionary principle. Anim. Sentience 2017, 2, 1–15.
[2] Horvath, K.; Angeletti, D.; Nascetti, G.; Carere, C. Invertebrate welfare: An overlooked issue. Ann. Ist. Super. Sanita 2013, 49, 9–17.
[3] Conte F, Voslarova E, Vecerek V, Elwood RW, Coluccio P, Pugliese M, Passantino A. Humane Slaughter of Edible Decapod Crustaceans. Animals (Basel). 2021 Apr 11;11(4):1089.
[4] Passantino A, Elwood RW, Coluccio P. Why Protect Decapod Crustaceans Used as Models in Biomedical Research and in Ecotoxicology? Ethical and Legislative Considerations. Animals (Basel). 2021 Jan 3;11(1):73

NEW INSIGHTS IN THE CLINICAL ASPECTS OF NATURALLY OCCURRING CANINE STRONGYLOIDIASIS

Serena Digiaro (1), Antonella Colella (1), Federica Lo Tempio (1), Alessandra Recchia (1), Rosanna Lacinio (1), Dora Buonfrate (2), Paola Paradies (1)

(1) Department of Precision and Regenerative Medicine and Jonian Area (DiMePre-J); University of Bari "Aldo Moro"
(2) Department of Infectious, Tropical diseases and Microbiology, IRCCS Sacro Cuore Don Calabria hospital

Corresponding Author: **Paola Paradies** - paola.paradies@uniba.it

Canine strongyloidiasis by *Strongyloides stercoralis* is a parasitic disease emerging in Europe, which represents both a veterinary clinical issue and a public health challenge because of the zoonotic potential [1, 2]. The disease, rarely described in Europe, could induce severe symptoms in dogs [3]; therefore, an early diagnosis and appropriate treatment are desirable. The aim of the present work is to retrospectively investigate the clinical and paraclinical findings in symptomatic dogs naturally infected by *S. stercoralis*, with particular attention to ultrasound changes at the gastrointestinal level and to discuss the critical points of diagnosis in clinical practice. Twelve symptomatic dogs presented at the Veterinary Teaching Hospital, University of Bari, Italy, were included in the study. The diagnosis was made by means of larval morphological identification and real-time PCR (IRCCS Verona) on faecal samples.
Data on signaling (breed, weight, age and sex), history, clinical signs, laboratory tests, and imaging results available at the time of diagnosis were obtained from the records. Most dogs presented with gastrointestinal signs; diarrhea and weight loss were the most common presenting complaint. Only one dog showed respiratory signs, associated to a parasitic cutaneous nodule. Hypoproteinaemia, anaemia, leucocytosis and an increase in α2-globulin fraction at serum protein electrophoresis were common (>50%) but not constant findings. All but two dogs showed an abnormal ultrasound examination of the gastrointestinal tract (82,3 %). The most common gastrointestinal findings were diffusely distended intestinal loops, the presence of a liquid pattern and altered peristaltic movements found in 7/10 dogs with gastroenteric signs (70 %), while the wall thickness commonly associated with chronic enteritis was uncommon. The combination of the described changes could increase the suspicion of canine strongyloidiasis and may direct clinicians to include strongyloidiasis in the differential diagnosis in dogs with diarrhea or gastrointestinal signs. Correct diagnosis in clinical practice is crucial, considering that the infection does not respond to common antiparasitic drugs. The histological examination at the intestinal level, available in 5 dogs, revealed the presence of parasites from the full-thickness biopsy, but not from the endoscopic biopsy. One dog draws our attention to the respiratory and cutaneous disease associated with *S. stercoralis* infection.

[1] A.V Eslahi., S. Hashemipour, M. Olfatifar, E. Houshmand, E. Hajialilo, R. Mahmoudi, M. Badri, J.K. Ketzis, Global prevalence and epidemiology of Strongyloides stercoralis in dogs: a systematic review and meta-analysis. Parasit Vectors. (2022) 15(1):2. https://doi.org/10.1186/s13071-021-05135-0
[2] L. Ottino, D. Buonfrate, P. Paradies, Z. Bisoffi, A. Antonelli, G.M. Rossolini, S. Gabrielli, A. Bartoloni, L. Zammarchi, Autochthonous Human and Canine Strongyloides stercoralis Infection in Europe: Report of a Human Case in An Italian Teen and Systematic Review of the Literature. Pathogens. (2020) 9(6):439. https://doi.org/10.3390/pathogens9060439
[3] P. Paradies, F. Iarussi, M. Sasanelli, A. Capogna, R.P. Lia, D. Zucca, B. Greco, C. Cantacessi, D. Otranto, Occurrence of strongyloidiasis in privately owned and sheltered dogs: clinical presentation and treatment outcome. Parasit Vectors. (2017) 10(1):345. https://doi.org/10.1186/s13071-017-2275-5.

STRONGYLOIDIASIS IN SOUTHERN ITALY IN HUMANS AND DOGS

Paola Paradies (1), Antonella Colella (1), Serena Digiaro (1), Beatrice Greco (1), Luigi De Rosa (1), Giuseppe Losurdo (1), Alfredo Di Leo (1), Fabio Formenti (2), Dora Buonfrate (2)

(1) Department of Precision and Regenerative Medicine and Jonian Area (DiMePre-J); University of Bari "Aldo Moro"
(2) Department of Infectious, Tropical diseases and Microbiology, IRCCS Sacro Cuore Don Calabria hospital

Corresponding Author: **Paola Paradies** - paola.paradies@uniba.it

Strongyloidiasis by *Strongyloides stercoralis* is ubiquitous in tropical and subtropical countries and occasionally reported in temperate countries both in humans and in dogs [1]. In dogs, *S. stercoralis* infection represents both a veterinary clinical issue and a public health challenge because of the potential role of dogs in the transmission to humans. In Italy the presence of human infection is known in northern regions [2] while no data are available from southern regions. Differently, canine strongyloidiasis was already documented in southern Italy [3,4]. The aim of the present work was to assess the extent of the *S. stercoralis* infection problem in southern Italy both in humans and dogs. The study was conducted in Southern Italy in contexts where humans and dogs share the same environment. Dogs and humans were selected from 3 different habitat: 1: Shelters (Group K); 2: livestock farms (Group L); 3: agricultural farms (Group A). For humans, a commercial ELISA test was used for screening. On dog's faecal samples Baermann test and PCR were performed. Based on fecal tests results in dogs and serological results in humans, this study showed in Southern Italy a proportion of *S. stercoralis* positivity of 4.13% and 5.75% respectively. In dogs the highest positivity was revealed in group K (6.7% against 2% and 0% in L and A). Differently in humans, similar positivity was registered in all groups. In brief, the six positive dogs were 4 males and 2 females from 2 to 10 years, five from two kennels and 1 from a livestock farm. Three out of the six positive dogs were healthy, in the other three dogs weight loss and/or diarrhea were reported. ELISA positive persons were 4 males and 4 females from 51 to 58 years. They were in health except for two referring chronic weight loss and/or pruritus. People and dogs resulted positive at any tests were treated with 200 µg/kg ivermectin per os [4]. In conclusion, we confirm that *S. stercoralis* is present in dogs in Southern Italy, particularly in those living in kennels. Moreover, we found cases in humans, although these were not confirmed by a faecal test. Positive dogs and humans did not share the same environment. Further screenings on larger cohorts of people are needed to estimate the prevalence of human strongyloidiasis in Southern Italy. Genotyping studies would be relevant to evaluate a possible zoonotic transmission.

[1] Ottino L, et al.Autochthonous Human and Canine Strongyloides stercoralis Infection in Europe. 2020 Jun 3;9(6):439.
[2] Buonfrate D, et al.Epidemiology of Strongyloides stercoralis in northern Italy: results of a multicentre case-control study, February 2013 to July 2014.Euro Surveill. 2016 Aug 4;21(31):30310.
[3] Paradies P, et al.Occurrence of strongyloidiasis in privately owned and sheltered dogs: clinical presentation and treatment outcome.Parasit Vectors.2017 Jul 20;10(1):345.
[4] Paradies P, et al.Efficacy of ivermectin to control Strongyloides stercoralis infection in sheltered dogs. Acta Trop. 2019 Feb; 190:204-209.

76° CONVEGNO SISVET
BARI, 21-22-23 GIUGNO 2023

THE RED BLOOD CELL MEMBRANE LIPIDOME OF DOMESTIC CATS

P.E. Crisi (1), M.V. Giordano (1), A. Gramenzi (1), A. Luciani (1), V. Rinaldi (1), A. Sansone (2), C. Ferreri (2), A. Boari (1)

(1) Department of Veterinary Medicine, University of Teramo, Strada Provinciale 18, 64100 Località Piano d'Accio, Teramo, Italy
(2) ISOF, Consiglio Nazionale delle Ricerche, Via Piero Gobetti, 101, 40129 Bologna, Italy

Corresponding Author: **Paolo Emidio Crisi** - pecrisi@unite.it

Analysis of red blood cell (RBC) membrane lipidome is a useful tool for assessing the quantity and quality of fatty acids (FA) in different conditions. The aim of this work is to create a benchmark of interval values of FA RBC membrane to start a systematic approach for the examination of metabolic and nutritional status in healthy and diseased cats.
Cats admitted to the VTH of Teramo for clinical check-up or spaying and neutering surgeries, without evidence of disease were included. EDTA-treated blood was utilized for extracting RBC and performing lipidome analyses, using Gas-Chromatography as previously described in dogs [1].
The RBC membrane lipidome in healthy cats was represented by saturated FA (SFA: stearic, palmitic), mono-unsaturated FA (MUFA: palmitoleic, oleic, vaccenic), polyunsaturated FA ω6 (PUFA ω6: linoleic [LA], dihomo-gamma-linolenic [DGLA], arachidonic) and PUFA ω3 (EPA, DPA, DHA).
Twentynine cats (15 males, 14 females) were included, ageing from 5 to 163 months (median 33), with a mean bodyweight (BW) of 3.6±1.0 Kg, being 14 indoor cats and 15 with outdoor access. The indoor and outdoor groups were matched for sex (p=0.75) and age (p=0.08), while outdoor cats had lower BW (p=0.004).
The RBC FA divided by the 3 families gave: SFA 45.7±6.0%, MUFA 11.4±1.2%, PUFA-ω6 40.2±5.9% and PUFA-ω3 2.5±1.1%. The SFA/MUFA and the ω6/ω3 ratios were 4.1±0.7 and 19.3±9.5 respectively. The DGLA/LA ratio resulted 0.03±0.01.
A higher SFA/MUFA ratio was found in cats (4.0±0.7) compared to dogs (3) and humans (2.7), together with ω6/ω3 ratio of 19.3±0.7%, in contrast to 33% and 4% observed in dogs and humans respectively [1,2].
The LA-to-DGLA transformation was minimal (0.03) compared to dogs (0.09) and humans (0.15) [1,2], confirming published data on low efficiency of Δ6 desaturase in cats [3].
Outdoor cats had lower palmitic (p=0.003), palmitoleic (p=0.0002) and vaccenic (p<0.0001) acid and increased LA (p=0.04), DGLA (p=0.03) and ω6 (p=0.03). This result suggests that cats free to hunt had less SFA and this is reflected into less MUFA metabolism. Enhanced ω6 fatty acid metabolism could indicate more availability of essential PUFA derived from prey or plants.
Using the FA-based RBC membrane lipidome as benchmark, a systematic comparison among species can be carried out, deepening the diversity of composition of cell membranes in mammals. Moreover, these results suggest that environment and hunting behaviour may have significant impact on lipid metabolism.

[1] Prasinou P, Crisi PE, Chatgilialoglu C, Di Tommaso M, Sansone A, Gramenzi A, Belà B, De Santis F, Boari A, Ferreri C. The Erythrocyte Membrane Lipidome of Healthy Dogs: Creating a Benchmark of Fatty Acid Distribution and Interval Values. Front Vet Sci. 2020 Aug 21; 7:502. doi: 10.3389/fvets.2020.00502.
[2] Amézaga J, Arranz S, Urruticoechea A, Ugartemendia G, Larraioz A, Louka M, Uriarte M, Ferreri C, Tueros I. Altered Red Blood Cell Membrane Fatty Acid Profile in Cancer Patients. Nutrients. 2018 Dec 1;10(12):1853. doi: 10.3390/nu10121853.
[3] Bauer JJE. Essential fatty acid metabolism in dogs and cats. R Bras Zootec. 2008 Jul; 37:20-27. doi: 10.1590/S1516-35982008001300004

76° CONVEGNO SISVET
BARI, 21-22-23 GIUGNO 2023

SEASONAL VARIATION OF LABORATORY ABNORMALITIES IN DOGS WITH LEISHMANIOSIS

Andrea Zatelli (1), Maria Alfonsa Cavalera (1), Annamaria Uva (1), Rossella Donghia (2), Roberta Iatta (3), Jairo Alfonso Mendoza-Roldan (1), Xavier Roura (4), Domenico Otranto (1,5)

(1) Department of Veterinary Medicine, University of Bari
(2) National Institute of Gastroenterology "S. de Bellis", Research Hospital
(3) Interdisciplinary Department of Medicine, University of Bari
(4) Hospital Clínic Veterinari, Universitat Autònoma de Barcelona
(5) Faculty of Veterinary Sciences, Bu-Ali Sina University

Corresponding Author: **Maria Alfonsa Cavalera** - mariaalfonsa.cavalera@uniba.it

Laboratory abnormalities are pivotal for diagnosing, clinical staging, and therapeutic monitoring of dogs affected by leishmaniosis. Recently, it was shown that anti-Leishmania infantum antibody titers varied between sand fly and non-sand fly transmission periods in dogs from an endemic area for leishmaniosis. To evaluate if laboratory alterations could be influenced by transmission season in seropositive dogs living in an endemic area for canine leishmaniosis, in September 2021(transmission season; T0) and January 2022 (non-transmission season; T1), L. infantum seropositive dogs were physically examined, a clinical sign-based score for canine leishmaniosis ranging from 0 (absence of clinical signs) to 19 (severely sick) was assigned, and a blood sampling was performed for laboratory tests. At both time points, dogs underwent routine hematology, a complete biochemical panel including acute phase proteins (C-reactive protein [CRP], ferritin) and total iron, erythrocyte sedimentation rate (ESR) measurement, serum capillary electrophoresis, and serology for L. infantum. Dogs were also tested for arthropod-borne and snail-borne pathogens as positivity was considered an exclusion criteria. Of the 18 dogs enrolled in the study, antibody titers decreased from T0 to T1 in 10 dogs (55.5%), with 2 becoming seronegative (20%), and remained constant in 8 dogs (44.4%). From T0 to T1, globulin percentage, ferritin, and ESR significantly reduced, while albumin percentage and total iron significantly increased. Even if not showing a statistical difference, total proteins, and CRP slightly reduced from T0 to T1. During the trial, dogs maintained a good state of health with a mean clinical score of 1.1 and 1.2 at T0 and T1, respectively. Eight out of 18 dogs experienced a slight increase in their clinical score, while 6 and 4 animals showed the same or a reduced clinical score, respectively. Vector seasonality seems to have a strong influence on laboratory alterations and anti-L. infantum antibody titers of dogs living in endemic areas for leishmaniosis. These results reinforce the importance of considering sampling season in the clinical evaluation and management of dogs affected by leishmaniosis.

[1] Cavalera MA, Iatta R, Panarese R, Mendoza-Roldan JA, Gernone F, Otranto D, Paltrinieri S, Zatelli A (2021) Seasonal variation in canine anti-Leishmania infantum antibody titres. Vet J. 271:105638. Doi: 10.1016/j.tvjl.2021.105638.
[2] Paltrinieri S, Solano-Gallego L, Fondati A, Lubas G, Gradoni L, Castagnaro M, Crotti A, Maroli M, Oliva G, Roura X, Zatelli A, Zini E (2010) Guidelines for diagnosis and clinical classification of leishmaniasis in dogs. J. Am. Vet. Med. Assoc. 236:1184-1191. doi: 10.2460/javma.236.11.1184.
[3] Villanueva-Saz S, Giner J, Verde M, Yzuel A, Ruiz H, Lacasta D, Riera C, Fisa R, Alcover MM, Fernández A (2021) Antibodies to Leishmania in naturally exposed domestic ferrets (Mustela putorius furo) in Spain. Vet Parasitol. 296:109492. doi: 10.1016/j.vetpar.2021.109492.

SERUM BICARBONATE DEFICIENCY IN DOGS WITH ACUTE AND CHRONIC KIDNEY DISEASE

Ilaria Lippi (1), Francesca Perondi (1), Eleonora Gori (1), Alessio Pierini (1), Lucrezia Bernicchi (1),, Veronica Marchetti (1)

(1) Dipartimento di Scienze Veterinarie - Università di Pisa

Corresponding Author: **Ilaria Lippi** – ilaria.lippi@unipi.it

Serum bicarbonate deficiency is a frequent disorder in human patients with acute (AKI) and chronic (CKD) kidney disease, due to abnormalities in kidney generation and reabsorption of bicarbonate [1]. Data regarding the frequency of bicarbonate disorders in AKI and CKD dogs are scarce [2]. The aim of the present study was to assess the frequency and the severity of bicarbonate deficiency in dogs with AKI, acute on chronic kidney disease (ACKD), and CKD, and its possible association with the IRIS grade/stage, and disorders of calcium-phosphate metabolism.
A retrospective evaluation of serum biochemical panels (serum creatinine, urea, ionized calcium, total calcium, phosphate, bicarbonate) of all dogs with diagnosis of AKI, ACKD, and CKD, referred to the nephrology service of the VTH of the University of Pisa, between January 2014 and January 2022 was performed. Serum biochemical panels were excluded in case of missing parameters, post-dialysis panels, and dogs on sodium bicarbonate supplementation. Bicarbonate deficiency was defined as serum bicarbonate <22 mEq/L,and classified as moderate (18-22 mEq/L), or severe (<18 mEq/L) [3]. Serum bicarbonate deficiency was found in 397/521 dogs (76%), of which 142/397 (36%) showed moderate deficiency, and 255/397 (64%) severe deficiency. Dogs with AKI and ACKD showed a significantly higher frequency of bicarbonate deficiency (p=0.004), and severe forms compared to CKD dogs (p=0.02). In AKI and ACKD dogs, a negative linear correlation was found among serum bicarbonate, and serum creatinine, urea, and phosphate. The frequency of bicarbonate deficiency was higher in later stages of the disease in both AKI (p=0.01), ACKD (p=0.0003), and CKD (p=0.009). Dogs with serum CaxP $\geq$70mg^2/dL^2 showed a higher frequency of bicarbonate deficiency (p=0.01), and severe forms (p=0.01), compared to dogs with CaxP <70mg^2/dL^2.
Serum bicarbonate deficiency seems to be a very frequent disorder in both AKI, ACKD and CKD dogs, with an increasing frequency and severity in more advanced stages of renal disease. The higher frequency and severity of bicarbonate deficiency in AKI, and ACKD may be caused by a more severe and sudden loss of renal function, or extra-renal factors. Finally, the association between frequency and severity of bicarbonate deficiency, and abnormal CaxP may suggest a potential connection between metabolic acidosis, and bone-mineral disorders, as previously demostrated in human medicine.

[1] Kim, H.J., et al. *Front Med* 2021
[2] Polzin, D.J. Chronic Kidney Disease. In Nephrology and Urology of Small Animals, 1st ed.; Bartges J., Polzin D.J., Eds.; Wiley-Blackwell: Iowa, USA, 2011; 433-471
[3] Navaneethan, S.D., et al. *Clin J Am Soc Nephrol* 2019

GENERATION OF INSULIN-PRODUCING CELLS FROM CANINE BONE MARROW-DERIVED MESENCHYMAL STEM CELLS

Antonella Colella (1), N. Marrano (1), G. Biondi (1), L. Fracassi (1), Antonio Crovace (1), Francesco Giorgino (1), Edda Francioso (1), Paola Paradies (1), Annalisa Natalicchio (1)

(1) Department of Precision and Regenerative Medicine and Jonian Area (DiMePre-J); University of Bari "Aldo Moro"

Corresponding Author: **Paola Paradies** - paola.paradies@uniba.it

Cell-based therapy using insulin-producing cells (IPCs) differentiated from stem cells is anticipated as an alternative treatment option to insulin injection or pancreatic islet transplantation for the treatment of diabetes mellitus in both human and veterinary medicine. Several protocols were reported for the differentiation of mesenchymal stem cells (MSCs) into IPCs; to date, glucose-responsive IPCs have only been obtained from canine adipose tissue-derived MSCs (cAD-MSCs) [1,2], but not from canine bone marrow-derived MSCs (cBM-MSCs) [3]. Therefore, this study is aimed to obtain in vitro-generated glucose-responsive IPCs from cBM-MSCs using two-step [3] or three-step differentiation protocols [1].

BM-MSCs from one dog were successfully cultured and expanded. Most of the cells isolated expressed the MSCs markers CD73, CD90, and CD105. At baseline, cells exposed to the two-step differentiation protocol had a very variable morphology and rarely grouped to form clusters (a differentiation feature). On day 10, cells proliferated compared to baseline and only a small group of cells appeared grouped to form small clusters. Accordingly, pancreatic mRNA analysis showed a slight increase of the pancreatic endoderm marker (Pdx1) and pancreatic beta-cell marker (Insulin) compared to basal condition, but no insulin secretion was detected either in the culture medium or following glucose stimulation.

Conversely, cells exposed to the three-step differentiation protocol under a three-dimensional (3D) culture system (Matrigel) formed colony-like structures starting from the eighth day of differentiation, without showing further proliferation. Interestingly, in cells exposed to the three-step differentiation protocol, insulin mRNA expression was significantly upregulated compared to undifferentiated controls. Besides, IPCs colonies secreted insulin in the culture medium, compared to basal condition, although insulin secretion was not enhanced by high-glucose (22 mM) compared to low-glucose (5.5 mM) culture conditions.

The results suggest that the three-step differentiation protocol can generate IPCs from cBM-MSCs, although with limited functional property.

Further studies are required to establish a more effective differentiation protocol, able to differentiate cBM-MSCs in glucose-responsive IPCs.

[1] Teshima T, et al. (2020). Generation of IPCs from canine adipose tissue-derived mesenchymal stem cells. Stem Cells International,1-10.
[2] Dang Le Q, et al. (2022). In vitro generation of transplantable IPCs from canine adipose-derived mesenchymal stem cells. Scientific reports,12(1),1-18.
[3] Rodprasert W, et al. (2021). Tailored generation of IPCs from canine mesenchymal stem cells derived from bone marrow and adipose tissue. Scientific reports,11(1),1-17.
[4] Gabr MM, et al. (2018). IPCs from adult human bone marrow mesenchymal stromal cells could control chemically induced diabetes in dogs. Cell transplantation,27(6),937-947.

EVALUATION OF BLOOD GROUP SALIVARY SECRETORY STATUS IN DOGS

Roberta Perego (1), Eva Spada (1), Luciana Baggiani (1), Martina Falco (1), Daniela Proverbio (1)

(1) Università degli Studi di Milano, Dipartimento di Medicina Veterinaria e Scienze Animali, Lodi, Italy

Corresponding Author: **Roberta Perego** - roberta.perego@unimi.it

Some people, called secretors, secrete ABO blood antigens in body fluids, particularly in saliva1. The salivary secretory status prevalence varies from 60 to 80%, with ethnic and sex differences2. Many studies have shown that nonsecretory status could be a risk factor for a number of infectious and non-infectious diseases3. In dogs there are no studies looking at salivary expression of blood antigens and it is not known if salivary secretory status exists in dogs.
The aim of this preliminary study was to look for DEA 1, DEA 4 and Dal antigens in canine saliva and define the prevalence of salivary secretory status in dogs. Thirty-six saliva samples were collected from healthy DEA1+, DEA4+ and Dal+ donor dogs of different age, sex and breed. A dedicated device was used for saliva collection (Salivette□) and the salivary secretory status determined through two methods: the tube inhibition test and the gel-based inhibition test. The presence (nonsecretory status) or absence (secretory status) of agglutination was macroscopically and microscopically evaluated for the tube method and only macroscopically for the gel method. Agglutination was graded from 0 (absence) to 4+ (strong). With tube, 25 and 32 canine saliva samples were tested for DEA 1 and DEA 4 antigens, respectively, while with gel, 25, 30 and 24 saliva samples were tested for DEA 1, DEA 4 and Dal antigens, respectively.
Furthermore, for each method, repeatability tests were carried out. The K coefficient was calculated to verify the concordance between macroscopic and microscopic results on tube method and between tube and gel methods.
All saliva samples tested showed agglutination with both tube and gel techniques, therefore prevalence of secretory status was 0% for the three tested antigens. The concordance between microscopic and macroscopic agglutination degrees with tube method was fair (K 0.33) for DEA1 and good (K 0.63) for DEA 4. The K coefficient between tube and gel methods was poor (K 0) for DEA 1, and good (K 0.67) for DEA 4. Repeatability was 100% for both methods.
This preliminary study did not identify canine salivary secretory status for blood antigens DEA1, DEA4 and Dal in any dog analyzed. As in canine blood typing studies, the tube method showed weak agglutinations, sometimes difficult to interpret for DEA 1 antigen, and the gel inhibition method represents the most effective and most easily interpreted test for further studies on secretory status in dogs.

[1] Motghare P, Kale L, Bedia A S, Charde S. Efficacy and accuracy of ABO blood group determination from saliva. J Indian Acad Oral Med Radiol. 2011; 23:163–167
[2] Saboor M, Ullah A, Qamar K, Mir A, Moinuddin. Frequency of ABH secretors and non secretors: A cross sectional study in Karachi. Pak J Med Sci. 2014 Jan;30(1):189-93
[3] Bakhtiari S, Mani Far S, Alibakhshi Z, Shirkhoda M, Anbari F.Salivary Secretor Status of Blood Group Antigens in Patients with Head and Neck Cancer.Open Access Maced J Med Sci.2019 Feb 15; 7(3):373-377.

76° CONVEGNO SISVET
BARI, 21-22-23 GIUGNO 2023

A RETROSPECTIVE COMPARATIVE EVALUATION OF SELECTED LEUKOCYTES RATIOS, ACUTE PHASE PROTEINS AND LEUKOCYTE CHANGES SUGGESTIVE OF INFLAMMATION IN CATS.

Giulia Donato (1), Marisa Masucci (1), Maria Flaminia Persichetti (1), Joy Archer (2), Maria Grazia Pennisi (1)

(1) Dipartimento di Scienze Veterinarie, Università degli Studi di Messina, Messina, Italy.
(2) Department of Veterinary Medicine, University of Cambridge, Cambridge, UK.

Corresponding Author: **Giulia Donato** - giudonato@unime.it

Neutrophil/lymphocyte (NLR) and platelet/lymphocyte (PLR) ratios have been recently proposed as diagnostic and prognostic markers for feline inflammatory and neoplastic conditions [1-4]. However, no information exists on the relationship between these ratios and other markers of inflammation in cats. The aims of this study were to evaluate the relationship between NLR, monocyte/lymphocyte ratio (MLR), PLR and serum amyloid A (SAA), hypoalbuminemia, hyperglobulinemia, presence of leukocyte alterations suggestive of inflammation ("LAI" = neutrophil left shift, toxic neutrophils, reactive lymphocytes). The study was conducted in 275 cats admitted to veterinary clinics located in Sicily and Calabria. For each cat results from complete blood count (CBC) with blood smears evaluation, albumin, globulins, and SAA measurement were statistically evaluated. Blood smears were always evaluated by the same operator. The absolute count of neutrophils, monocytes or platelets divided by the absolute count of lymphocytes, were defined as NLR, MLR and PLR respectively. Data distribution was assessed by the D'Agostino-Pearson omnibus normality test. Mann-Whitney test or Spearman's Rho test were performed where appropriate. P <0.05 was considered significant. Positive correlations between SAA and NLR (P=0.0002, rs=0.2261) and MLR (P<0.0001, rs=0.2881) and between globulins values and NLR (P<0.0001, rs=0.3111) and MLR (P=0.0002, rs=0.2193) were found. A negative correlation between albumin values and NLR (P<0.0001, rs=-0.438), MLR (P<0.0001, rs=-0.3638) and PLR (P=0.0004, rs=-0.2124) was found. Cats with LAI showed significantly higher NLR (P=0.0001), MLR (P=0.0236) and PLR (P=0.0439) compared to cats without LAI. The results of this study suggest that NLR, MLR and PLR values could be usefully added in the CBC report for obtaining information on any ongoing inflammatory process in examined cats without additional costs and tests. However, reference values for these ratios in healthy animals need to be established first.

[1] Gori E, Pierini A, Lippi I, Lubas G, Marchetti V. Leukocytes Ratios in Feline Systemic Inflammatory Response Syndrome and Sepsis: A Retrospective Analysis of 209 Cases. Animals (Basel). 2021 Jun 1;11(6):1644.
[2] Neumann S. Neutrophil-to-lymphocyte and platelet-to-lymphocyte ratios in dogs and cats with acute pancreatitis. Vet Clin Pathol. 2021 Mar;50(1):45-51
[3] Petrucci GN, Lobo L, Queiroga F, Martins J, Prada J, Pires I, Henriques J. Neutrophil-to-lymphocyte ratio is an independent prognostic marker for feline mammary carcinomas. Vet Comp Oncol. 2021 Sep;19(3):482-491.
[4] Naito E, Yuki M, Hirano T, Kainuma D, Aoyama R. Prognostic utility of preoperative neutrophil-lymphocyte ratio in cats with malignant mammary tumors. Res Vet Sci. 2021 Mar; 135:349-354.

76° CONVEGNO SISVET
BARI, 21-22-23 GIUGNO 2023

RED BLOOD CELL LIPIDOMIC PROFILE IN SEPTIC DOG AFFECTED BY PARVOVIRUS ENTERITIS

R. Di Prinzio (1), C. De Rose (1), A. Sansone (2), C. Ferreri (2), G. Aste (1)

(1) Università degli studi di Teramo, Dipartimento di Medicina veterinaria. SP 18, Loc. Piano D'Accio, 64100 Teramo, Italia
(2) ISOF, Consiglio Nazionale delle Ricerche. Via piero Gobetti 101, 40129 Bologna, Italia

Corresponding Author: **Roberta Di Prinzio** - rdirpinzio@unite.it

Inflammation involves a multitude of cell types, chemical mediators and interactions. Fatty acids play a key role in inflammatory processes (1). The lipidomic profile of red blood cells (RBC) has been recently evaluated in dogs in order to monitor different health and disease conditions. Parvovirus (CPV-2) enteritis in dogs is a predisposing factor for sepsis and we hypothesized that the erythrocyte membrane lipidome could reflect the animal's acute sepsis status. Dogs admitted to the Veterinary Teaching Hospital of the University of Teramo (2020-2022) were prospectively enrolled in the study. Symptomatic dogs with PCR-confirmed CPV-2 infection were eligible for enrollment. Healthy dogs, younger than 24 months, feeded with a commercial diet were assigned to the healthy group. Blood samples (1 ml) were collected in EDTA tubes, refrigerated and stored. RBC lipidome analysis was performed by Research National Council laboratories. The analysis allows the quantification of 10 fatty acids detected by gas chromatographic analyses of the FAMEs1 (fatty acid methyl esters), 3 indexes were also calculated [unsaturation index (UI), peroxidation index (PI), PUFA balance balance] (2). All datasets were tested for normal distribution (Shapiro-Wilk test). The comparisons between the values of fatty acid families and lipid indexes of the two groups were carried out using the Mann-Whitney test. Statistical analysis was performed using GraphPad Prism. In the Healthy (H) group were 16 dogs (13 male, 3 female). The Parvovirus (P) group was composed of 19 puppies (13 male, 16 female). The median age was 6 months (range:1-21) in H dogs and 5 months in P dogs (range: 2-11). The median weight was 26 kg (range:15-42) in H dogs and 7 kg in P dogs (range: 1-21). Vaccenic acid showed a significant decrease in the P group (2.557 µg/ml group H, 1.990µ g/ml group P, p:0,0031). Short-chain fatty acids are also represented in significantly lower p: 0.0092) amounts in dogs with parvovirus (38.03 µg/ml) in comparison of H group (42.17 µg/ml). PI is higher (p:0.0406) in group P (164) compared to H group (147), even if both values fall within the reference range present in the literature. The present work highlights differences in the composition of the erythrocyte phospholipid membrane in septic puppies affected by Parvovirus enteritis. Further studies are needed to gain new insights related to the balance among fatty acid types that regulate signaling cascades.

[1] Calder PC. Omega-3 fatty acids and inflammatory processes: from molecules to man. Biochem Soc Trans. 2017 Oct 15;45(5):1105-1115. doi: 10.1042/BST20160474. Epub 2017 Sep 12. PMID: 28900017.
[2] Prasinou P, Crisi PE, Chatgilialoglu C, Di Tommaso M, Sansone A, Gramenzi A, Belà B, De Santis F, Boari A, Ferreri C. The Erythrocyte Membrane Lipidome of Healthy Dogs: Creating a Benchmark of Fatty Acid Distribution and Interval Values. Front Vet Sci. 2020 Aug 21; 7:502. doi: 10.3389/fvets.2020.00502. PMID: 32974399; PMCID: PMC7472600.

76° CONVEGNO SISVET
BARI, 21-22-23 GIUGNO 2023

ZOOMICS: IMPACT OF LEUKOREDUCTION ON THE OMICS PHENOTYPES OF CANINE PACKED RED BLOOD CELLS DURING REFRIGERATED STORAGE

Arianna Miglio (1), Francesca Rocconi (2), Maria T. Antognoni (1), Valentina Cremoni (1), Giorgio Tiscar (1), Julie A. Reisz (3), Mark Maslanka (3), Ian S. Lacroix (3), Angelo d'Alessandro (3), Morena Di Tommaso (1)

(1) Department of Veterinary Medicine, University of Perugia, Via San Costanzo 4, 06126 Perugia, PG, Italy
(2) Department of Veterinary Medicine, Veterinary University Hospital, University of Teramo, Località Piano D'Accio, 64100 Teramo, TE, Italy
(3) Department of Biochemistry and Molecular Genetics, University of Colorado Denver - Anschutz Medical Campus, Aurora, CO, United States of America

Corresponding Author: **Arianna Miglio** - arianna.miglio@unipg.it

Background: Red blood cell (RBC) storage in the blood bank promotes a series of biochemical and morphological alterations, collectively referred to as the storage lesion. Extensive studies in humans have identified leukoreduction as a critical processing step that mitigates the storage lesion. While transfusion of packed RBCs is a life-saving interventions in veterinary species as well, to date no study has characterized the impact of leukoreduction on the storage lesion of canine blood units through omics technology.
Objective: To evaluate the lipid and metabolic profiles in canine packed RBC units as a function of leukoreduction in fresh and hypothermically-stored (1°-6°C) RBCs for up to 42 days.
Animals: pRBCs were obtained from eight donor dogs referred to two different Italian blood banks.
Methods: A volume of 450 ml of whole blood was collected using a CPD-SAGM transfusion bags with a leukoreduction (LR) filter in-process, to produce two pRBC for each donor, before (nLR-pRBC) and after (LR-pRBC) LR. Units were then stored in blood bank refrigerator at 4°C. Following sterile weekly sampling of each unit on days 0, 7, 14, 21, 28, 35, and 42, all pRBC samples were centrifuged (1.500 rpm) to separate RBCs pellet (P) and supernatant (S) for omics analyses.
Results: Metabolomics analyses reveal a significant impact of leukoreduction on fresh and stored RBC metabolic phenotypes. Specifically, nLR units were characterized by significant higher levels of free short and medium-chain fatty acids, carboxylic acids (especially pyruvate, lactate, alpha-ketoglutarate), amino acids (arginine, cystine, lysine). LR RBC had instead higher levels of glycolytic metabolites, high energy phosphate compounds (the whole adenylate pool, including ATP, ADP, AMP), and antioxidant metabolites, including markers of the activation of the pentose phosphate pathway and total glutathione pools.
Conclusion and Clinical Importance: Leukoreduction significantly ameliorates the metabolic storage lesion of canine packed RBCs, by preserving energy metabolism and preventing oxidative lesions.

[1] Weinberg JA, McGwin G, Vandromme MJ, Marques MB, Melton SM, Reiff DA, et al. Duration of Red Cell Storage Influences Mortality After Trauma. J Trauma Inj Infect Crit Care. 2010 Dec;69(6):1427–32.
[2] Koch CG, Figueroa P, Blackstone EH. Duration of Red-Cell Storage and Complications after Cardiac Surgery. N Engl J Med. 2008;
[3] Spinella PC, Carroll CL, Staff I, Gros

76° CONVEGNO SISVET
BARI, 21-22-23 GIUGNO 2023

IN VITRO EVALUATION OF COMPATIBILITY OF FELINE TYPE-B BLOOD WITH CANINE AND FELINE BLOOD OF DIFFERENT BLOOD TYPE

Eva Spada (1), Roberta Perego (1), Federica Bruno (2), Germano Castelli (2), Fabrizio Vitale (2), Vito Biondi (3), Luciana Baggiani (1), Alejandro Zuliani (1), Daniela Proverbio (1)

(1) Università degli Studi di Milano, Dipartimento di Medicina Veterinaria e Scienze Animali, Lodi, Italy
(2) Istituto Zooprofilattico Sperimentale della Sicilia, Palermo, Italy
(3) Università degli Studi di Messina, Dipartimento di Scienze Veterinarie, Messina, Italy

Corresponding Author: **Eva Spada** - eva.spada@unimi.it

Cats have no naturally occurring antibodies against canine red blood cell (RBC) antigens, therefore, xenotransfusion of canine blood to a feline recipient is still used in emergencies [1,2]. This practice is particularly helpful in type-B recipients for which it is sometimes difficult to find a compatible feline donor, due to their rarity in feline population (from 5.2% to 12.1% in North and South Italy, respectively) [3]. However, data on compatibility of canine blood with type B cats are limited. The aim of this study was to evaluate *in vitro* compatibility of feline type-B blood with canine blood in comparison to type-A or type-B blood. Forty-nine type-B cats were crossmatched using a slide crossmatch (XM) technique with at least one DEA1+, DEA1- canine blood and one type-A feline blood. Both major and minor XMs were performed with macro- and microscopic evaluation of agglutination. Of 49 type-B cats, 35 were also major crossmatched with at least one type-B cat. A total of 214 major XMs were performed, 165 with canine blood and 49 with type-A feline blood, of which 91 were incompatible: 43/165 (26%) with canine blood samples and 48/49 (98%) with type-A feline samples. Only the species had a statistically significant association (P<0,0001) with incompatible XM results, with type-A feline blood having a significant relative risk (RR) of 3.7 (95%CI:2.8-4.8, P<0.0001) of incompatibility with type-B feline blood. Of 201 minor XMs performed, 153 were with canine blood and 48 with type-A feline blood. Incompatibility was found in 150 minor XMs, 131/153 (86%) canine samples and 19/48 (40%) type-A feline samples. Species (P<0.0001) and DEA blood type (P=0.0001) were significantly associated with incompatibility, with canine blood and DEA1+ type having respectively a RR of 2.1 (95%CI:1.5-3.0, P<0.0001) and 1.3 (95%CI:1.1-1.5, P=0.0006) of incompatible minor XM. Relative to the 35 type-B cats crossmatched with type-B cats, of 71 major XMs performed, 25 (35%) were incompatible. No significant difference (P=0.1555) was found for type-B blood major XM incompatibilities between canine blood and feline type-B blood. In emergencies, when compatible feline blood is not readily available, type-B cats could be transfused with canine blood, if possible, using DEA1- packed RBCs. Due to incompatibility outside the AB feline blood group system, major XM should be always performed before feline transfusions, even when blood type compatible blood is being transfused.

[1] Le Gal, A., Thomas, E. K. & Humm, K. R. Xenotransfusion of canine blood to cats: a review of 49 cases and their outcome. J. Small Anim. Pract. 61, 156–162 (2020).
[2] Tinson, E., Talbot, C. T. & Humm, K. Incidence of acute haemolysis in cats receiving canine packed red blood cells (xenotransfusions). J. Feline Med. Surg. 24, e628–e635 (2022).
[3] Spada, E. et al. Prevalence of Blood Types and Alloantibodies of the AB Blood Group System in Non-Pedigree Cats from Northern (Lombardy) and Southern (Sicily) Italy. Animals 10, 1129 (2020).

76° CONVEGNO SISVET
BARI, 21-22-23 GIUGNO 2023

ASSESSMENT OF THE CLINICAL EFFICACY OF A DIETARY MINERAL COMPLEMENTARY FEED CONTAINED IN AN INTRARUMINAL SLOW-RELEASE BOLUS ON UDDER HEALTH IN DAIRY COWS

Jacopo Guccione (1), Maria Chiara Alterisio (1), Sergio Esposito (1), Sebastiano Tinelli (2), Giovanni D'Onghia (2), Paolo Ciaramella (1)

(1) Department of Veterinary Medicine and Animal Productions, University of Napoli Federico II, Via Delpino 1, 80137 Napoli, Italy
(2) Veterinary Practitioner, Bari District, Puglia Region, Italy

Corresponding Author: **Maria Chiara Alterisio** - mariachiara.alterisio@unina.it

The aim of the study was to evaluate the effects on udder health of a dietary mineral complementary feed contained in slow-release intraruminal bolus (Dalmaglass®, Fatro S.p.a., IT) administered in dairy cows at drying-off.
Fifty-two pluriparous cows were enrolled as treated group (TG, receiving two boluses as labelled) and 53 as control group (CG, left untreated). From all of them, composite milk samples were monthly (MO) collected from 30 to 300 days in milk (DIM) to perform somatic cell count (SCC) and milk production were recorded. As reported by Bradley et al. (1), cows with SCC>200*103 cells/mL were considered as affected by mastitis. The effects on udder health were assessed by: (i) average (AV) daily production; (ii) AV peak production; (iii) AV persistency of production; (iv) overall SCC and (vi) MO AV; (vii) overall and (viii) MO numbers of mastitis- or (ix) non-mastitis-cases. Finally, (x) MO proportions of new mastitis, (xi) MO cured mastitis, and (xii) MO failure of existing mastitis to cure, (xiii) MO number of chronic cases were calculated. Variables were analysed by parametric and non-parametric tests. Probabilities<0.05 were considered as significant.
The AV daily production was 38.1 Litres (L) in TG and 36.2 L in CG (P<0.001). The overall SCC observed in TG was 332.3*103 cells/mL while 513.8*103 in CG (P<0.05). The TG showed both a smaller overall number of mastitis-cases as compared to CG (91 vs. 133, P<0.001) and a greater number of non-mastitis-cases (381 vs. 317, P<0.001). In TG a smaller number of cows MO affected by mastitis was observed at 60 (4 in TG vs. 14 in CG, P<0.01) and 270 DIM (5 in TG vs. 12 in CG, P<0.05), as well as a greater those non-affected by mastitis at 60 (50 in TG vs. 39 in CG, P<0.01), 150 (44 in TG vs. 33 in CG, P<0.05), and 270 DIM (27 in TG vs. 20 in CG, P<0.05). For the MO proportion of failure of existing mastitis to cure, lower values were found in TG at 60 (3.6% in TG vs. 15.7% in CG, P<0.05), 90 (0.0% in TG vs. 17.6% in CG, P<0.001), and 120 DIM (3.6% in TG vs. 17.6% in CG, P< 0.05). Finally, similar findings were found for the number of chronic cases at 90 (3 in TG vs. 14 in CG, P<0.001) and 120 DIM (3 in TG vs. 12 in CG, P=0.01).
Preliminary data shows overall potential beneficial effects on udder health. Its use might be hypothesized as support to a complete clinical udder health management program. Nevertheless, further studies are necessary to confirm the encouraging outcomes observed.

[1] Bradley A, Barkema H, Biggs A, Green M, Lam T. Control of Mastitis and Enhancement of Milk Quality. In: Green M, editor. Dairy herd health. Oxford: CABI International; 2012. Chapter 5. p. 117–68

ASSESSMENT OF THE CLINICAL EFFICACY OF A DIETARY MINERAL COMPLEMENTARY FEED CONTAINED IN AN INTRARUMINAL SLOW-RELEASE BOLUS ON DAIRY COWS' POST-PARTUM DISEASES AND COLOSTRUM QUALITY

Maria Chiara Alterisio (1), Sergio Esposito (1), Sebastiano Tinelli (2), Giovanni D'Onghia (2), Paolo Ciaramella (1), Jacopo Guccione (1)

(1) Department of Veterinary Medicine and Animal Productions, University of Napoli Federico II, Via Delpino 1, 80137 Napoli, Italy
(2) Veterinary Practitioner, Bari District, Puglia Region, Italy

The aim of the study was to evaluate the effects on post-partum diseases and colostrum quality of a dietary mineral complementary feed contained in slow-release intraruminal bolus (Dalmaglass®, Fatro S.p.a., IT) administered in dairy cows at drying-off.
Fifty-two pluriparous cows were enrolled as treated group (TG, receiving two boluses as labelled and containing Mg<1%, Na=19%, P=27%, Ca<1%) and 53 as control group (CG, left untreated). All of them received complete clinical examinations at different times to assess the presence of the production diseases classified according to Breen et al. (1), as follows: type of calving (eutocic and dystocic), retain foetal membranes (within 24 hours after calving) puerperal metritis (from 24 hours to 10 days after calving), clinical metritis (within 21 days after calving), clinical endometritis (at 21 or more days after calving), ketosis and milk fever (within 21 days after calving). Moreover, effects on colostrum quality were assessed by colostrometer (0-32.0% Brix Refractometer), as well as cases of diarrhoea (within 2 months after birth) and pneumonia (within 6 months after birth) affecting the progeny born by the cows enrolled. Variables were analysed by appropriate non-parametric tests. Probabilities<0.05 were considered significant.
The percentage of cows affected by retain foetal membranes was 8.9 in TG and 38.2 in CG (P<0.001), while that of affected by puerperal metritis was 3.6 in TG and 20.0 in CG (P<0.01). The TG showed a smaller percentage of clinical metritis-cases as compared to CG (7.1% vs. 30.9%, P<0.01) as well as of clinical endometritis (3.6% vs. 21.8%, P<0.01). Moreover, a smaller percentage of cows were affected by ketosis in TG (0.0% in TG vs. 9.1% in CG, P<0.05). Regarding the colostrum, the TG showed a higher percentage of cows with the best quality (42.9% in TG vs. 5.5% in CG for Brix values>25%, P<0.0001). Finally, considering the effects on progeny, a lower percentage of calves developed diarrhoea when born from treated animals as compared to those born from left untreated (10.7% in TG vs. 47.3% in CG, P<0.0001).
Preliminary data shows overall potential beneficial effects on some post-partum diseases of dairy cows and calves as well as on colostrum quality. Its use might be hypothesized as support for a complete clinical dairy herd management program. Nevertheless, further studies are necessary to confirm the encouraging outcomes observed.

[1] Breen J, Down P, Kerby M, Bradley A. Restoring the dairy herd: rearing youngstock and replacing cows. In: Green M, editor. Dairy herd health. Oxford: CABI International; 2012. Chapter 3. p. 35–77

76° CONVEGNO SISVET
BARI, 21-22-23 GIUGNO 2023

LUNG ULTRASONOGRAPHY EVALUATIONS AFTER INTRANASAL AND/OR PARENTERAL VACCINATION FOR BOVINE RESPIRATORY DISEASE IN DAIRY CALVES

Anastasia Lisuzzo (1), Giacomo Catarin (1), Elisa Mazzotta (2), Matteo Gianesella (1), Nicola Morandi (3), Eliana Schiavon (2), Giulia Cento (2), Chiara Tommasoni (1), Enrico Fiore (1)

(1) Department of Animal Medicine, Production, and Health, University of Padua, 35020 Legnaro (PD), Italy
(2) Experimental Zooprophylactic Institute of Venezie, 35020 Legnaro (PD), Italy
(3) Boehringer Ingelheim Italia, 20139 Milan (MI), Italy

Corresponding Author: **Anastasia Lisuzzo** - anastasia.lisuzzo@phd.unipd.it

The bovine respiratory disease (BRD) can significantly reduce the health and welfare of dairy calves. Vaccination is a common practice to minimize the incidence of BRD both intranasal and parenteral. The aim of this study was to evaluate the lung ultrasound response of calves undergoing intranasal, parenteral, or both vaccination for BRD. Two-hundred one Holstein Friesian calves were enrolled and divided into four group: control group (Group A, n=41, without vaccination); intranasal-vaccination group (Group B, n=46, intranasal vaccination); parenteral-vaccination group (Group C, n=52, subcutaneous vaccination); double-vaccination group (Group D, n=62, intranasal and subcutaneous vaccinations). All animals received a clinical examination and lung ultrasonographic evaluation at 10-15 days of life (day of recruitment: T0), 17-22 (T1), 31-38 (T2), and 45-52 (T3) days of life. The intranasal vaccination was performed after lung ultrasonography at T0 in Group B and D, while parenteral vaccination was performed in Group C. Furthermore, a parenteral vaccination was performed at T1 in Group D, and the booster was administered at T2 in Group C and D. Animals were identified as affected by BRD according to ultrasonography score (0: healthy; 1: comet-tail without consolidation; 2: lobular consolidation; 3: lobar consolidation; 4: two lobar consolidations; 5: three or more lobar consolidation). The Kruskal-Wallis and the Dunn tests were performed to assess differences between groups and over time, while the Chi-squared test was used to evaluate the differences between proportions. All vaccinated groups showed a lower ultrasonography score over time compared to Group A except for Group B at T3. Groups B and D presented a lower percentage of diseased animals compared to Group A at T1 and T2, while groups C and D were lower at T3. The odds ratio showed a lower risk of BRD in all vaccinated groups at T1 and T2, but only Group D continued to T3. Group D also showed a lower risk compared to Group C at T1, and groups B and C at T2. All vaccinated groups showed similar and lower mortality compared to the control group. Our results suggest that the lung ultrasonography was more effective in identifying cases of BRD. Furthermore, the association of intranasal and parenteral vaccinations was more effective in reducing the risk of BRD.

SALMONELLA ENTERICA SEROVAR DUBLIN INFECTION IN DAIRY CATTLE: A CASE STUDY ON THE MANAGEMENT OF AN OUTBREAK IN VENETO REGION

Chiara Tommasoni (1), Eliana Schiavon (2), Anastasia Lisuzzo (1), Matteo Gianesella (1), Marianna Merenda (3), Patrizio Coin (4), Tommaso Patregnani (5), Sebastiana Tola (6), Salvatore Catania (3), Antonio Barberio (2)

(1) Department of Animal Medicine, Production and Health, University of Padua, 35020 Legnaro, Italy
(2) Istituto Zooprofilattico Sperimentale delle Venezie, 35020 Legnaro (PD), Italy
(3) Istituto Zooprofilattico Sperimentale delle Venezie, 37060 Buttapietra (VR), Italy
(4) Dipartimento veterinario e sicurezza degli alimenti di origine animale "ATS Brescia", 25124 Brescia, BS, Italy
(5) Veterinary Services, Local Health Unit "AULSS 9 Scaligera", 37057 Verona, VR, Italy
(6) Istituto Zooprofilattico Sperimentale della Sardegna, 07100 Sassari, Italy

Corresponding Author: **Chiara Tommasoni** - chiara.tommasoni@phd.unipd.it

Salmonella enterica, subsp. *enterica serovar Dublin* (S. Dublin), is a serovar adapted to cattle, causing both intestinal and systemic infections. The introduction of the bacterium leads to serious economic losses due to abortions, high mortality in calves and persistent infections, also representing a major health problem as zoonotic agent.
This case study describes an outbreak of S. Dublin on a farm of 210 Holstein-Friesian lactating cows. Clinical signs were observed in calves younger than 6 months; deaths occurred in animals younger than 30 days. Following the conferral at the IZSVe of a newborn calf that died from enteric syndrome, S. Dublin was diagnosed. At the autoptic exam pathological findings were observed in gut, liver, pericardium, lungs, joints, lymph nodes and abomasum. Considering the pathogenesis of S. Dublin, authors decided to apply a protocol prepared by the IZSVe based both on direct and indirect prophylaxis. Screening tests were performed both on fecal and milk samples. Feces were collected from 80 animals (dry-off cows and calves), in 7 times; 25 milk samples were collected from bulk tank milk (BTM). 2 environmental swabs were also collected from lactating and dry cows' boxes. All lactating cows were tested with fecal swabs 2 times for a total of 220 and 223 dairy cows.
Considering the difficulty to find an effective commercial vaccine against S. Dublin, an autogenous vaccine prepared by the Istituto Zooprofilattico Sperimentale della Sardegna (IZS Sardegna) was administered. A pre and post-vaccination screening was performed to assess the immunization of cows and the effectiveness of the protocol itself. A total of 52 cattle randomly selected among cows and heifers were enrolled for a 3-time sampling (T0, T1, T2), the first 1 day prior immunization, the second and the third 2 and 11 months later respectively, both on feces and serum, collecting blood samples from the coccygeal vein. 25 BTM samples were also weekly bacteriologically analyzed.
Serological examination identified 13/52 (25%), 49/49 (100%) and 32/44 (73%) positive animals at T0, T1 and T2 respectively. No fecal sample in all time-points was found positive. After vaccination only 1 milk sample turned out positive. Considering the pathogenesis of S. Dublin, the negativity of the bacteriological exams suggests a positive effect of the protocol in the reduction of clinical cases, circulation of the etiological agent and biocontainment of the infection.

[1] Egle Kudirkiene, Gitte Sørensen, Mia Torpdahl, Leonardo V. de Knegt, Liza R. Nielsen ER, Shahana Ahmed JEO. 2020. Epidemiology of Salmonella enterica Serovar Dublin in Cattle. 86(3):1–12
[2] Henderson K, Mason C. 2017. Diagnosis and control of Salmonella Dublin in dairy Herds. In Pract. 39(4):158–168. doi:10.1136/inp.j1160
[3] Lynne AM, Dorsey LL, David DE, Foley SL. 2009. Characterisation of antibiotic resistance in host-adapted Salmonella enterica. Int J Antimicrob Agents. 34(2):169–172. doi: 10.1016/j.ijantimicag.2009.02.018

76° CONVEGNO SISVET
BARI, 21-22-23 GIUGNO 2023

DIFFERENCES IN THE *SERUM METABOLOME* PROFILE OF DAIRY COWS AFFECTED BY SUBCLINICAL MASTITIS

Anastasia Lisuzzo (1), Luca Laghi (2), Chiara Tommasoni (1), Enrico Fiore (1), Rossella Tessari (1), Alessio Cecchinato (3), Vittoria Bisutti (3), Matteo Gianesella (1)

(1) Department of Animal Medicine, Productions and Health, University of Padua, Viale dell' Università, 16, 35020, Legnaro (PD), Italy
(2) Department of Agricultural and Food Science, University of Bologna, 47521 Cesena, Italy.
(3) Department of Agronomy, Food, Natural Resources, Animals and Environment (DAFNAE), University of Padova, Viale dell' Università 16, 35020, Legnaro (PD), Italy

Corresponding Author: **Chiara Tommasoni** - chiara.tommasoni@phd.unipd.it

Mastitis is an inflammatory response of mammary tissue caused by physical trauma or infectious agents, representing one of the main diseases in dairy cows' farms leading to significant economic losses due to reduced milk production, treatment costs, milk wastage due to drug withdrawal times and prematurely reformed animals [1][2].
This study was part of the LATSAN project performed in three Friesian-Holstein Italian selected dairy farms with the presence of contagious mastitis.
The aim of this study was to evaluate serum metabolome differences in animals affected by subclinical intramammary infection (sIMI) caused by *Streptococcus agalactiae* and *Prototheca spp.*
After an initial blood sampling and bacteriological milk screening (T0) performed on all animals (n = 450), aimed at detecting cows with sIMI and at obtaining serum (n.75), the animals were followed up after two (T1) weeks from T0.
For this study 40 out of 75 sIMI animals were selected and grouped according to the infectious status confirmed in both period (T0 and T1): G0 for negative animals (n=16), G1 for animals affected by Streptococcus agalactiae mastitis (n=17), G2 for animals affected by *Prototheca spp*. (n=7).
Serum samples of T0 were processed to perform metabolomic analysis through 1H-NMR.
Statistical differences between groups of the main characteristics as parity, somatic cell count (SCC), milk composition and blood metabolites were evaluated by one-way ANOVA. A p-value < 0.05 was considered as significant, while a trend for significance was considered with $0.05 \leq$ p-value ≤ 0.10.
According to the statistical analysis, SCC significantly differed among the 3 groups: in G1 the highest value was observed, in G0 the lowest instead. From serum samples, 43 metabolites were identified, of which 10 (3-Hydroxybutyrate, Acetate, Acetone, Allantoin, Asparagine, Carnitine, Citrulline, Ethanol, Lactose, Methylguanidine) showed significant differences among groups and 4 (Citrate, Dimethylamine, Histidine, Valine) a trend for significance.
In conclusion, sIMI may influence blood metabolome also according to different etiological pathogens.
Acknowledgments: This study was part of the LATSAN project funded by MIPAAF.

[1] A. J. Bradley, "Bovine Mastitis: An Evolving Disease," Vet. J., vol. 164, no. 2, pp. 116–128, Sep. 2002, doi: 10.1053/TVJL.2002.0724.
[2] P. Martin, H. W. Barkema, L. F. Brito, S. G. Narayana, and F. Miglior, "Symposium review: Novel strategies to genetically improve mastitis resistance in dairy cattle," J. Dairy Sci., vol. 101, no. 3, pp. 2724–2736, Mar. 2018, doi: 10.3168/JDS.2017-13554.

76° CONVEGNO SISVET
BARI, 21-22-23 GIUGNO 2023

PAIN ASSESSMENT IN ADULT CATTLE UNDERGOING DIFFERENT TYPES OF ABDOMINAL SURGERY AT A VETERINARY TEACHING HOSPITAL USING THE UNESP-BOTUCATU SCALE: FEASIBILITY STUDY

Giovanni Armenia (1), Giulia Sala (1,2), Irene Nocera (3), Antonio Boccardo (4), Davide Pravettoni (4), Simonetta Citi (1), Vincenzo Ferrulli (4), Micaela Sgorbini (1,2), Francesca Bonelli (1,2)

(1) Department of Veterinary Sciences, University of Pisa, Viale delle Piagge 2, 56124, Pisa, Italy
(2) Centro di Ricerche Agro-Ambientali "E. Avanzi", University of Pisa, via Vecchia di Marina 6, 56122, San Piero a Grado, Italy
(3) Institute of Life Sciences, Sant'Anna School of Advanced Studies, 33 Martiri della Libertà Square, 56127, Pisa, Italy
(4) Department of Veterinary Medicine and Animal Science, University of Milan, Via dell'Università 6, 26900 Lodi, Italy

Corresponding Author: **Giovanni Armenia** - giovanni.armenia@phd.unipi.it

Recognize pain in cattle is important for prompt pharmacological treatment, ensure animal welfare, and improving health and productivity [1]. An UNESP-Botucatu pain scale was developed to evaluate postoperative pain after orchiectomy [2].
The aims of the study were to evaluate UNESP-Botucatu pain scale in cattle under surgery; to analyse variations in pain score through different length periods of observation; to assess the intra-observer agreement. Eleven healthy cows from the University of Pisa were included as control group (CG) and 11 cows (different breeds) hospitalized for surgery (LDA, RDA, TRP) at VTH of the University of Milan were included as sick group (SG). Surgery was made in standing and under local anaesthesia. CG cows were continuously filmed for 25 minutes once, while in the SG, videos were recorded at different time points for 25 minutes: before surgery (T0); 2 hours (T1), 6 hours (T2), and 24 hours after surgery (T3). To evaluate the best time length of the observation period, each video was edited and shortened to obtain three different lengths: 10, 15, and 25 minutes. The same clinician assessed postoperative pain with the UNESP-Botucatu pain scale twice, one month apart. Pain scores difference between groups and within group at different time points was evaluated with Mann-Whitney U test and Kruskal-Wallis test. Intra-observer agreement was tested with Cohen's K test. In the CG, the median UNESP-Botucatu score resulted 2, while in the SG the median score resulted 3 at T0, 5.5 at T1 and T2, and 4 at T3 (p-value <0.001). The observation minutes showed no statistically significant differences in pain measurement, either between the CG and SG or at the different time points. The intra-observer agreement resulted moderate for the total score (K= 0.58), substantial for the indicators "activity" (K= 0.79) and "miscellaneous behaviours" (K=0.71), and almost perfect for "locomotion" (K=0.89), "interactive behaviour" (K=0.93) and "appetite" (0.98) indicators, respectively. This preliminary investigation highlights the feasibility of the UNESP-Botucatu scale to assess pain in cattle following different abdominal surgery, suggesting it can be a simple, useful, reliable, and responsive tool to ensure animal welfare. Moreover, this study verified that recognition and grading of pain are possible by observing animals for as little as 10 min instead of 25 min as was done in the scale validation study.

[1] Prunier, A., Mounier, L., Le Neindre, P., Leterrier, C., Mormède, P., Paulmier, V., Prunet, P., Terlouw, C., & Guatteo, R. (2013). Identifying and monitoring pain in farm animals: a review. Animal, 7(6), 998-1010.
[2] de Oliveira, F. A., Luna, S. P. L., do Amaral, J. B., Rodrigues, K. A., Sant'Anna, A. C., Daolio, M., & Brondani, J. T. (2014). Validation of the UNESP-Botucatu unidimensional composite pain scale for assessing postoperative pain in cattle. BMC Veterinary Research, 10(1), 1-14.

76° CONVEGNO SISVET
BARI, 21-22-23 GIUGNO 2023

BLOOD GAS VARIABLES, ACID-BASE STATUS, AND METABOLIC PARAMETERS IN CALVES WITH BRONCHOPNEUMONIA DIAGNOSED BY CLINICAL SCORES AND THORACIC ULTRASONOGRAPHY

Antonio Boccardo (1), Vincenzo Ferrulli (1), Giulia Sala (2), Donatella Scavone (1), Saverio Paltrinieri (1), Davide Pravettoni (1)

(1) Dipartimento di Medicina Veterinaria e Scienze Animali (DIVAS), Università degli Studi di Milano, Via dell'Università 6, 26900 Lodi, Italy.
(2) Dipartimento di Scienze Veterinarie, Università degli Studi di Pisa, Via Livornese SNC, 56122 San Piero a Grado (PI), Italy.

Corresponding Author: **Antonio Boccardo** - antonio.boccardo@unimi.it

The objective of this study was to describe 1) blood oxygenation status, 2) acid-base abnormalities, and 3) biochemical findings in calves affected by bronchopneumonia (BP) detected with clinical scores, thoracic ultrasonography (TUS), and different combinations of clinical scores and TUS.
Two hundred thirty-one Holstein-Friesian female calves aged between 12 to 94 days old were randomly assessed with the same one-gate design protocol. After the arterial blood sample evaluation, calves were examined and assigned a clinical score from Wisconsin [1] and California [2] respiratory scoring charts. Finally, TUS was interpreted in two different ways, and calves with a consolidation of ≥1 cm (TUS1cm) or ≥3 cm (TUS3cm) were considered positive [3]. Based on the combination of TUS and clinical scoring, enrolled calves were categorized as follows: healthy (negative at the clinical score without lesions on TUS), upper tract infection (positive at the clinical score but no lesions on TUS), subclinical BP (negative at the clinical score, positive on TUS), clinical BP (positive at the clinical score and TUS).
Differences between parameters studied were analysed first for the single clinical scores and TUS methods with the Mann-Whitney U test and then in the combinations between clinical scores and TUS with the Kruskal Wallis test. Statistical significance was set for a p-value < 0.05.
The blood oxygenation status differs only for the alveolus-arterial difference for oxygen in calves with a 1cm TUS lesion (p 0.040). Traditional acid-base abnormalities were not statistically different between healthy and diseased calves. On the other hand, with biochemical parameters and the simplified strong ion difference approach, many significant differences were shown between healthy, TUS-positive calves with and without clinical signs. Systematically, blood glucose ($p < 0.001$, serum paraoxonase-1 activity ($p < 0.001$), and unmeasured strong ion ($p < 0.001$) differed significantly within the categories of disease, revealing greater severity in calves with ultrasonographic lesions but not detecting increased severity in calves with coexisting clinical signs. This observation supports the hypothesis that lung lesions detected by TUS were more pathologically relevant than the mere observation of clinical signs during BP episodes. Using a TUS cutoff≥1 cm seems promising for describing the severity of metabolic changes during a BP episode.

[1] McGuirk, S. M., & Peek, S. F. (2014). Timely diagnosis of dairy calf respiratory disease using a standardized scoring system. Animal Health Research Reviews, 15(2), 145-147.
[2] Love, W. J., Lehenbauer, T. W., Kass, P. H., Van Eenennaam, A. L., & Aly, S. S. (2014). Development of a novel clinical scoring system for on-farm diagnosis of bovine respiratory disease in pre-weaned dairy calves. PeerJ, 2, e238.
[3] Buczinski, S., & Pardon, B. (2020). Bovine respiratory disease diagnosis: What progress has been made in clinical diagnosis? Veterinary Clinics: Food Animal Practice, 36(2), 399-423.

ENDOGENOUS PROCALCITONIN AND CARBONYLATED PROTEIN LEVELS IN HEALTHY COWS AND COWS AFFECTED BY SUBCLINICAL MASTITIS

Giulia Sala (1,2), Chiara Orsetti (1,2), Valentina Meucci (1), Micaela Sgorbini (1,2), Francesca Bonelli (1,2)

(1) Università degli Studi di Pisa, Dipartimento di Scienze Veterinarie
(2) Università degli Studi di Pisa, Centro di Ricerche Agro-Ambientali "E. Avanzi"

Corresponding Author: **Francesca Bonelli** - francesca.bonelli@unipi.it

Procalcitonin (PCT) and carbonylated proteins (CP) are biomarkers of bacterial infection and inflammation [1-2]. The study evaluates plasma PCT and CP levels in healthy cows (H) and cows with subclinical mastitis (SCM) (Institutional Animal Care and Use Committee of the University of Pisa, prot. N: 2825/2014). Cows were included in H group based on clinical examination and somatic cell count (SCC) < 250.00 cells/ml, while cows in SCM group had SCC ≥ 250.000 cells/ml and no signs of mastitis. A total of 92 (43 H and 49 SCM) and 115 (40 H and 75 SCM) cows were included for plasma PCT and CP evaluation, respectively. Blood samples were collected in lithium heparin tubes. The harvested plasma was frozen at −80 °C. For PCT assessment, the bovine procalcitonin ELISA kit (Cusabio, Houston – TX, USA) was used, while CP was evaluated as previously described [2]. Data distribution was assessed with the Kolmogorov-Smirnov test. A Man-Whitney U test was performed to evaluate differences among the groups (H vs SCM). If the difference between healthy and diseased animals was significant ($p < 0,005$), the cut-off was calculated by Receiver Operating Characteristic (ROC) curve along with the sensitivity and specificity. The median PCT concentration was: 71,0 pg/ml (20,0–116,9) for H and 108,7 pg/ml (74,4–222,0) for SCM. This difference resulted statistically significant ($p < 0,001$). The median CP concentration was 0,054 nmol/ml/mg (0,03-0,08) in H and 0,047 nmol/ml/mg (0,02-0,09) in SC. No statistically significant differences were found for CP among the groups ($p = 0,88$). The PCT cut-off for distinguishing between healthy and subclinical mastitis animals was > 90 pg/ml (AUC 0,72) with a sensitivity and specificity of 74% and 65%, respectively. PCT concentrations were higher in SCM compared to H cows, in line with studies performed in bovine affected by different pathological conditions [1,3-4]. The result of CP is in contrast with studies performed in other species [2,5], maybe because localized mastitis doesn't cause systemic oxidative stress, thus, do not lead to increased CP levels in plasma. In conclusion, PCT might be used as an endogenous marker of mastitis in cows, while CP seems not promising.

Acknowledgment: Agritech National Research Center funding.

[1] Bonelli et al., 2023. Res Vet Sci, 155:56-61.
[2] Nocera et al., 2020. Front Vet Sci, 7:582886.
[3] Bonelli et al., 2018. Vet J, 234 :61-5.
[4] El-Deeb et al., 2020. Acta Trop, 204 :105336.

CAN ENDOGENOUS PROCALCITONIN DISTINGUISH HEALTHY CALVES FROM CALVES AFFECTED BY BRONCHOPNEUMONIA?

Giulia Sala (1,2), Davide Pravettoni (3), Valentina Meucci (1), Micaela Sgorbini (1,2), Vincenzo Ferrulli (3), Antonio Boccardo (3), Matteo Castelli (1), Francesca Bonelli (1,2)

(1) Università degli Studi di Pisa, Dipartimento di Scienze Veterinarie
(2) Università degli Studi di Pisa, Centro di Ricerche Agro-Ambientali "E. Avanzi"
(3) Università degli Studi di Milano, Dipartimento di Medicina Veterinaria e Scienze Animali

Corresponding Author: **Giulia Sala** - giulia.sala@unipi.it

Procalcitonin (PCT) is an inflammatory marker able to distinguish between infections from bacterial origin or not [1] and to guide the antimicrobial therapy in humans [2]. This study aims to assess if PCT can distinguish healthy calves from those with bronchopneumonia (BP) diagnosed by thoracic ultrasonography (TUS) (Institutional Animal Care and Use Committee of the University of Milan, prot. N: 47/2017 of 28.11.2017). TUS was performed in 80 pre-weaning Holstein Friesian calves from herds with a history of BP by the Ruminant and Swine Clinic service (University of Milan). Calves were considered healthy based on the clinical examination and a negative TUS. To be included as BP calves, they must show at least a pulmonary consolidation area of ≥1 cm [3]. Blood sample in lithium heparin tubes was collected for each calf included. Samples were centrifuged and the harvested plasma was frozen at −80 °C. PCT concentrations were measured using the bovine procalcitonin ELISA kit (Cusabio, Houston – TX, USA). Data were not normally distributed (Shapiro-Wilk test), thus expressed as median, 1st, and 3rd quartile. The difference in PCT concentrations between healthy and affected calves was assessed with the U Mann-Whitney test. If the difference was significant ($p < 0.005$), the cut-off was calculated using the Receiver Operating Characteristic (ROC) curve along with the sensitivity and specificity.
Of the 80 animals included in the study, 16 (20%) were deemed healthy, while 64 (80%) showed BP. The median PCT in healthy calves was 81.5 pg/ml (73.8 pg/ml – 89.5 pg/ml), while the median PCT in BRD calves was 110.2 pg/ml (87.9 pg/ml – 137.3 pg/ml). This difference was statistically significant ($p < 0.001$). The PCT cut-off calculated with the ROC curve was > 87 pg/ml (AUC 0.78). The PCT sensitivity and specificity resulted in 78% and 75%, respectively.
The results of this study are in line with other studies that investigated the relationship between PCT and bovine diseases [1,4]. There is not yet a practical and affordable gold-standard test for diagnosing BP [3]. Diagnostic accuracy results suggest that PCT could be useful to identify animals needing antibiotic treatment, thus helping to reduce unnecessary antimicrobial therapies. Further research in this field is required to identify valuable biomarkers using more practical and feasible solutions that can be applied in future routine clinical practice.
Acknowledgment: Agritech National Research Center funding.

[1] Bonelli, Francesca, et al. "Case–Control study: Evaluation of plasma procalcitonin concentration as an indicator of inflammation in healthy and sick cows." Research in veterinary science (2023).
[2] Schuetz, Philipp, Yannick Wirz, and Beat Mueller. "Procalcitonin testing to guide antibiotic therapy in acute upper and lower respiratory tract infections." Jama 319.9 (2018): 925-926.
[3] Buczinski, S., et al. "Comparison of thoracic auscultation, clinical score, and ultrasonography as indicators of bovine respiratory disease in preweaned dairy calves." Journal of veterinary internal medicine 28.1 (2014): 234-242.
[4] El-Deeb, Wael, et al. "Use of procalcitonin, neopterin, haptoglobin, serum amyloid A and proinflammatory cytokines in diagnosis and prognosis of bovine respiratory disease in feedlot calves under field conditions." Acta tropica 204 (2020): 105336.

76° CONVEGNO SISVET
BARI, 21-22-23 GIUGNO 2023

EVALUATION OF A PANEL OF OXIDATIVE STRESS BOMARKERS IN HEALTHY AND COLIC HORSES: PRELIMINARY STUDY

Bindi F. (1), De Marchi L (1), Vernaccini M (1), Spadari A (2), Rinnovati A (2), Nocera I (3), Meucci V (1), Bonelli F (1), Sgorbini M (1)

(1) Ospedale Didattico Veterinario "Mario Modenato", Dipartimento Scienze Veterinarie, San Piero a Grado (PI), Italy
(2) Department of Veterinary Medical Sciences, Ozzano dell'Emilia, Bologna, Italy
(3) Institute of Life Sciences, Sant'Anna School of Advanced Studies, Pisa, Italy

Corresponding Author: **Francesca Bindi** - francesca.bindi@phd.unipi.it

During Systemic Inflammatory Response Syndrome (SIRS), neutrophils produce reactive oxygen species (ROS). Oxidative stress occurs when ROS level is not adequately balanced by antioxidant system (1-3). The aim was to dose a panel of biomarkers (BIOs) in healthy (H) and colic (C) horses to assess differences between groups and performances in identifying SIRS positive and negative horses. The study was approved by OPBA (3/23) and involved 23 horses. SIRS score was evaluated (4); blood samples were collected once in H, while at admission (T0), 24(T1), 48(T2), 72(T3), 96(T4) h after admission in C horses. BIOs were analyzed as indicated by manufacturers. Data distribution was assessed. Differences between H vs C horses were verified for sampling times using non-parametric tests. If the difference was significant ($p < 0.005$), the cut-off was calculated using the Receiver Operating Characteristic (ROC) curve along with the sensitivity (Se), specificity (Sp) and likelihood ratio (LR). Ten out of 23 were H warmblood mares aged 7-15y; 13/23 were C horses referred to two different hospitals: 7/13 female and 6 gelding, aged 5-25y; 11/13 Warmblood and 2/13 hotblooded horses. The C horses were grouped as follow: obstructive non strangulative (n=9) and strangulative colic (n=4). Five out of 13 C horses were SIRS negative, 8/13 SIRS positive. The SIRS negative horses were affected by obstructive non strangulative (4/5) or strangulative colic (1/5). The SIRS positive horses were affected by obstructive non strangulative (5/8) and strangulative (3/8) colic. None of the C horses spontaneously died, 10/13 were discharged (8/10 obstructive non strangulative, 2/10 strangulative colic) and 3/13 horses were euthanized (2/3 strangulative and 1/3 obstructive non strangulative colic). Of the 3 horses euthanized, 1/3 was negative, and 2/3 positive to SIRS; 1/3 horses were euthanized after the first sampling, 2/3 after the last sampling. POase activity in C horses was statistically higher vs H ones ($p<0.0001$) for all sampling times (T0-T96). BChE activity in C horses was lower vs H ones ($p=0.0024$) starting from T1-T96. A cut off of 8.3 µmol/mL/min was found for POase (100% Se, 90% Sp, 10 LR) both for C SIRS positive and negative horses. A cut off of 8.1 µmol/mL/min was found for BChE (100% Se, 75% Sp, 4 LR) were found for C SIRS positive and 12.3 µmol/mL/min (80% Se, 80% Sp, 4 LR) for C negative horses. POase and BChE seemed good BIOs in distinguishing SIRS vs healthy horses.

[1] Moore JN, Vandenplas ML (2014). Is the systemic inflammatory response syndrome or endotoxemia in horses with colic? Vet Clin Eq Pract, 30_337-51.
[2] Mittal M et al. (2014). Reactive oxygen species in inflammatory and tissue injury. Antiox Redox Signal 20(7): 1126-67.
[3] Dalle-Donne I et al (2003). Protein carbonylation in human diseases. Trends Mol Med, 9:169-76.
[4] Roy MF et al. (2017). Prognostic value and development of a scoring system in horses with systemic inflammatory response syndrome. JVIM, 31: 582-92.

76° CONVEGNO SISVET
BARI, 21-22-23 GIUGNO 2023

TRANSCRANIAL MAGNETIC MOTOR EVOKED POTENTIALS TEST IN THE DIAGNOSIS OF SPINAL MOTOR DYSFUNCTION IN THE HORSE WITH ATAXIA: DETERMINATION OF PERFORMANCE, CUT-OFF AND ASSOCIATION WITH OTHER DIAGNOSTIC TESTS

Forni G. (1), Menchetti L. (2), Cerri S. (3), Mainardi C. (1), Gandini G. (1), Freccero F. (1)

(1) Department of Veterinary Medical Sciences, University of Bologna, Bologna, Italy
(2) School of Biociences and Veterinary Medicine, University of Camerino, Camerino, Italy
(3) Equitom Equine Hospital, Meldert, Belgium

Corresponding Author: **Giulia Forni** – giulia.forni6@unibo.it

Proprioceptive ataxia is a common disease in horses and even mild cases can cause a loss in sport performance. The most common cause of 222yelography222ve ataxia is cervical vertebral stenotic myelopathy (Wobbler Syndrome). Transcranial magnetic stimulation test with motor evoked potentials (TMMEP) recording at the level of peripheral effector muscles seems promising to assess the function of descending motor pathways in horses [1]. The aim of this retrospective study is to evaluate the performance and the association of the TMMEP test with the neurological gait exam and myelographic CT scan of the neck (treated as the ante- mortem gold standard) for the diagnosis of compressive and non-compressive spinal dysfunction. Twenty-two horses presented at a referral equine clinic for gait abnormalities and submitted to the diagnostic work up for ataxia were included in the study. This consisted of: neurological examination, orthopedic examination where necessary, radiographic study of the cervical region, TMMEP test, and static and dynamic CT scan myelography. Clinical reports were reviewed by certified specialists and findings categorized and subjected to statistical analysis. All horses had gait abnormalities consistent with proprioceptive ataxia. Results showed a positive association between the 0-5 score of ataxia attributed at the neurological examination, and TMMEP values for pelvic limbs (OR=1.052, 95%IC=1.005-1.101, $p<0.05$). The Positive Percent Agreement between the diagnosis of spinal dysfunction based on neurological exam and TMMEP test ranged from 77 to 88%, according to the TMMEP cut-off values that were used for the classification [2]. As regards the CT 222yelography, horses with cervical spinal compression had higher TMMEP values for pelvic limbs compared to horses that did not show compression ($p<0.01$). Moreover, compressions in a dorsal or dorso-lateral direction were characterized by higher TMMEP values ($p<0.05$). Finally, new cut-off were determined to diagnose spinal abnormalities in our populations achieving a sensitivity of 67% and a specificity of 75%.
In conclusion, given its reliability and relatively low invasiveness compared to advanced diagnostic imaging, the TMMEP test might be a useful aid in the diagnosis of spinal motor dysfunction in horses with gait abnormalities.

[1] Rijckaert, J., Pardon, B., Saey, V., Raes, E., Van Ham, L., Ducatelle, R., van Loon, G., Deprez, P. (2019). Determin ation of magnetic motor evoked potential latency time cutoff values for detection of spinal cord dysfunction in horses. Journal of Vet erinary Internal Medicine, Sep;33(5): 2312-2318
[2] Rijckaert, J., Raes, E., Buczinski, S., Dumoulin, M., Deprez, P., Van Ham, L., van Loon, G., Pardon, B. (2019). Accuracy of transcranial magnetic stimulation and a Bayesian latent class model for diagnosis of spinal cord dysfunction in horses. Journ al Veterinary Internal Medicine, Mar;34(2): 964-971

76° CONVEGNO SISVET
BARI, 21-22-23 GIUGNO 2023

PREVALENCE AND RISK FACTORS FOR EQUINE GASTRIC ULCER SYNDROME (EGUS) IN PLEASURE AND BREEDING HORSES IN ITALY

Sara Busechian (1), Micaela Sgorbini (2), Simona Orvieto (3), Francesca Bindi (2), Francesco Zappulla (4), Fabrizio Rueca (1)

(1) Department of Veterinary Medicine, University of Perugia, Italy
(2) Department of Veterinary Sciences, University of Pisa, Italy
(3) Private practitioner, Perugia, Italy
(4) SIAV A, ASL Novara, Italy

Corresponding Author: **Sara Busechian** - sarabusechian@gmail.com

Equine gastric ulcer syndrome (EGUS) is worldwide disease with high prevalence in various categories of horses. It includes two diseases, one related to the presence of ulcers and erosions in the squamous mucosa (Equine Squamous Gastric Disease, ESGD) and one in the glandular mucosa (Equine Glandular Gastric Disease, EGGD). Risk factors have been recognized for horses in active training, but little is known about the pathophysiology of the diseases in pleasure and breeding horses. Aim of this study was to determine the prevalence of the disease, to identify risk factors related to breed, age, sex and activity of pleasure and breeding horses and to correlate presence of lesions to clinical signs. Gastroscopy was performed according to the literature using a 3meters long scope and a portable processor (Karl Storz, Germany). ESGD was graded according to the scoring system proposed by the ECEIM Consensus Statement [1], while EGGD was only labelled as present/absent. Descriptive statistical analysis, Pearson's correlation, and logistical regression ($p<0.05$) were performed to determine prevalence of the diseases, correlation between clinical signs and ESGD and EGGD and possible risk factors. 925 adult horses at least 2 year old, were included. Most were females (533/925, 58%), geldings were 263/925 (28%) and males 129/925 (14%). There was no restriction based on age or breed for the enrolment, and the sport horses were not in heavy training. ESGD at least grade 2 was present in 560/925 (61%); EGGD in 285/925 (31%). Clinical signs were seen in 85/925 (9%), mostly as poor Body Condition Score and they were related to ESGD and to ESGD and EGGD together, but not to EGGD on its own. Risk factors for EGGD were activity (English riding especially), age (colts) and sex (males), while for ESGD, activity (Western riding especially) and age (old horses were less likely to present the disease). Activity (English riding), age (colts) and sex (males) were related to the presence of ESGD and EGGD seen at the same time. These results can give indications about possible categories of horses to be considered at risk, even if not in active training: these animals should be investigated for the presence of gastric ulcers, and treated and monitored accordingly, to avoid the development of more serious clinical signs, such as colic or reduced performance.

[1] Sykes BW, et al. European College of Equine Internal Medicine Consensus Statement—Equine Gastric Ulcer Syndrome in Adult Horses. J Vet Intern Med 2015; 29:1288–99.
[2] Rendle D, et al. Recommendations for the management of equine glandular gastric disease. UK-Vet Equine 2018; 2:2–11.
[3] Sykes BW, et al. Management factors and clinical implications of glandular and squamous gastric disease in horses. J Vet Intern Med 2019; 33:233–40.
[4] van den Boom R. Equine gastric ulcer syndrome in adult horses. Vet J 2022;283–284:105830.

76° CONVEGNO SISVET
BARI, 21-22-23 GIUGNO 2023

BRONCHOALVEOLAR FLUID CYTOLOGY IN THE DONKEY: COMPARISON OF DIFFERENT STAININGS

Vitale V. (1), Parietti C. (2), Bindi F. (2), Briganti A. (2), Bonelli F. (2), Sgorbini M. (2)

(1) Departamento de Medicina y Cirugía Animal Unidad de Équidos Hospital Clínico Veterinario CEU Universidad CEU Cardenal Herrera, Valencia, Spain
(2) Ospedale Didattico Veterinario "Mario Modenato", Dipartimento Scienze Veterinarie, San Piero a Grado (PI), Italy

Corresponding Author: **Valentina Vitale** - valentina.vitale@uchceu.es

The aim of this study, approved by OPBA University of Pisa (3/21), was to assess the cytology results of donkey Broncho Alveolar Lavage fluid (BALF) obtained with different staining. The BAL was performed on 9 female Amiata donkeys aged 8-17 years. The procedure was performed as previously described (1). Total nucleated cell count (TNCC) was evaluated on EDTA samples with an automated cell counter machine. Slides were prepared by cytocentrifugation and air dried. Slides were stained using 4 stains: modified May Grunwald Giemsa (automatic stainer machine), Diff Quick, toluidine blue and Perls stains. The differential cell count was manually performed by counting 400 cells/smear at 100X for macrophages (M), lymphocytes (L), neutrophils (N), eosinophils (E) and mast cells (MC). Hemosiderophages (HE) were also counted. Data distribution was verified; data with Gaussian distribution were expressed as mean□standard deviation (M, L, E, MC) while data not normally distributed were expressed as median, minimum, and maximum values (N, HE). Student's T test (M, L, E, MC) or Mann-Whitney test (N) were applied to compare differential count between automatic stainer vs Diff Quick. Kruskall-Wallis test was applied to compare differential count found among automatic stainer vs Diff Quick vs Perls stain. Correlation (Spearman test) was assessed between MC found with automatic stainer vs toluidine blue. No statistically significant differences were found between the readings on stained preparations with automatic stainer and Diff Quick for M (p=0.49), L (p=0.47), N (p=0.71), E (p=0.80), while statistically significant difference was found for MC (p=0.007). Statistically significant differences were found between the readings on stained preparations with automatic stainer and Diff Quick vs Perls stain (0.03). No correlation was found between automatic and toluidine blue staining (p=0.08, r=0.60) for MC. The results agree with the current literature on horses: Diff-Quik stain is indicated for differential cell counts, but underestimates MC, so separate enumeration with specific staining or toluidine blue is required (2-4). The May Grunwald Giemsa staining method is preferred for general cytology because it allows for a complete differential cell count, including MC detection. Finally, even though donkeys included in the study were non-athletic patients, it was possible to find HE visible only with Perls stain.

[1] Vitale et al. (2021). Bronchoalveolar lavage fluid cytological findings in healthy Amiata donkeys. Open Vet J, 11(1): 160-4.
[2] Hansen S etal (2019). Reliability of cytological evaluation of mast cells from bronchoalveolar lavage fluid in horses: Intraobserver agreement and mast cell identification. Equine Vet Educ, 32: 47-52.
[3] Hoffman AM (2008). Bronchoalveolar lavage: sampling technique and guidelines for cytologic preparation and interpretation. Vet Clin North Am Equine Pract, 24(2):423-35.
[4] Leclere M et al. (2006). Comparison of four staining methods for detection of mast cells in equine bronchoalveolar lavage fluid. JVIM, 20(2): 377-81.

IN VITRO PRELIMINARY INVESTIGATION OF THE TOXIC POTENTIAL OF EQUINE RIDING SURFACES: LET THE MINERAL HUNT BEGIN!

Alessandra Romolo (1), Beatrice Sica (1), Cristina Pavan (2), Maura Tomatis (1), Matteo Ardito (3), Francesco Di Benedetto (3), Silvana Capella (4), Elena Belluso (4), Francesco Turci (2), Michela Bullone (1)

(1) Department of Veterinary Sciences, University of Turin, Italy
(2) Department of Chemistry, University of Turin, Italy
(3) Department of Physics and Earth Sciences, University of Ferrara, Italy
(4) Department of Earth Sciences, University of Turin

Corresponding Author: **Michela Bullone** – michela.bullone@unito.it

Among respirable inorganic dusts with a toxic potential there are mineral particles. In particular, crystalline silica particles have been postulated as a possible matter of concern for equine respiratory health as many equine racing and riding surfaces are made mainly of silica (quartz or composite minerals) and the respiratory toxicity of silica is well recognized in humans [1]. Dusts generated during equine riding and racing can reach such a small size they can enter the alveoli and promote inflammation. Any experimental protocol on living animals in this respect would raise ethical issues and should be avoided if not strictly necessary. According to the 3R principle, *in vitro* or *ex vivo* tests should be implemented first. We present here the first results of a toxicity assay performed on sheep red blood cells (RBC) to test the membranolytic potential of dust from equine riding surface. RBC membranolysis is recognized in silica-associated research as a good predictor of silica inflammogenic activity [2]. The tests have been performed on three different surface types with variable amounts of quartz (from 50 to 98%, assessed by means of X-ray powder diffraction and electron microscopy before size selection), after selection of the smallest particulate portion (<30 um). Dust from riding surfaces was dried at 70°C before sieving. Membranolytic assay has been performed with a dose-response curve ranging from 0.3 to 10 mg/ml. A standard quartz dust (Min- U-Sil 5) was used as positive control. Results showed that membranolytic potential of the dusts from riding surfaces (<30 um) is not directly associated with their crystalline silica content, as the hemolytic activity decreases with increasing the amount of silica. These counterintuitive results can be due to the different distribution of mineral particles in different particle size fraction, as well as to the hemolytic activity of mineral species other than quartz [3]. Unfortunately, the available knowledge on the toxic potential of inhaled mineral particles is limited to a few of them, and future research in this field is warranted to gain a complete view of mineral exposome on human and animal health.

[1] Dhooria et al (2023) Silica-associated lung disease in developing countries. 29(2):65-75.
[2] Pavan et al (2020) Nearly free surface silanols are the critical molecular moieties that initiate the toxicity of silica particles. Proc Natl Acad Sci USA; 117(45):27836-27846.
[3] Canànà et al. (2022) Interaction of Layered Silicates with Biomembranes: Ion Exchangers and Non-Exchangers. Adv Mater Interfaces; 9, 2201347.

76° CONVEGNO SISVET
BARI, 21-22-23 GIUGNO 2023

AORTIC VALVE DISEASE IN DONKEYS

C. Bozzola (1), V. Ronaghi (2), E. Alberti (3), E. Zucca (1)

(1) Department of Veterinary Medicine and Animal Science, University of Milan
(2) Veterinary practitioner (BS)
(3) Veterinary practitioner (VA)

Corresponding Author: **Chiara Bozzola** - chiara.bozzola@unimi.it

Donkeys are important animals worldwide. Nowadays, they are kept for different human purposes, such as work, milk production and companion animals. Despite the increasing interest in donkeys' welfare, cardiac diseases are still not well-known in this species [1]. Therefore, the aim of this study was to investigate the prevalence of cardiovascular diseases in donkeys.
This study was approved by the Institutional Animal Care Committee of the University of Milan (OPBA_15_2022). Fifty-four donkeys of different age, sex, size, and bodyweight underwent physical, electrocardiographic, echocardiographic examinations and a blood sample was obtained. A mild heart murmur (I-II/VI degree) was identified in 5 donkeys. The predominant heart rhythm was sinus rhythm, except for physiological arrhythmias (wondering pacemaker, sinus arrhythmia and second-degree atrioventricular blocks) detected in 5 donkeys. By echocardiography, aortic abnormalities were observed in 6/54 donkeys (prevalence of 11.1%, one 3 years old and five of > 9 years old donkeys) associated with mild aortic regurgitation (2 subjects) and moderate aortic regurgitation (1 subject). The other three animals did not present any sign of aortic regurgitation. Nodular and band-like lesions, thickening of the aortic valve, and aortic cusps prolapse, compatible with degenerative changes, were the main lesions detected.
In this study, we observed a higher prevalence of cardiac murmurs (5/54 donkeys, prevalence of 9.4%) and a lower prevalence of ejection murmurs (2/54 donkeys, prevalence of 3.7%) compared to those already reported in donkeys [2,3]. According to previous studies, the aortic valve lesions were mainly detected in middle-aged and older donkeys, except for a three-year-old donkey. Since no clinical or hematological abnormalities were associated with the aortic nodular lesions observed in this donkey, we suspected a congenital valve disease, as reported in a foal with similar valvular lesions [4].
In conclusion, this study showed that donkeys are mainly affected by aortic valve diseases, and cardiac murmurs are not uncommon. Furthermore, we found that aortic valve lesions are not associated with hemodynamically significant alterations in most cases. Finally, none of them has shown signs of cardiac insufficiency or other cardiovascular diseases.

[1] A. Decloedt "Prevalence of cardiovascular disease in donkeys", Veterinary Record, 2016: 382-383
[2] S. L. Roberts and J. Dukes-McEwan "Assessment of cardiovascular disease in the donkey: clinical, echocardiographic and pathological observations", Veterinary record, 2016, 179(15)
[3] H. Amory et al. "Doppler echocardiographic reference values in healthy donkeys", Veterinary Care of Donkeys, 2004
[4] S. E. Taylor et al. "Congenital aortic valve dysplasia in a Clydesdale foal", Equine Vet Educ, 2007, 19(9): 463-468

76° CONVEGNO SISVET
BARI, 21-22-23 GIUGNO 2023

STRONGYLUS VULGARIS DETECTION IN A MINIATURE HORSE WITH COMPATIBLE CLINICAL SIGNS: A CASE REPORT AND CLINICO/PARASITOLOGICAL CONSIDERATIONS

Giulia Memoli (1), Stefania Zanet (1), Michela Bullone (1)

(1) Department of Veterinary Sciences, University of Turin

Corresponding Author: **Michela Bullone** – michela.bullone@unito.itc

Large strongyles are a group of parasitic worms once considered as one of the most prevalent and pathogenic parasites of the horse. In particular, Strongylus vulgaris was once considered the major threat to equine health [1] as its larval migration through mesenteric vessels to different organs led to severe clinical and pathological consequences. Parasite migration in the CNS is a rare, but possible, cause of neurological disease in horses [2]. Although S. vulgaris has progressively declined in Italy since 1990s, and by some considered an "eradicated problem", it is well advised to consider this kind of infestation during equine clinical practice also in current days, especially in the absence of adequate prophylaxis. We report here a case of a miniature horse referred to our Veterinary Teaching Hospital for abrupt presentation of severe neurological signs, which we finally ascribed to parasite migration in CNS.

A 3-yr old Falabella pony was presented for acute onset of vestibular syndrome with head rotation on the left side, severe ataxia and inability to feed on his own. On arrival, the pony was conscious, lying on its left side, with severe left head tilt, intermittent horizontal and rotary nystagmus (worsened upon stimulation), trismus, severe hindlimbs weakness causing inability to stand but volontary mobility preserved. Urination and defecation were maintained. The pony presented intermittent tachicardia, while other clinicals were normal. Skull and back RX examination excluded significant traumatic lesions. Haematology was unremarkable. The pony never had vaccine prophylaxis nor antiparasite treatment before. Nasopharyngeal swab and serology did not support EHV1-4 or WNV infection. Serum was sent for troponine I dosage and revealed concurrent cardiomyopathy, possibly immuno-mediated in origin. Fluidotherapy was started with mannitol integration, and a broad spectrum antibiotic treatment with good nervious tissue penetration was startedand together with anti-infiammatory therapy. Cerebrospinal fluid (CSF) cytology evealed erythrophagocytosis. Faecal worm egg count and fecal colture revealed 900 EPG Strongylus vulgaris. Moxidectin (0.4 mg/kg) was added to the treatment [3]. The clinical condition of the pony gradually improved and he was discharged after approximately 15 days with mild neurological deficits still detectable (grade 1 ataxia).

[1] Pilo (2012) Strongylus vulgaris (Looss, 1900) in horses in Italy: Is it still a problem? Vet Parasitol; 184(2-4):161-7.
[2] Wilford (2013) Successful treatment of a horse with presumed parasitic encephalitis. Equine Vet Educ; 25 12):601-604.
[3] ESCCAP Guideline 08 Second Edition- A guide to treatment and control of equine gastrointestinal parasite infections (available at: https://www.esccap.org/uploads/docs/kvhqncl2_0796_ESCCAP_Guideline_GL8_v9_1p.pdf)

76° CONVEGNO SISVET
BARI, 21-22-23 GIUGNO 2023

SUBCLINICAL MEDICAL DISORDERS ASSOCIATED WITH RACING RESULTS IN STANDARDBRED RACEHORSES

C.M. Lo Feudo (1), L. Stucchi (1), C. Bozzola (1), G. Stancari (1), B. Conturba (1), E. Zucca (1), F. Ferrucci (1)

(1) Department of Veterinary Medicine and Animal Sciences, University of Milan

Corresponding Author: **Chiara Maria Lo Feudo** - chiara.lofeudo@unimi.it

Poor performance is a multifactorial disorder of racehorses, commonly associated with subclinical diseases. This study aims to evaluate the associations between subclinical medical disorders and racing results in Standardbred racehorses. The clinical records of 248 Standardbreds referred to our hospital for poor performance were retrospectively reviewed; all horses underwent physical examination, laboratory analyses, and electrocardiogram (ECG), while further ancillary diagnostic procedures were selected based on diagnostic suspicion, including dynamic endoscopy on treadmill, post-exercise tracheobronchoscopy, bronchoalveolar lavage (BAL), ECG during exercise, post-exercise creatine-kinase (CK) assessment and gastroscopy. The racing results (starts, wins, placings, earnings) of included horses were extracted from the online database HippoWeb and divided into three periods: 3 months before and 6 months after hospitalization, and lifetime. The associations between disorders and racing results were evaluated by parametric and non-parametric tests in the whole population and in different age groups. The BAL neutrophilia was inversely correlated with lifetime number of wins and placings in 2-year-old horses, and with number of wins before hospitalization in adult horses; the BAL mastocytosis was inversely correlated with number of wins, and with wins/starts and placings/starts ratios before hospitalization in 3-year-old horses. In 2- and 3-year-old horses, the severity of exercise induced pulmonary hemorrhage (EIPH) was positively associated with number of starts and wins and with wins/starts ratio before hospitalization. Serum CK was inversely correlated with lifetime starts, wins, placings, and earnings. Finally, squamous gastric ulcers severity was associated with the number of placings and placings/starts ratio before hospitalization in 3-year-old horses. Upper airway obstructions and cardiac arrhythmias were not associated with any racing results. In conclusion, the association between lower airway inflammation and poor racing results in the period before hospitalization confirms its role in performance decrease. Gastric ulcers and EIPH were associated with a better performance in the period before hospitalization, probably because their pathogenesis is strictly related to exercise intensity. Finally, rhabdomyolysis, reflected by high CK values, was the only disease having an impact on long-term performance, by reducing athletic longevity and quality.

[1] Richard EA, Fortier GD, Pitel PH, et al. Subclinical diseases affecting performance in Standardbred trotter: diagnostic methods and predictive parameters. Vet J. 2010; 185:282-289.
[2] Lo Feudo CM, Stucchi L, Conturba B, et al. Impact of lower airway inflammation on fitness parameters in Standardbred racehorses. Animals. 2022; 12:3228.
[3] Martin BJ, Reef VB, Parente EG, et al. Causes of poor performance of horses during training, racing, or showing: 348 cases (1992–1996). J Am Vet Med Assoc. 2000; 216:554-558.

76° CONVEGNO SISVET
BARI, 21-22-23 GIUGNO 2023

DAILY VARIABILITY OF SELECTED RENAL BIOCHEMICAL PARAMETERS IN HEALTHY ADULT MARTINA FRANCA DONKEYS

A. Luciani (1), P.E. Crisi (1), R.E. Peli (1), B.A. Giangaspero (1), A. De Camillis (1), A. Boari (1)

(1) Department of Veterinary Medicine, University of Teramo, Strada Provinciale 18, 64100 Località Piano d'Accio, Teramo, Italy

Corresponding Author: **Alessia Luciani** - aluciani@unite.it

The normal values of the serum and urine biochemical parameters are well-known in horses but knowledge of the reference values of these parameters is still limited in donkeys. Recently, reference range of selected renal biochemical parameters in donkeys have been established (1) but no data are available in Martina Franca (MF) donkeys, considered as endangered breed. Therefore, the aim of this study was to determine the value of selected renal biochemical parameters in healthy MF donkeys. Thirty adult female healthy donkeys were included based on a clinical examination, a complete biochemical profile, and urynalisis. On serum samples, concentrantions of creatinine (sCr), BUN, total protein (TP), albumin, calcium, inorganic phosphate, sodium (Na), potassium (K) and chloride values were obtained. On voided urine sample, collected from each donkeys, in the morning and in the afternoon, the concentration of the following parameters were detected: urine specific gravity (USG), pH, urine creatinine (uCr), urine protein (uP), sodium and potassium concentration, the urine gamma-glutamyl transferase (GGTP)-to-creatinine ratio, uP/uCr ratio (UPCR), and sCr/uCr ratio. In addition, the fractional electrolyte excretion of sodium (FENa) and potassium (FEK) were calculated. All the parameters were obtained using a clinical chemistry automatic analyzer (Olympus AU 680, Beckman Coulter). Computer software was used to perform the analysis (Graphpad Prism version 6.01, La Jolla, CA, USA). Normality was checked using the D'Agostino Pearson test. The differences between urinary samples collected in the two daily time points were performed using the paired t-test or the Mann-Whitney test, according to data distribution. A p value <0.05 was considered significant. Renal biochemical parameters were within the normality range established in MF donkeys (2). Several measured urinary parameters were comparable to the results obtained in donkeys (1). In our study, only UPCR showed lower values, as they ranged from 0.12 to 0.34. FeK and GGTP/uCr ratio showed a reduction in urinary samples collected in the morning compared to samples collected in the afternoon ($p=0.0009$ and $p=0.0006$, respectively) while GGTP values showed an opposite trend ($p=0.0228$). Our results could be related to the fed diet and to the urine flow rate at the time of sampling. The exact knowledge of the parameters of organ function is indispensable in supporting the conservation strategies adopted for this breed.

[1] Fraczkowska et al. Measurement of selected renal biochemical parameters in healthy adult donkeys considering the influence of gender, age and blood freezing. Animals, 11:1-10, 2021.
[2] Trimboli et al. Reference ranges for hematological and biochemical profile of Martina Franca donkeys, Frontiers in Veterinary Science, 7:1-7, 2020.

ANTI-DOPING VIOLATIONS IN RACEHORSES IN ITALY FROM 2014 TO 2022

Mariana Roccaro (1), Federica La Rocca (2), Riccardo Rinnovati (2), Angelo Peli (1)

(1) Alma Mater Studiorum - Università di Bologna, Dipartimento di Scienze per la Qualità della Vita
(2) Alma Mater Studiorum - Università di Bologna, Dipartimento di Scienze Mediche Veterinarie

Corresponding Author: **Mariana Roccaro** - mariana.roccaro2@unibo.it

Anti-doping controls aim to guarantee the integrity of horseracing and to protect horse welfare. The Italian regulation for the control of prohibited substances defines prohibited drugs and controlled substances and establishes a minimum of pre-race and post-race samples, the latter usually from the first placed horses, which are analysed by the official laboratory of the Ministry of Agriculture, Food Sovereignty and Forestry (MASAF). Literature on anti-doping violations in horses is scarce [1–4]. This study aimed to provide information on the most common drug violations in racehorses in Italy. Data on horses tested positive to anti-doping controls between 2014 and 2022 were collected from a publicly available database held by the MASAF, including horse's sex and age, type of race (flat galloping or trotting), race location (Northern, Central or Southern Italy), detected substance(s), frequency and results of retests. Data on 549 horses were retrieved during the considered 9-year timeframe. Retests were requested in 179 cases; 9 were negative or inconclusive and 10 are still ongoing. Overall, 530 horses confirmed to be positive, of which 71% competed in trotting races. The 48% were males, 35% females and 17% geldings. Median age was 4 years. Positive results were evenly distributed throughout the country. Seventy-nine parent drugs, belonging to 29 different drug classes, were detected. Forty-nine horses (9%) tested positive for more than one parent drug. The 5 most represented drug classes (n=549) were anti-inflammatory steroids (18%), stimulants (16%), NSAIDs (15%), anabolic steroids (10%) and sedatives (9%). The 5 most frequent substances (n=586) were dexamethasone (8%), cocaine (6%), testosterone (6%), caffeine (5%) and theophylline (4%). A wide variety of substances was detected, most of which were prohibited (79%; 464/586). Ninety horses (17%) were doped with anabolic steroids or narcotics, resulting in harsher punishments. Anabolic steroids were reported only in two studies [1,4], whilst common stimulants included caffeine and cocaine [1–3]. Differently from other studies, anti-inflammatory steroids were more used than NSAIDs such as phenylbutazone [1–4], whereas the use of morphine, clenbuterol and methocarbamol was very limited in our sample population [2,4]. Data on the total number of horses starting at races and tested have been requested to the competent authorities in order to analyze prevalence and perform inferential statistics.

[1] Moss et al. Survey of positive results from racecourse antidoping samples received at Racehorse Security Services' Laboratories. Equine Veterinary Journal, 16:39–42, 1984.
[2] Lotfollahzadeh et al. A survey on two years of medication regulation in horse races in Iran, Equine Veterinary Journal, 42:161–163, 2010.
[3] Taddei et al. Detection of various performance enhancing substances in specimens collected from race horses in Illinois: a five-year experience. Journal of Analytical Toxicology, 35:438–443, 2011.
[4] Waller et al. The medication violations in racehorses at Louisiana racetracks from 2016 to 2020. Veterinary Medicine and Science, 8:553–560, 2022.

EXAMINING THE VETERINARY ELECTRONIC ANTIMICROBIAL PRESCRIPTION FOR DOGS AND CATS IN CAMPANIA REGION, ITALY: CORRECTIVE STRATEGIES ARE IMPERATIVE

Peruzy F. (1), Foglia Manzillo V. (1), Gizzarelli M. (1), Izzo B. (2)., Sarnelli P. (2), Carrella A. (2), Chirollo C. (2), Balestrino I. (1), Oliva G. (1)

(1) Dipartimento Medicina Veterinaria e Produzioni Animali, Università degli Studi di Napoli Federico II
(2) Regione Campania - Centro di Farmacovigilanza Veterinaria

Corresponding Author: **Gaetano Oliva** - gaetano.oliva@unina.it

Antimicrobial Resistance (AR) represents one of the most important human and animal health-threatening issues worldwide. Companion animals are increasingly being recognized as important contributors to the spreading of resistant bacteria (1) and therefore with the EU Reg. 2019/6, they have been included in the surveillance. To date, few data on antimicrobial use in dogs and cats are available (2). The present work aimed to measure the antimicrobial drug prescribing in veterinary practice in dogs and cats in the Campania region, southern Italy by analyzing the Veterinary Electronic Prescriptions (VEPs) between April 2019 to December 2020 collected from "VET INFO". The medical record of each dog and cat associated with antimicrobial drug prescription were collected considering the drug delivery (systemic or local) and the rationale for the treatment chosen: skin, respiratory, gastrointestinal, genitourinary, mammary, metabolic, cardiovascular, neurological, oncological, orthopedic, parasitic, ear and eye diseases, sepsis and general surgery. The collected data were recorded using spreadsheet software (Microsoft® Excel® 2018) and differences in the frequency of antibiotics prescriptions were assessed using the chi-square test ($p < 0.05$). In the period under investigation, a total of 166879 drugs were prescribed through the VEPs to companion animals of which 129116 (73.37 %) were antimicrobial. A total of 83965 (65.03 %) antibiotics were prescribed to dogs, 40477 (31.35 %) to cats, and 4674 (3.62 %) to other companion animals not included in the present analysis. In dogs a total of 56951/62941 (90.48 %) VEPs prescribed for systemic treatment included an antimicrobial classified by WHO (2018) either as Critically Important or Highly Important or Important for human medicine. In general, most of VEPs contained one single-active compound and within them the most widely commonly prescribed class both in dogs and in cats were fluoroquinolones (dogs = 44.47 %; cats = 50.31 %). In summary, the main antimicrobials that were prescribed were the metronidazole-spiramycin (29.71% - $p < 0.05$), amoxicillin-clavulanic (19.58%), enrofloxacin and cephalexin in dogs (16.52 % - $p < 0.05$) and enrofloxacin (22.64%) and amoxicillin-clavulanic acid (21.37 % $p < 0.05$) in cats. Based on the results the wide use of broad-spectrum or second-line antibiotics is emerged. The focus should be on performing the proper diagnostic steps, and the treatment of suspected infection without culture and antibiogram should be avoided along with the use of the critically important antimicrobials for human medicine.

[1] Singleton, D. A., Rayner, A., Brant, B., Smyth, S., Noble, P. J. M., Radford, A. D., et al. (2021). A randomised controlled trial to reduce highest priority critically important antimicrobial prescription in companion animals. Nat. Commun. 12, 1–14. doi: 10.1038/s41467-021-21864-3.

[2] Goggs, R., Menard, J. M., Altier, C., Cummings, K. J., Jacob, M. E., Lalonde-Paul, D. F., et al. (2021). Patterns of antimicrobial drug use in veterinary primary care and specialty practice: A 6-year multi-institution study. J. Vet. Intern. Med. 35, 1496–1508. doi: 10.1111/jvim.16136.

OVERVIEW ON ANTIMICROBIAL PRESCRIPTION HABITS IN CATS AT DIFFERENT SERVICES OF THE VETERINARY TEACHING HOSPITAL OF THE UNIVERSITY OF PARMA

Fidanzio Francesca (1), Rega Martina (1), Bertini Simone (1), Corsini Andrea (1), Crosara Serena (1), Quintavalla Cecilia (1)

(1) University of Parma, Dept. of Veterinary Sciences, Strada del Taglio, 10, Parma (PR)

Corresponding Author: **Francesca Fidanzio** - francesca.fidanzio@unipr.it

Antimicrobials are frequently administered for therapeutic and prophylactic purposes in companion animals [1]. Their use is closely monitored as related to antimicrobial resistance both in human and veterinary medicine [2]. This retrospective study aims to describe the antimicrobial prescription, at different services, in cats visited at the Veterinary Teaching Hospital of the University of Parma in 2022. Overall, antibiotics were prescribed in the 35% (420/1200) of visited cats. The emergency service prescribed antibiotics in the 25.9% (79/305) of the cases, with the 11.4% of the prescriptions supported by culture and susceptibility testing (CST); the primary care service prescribed no antibiotics (0/71). Ophtalmology prescribed topic antibiotics in the 78,3% of cats (36/46), not guided by CST. Internal medicine prescribed antibiotics in 27.9% (86/308) of its patients, with 61.2% of CST performed. Neurology prescribed antibiotics in the 12.2% (11/90), only 1/11 (9.1%) CST performed. Cardiology made one prescription (1.1%, 1/89) supported by CST. Oncology and dermatology prescribed antibiotics in 3.2% (2/63) and 6.2% (1/16) of their patients, respectively, none guided by CST. Surgery (reproduction, soft tissue surgery and orthopaedics) prescriptions were made for prophylactic use. Antibiotics were given only intraoperative in the 100% of reproduction interventions and in 85.4% (47/55) of soft tissue surgeries. Orthopaedics routinely prescribed a 7-day course of antibiotic therapy. Most of the prescriptions (89.5%, 420/475) belonged to Category C "Caution" and D "Prudence" antibiotics [3]. Category B "Restrict" antibiotics [3] represented 11.5% (55/475) of total antimicrobials prescribed, guided by CST in 69.1% of cases. In particular, the percentage of CST guided prescription was 100% (1/1) cardiology, 90.6% (29/32) internal medicine, 50% (2/4) soft tissue surgery, 45.5% (5/11) emergency, 14.3% (1/7) neurology. The low CST percentage performed at the neurology service is due to the inherent difficulty in sampling cerebrospinal fluid. This study shows that adhering to guidelines for the prudent use of antibiotics is feasible in feline medicine and surgery. The use of "Restrict" antibiotics was limited to a small number of selected feline patients in our hospital. The use of CST could strongly reduce the use of antibiotics; particular attention should be paid to use not-critical categories of antimicrobials for therapeutic and prophylactic use.

[1] Pomba C, Rantala M, Greko C, Baptiste KE, Catry B, van Duijkeren E, Mateus A, Moreno MA, Pyörälä S, Ružauskas M, Sanders P, Teale C, Threlfall EJ, Kunsagi Z, Torren-Edo J, Jukes H, Törneke K. Public health risk of antimicrobial resistance transfer from companion animals. J Antimicrob Chemother. 2017 Apr 1;72(4):957-968. doi: 10.1093/jac/dkw481. PMID: 27999066.
[2] Tompson AC, Mateus ALP, Brodbelt DC, Chandler CIR. Understanding Antibiotic Use in Companion Animals: A Literature Review Identifying Avenues for Future Efforts. Front Vet Sci. 2021 Oct 8;8:719547. doi: 10.3389/fvets.2021.719547. PMID: 34692805; PMCID: PMC8531418.
[3] European Medicine Agency (EMA), Committee for Medicinal Products for Veterinary use (CVMP) Committee for Medicinal Products for Human Use (CHMP). Categorization of antibiotics in the European Union Answer to the request from the European Commission for updating the scientific advice on the impact on public health and animal health of the use of antibiotics in animals. 2019, https://www.ema.europa.eu/en/documents/report/categorisation-antibiotics-european-union-answer-request-european-commission-updating-scientific_en.pdf

76° CONVEGNO SISVET
BARI, 21-22-23 GIUGNO 2023

CLASSIFICATION OF SEPTIC SHOCK PHENOTYPES BASED ON THE PRESENCE OF HYPOTENSION AND HYPERLACTATEMIA IN DOGS

Francesca Buzzurra (1), Elena Ciuffoli (1), Ilaria Bergamin (1), Massimo Giunti (1), Roberta Troìa (1)

(1) Alma Mater Studiorum - Università di Bologna, Dipartimento di Scienze Veterinarie

Corresponding Author: **Francesca Buzzurra** - francesca.buzzurra@gmail.com

Septic shock is considered a subset of sepsis associated with a greater risk of mortality than sepsis alone [1]. Prognostic value of septic shock phenotypes based on persistent fluid-refractory hyperlactatemia alone (cryptic shock, CS), fluid-refractory hypotension alone (vasoplegic shock, VS) and the combination of both these criteria (dysoxic shock, DS) is reported in people [2, 3]. In dogs, septic shock is associated with a poor prognosis [4], but the clinical and prognostic implications of these phenotypes have not yet been investigated.
This study analyzed the prevalence, characteristics, and mortality rates of cryptic, vasoplegic and dysoxic shock in a population of dogs with sepsis hospitalized at a veterinary teaching hospital.
Dogs with septic shock were prospectively included and grouped according to the presence of hypotension (systolic blood pressure <90 mmHg) requiring vasopressor support, hyperlactatemia (>2 mmol/L), or both. Clinical and clinicopathological data including the full Acute Patient Physiologic and Laboratory Evaluation (APPLEfull), the Sequential Organ Failure Assessment (SOFA) scores, the shock index, multi-organ dysfunction syndrome (MODS), and outcome were compared among groups. Significance was set at $P < 0.05$.
The study enrolled 90 dogs with uncomplicated sepsis (US) and 56 dogs with septic shock (DS n=35; CS n=10; VS n=11). Mortality rates, MODS occurrence, APPLEfull and SOFA scores were significantly lower in US (21%; 29%; 31, 13-52; 1, 0-11; respectively) compared to CS (50%; 70%; 37, 29-55; 3, 0-9; respectively), DS (57%; 88%; 44, 24-66; 6, 2-14; respectively) and VS (64%; 100%; 45, 28-53; 6, 3-11; respectively) ($P<0.01$). Significantly higher values of SOFA score, and mortality risk were reported in VS and DS compared to CS ($P<0.05$). The latter was significantly associated with a lower rate of renal dysfunction (30%) compared to VS (64%) and DS (77%) ($P<0.02$). DS was associated with a greater risk of MODS occurrence ($P<0.05$), higher values of shock index ($P<0.01$) and a higher need of fluid resuscitation compared to CS and VS ($P<0.01$).
Our results report that canine septic shock, based on persistent fluid-refractory hypotension and/or hyperlactatemia, is associated with higher disease severity than sepsis alone. However, presence of DS or VS is associated with a poor outcome, specifically, DS has a higher risk of development of MODS.

[1] Singer M, Deutschman CS, Seymour CW, Shankar-Hari M, Annane D, Bauer M, Bellomo R, Bernard GR, Chiche JD, Coopersmith CM, Hotchkiss RS, Levy MM, Marshall JC, Martin GS, Opal SM, Rubenfeld GD, van der Poll T, Vincent JL, Angus DC. The Third International Consensus Definitions for Sepsis and Septic Shock (Sepsis-3). JAMA. 2016 Feb 23;315(8):801-10. doi: 10.1001/jama.2016.0287. PMID: 26903338; PMCID: PMC4968574.
[2] Ranzani OT, Monteiro MB, Ferreira EM, Santos SR, Machado FR, Noritomi DT; Grupo de Cuidados Críticos Amil. Reclassifying the spectrum of septic patients using lactate: severe sepsis, cryptic shock, vasoplegic shock and dysoxic shock. Rev Bras Ter Intensiva. 2013 Oct-Dec;25(4):270-8. doi: 10.5935/0103-507X.20130047. PMID: 24553507; PMCID: PMC4031869.
[3] Swenson KE, Dziura JD, Aydin A, Reynolds J, Wira CR 3rd. Evaluation of a novel 5-group classification system of sepsis by vasopressor use and initial serum lactate in the emergency department. Intern Emerg Med. 2018 Mar;13(2):257-268. doi: 10.1007/s11739-017-1607-y. Epub 2017 Jan 28. PMID: 28132131.
[4] Summers AM, Vezzi N, Gravelyn T, Culler C, Guillaumin J. Clinical features and outcome of septic shock in dogs: 37 Cases (2008-2015). J Vet Emerg Crit Care (San Antonio). 2021 May;31(3):360-370. doi: 10.1111/vec.13038. Epub 2020 Dec 31. PMID: 33382202.

ACUTE ONSET OF HYPERTENSIVE ENCEPHALOPATHY IN A DOG WITH RIGHT ADRENAL PHEOCHROMOCYTOMA AND NEOPLASTIC INVASION OF THE CAUDAL VENA CAVA

Alessandra Recchia (1), Serena Digiaro (1), Antonella Colella (1), Italia Mele (1), Beatrice Greco (1), Paola Paradies (1)

(1) Department of Precision and Regenerative Medicine and Jonian Area (DiMePre-J); University of Bari "Aldo Moro"

Corresponding Author: **Paola Paradies** - paola.paradies@uniba.it

Pheochromocytomas (PCC) are endocrine tumours arising from chromaffin cells of the adrenal gland. They affect middle-aged to older dogs with no apparent sex or breed predilection. Most of the clinical signs result from the space-occupying nature of the tumour or the excessive secretion of catecholamines [1]. A 12-year-old female Épagneul Breton was examined at the Veterinary Hospital of University of Bari for acute neurological signs. The patient had a history of PU/PD referred to a Iris stage II CKD with hypertension diagnosed one year before. Mild progressive weight loss and tremors in the last 2 months were referred. The day before presentation the dog showed acute onset of ataxia with right head tilt,right drifting, cyrcling, loss of balance and head pressing with the evidence of an abnormal mental status,disorientation and vocalizations. At the time of presentation, the dog was severely depressed. Blood pressure was serially evaluated showing constant high values (systolic Bp>180 and diastolic BP>120) with mild change in renal biochemical tests (Urea 193 mg/dl; Crea 2.89). The dog was included in the risk category 4 corresponding to a severe risk of Target Organ Damage.Based on these findings and a negative brain TC a hypertensive encephalopathy was suspected. An emergency treatment was started and an abdominal ultrasonographic examination was requested to investigate adrenal glands. An increased right adrenal gland (with normal left) and the partial invasion of caudal vena cava(CVC) was documented at ultrasound and confirmed at TC.After an initial response to the medical treatment the animal's general conditions deteri orated.A necropsy was performed and histological examination confirmed the diagnosis of right adrenal PCC with neoplastic invasion of the CVC.This case report describes the acute onset of hypertensive encephalopathy signs in a dog with PCC.PCC is a rare tumour in dogs, it is usually a solitary, slow-growing tumour that extend into the lumen of adjacent vessels, particularly the CVC (in approximately 50% of cases) and when active it produces catecholamines.The variety of clinical signs coupled with the paroxysmal nature of the disease make the ante-mortem diagnosis extremely difficult [2].In our patient it is not possible to exclude that the disease was already present one year before when CKD was diagnosed and emphasize the importance to monitor blood pressure and investigate adrenal glands in elderly dogs with acute onset of neurological signs.

[1] Maher Jr, E. R., & McNiel, E. A. (1997). Pheochromocytoma in dogs and cats. Veterinary Clinics of North America: Small Animal Practice, 27(2), 359-380.
[2] Enright, D., Dickerson, V. M., Grimes, J. A., Townsend, S., & Thieman Mankin, K. M. (2022). Short-and long-term survival after adrenalectomy in 53 dogs with pheochromocytomas with or without alpha-blocker therapy. Veterinary Surgery, 51(3), 438-446.

VINORELBINE AS FIRST LINE TREATMENT IN STAGE IV CANINE PRIMARY PULMONARY CARCINOMA

V. Rinaldi (1), PE. Crisi (1), R. Finotello (2), E. Cabibbo (3), A. Boari (1)

(1) Dipartimento di Medicina Veterinaria, Università degli studi di Teramo 64100, Teramo
(2) Dipartimento di Medicina Veterinaria, Università di Bari, 70010 Valenzano (Ba)
(3) Clinica Veterinaria Jenner, VetPartner, 43126 Parma

Corresponding Author: **Valentina Rinaldi** - vrinaldi@unite.it

Primary pulmonary tumors represent 1% of neoplastic diseases in companion animals, with approximatively 85% being epithelial in origin. Low-grade tumors are associated with good prognosis; however, aggressive phenotypes tend to present with advanced regional disease and are associated with poor prognosis. Vinorelbine (VRL), a semi-synthetic vinka alkaloid, commonly used in humans with advanced lung cancer, reaches high concentrations in the lung tissue, has proven antineoplastic activity and low toxicity profile in dogs. Treatment naïve, client owned dogs with, a cyto/histological diagnosis of advanced pulmonary carcinoma [stage IV: a tumor with lymph node involvement and intra/extra thoracic metastasis (including malignant effusion)] (1), laboratory database and imaging (radiographs-US or TC), were enrolled in the study. Vinorelbine (15mg/m2) was administered weekly for 4 weeks and then fortnightly until progressive disease (PD) was documented. Staging work-up was repeated by means of diagnostic imaging, after the fourth VRL (i.e., 28 days) and monthly thereafter; response to treatment was evaluated according to the Response Evaluation Criteria for Solid Tumors in dogs (2). Toxicity was graded following the Veterinary Cooperative Oncology Group-Common Terminology Criteria for Adverse Events (3). Ten dogs were enrolled. One dog received only one VRL and died after seven days due to PD; the remaining dogs received a minimum of 8 VRL. In 3 dogs Piroxicam 0.3mg/Kg PO was also given. The most common side effect was neutropenia: 2 grade I, 2 grade II and 1 grade IV, requiring hospitalization. One dog developed grade II anorexia while 2 dogs had grade I vomiting. Eight dogs achieved partial remission (PR), one maintained stable disease (SD) for 56 days and one died 7 days after the first VRL due to worsening of respiratory signs. Median time to progression was 88 days (range: 7–112). One of the dogs that achieved PR, at the time of PD (90 days) had carboplatin followed by metronomic chemotherapy and is still alive and in SD 400 days post diagnosis. Median survival time for all dogs was 100 days (range 7-400). This study presents several limitations: the small cohort, its retrospective nature as well as the lack of histological diagnosis for all cases. Vinorelbine may offer a modest benefit in the management of advanced pulmonary carcinoma in dogs and should be revaluated in a phase II clinical trial.

(1) Lee et al, "Retrospective evaluation of a modified human lung cancer stage classification in dogs with surgically excised primary pulmonary carcinomas", Vet Comp Oncol. 2020;1–9.
(2) Ref Nguyen SM, Thamm DH, Vail DM, London CA. Response evaluation criteria for solid tumours in dogs (v1.0): a Veterinary Cooperative Oncology Group (VCOG) consensus document. Vet Comp Oncol. 2015 Sep;13(3):176-83.
(3) Veterinary cooperative oncology group – common terminology criteria for adverse events (VCOG-CTCAE) following chemotherapy or biological antineoplastic therapy in dogs and cats

HOSPITALIZATION DISRUPTS THE DAILY RHYTHM OF TEAR PRODUCTION IN DOGS

Marco Tabbì (1), Claudia Giannetto (1), Francesco Macrì (1), Elisabetta Giudice (1), Giuseppe Piccione (1), Simona Di Pietro (1)

(1) Department of Veterinary Sciences, University of Messina. Viale Palatucci snc 98168 Messina

Corresponding Author: **Simona Di Pietro** - simona.dipietro@unime.it

Tears carry out an important role in maintaining the health status of the ocular surface. Reduction in tear production can cause ocular damage that may lead to blindness. Hospitalization has been demonstrated to have an influence on tear production. In cats, the exposure to constant light during the hospitalization causes the loss of the daily rhythmicity of Schirmer Tear Test 1 (STT-1). In dogs, STT-1 values decreased in patients hospitalized in the intensive unit. In order to verify if the exposure to a constant light during the hospitalization had an influence on the tear production and its circadian rhythmicity in canine specie, the STT-1 values of hospitalized owned dogs (HD, n=6) exposed to a 24/0 Light/Dark cycle, were compared to a staff-owned canine control group (CD, n=6), living in their owner house, exposed to a natural photoperiod of 12/12 L/D cycle. The dogs were hospitalized for monitoring of a suspected rodenticide poisoning. All animals enrolled were free of signs of ocular diseases. Both eyes were tested (n= 24 eyes). Starting from the second day of hospitaliztion, the STT-1 was performed at 4 h intervals over a 48-h period (starting at 8:00 a.m. on Day 1 and finishing at 8:00 a.m. on Day 3). Department's Animal Ethics Council approved the study (protocol n.: 44/2020). Owner consent was obtained. The application of multivariate repeated measure ANOVA showed no statistical difference in the tear production between left and right eyes ($p = 0.90$), the two days of monitoring ($p=0.32$) and the two different experimental conditions ($p = 0.23$). A statistically significant effect of time of day was found ($p<0.0001$). The application of single cosinor procedure showed a robust daily rhythmicity of STT-1 in both eyes during the entire monitoring period in control dogs; circadian rhythm was lost in both eyes during the constant light period in hospitalized dogs.

Our results showed that in HD group the 24 hours mean value of tear production in left eye (Day 1: 21.46±2.89 mm/min; Day 2 18.86±5.47) and right eye (Day 1: 19.39±3.29 mm/min; Day 2: 19.46±2.62) were within reference range for canine specie.

Since it can be excluded a stress response due to animals handling that was the same in both groups, the loss of rhythmicity of tear production in hospitalized dogs is likely related to the change in the photoperiod.

Despite this finding, the absence of a reduction in STT-1 values could not predispose the hospitalized dogs to ocular surface disorders.

[1] Chandler et al., JVECC 23(3): 274–279, 2013.
[2] Refinetti R. Circadian physiology. II Edition, Saunders Ed., 2006.
[3] Di Pietro et al., Vet Ophthalmol. DOI: 10.1111/vop.13037.

76° CONVEGNO SISVET
BARI, 21-22-23 GIUGNO 2023

LIPEMIA-RELATED PSEUDOTHROMBOCYTOSIS IN CANINE BLOOD SAMPLES ANALYZED WITH AN AUTOMATED HEMATOLOGY ANALYZER (ADVIA 2120)

Sabrina Fasoli (1), Maria Giulia Ferrari (1), Kateryna Vasylyeva (1), Francesco Lunetta (1), Martina Baldin (1), Elisa Brini (1), Chiara Agnoli (1), Francesco Dondi (1)

(1) Department of Veterinary Medical Sciences (DIMEVET), Alma Mater Studiorum-University of Bologna

Corresponding Author: **Sabrina Fasoli** - sabrina.fasoli2@unibo.it

Pseudothrombocytosis is anecdotally reported to be associated with lipemia in blood samples analyzed with laser-based hematology analyzers (HA). Lipid droplets with high refractive index can be erroneously counted as platelets (PLT) similarly to cellular fragments, microorganisms or cryoglobulins. [1,2] Aims of this study were 1) to characterize cases of pseudothrombocytosis associated with lipemia in canine blood samples evaluated by a flow cytometry-based HA (ADVIA 2120, Siemens Healthineers); 2) to evaluate the association between PLT count and lipemia, serum triglyceride (TG) and cholesterol (TC) concentration. Cases of pseudothrombocytosis, defined as concurrent presence of increased automated PLT count (APLT-C; >500x10^3/µl; R.I. 150-500) and normal microscopic manual PLT estimate (MPE) (10-30 PLT in ≥5 fields at 1000X) [3] in EDTA samples, were examined and their medical records reviewed (2020-2022). Dogs with a simultaneous CBC and serum chemistry performed during the study period (n=8990) were also retrospectively evaluated, for comparative purpose. Dogs with thrombocytopenia were excluded from this population. Blood smear was microscopically evaluated and presence of lipemia-related erythrocyte lysis [4] recorded. Macroscopic lipemia in serum samples was graded from 0 to 4, based on its severity, and dogs were grouped accordingly. PLT count was compared among groups and correlated with TG and TC. Data were reported as median and range and compared with nonparametric statistics ($P<0.05$ considered significant). In the study period, 12 dogs had pseudothrombocytosis and APLT-C significantly increased if compared to MPE (759, 690-1663 vs. 416, 192-469 x10^3/µl; $P<0.01$). All these dogs had macroscopic lipemia in serum samples and signs of lipemia-related erythrocyte lysis. Conditions able to artifactually increase APLT-C other than lipemia, were excluded. Final diagnoses were pancreatitis (n=4), cardiac disease (n=2), neurological disease (n=2), neoplasia (n=1), others (n=3). In dogs selected for comparative purpose, APLT-C was significantly increased in lipemic samples if compared with the non-lipemic ones (343, 155-1899 vs 313, 150-3397 x10^3/µl; $P<0.01$). A significant correlation was detected between APLT-C and respectively grade of lipemia ($r=0.08$; $P<0.01$), TG ($r=0.21$; $P<0.01$) and TC ($r=0.12$; $P<0.01$). Lipemia can significantly affect APLT-C obtained with flow cytometry-based automated HA. MPE remain mandatory if hyperlipemia is suspected in dogs.

[1] Zandecki, M., Genevieve, F., Gerard, J. & Godon, A., 2007. Spurious counts and spurious results on haematology analysers: a review. Part I: platelets. Int. Jnl. Lab. Hem., Volume 29, pp. 4-20.
[2] Lippi, G., Lima-Oliveira, G., Salvagno, G.L., et al., 2010- Influence of a light meal on routine haematological tests. Blood Transfus, 8, pp. 94-99.
[3] Harvey, J.W., 2012. Hematology Procedures. In: Harvey, J.W., Veterinary Hematology. Ed Harvey, J.W. Elsevier Saunders, Missouri, pp. 11-32.
[4] Harvey, J.W., 2012. Evaluation of Erythrocytes. In: Harvey, J.W., Veterinary Hematology. Ed Harvey, J.W. Elsevier Saunders, Missouri, pp. 49-121.

SICV

METABOLOMICS PROFILE OF KNEE SYNOVIAL FLUID AFTER OSTEOCONDRAL SCAFFOLD IMPLANTS IN AN OVINE MODEL

A.M .Crovace (1), C.R. Girelli (2), A. Imperante (3), D. Monopoli (4), F. Fanizzi (2), L.F. Staffieri (3), L. Lacitignola (3)

(1) Dipartimento di Scienze Veterinarie Università di Sassari
(2) Unisalento
(3) DiMePRe-J ,Università di Bari -Aldo Moro ,Dottorato di Ricerca in "Trapianti di Tessuti e Organi e Terapie Cellulari
(4) Istituto Tecnologico delle Canarie (ITC)

Corresponding Author: **Luca Lacitignola** - luca.lacitignola@uniba.it

There are two general categories of OA regarding its origin: primary OA and secondary OA which occurs in patients that suffer traumatic injury to an articular joint. It is generally accepted that mechanical alterations can produce articular modifications of synovial fluid. In this study we investigated with 1H nuclear magnetic resonance (NMR) spectroscopy, the post traumatic joint changes of synovial fluid after titanium+Polycaprolactone (T/PCL) scaffold and Polycaprolactone (PCL) scaffold implants of femoral condyle. A total of 21 synovial fluid samples (10 healthy, 5 PCL, and 6 T+PCL) were stored at a temperature of −20 °C, until the NMR measurements. The NMR spectra were recorded at 300 K on a Bruker Avance III NMR spectrometer (Bruker, Karlsruhe, Germany), operating at 600.13 MHz for1H observation, equipped with a TCI cryoprobe incorporating a z axis gradient coil and automatic tuning-matching (ATM). The various spectra were recorded using the Bruker's standard pulse program sequences. After preliminary optimizations of experimental parameters, the metabolite assignment of spectral peaks of the 1D 1H NMR spectra was performed by comparison with literature data and with the help of 2D 1H Jres, 1H-1H COSY, 1H-13C HSQC, and 1H-13C HMBC spectra. Then a series of pair wise PLS-DA analyses were performed by comparing healthy samples with PCL and T+PCL synovial fluid samples respectively. In both case the analysis gave a good model (4 components gave RX= 0.99, Q2= 0.82 and RX=0.99 Q2= 0.91 for healthy samples with PCL and T+PCL synovial fluid samples respectively). The 2D scores plot showed a certain degree of separation between T+PCL and PCL samples along t1 component and marked separation of the healthy samples from the other two classes (PCL and T+PCL). The observed separation suggested variations in metabolic profiles of the three classes. The variables (bucket reduced NMR signals) responsible for the observed separation were identified in the corresponding variable importance in projection (VIP) plot.The most contributory variables in class discrimination in the PLS-DA model are lactate and acetate, with higher concentration in T+PCL and glucose and creatine/creatine with higher concentration in PCL. In both cases a higher concentration of glucose, acetate and creatine/creatinine was observed in the healthy group. On the other hand, both PCL and T+PCL groups showed higher concentration of lactate.

[1] Anderson, J. R.; Phelan, M. M.; Clegg, P. D.; Peffers, M. J.; Rubio-Martinez, L. M. Synovial Fluid Metabolites Differentiate between Septic and Nonseptic Joint Pathologies. J Proteome Res 2018, 17 (8), 2735–2743. https://doi.org/10.1021/acs.jproteome.8b00190.
[2] Mickiewicz, B.; Heard, B. J.; Chau, J. K.; Chung, M.; Hart, D. A.; Shrive, N. G.; Frank, C. B.; Vogel, H. J. Metabolic Profiling of Synovial Fluid in a Unilateral Ovine Model of Anterior Cruciate Ligament Reconstruction of the Knee Suggests Biomarkers for Early Osteoarthritis. Journal of Orthopaedic Research 2015, 33 (1), 71–77. https://doi.org/10.1002/jor.22743.
[3] Dubey, D.; Chaurasia, S.; Guleria, A.; Kumar, S.; Modi, D. R.; Misra, R.; Kumar, D. Metabolite Assignment of Ultrafiltered Synovial Fluid Extracted from Knee Joints of Reactive Arthritis Patients Using High Resolution NMR Spectroscopy. Magnetic Resonance in Chemistry 2019, 57 (1), 30–43. https://doi.org/10.1002/mrc.4763.
[4] Maher, A. D.; Coles, C.; White, J.; Bateman, J. F.; Fuller, E. S.; Burkhardt, D.; Little, C. B.; Cake, M.; Read, R.; McDonagh, M. B.; Rochfort, S. J. 1H NMR Spectroscopy of Serum Reveals Unique Metabolic Fingerprints Associated with Subtypes of Surgically Induced Osteoarthritis in Sheep. J Proteome Res 2012, 11 (8), 4261–4268. https://doi.org/10.1021/pr300368h.

76° CONVEGNO SISVET
BARI, 21-22-23 GIUGNO 2023

EX-VIVO EVALUATION OF THE SINGLE PELVIC OSTEOTOMY ON THE ACETABULAR VENTROVERSION IN CANINE HIP

Amanda Bianchi (1), Francesco Collivignarelli (1), Andrea Paolini (1), Massimo Vignoli (1), Andrea De Bonis (1), Sara Canal (1), Martina Rosto (1), Francesca Del Signore (1), Roberto Tamburro (1)

(1) University of Teramo - Department of Veterinary Medicine

Corresponding Author: **Amanda Bianchi** - abianchi@unite.it

The double pelvic osteotomy (DPO) is a prophylactic surgical procedure that aims to increase the dorsal acetabular coverage in dogs affected by hip dysplasia. The DPO technique includes an iliac osteotomy and a pubic ostectomy. The consequent acetabular rotation is fixed using a preangled plate (1,2). In order to reduce the surgical invasivity we hypothesized that a single iliac osteotomy may be sufficient to achieve a proper acetabular ventroversion. This peocedure is called single pelvic osteotomy (SPO). The aim of this ex-vivo study was to assess the feasibility of the SPO and to compare the amount of acetabular rotation (AR) following SPO with that obtained following DPO on the same hip. Inclusion criteria were large breed dogs euthanized for reasons unrelated with this study. A signed consent form was obtained by the owners. Each dog underwent radiographic evaluation to exclude any previous bone pathologies. Each pelvis was carefully dissected from the soft tissues and extracted from the cadaver. An SPO was performed on the right hip, a DPO was performed on left one. Two groups were obtained. The amount of AR was measured according to Petazzoni et al (3). A 20° DPO plate was used to fix the osteotomy. A statistical comparison between the two groups were carried out and $p < 0.05$ was considered significant. Five dogs were enrolled in the present study. The median body-weight was 28 kg (range 26-32,4 kg) . The median age was 7 year-old (range 4-9 year-old). The mean AR following SPO was 8,35±5.45, the mean AR following DPO was 11±4.37. Statistical correlation between the groups was not significan ($p>0.05$).
The results showed the feasibility of the SPO procedure. The mean AR following SPO was lower than DPO, but no statistical correlation was observed. The main SPO advantage is to avoid the pubic osteotomy, reducing the surgical invasivity and preventing potential complications such local hemorraghe and obturator nerve damage (3). We believed that the SPO must be performed in growing dogs in which the elastic bone may be able to absorbe the pastic deformation related to acebular rotation. However, pubic and/or ischial fractures and above all implant failure cannot be exclued. The main limitation of the present study was its ex-vivo nature. Moreover ex vivo evaluation are commonly used in veterinary medicine literature to preliminary assess a surgical technique. For this reason further clinical studies are stricly required to evaluate the SPO outcomes.

[1] Vezzoni, A., Boiocchi, S., Vezzoni, L., Vanelli, A. B., & Bronzo, V. Double pelvic osteotomy for the treatment of hip dysplasia in young dogs. VCOT 2010, 23(06), 444–452.
[2] Petazzoni M, Tamburro R, Nicetto T, Kowaleski MP. Evaluation of the dorsal acetabular coverage obtained by a modified triple 2.5 pelvic osteotomy). VCOT 2012; 25(5): 385-389
[3] Tong K, Hayashi K. Obturator nerve impingement as a severe late complication of bilateral triple pelvic ostotomy. VCOT 2012; 25(1):67-70.

76° CONVEGNO SISVET
BARI, 21-22-23 GIUGNO 2023

PATIENT SPECIFIC IMPLANTS (PSI) FOR BONE AND CARTILAGE RECONSTRUCTIONS IN VETERINARY APPLICATIONS

A.M. Crovace (1), A. Monopoli (2), N. Columbano (1), G. Masala (1), N.S. Romeo (1), Adrian Berrel (2), D. Monopoli (2), E. Sanna Passino (1)

(1) Dipartimento di Scienze Veterinarie Università di Sassari,Italy
(2) Istituto Tecnologico delle Canarie, Spain

Corresponding Author: **Alberto Crovace** - acrovace@uniss.it

Patient Specific Implants (PSI) emerged in the 1990s for human application in consequence of CT introduction but have been rarely used, during the next 20 years, due to their high cost and long development time. The manufacturing technology (chip removal) in use at that time and the lack of efficient surgeon-engineer communication tools are the main reason of that poor success. With the introduction of 3D metal printers in the 2007, the use of PSI has been increasing, thanks also to the advancement of software for 3D reconstruction and surgical planning and more efficient communications. Nowadays PSI are also in use in veterinary applications and are demonstrating some clear advantages: reduce risks and surgical times, preserve surrounding tissues, allow a better surgical planning and simplify the surgical steps.The workflow for PSI include: 3D-CT-reconstruction (MIS, Materialize NV, Invesalius), 3Dprinting (stereolithography, fused deposition) of anatomical plastic models, virtual surgical planning, implant/instrument design and manufacturing (3Dprinting, power bed fusion, Láser or EBM) validation and surgical training with models. Surgeon-engineer Communication is very easy using standard tools, e-mail, whatsapp, wetransfer, videocall, dropbox, etc. Main results are in cranio-maxillofacial and orthopedic surgery focused on: correction of angular deformities, joint resurfacing of focal defects, severe bone loss reconstruction (revisions, tumors, infections). In this paper we show and discuss the results obtained with the use of this techniques for the treatment of severe bone loss reconstruction and for the planning of osteochondral scaffolds to be used on experimental models of large animals for the treatment of joint injuries.Surgeon-engineer communication greatly conditions the development time and surgeons who perform a first case tend to repeat and attract others.Development times are often shortened when more cases are done with the same surgeonPSI are an effective solution in difficult surgical cases, supporting technology is already mature and continues to improve. It remains to remove some old prejudices about PSI (expensive and long time demanding) and to transmit to surgeon motivation to do it at least one time.

[1] A.Herrera e coll. Computational study and experimental validation of porous structures fabricated by electron beam melting: A challenge to avoid stress shielding. Materials Science and Engineering C 45 (2014) 89–93.
[2] A Cuadrado, A. Yánez, O. Martel, S. Deviaene, D. Monopoli. Influence of load orientation and of types of loads on the mechanical properties of porousTi6Al4V biomaterials, Materials & Design (2017). doi: 10.1016/j.matdes.2017.09.045
[3] Benady, A., Sam Meyer, J., Golden, E., Dadia, S., Katarivas Levy, G.. Patient-specific Ti-6Al-4V Lattice Implants for Critical-sized Load-bearing Bone Defects Reconstruction, Materials & Design(2023). doi: https://doi.org/10.1016/j.matdes.2023.111605
[4] A. Yánez, A. Cuadrado , O. Martel , H. Afonso, D. Monopoli. Gyroid porous titanium structures: A versatile solution to be used asscaffolds in bone defect reconstruction. Materials and Design 140 (2018) 21–29.

RETROSPECTIVE STUDY OF LAMENESS IN BEEF CATTLE IN NORTHEASTERN SARDINIA, ITALY

Sarah Morrone (1), Antonio Scanu (1), Gerolamo Masala (1), Alberto Maria Crovace (1), Stella Maria Teresa Romeo (1), Laura Saderi (2), Giovanni Sotgiu (2), Nicolò Columbano (1)

(1) Department of Veterinary Medicine, University of Sassari, Via Vienna No. 2, Sassari, 07100, Italy
(2) Clinical Epidemiology and Medical Statistics Unit, Department of Medical, Surgical and Experimental Sciences, University of Sassari, Sassari, Italy

Corresponding Author: **Nicolò Columbano** - ncolumbano@uniss.it

Lameness is one of the most prevalent diseases affecting dairy and beef cattle, resulting in decreased animal performance, decreased animal welfare, and substantial economic loss. In extensive beef cattle farming, the risk factors for this multifactorial disease are largely unexplored. This study aims to conduct a preliminary epidemiological survey of risk factors in beef cattle in extensive breeding, evaluate the farmer's perception of lameness, and determine the recurrence frequency of the pathologies under investigation in treated animals. The study was conducted in Sardinia, Italy. The population of the study consisted of 14379 cattle from 230 farms. An ad hoc questionnaire was developed to collect all the necessary data. A strong association was found between breed and the occurrence and recurrence of lameness ($p < 0.0001$). In addition, the Country of origin of both bulls and cows was found to be correlated with the incidence of lameness ($p < 0.0001$ and 0.0001, respectively). Farmers who indicated on the questionnaire that lameness was not important on their farm had more animals with recurrences ($p < 0.0001$) than other farmers. The veterinarian's treatment choice differed significantly by farmer concern ($p = 0.007$) and was associated with less disease recurrence ($p < 0.0001$), resulting in greater farmer satisfaction ($p < 0.007$). Pure cow breed, French bull origin, and farmer's age were detected as significant predictors of lameness issues, with pure cow breed and French bull origin having the strongest associations (odds ratio = 2.37; $p = 0.009$). Even though the results of this study are preliminary, they indicate that breed selection is crucial in extensive beef farms to reduce lameness prevalence. In addition, it would be reasonable to train breeders to prevent and diagnose lameness early in order to collaborate with veterinarians to prevent recurrence.

[1] Murray RD, Downham DY, Clarkson MJ, Faull WB, Hughes JW, Manson FJ, et al. Epidemiology of lameness in dairy cattle: Description and analysis of foot lesions. Veterinary Record. 1996. doi:10.1136/vr.138.24.586;
[2] Ózsvári L. Economic Cost of Lameness in Dairy Cattle Herds. Journal of Dairy, Veterinary & Animal Research. 2017;6. doi:10.15406/jdvar.2017.06.00176;
[3] an De Gucht T, Saeys W, Van Meensel J, Van Nuffel A, Vangeyte J, Lauwers L. Farm-specific economic value of automatic lameness detection systems in dairy cattle: From concepts to operational simulations. J Dairy Sci. 2018. doi:10.3168/jds.2017-12867;

76° CONVEGNO SISVET
BARI, 21-22-23 GIUGNO 2023

COMPARISON OF ANALGESIC EFFICACY BETWEEN INTRATESTICULAR AND INTRAFUNICULAR INFILTRATION OF LIDOCAINE IN HORSES UNDERGOING ELECTIVE ORCHIECTOMY

Alessio Angorini (1), Nicola Pilati (1), Galosi Margherita (1), Serino Federica (1), Troisi Alessandro (1), Palumbo Piccionello Angela (1), Di Bella Caterina (1)

(1) University of Camerino, School of Biosciences and Veterinary Medicine

Corresponding Author: **Alessio Angorini** - alessio.angorini@unicam.it

The aim of this study is to compare the analgesic effect of intratesticular versus intrafunicular infiltration of lidocaine in horses undergoing orchiectomy. Ten male horses were selected for this clinical, blinded, prospective and randomized study. All patients were premedicated with acepromazine (20 mcg/kg IV) and then a jugular catheter was applied; subsequently the horses were induced with xylazine (0.4 mg/kg IV), ketamine (2 mg/kg IV) and midazolam (0.1 mg/kg IV). After the orotracheal intubation, general anesthesia was maintained with isoflurane in pure oxygen. Five patients (Testis group) received 2 ml/100 kg of lidocaine 2% intratesticular, and the same volume of NaCl 0.9% was infiltered in the funiculus. Conversely, in the Funicular group (five horses) the drugs were administered in reverse. The infiltrations and surgical procedures were carried out by the same blind operator. During the intraoperative period, the main cardiovascular and respiratory parameters were recorded 10 minutes before the start of the surgery (BASELINE), during the skin incision of the two testis (SKIN1 and SKIN2, respectively) and during the traction of the funiculus (TRACTION1 and TRACTION2, respectively). The same surgeon assigned a score from 0 (no tension) to 5 (high tension) as indication of the degree of tension of the cremaster muscle during the funiculus traction phase. An increase in heart rate, respiratory rate and mean arterial pressure equal to or greater than 20% compared to baseline was considered as a nociceptive stimulus, and the infusion of xylazine would be increased from 1 mg/kg/h to 2 mg/kg/h. Data with $p<0.05$ were considered statistically significant. There were no statistically significant differences in physiological parameters among study phases and between the two groups; however, the tension score was significantly lower in the Testis group than in the Funicular group. These results agree with another study in pigs (1). Moreover, Haga et al. reported that the injection of intratesticular lidocaine induces a lower cardiovascular response and a greater relaxation of the cremaster muscle compared to traditional castration without the local infiltration (2). In conclusion, the administration of intratesticular lidocaine could perform a greater analgesic action compared to funicular infiltration.

[1] Haga HA, Ranheim B. Castration of piglets: the analgesic effects of intratesticular and intrafunicular lidocaine injection. Vet Anaesth Analg. 2005 Jan;32(1):1-9. doi: 10.1111/j.1467-2995.2004.00225.x. PMID: 15663733.
[2] Haga HA, Lykkjen S, Revold T, Ranheim B. Effect of intratesticular injection of lidocaine on cardiovascular responses to castration in isoflurane-anesthetized stallions. Am J Vet Res. 2006 Mar;67(3):403-8. doi: 10.2460/ajvr.67.3.403. PMID: 16506900.

76° CONVEGNO SISVET
BARI, 21-22-23 GIUGNO 2023

ABDOMINAL SURGERY UNDER EPIDURAL ANAESTHESIA IN CALVES

Gessica Giusto (1), Claudio Bellino (1), Anna Cerullo (1), Alberto Ceppa (1), Marco Gandini (1)

(1) Department of Veterinary Sciences, University of Turin, L.go Paolo Braccini 2-5, Grugliasco, 10095, Turin (Italy)

Corresponding Author: **Gessica Giusto** - gessica.giusto@unito.it

Epidural anaesthesia (EA) is widely described for several surgical procedures in cattle [1,2]. It is mainly used for surgeries of perineum, tail or pelvis, but is less frequently described for major abdominal surgery.EA has the advantage of being easy to perform and rapidly metabolized, with limited systemic effects. Furthermore, the use of EA avoids general anaesthesia, which is generally associated with several systemic side effects such as regurgitation, bloating and muscle damage [3]. The aim of this study is to describe laparotomies in calves after the administration of EA in association with local block to evaluate if it is a useful protocol in abdominal surgery of calves. Seventy-one calves referred for gastrointestinal, urogenital and umbilical diseases and that were submitted to exploratory laparotomy under epidural anaesthesia were included in the study. Fifty-three were beef breed,10 were dairy calves, and 8 were crossbreed. Thirty-nine were male and 32 were female with median age of 12 days (range 1-180 days) and median weight of 63 kg (range 30-350 Kg). Thirty-nine out of 71(55%) cases included in the report had omphalitis or urachal problems, 27(38%) calves had gastrointestinal problems and 5(7%) calves had other genitourinary problems. Duration of surgery were recorded in all cases. Only epidural anaesthesia with xylazine/procaine combination was used in 43 calves (61%) and median duration of surgery was 45 minutes. In 28 calves, IV administration of xylazine (0.05 mg/kg) was required to complete the surgical procedure with a median duration of 80 minutes.EA was sufficient to ensure adequate analgesia in most surgeries while in surgery of longer duration, intravenous administration of xylazine was necessary. All calves reached the quadrupedal station without difficulty after surgery. Short-term survival was 88.7%. Three calves underwent intraoperative euthanasia for prediction of poor prognosis due to severe peritonitis and 1 for excessive extension of intestinal pathology. Three calves with atresia coli underwent postoperative euthanasia for peritonitis and postoperative ileus and one calf with omphalitis died postoperatively for severe peritonitis.All discharged animals reached slaughtering age or had a normal reproductive career.EA can be used safely for calf laparotomy, with the advantage of being easy to perform, rapidly metabolized and with limited systemic effects. Complications are limited and rarely associated with anaesthesiologic protocol.

[1] Rush J., Stockler J. et al, (2022): Local and Regional Anesthesia in Food Animal. In Lin H.C., Passler T., Clark-Price S.: Farm Animal Anesthesia, Wiley Blackwell, p159-183
[2] Skarda R.T., (1996). Local and regional anesthesia in ruminants and swine. Vet Clin North Am Food Anim Pract; 12:579–626
[3] Kamiloglu, A., Kamiloglu, N.N., Ozturk, S. et al. Clinical assessment of epidural analgesia induced by xylazine-lidocaine combination accompanied by xylazine sedation in calves. Ir Vet J 58, 567 (2005). https://doi.org/10.1186/2046-0481-58-10-567

76° CONVEGNO SISVET
BARI, 21-22-23 GIUGNO 2023

INFLAMMATORY OXIDATIVE STRESS INDUCED BY OVARIECTOMY IN DOGS UNDERWENT PERIOPERATIVE ANALGESIA WITH MELOXICAM

Giovanna Costa (1), Patrizia Licata (1), Nicola M. Iannelli (1), Diego Iannelli (3), Vito Angileri (2), Francesco Macrì (1), Filippo Spadola (1), Simona Di Pietro, Federica De Paoli (3), Fabio Leonardi (3), Giuseppe Bruschetta (1), Claudia Interlandi (1)

(1) University of Messina
(2) University of Parma
(3) Freelance

Corresponding Author: **Giovanna Costa** - glcosta@unime.it

In recent years, oxidative stress markers have been measured in dogs after spaying, as a traumatic event causing an inflammatory oxidative stress. A balanced anaesthesia regimen could reduce the side effects induced by surgical. The aim was to evaluate the response to inflammatory oxidative stress induced by ovariectomy in dogs underwent perioperative analgesia with meloxicam. Ten female dogs were enrolled in the study. Dogs received meloxicam (0,2 mgkg-1 SC) and atropine (0,03 mgkg-1 IM); propofol was administered to effect; maintenance under anaesthesia was performed using sevofluorane. After surgery, meloxicam (0.1 mg/kg OS, every 24 hours) was administered. Heart rate, respiratory rate, non-invasive blood pressure, temperature (T°); end-tidal CO2 (EtCO2, mmHg) arterial hemoglobin oxygen saturation (SpO2, %) and concentration of inspired and expired isoflurane (CSI and CSE) were recorded. To determination of oxidative stress, malondialdehyde (MDA), α, δ and γ-tocopherol were determined at baseline, 12, and 24 hours after the last administration of meloxicam. The evaluation of the intraoperative response to the surgical stimulus was evaluated using a cumulative pain scale (CPS) with a score from 0 to 4 (cut off point for a rescue analgesia: 10). Postoperative pain score was evaluated using Canine acute pain scale (CAPS) with a score from 0 to 4 (cut off point for a rescue analgesia: 2). The parameters recorded during general anaesthesia remaining within the physiological canine ranges. SPO2 was 96/100%. ETCO2 decreased from 45 to 36 mmHg. CSI and CSE varied from 5 to 3% and from 4 to 7%, respectively. CPS scores assigned were 0–6; CAPS score, were 0. No rescue analgesia was required. MDA levels increased from baseline at 12 and 24 hours after meloxicam discontinuation: 33.18 (2.44/72.20) µg\mL; 52.70(15.20/99.90) µg\mL; 52(15.13/98.55) µg\mL P=0.000. Tocopherols concentration decreased from baseline at 12 and 24 hours after discontinuation of meloxicam: α-tocopherols 13.7(6.48/19.80) mg/L; 12.5 (6.25 /19.35) mg/L; γ- tocopherols 3.0(2.72/ 6.20) mg/L, 2.71(2.50/3.47) mg/L; δ- tocopherols 92(87/1.80) mg/L, 84(76/86) mg/L P=0.000. The results showed that ovariectomy induced oxidative stress in the patients, despite the intra and post-operative clinical monitoring showing a good analgesic plan. Moreover, the reduced endogenous tocopherol levels could be due to their consumption to cope with the stress induced by surgery.

[1] Sina Salavati, Asghar Mogheiseh, Saeed Nazifi, Atefeh Amiri and Behrooz Nikahval. The effects of melatonin treatment on oxidative stress induced byovariohysterectomy in dogs. BMC Veterinary Research (2021) 17:181
[2] O. Yilmaz, M. Korkmaz, J.J. Jaroszewski, E. Yazici, E. Ulutas, Z.K. Saritas Comparison of flunixin meglumine and meloxicam influence on post operative and oxidative stress in ovariohysterectomized bitches. Polish Journal of Veterinary Sciences Vol. 17, No. 3 (2014), 493–499

EFFECTS OF INTRAOPERATIVE HAEMODYNAMIC INSTABILITY IN DOGS

Chiara di Franco (1), Bianca Maria Bigi (1), Elisa Bonafè (1), Flavia Evangelista (1), Angela Briganti (1)

(1) Department of Veterinary Sciences, University of Pisa

Corresponding Author: **Chiara Di Franco** - chiara.difranco@phd.unipi.it

Haemodynamic instability refers to alterations in blood pressure which can lead to inadequate blood flow and organ injury (1,2). The aim of this prospective observational study was to evaluate intraoperative hemodynamic instability and the potential onset of a renal and/or myocardial injury (OPBA 20/2022). Dogs of different breeds, ages and weight, conducted to the Veterinary Teaching Hospital to undergo surgery and hospitalization in the Intensive Care Unit for at least 24 hours after surgery, were enrolled. Preoperative clinical evaluation and blood exams were done before the surgery. The day of the procedure before anaesthesia (Tpre) troponin and venous blood gas analysis (including the measurement of BUN and creatinine) were repeated on each patient; blood gas analysis was repeated after surgery (Tpost), 12 hours from the end of surgery (T12) and 24 hours from the end of surgery (T24), while troponin was repeated at T24. The anaesthetist in charge of the case chose the anaesthetic protocol, according to the patient's clinical conditions. After induction an arterial catheter on dorsal metatarsal artery was positioned to measure invasive mean arterial pressure (MAP), systolic arterial pressure (SAP), cardiac output (CO), heart rate (HR) and oxygen delivery (DO2), with a haemodynamic monitor (MostCareup® Vytech®, Vygon Group Italia). Haemodynamic supportive drugs have been used in order to manage alteration of blood pressure. Patients were retrospectively divided in 2 groups: a "No-Injury" group (with no renal or myocardial injury) and a "RM-Injury" group (with renal and/or myocardial injury) by evaluating an abnormal troponin increase in 24 h or an increase of serum creatinine of 0,3 mg/dL in the 24 h after surgery. Thirty-three dogs were included, 16 were in RM-Injury group and 17 in No-Injury group. Median value of ASA classification was higher ($p=0.002$) in the RM-Injury group [3(1-5)] compared to the No-Injury group [2(1-3)]. In RM-Injury group mean value of SAP, MAP, CI, DO2 resulted significantly lower ($p<0.0001$) in comparison to No-Injury group, while HR was significantly higher ($p=0.003$). Lactate in RM-Injury were significantly higher ($p=0.02$) at Tpost compared to Tpre and were higher in comparison to No-injury group at Tpost ($p=0.02$).

The results of this study showed a correlation between hemodynamic instability and the possible onset of acute renal and/or myocardial injury and that postoperative lactate increase can be a clue of haemodynamic instability. Patients with high ASA status are more prone to haemodynamic instability.

[1] Salmasi V, Maheshwari K, Yang D, et al. Relationship between Intraoperative Hypotension, Defined by Either Reduction from Baseline or Absolute Thresholds, and Acute Kidney and Myocardial Injury after Noncardiac Surgery: A Retrospective Cohort Analysis. Anesthesiology. 2017;126(1):47-65. doi: 10.1097/ALN.0000000000001432

[2] Buitenwerf E, Boekel MF, van der Velde MI, et al. The haemodynamic instability score: Development and internal validation of a new rating method of intra-operative haemodynamic instability. Eur J Anaesthesiol. 2019;36(4):290-296. doi:10.1097/EJA.0000000000000941

76° CONVEGNO SISVET
BARI, 21-22-23 GIUGNO 2023

EVALUATION OF ULTRASOUND GUIDED INSERTION OF A REBOA CATHETER IN DOGS: A CADAVERIC STUDY

Chiara Di Franco (1), Camilla Flori (1), Caterina Puccinelli (1), Simona Citi (1), Angela Briganti (1)

(1) Department of Veterinary Sciences, University of Pisa

Corresponding Author: **Chiara Di Franco** - chiara.difranco@phd.unipi.it

Resuscitative endovascular balloon occlusion of the aorta (REBOA) is an endovascular technique for the control of haemorrhage. In Veterinary medicine this technique has not been yet applied in clinical setting but 2 cadaveric studies in which the insertion of the catheter has been obtained with a cut down technique and the positioning of the balloon was guided by fluoroscopy were published (1,2).The aim of the present cadaveric study was to evaluate the ultrasound (US)-guided insertion of the REBOA catheter and the use of the xiphoid process as landmark for the balloon positioning.
Eight cadavers of various breeds and genders, weighing at least 15 kg were enrolled. All dogs underwent brachial artery catheterization using cut-down and Seldinger technique to inject 0.9% NaCl solution to facilitate US-guided access of the femoral artery for the REBOA technique. The cadavers were placed in dorsal recumbency and the medial aspect of the limbs was prepared for the catheterization of femoral artery. The arterial access was performed by US-guided by an unskilled student and the catheter was inserted transcutaneously using the Seldinger technique; randomization was used to decide from which artery starting (right or left). The catheter was positioned in zone 1 (segment of the aorta between the left subclavian artery and the celiac trunk), taking the xiphoid process as the anatomical reference for measuring the length of the catheter from the insertion point in the femoral artery. Once positioned, the balloon was inflated, and a radiographic check was carried out to verify correct positioning. The times for placement were recorded, if 20 minutes were exceeded to position the US-guided vascular access, a more experienced doctor support was requested, or it was performed through a surgical incision.
The catheter was placed in all the animals for a total of 16 positioning. The average weight of the cadavers recruited for the study was 27.2 □ 10 kg. The total time to perform the technique was 9.5 □ 5 minutes, no differences were found between the execution times in one limb compared to the other. The balloon placement in zone 1 was correct in all cases.
This study demonstrated that US-guided catheterization of the femoral artery for the REBOA catheter application is feasible, and that positioning times are short despite the operator was inexperienced. Moreover, it is possible to use the xiphoid process as landmark to guide the positioning of the balloon in zone 1.

[1] Loewen JM, Blume LM, Bach JF. Placement of a balloon for resuscitative endovascular balloon occlusion of the aorta without fluoroscopic guidance in canine cadavers. Vet Surg. (2019) 48:592–6. doi: 10.1111/vsu.13145
[2] Reich CF, Levy NA, Beal MW. Evaluation of resuscitative endovascular balloon occlusion of the aorta catheter placement and comparison to resuscitative thoracotomy with aortic clamping in cadaver dogs. J Vet Emerg Crit Care (San Antonio). 2022 Sep;32(5):623-628. doi: 10.1111/vec.13197. Epub 2022 Jun 10. PMID: 35687424.

76° CONVEGNO SISVET
BARI, 21-22-23 GIUGNO 2023

EFFECTS OF THE CANINE MONOCLONAL ANTIBODY ANTI-NGF (BEDINVETMAB) ON THE MOBILITY IMPAIRMENT IN DOG AFFECTED BY SEVERE OSTEOARTHRITIS

Stabile M. (1), Fracassi L. (1), Lacitignola L. (1), Crovace A. (1), Staffieri F. (1)

(1) Section of Veterinary Clinics and Animal Production - Veterinary Surgery, Department of Precision and Regenerative Medicine and Jonic Area; University of Bari "Aldo Moro".

Corresponding Author: **Marzia Stabile** - stabilemarzia@gmail.com

The Nerve Growth factor (NGF) appears to play an important role in osteoarthritic pain.(1) Bedinvetmab, a canine monoclonal antibody (mAb) directed against NGF, was recently proposed as a novel analgesic therapy for the treatment of osteoarthritis (OA)in dogs.(2) The objective of this study was to evaluate the effects of bedinvetmab (Librela; Zoetis Inc., Italy), on the mobility impairment in dogs affected by severe OA. Client-owned dogs with mobility problems conducted at the veterinary surgical unit of University of Bari were enrolled in this observational study. For each dog an informed consent was signed by the owner. All dogs underwent a complete physical, orthopaedic and x-ray examination. Only dogs with COAST stage 4 (3) were included and treated with Bedinvetmab (0.5–1.0 mg kg–1) subcutaneously, administrated monthly, for a total of two consecutive administrations (D0, D28). The follow up visits were scheduled each 28 days (D28 – D56). At every visit, owners completed the Liverpool Osteoarthritis in dog (L.O.A.D) survey and the veterinarian performed a physical examination and the quantitative gait analysis (GAIT4 Dog R walkway, CIR Systems Inc., Sparta, NJ). Variation in L.O.A.D score, Gait Lameness Score (GLS) and latero-lateral symmetry ratio were evaluated as outcomes to test the efficacy of the treatment. Normality was assessed by shapiro-wilk test. Paired t-student test was used to capture the mean difference in L.O.A.D, GLS score and symmetry ratio before and after treatment. Twenty dogs were included in the study. Data from 15 dogs (12 M/3 F, 9.9 ± 3 years, 34.5 ± 15 kg) that completed the study were analyzed. L.O.A.D score was found significant lower at D56 when compared to D0 (23.7 ± 6.7 Vs 28 ± 6.8; p= 0.008). GLS score of the most affected limb was significantly higher at D56 compared to baseline (93.8 ± 9.4; 85.1 ± 5.2; p=0.01). Symmetry ratio of the most affected side was significantly lower compared to the contralateral (0.87 ± 0.08; Vs 1.14 ± 0.11, p<0.001) at D0; after first administration (D28) it was higher compared to D0 (0.92 ± 0.1 Vs D0 0.87 ± 0.08); at D56 it archived comparable values to the contralateral (0.98 ± 0.14 Vs 1.03 ± 0.15. p=0.2). The results prove that the Bedinvetmab is able to improve mobility and restore the gait symmetry already after two administrations, in dogs with severe OA.

[1] Enomoto M, Mantyh PW, Murrell J, Innes JF, Lascelles BDX. Anti-nerve growth factor monoclonal antibodies for the control of pain in dogs and cats. Vet Rec. 2019 Jan 5;184(1):23. doi: 10.1136/vr.104590. Epub 2018 Oct 27. PMID: 30368458; PMCID: PMC6326241.

[2] Lascelles BD, Knazovicky D, Case B, Freire M, Innes JF, Drew AC, Gearing DP. A canine-specific anti-nerve growth factor antibody alleviates pain and improves mobility and function in dogs with degenerative joint disease-associated pain. BMC Vet Res. 2015 Apr 30; 11:101. doi: 10.1186/s12917-015-0413-x. PMID: 25926287; PMCID: PMC4419463.

[3] Stabile M, Van Ryssen B, Minei S, Coppieters E, Crovace A, Lacitignola L, Staffieri F. Observational study of the clinical value of the canine osteoarthritis staging tool. Vet J. 2022 May-Jun;283-284:105832. doi: 10.1016/j.tvjl.2022.105832. Epub 2022 Apr 27. PMID: 35487477.

76° CONVEGNO SISVET
BARI, 21-22-23 GIUGNO 2023

SUPERFICIAL SENTINEL LYMPH NODE BIOPSY IN 23 TUMOR-BEARING DOGS: PRE- AND INTRA-OPERATIVE INTRAPATIENT COMPARISON BETWEEN LYMPHOSCINTIGRAPHY AND NEAR-INFRARED FLUORESCENCE

Gariboldi Elisa Maria (1), Ubiali Alessandra (1), De Zani Donatella (1), Ferrari Roberta (1), Zani Davide Danilo (1), Auletta Luigi (1), Grieco Valeria (1), Giudice Chiara (1), Recordati Camilla (1), Stefanello Damiano (1)

(1) Dipartimento di Medicina Veterinaria e Scienze Animali, Università degli Studi di Milano, 26900, Lodi, Italy

Corresponding Author: **Elisa Maria Gariboldi** - elisamaria.gariboldi@unimi.it

Near Infrared Fluorescence (NIRF) with Indocyanine Green (ICG) can replace and improve lymphoscintigraphy (LPS) during sentinel lymph node (SLN) biopsy in human [1-3]. The paucity of data in veterinary medicine [4] led us to compare LPS and NIRF into sentinel lymphocentrum (SLC) mapping and SLN extirpation in canine patients bearing different solid tumors, during the first 6 month of a Ph.D. project. Tumor-bearing dogs were prospectively enrolled for SLN excision guided by handheld gamma probe (HGP) and NIRF. The same surgeon performe all the procedures.
Tc99m and ICG were injected peritumorally; for standard practice, methylene blue was also injected. Data collected were: tumor histotype, histological nodal status, number of SLCs, correspondence with the regional lymphocentrum, number of SLNs and SLN detection rate. Intrapatient comparison between LPS and NIRF performance was recorded in pre-operative phase (planar LPS and NIRF during mapping and SLC detection; HGP and NIRF during incisional site detection) and intra-operative phase (HGP and NIRF during SLC surgical exploration for SLNs excision). In the latter phase, SLN radioactivity, fluorescence, blue dyeing, and the surgeon's perception in helping the SLN detection were recorded. Forty-four SLNs were excised from 32 SLCs, in 23 dogs with 27 tumors (21 mast cell tumors, 2 soft tissue sarcomas, one oral melanoma and 3 mammary tumors). The SLN detection rate of the 2 techniques combined was 100%. In 59% of cases, SLC did not correspond to the regional lymphocentrum. Pre-operatively planar LPS and NIRF failed mapping respectively in one and two tumors. In 2 tumors, both HGP and NIRF failed in the incisional site detection due to "shine through" effect. Radioactivity, fluorescence and blue dying were found respectively in 40, 41 and 39 of 44 excised SLNs. Surgeon's perception in SLNs detection was: HGP was better in 21/44, NIRF was better in 8/44 SLNs, and no differences in 15/44. In 11/11 axillary SLCs, the HGP was judged more useful than NIRF. In other regions, the 2 techniques had equal performance. Ten (45%) dogs had histological nodal metastasis. In conclusion, the performance during the pre-operative mapping phase for superficial SLC detection was equal in LPS and NIRF. When considering SLCs incisional site detection and their surgical exploration, NIRF performance was negatively influenced by SLC anatomical location (such as in the axillary region) and deeper lymphatic networks.

[1] Verbeek FP, Breast Cancer Res Treat. 2014; 143(2): 333-42. doi: 10.1007/s10549-013-2802-9.
[2] Kedrzycki MS, Ann Surg Oncol. 2021; 28(7): 3738-3748. doi: 10.1245/s10434-020-09288-7
[3] van den Berg NS, Eur J Nucl Med Mol Imaging. 2012; 39(7):1128-36. doi:10.1007/s00259-012-2129-5
[4] Beer P, Vet Comp Oncol. 2018; 16(2):178-187. doi: 10.1111/vco.12372

EXTERNAL SKELETAL FIXATION FOR TREATMENT OF HIND LIMB FRACTURES IN NINE FERAL PIGEONS (*COLUMBA LIVIA*)

Giuseppe Bonaffini (1), Matteo Serpieri (1), Chiara Ottino (1), Giuseppe Quaranta (1), Mitzy Mauthe von Degerfeld (1)

(1) Centro Animali Non Convenziali - Dipartimento di Scienze Veterinarie - Università di Torino, Grugliasco (TO), 10095, Italia

Corresponding Author: **Giuseppe Bonaffini** - giuseppe.bonaffini@unito.it

Traumatic fractures of the limbs are frequent causes of admission to wildlife rescue centers [1]. Even if the general principles and goals in fracture treatment in conventional species could be applicable, anatomical, and physiological peculiarities of birds should be considered [2]. External fixation is considered the gold standard for hind limb fractures in birds, because it is minimally invasive, preserves the bony vasculature and surrounding tissues, and allows rapid compensation of weight-bearing forces. Literature regarding the outcome of this kind of treatment for traumatic fractures in feral pigeons (*Columba livia*) is lacking, with a case report only [3]. The aim of this study was to evaluate the outcome of the treatment of pelvic limb fractures with an acrylic resin external fixator in 9 wild pigeons. 3 pigeons had fractures of the tibiotarsus and 6 of the tarsometatarsus. Surgery was performed, with anesthesia induced with a mix of ketamine and medetomidine (IM) and maintained with isoflurane. The diameter of the K-wires ranged from 0.8 to 1 mm. The tibiotarsal fractures were treated with tie-in and type 2B fixators and the tarsometatarsal fractures with type 2B and type 3 fixators. Fracture reduction was achieved by skeletal traction; the k-wires were connected using acrylic resin injected into a plastic straw of 4 mm diameter; for type 2B external fixators, the reduction was temporarily maintained, on one side, with Fixateur Externe du Service de Santé des Armées (FESSA), then removed after polymerization of the resin. The fixators were removed, following radiographic control, between the fifth and seventh week after surgery. All subjects were released into the wild after bone healing and functional recovery. Compared to other k-wire connection methods, the acrylic resin is light, rigid, inexpensive, highly adaptable in its application and well tolerated by the animal [4]. External fixators can be considered as a therapeutic method in the treatment of pelvic limb fractures in medium-sized wild birds.

[1] Ozsemir, K. G., & Altunatmaz, K. (2021). Treatment of extremity fractures in 20 wild birds with a modified Meynard external fixator and clinical assessment of the results. Veterinární medicína, 66(6), 257-265.
[2] Carrasco, D. C. (2019). Fracture management in avian species. Veterinary Clinics: Exotic Animal Practice, 22(2), 223-238.
[3] Rui, L. A., Viana, D. C., Dora, A. B., & Fratini, P. (2017). External fixation to correct tarsal-metatarsal fracture in rock pigeon (Columba livia). Revista Ceres, 64, 25-30.
[4] Staudte, K. L., Gibson, N. R., Read, R. A., Day, R., & Robertson, I. D. (2004). Evaluation of a metal acrylic repair product as a clamp for an external fixator. Veterinary and Comparative Orthopaedics and Traumatology, 17(02), 97-103.

EVALUATION OF SHEAR WAVE ELASTOGRAPHY TO ASSESS CANINE LENS STIFFNESS IN AGING SUBJECTS AND IN CASE OF CATARACT

Martina Rosto (1), Massimo Vignoli (1), Giovanni Aste (1), Andrea De Bonis (1), Cristina Pincelli (1), Andrea Paolini (1), Roberto Tamburro (1), Amanda Bianchi (1), Francesca Del Signore (1)

(1) Department of Veterinary Medicine, University of Teramo, Località Piano d'Accio - Teramo, Italy

Corresponding Author: **Martina Rosto** - martina_rosto@hotmail.it

Introduction. 2-D shear wave elastography (SWE) is a widely applied tool to assess tissue mechanical properties in healthy and diseased organs in human medicine.
This study aimed to investigate the repeatability and reproducibility of SWE and its feasibility to assess lens stiffness difference in aging dogs.
Methods. Dogs were prospectively enrolled divided in 4 groups, young (< 1.5 years), adult (1.5 -7 years), old (> 7 years) and cataract affected dogs.
Trans-corneal B-mode US and elastography were performed under physical restraint with a Logiq S8 (GE Healthcare) and a linear probe (9L/10 MHz) with a 2-D shear wave elastography software after owner consent. Triplicate kPa and m/s measurements were collected by two operators. Statistical analysis was performed with MATLAB R2020a, p value set < 0.05.
Results.44 dogs were included, respectively group 1 with 10 dogs (mean age 6 ± 3.07 months), group 2 with 12 dogs (mean age 4 ± 1.8 years), group 3 with 11 dogs (mean age 11.3 ± 1.3 years) and group 4 with 11 dogs (mean age 9.7 ± 5.1 years).
Slight differences of kPa (74.9±43.7 vs. 74.2±43.6 p= 0.06) and m/s (4,78±1,48 vs 4,75±1.48 p=0.0.3) were found between two operators. Intra-observer CV% for kPa and m/s were very low (2.86±3.04 and 1.39±1.45, mean±SD; p=0.001), respectively, with a significant difference between operators (kPa: 3.40±3.54 vs. 2.32±2.34, p<0.001; m/s: 1.64±1.66 vs. 1.16±1.17; p=0.001). kPa and m/s showed similar very high overall intra-observer ICC (0.996 for both). Inter-observer CV% was also very low (kPa: 3.46±3.98; m/s: 1.77±2.01; p=0.061) and ICC was very high (0.994 for both).
Mean stiffness expressed in m/s and kPa was respectively 3.1±0.5 and 28.9±9.2 for group 1, 4.6±0.6 and 65±18.4 for group 2, 6.46±0.36 and 126±14.6 for group 3 and 103±24.1 for group 4. Significant differences (p<0.05) were evidenced between all the groups for both m/s and kPa measurements.
Conclusions. These results evidence that SWE, is a reproducible tool to assess age-related stiffness in helthy dog and in case of cataract; some data obtained are operator dependent and m/s measures were slightly more reproducible than kPa.

76° CONVEGNO SISVET
BARI, 21-22-23 GIUGNO 2023

DIAGNOSTIC IMAGING FINDINGS OF SUBCHONDRAL BONE PATHOLOGY IN DOGS WITH OCCULT FORELIMB LAMENESS

Donatella De Zani (1), Jessica Bassi (1), Vanessa Rabbogliatti (1), Maurizio Longo (1), Davide Danilo Zani (1)

(1) Dipartimento di Medicina Veterinaria e Scienze Animali, Università degli Studi di Milano

Corresponding Author: **Donatella De Zani** - donatella.dezani@unimi.it

Bone scan is a sensitive tool for detecting early bone changes and it is indicated when the source of pain cannot be localized and/or when plain radiographs and/or Computed Tomography (CT) are inconclusive or suggestive of structural changes of uncertain clinical significance. Bone marrow lesion (BML) was observed on magnetic resonance imaging (MRI) of dogs with pain localized to the elbow, associated or not with medial coronoid (MCP) disease or osteoarthritis. In this study have been included dog referred for occult forelimb lameness that underwent to bone scan, CT and/or MRI. In the study were included 10 dogs. Scintigraphy recognized the source of pain in 9/10 dogs. In one case was impossible to identify the source of pain. MRI revealed the presence of BML of the proximal metaphysis of the ulna or humeral condyles in 7/10 patients. In 2/10 dogs CT findings suggested a definitive diagnosis of MCP disease while in 4/10 alterations of the MCP were observed bilaterally. Four of ten dogs did not show significant alterations on CT. In our study bone scan allowed a discrimination between inactive structural lesions, common in dog with elbow disease and osteoarthritis and areas of increased osseous activity. Even in dogs with unremarkable MRI and/or CT findings, scintigraphy revealed subchondral bone changes compatible with elbow disease, thanks to the high sensitivity of bone scan in detecting early stage of bone pathology. In no subjects BMLs were observed on MRI in absence of scintigraphic uptake even if it has been reported that a persistency of MRI signal alterations can be present after clinical and scintigraphic improvement.BML can be observed also in correlation with osteoarthrosis but, in our study, in the four dogs with bilateral structural changes of the elbow, BML, scintigraphic uptake were localized only to the lame limb. In conclusion, in 9/10 cases, bone scan allowed to recognize the source of pain and demonstrated a good correlation with clinical signs.In dogs with a combined findings of subchondral disease on MRI and bone scan, scintigraphy localized the lameness and the presence of bone remodeling while MRI evaluated the exact extent of BML.On the basis of these preliminary results, scintigraphy should be considered an aim in case of occult lameness also in dogs with structural changes that cannot explain alone the clinical signs. In fact, it has been demonstrated by several studies that subchondral bone lesions themselves could be a source of pain.

[1] Schwarz T et al. Bone scintigraphy in the investigation of occult lameness in the dog. Journal of Small Animal Practice (2004), 45, 232-237
[2] Van Bruggen LWL et al. Bone scintigraphy for the diagnosis of an abnormal medial coronoid process in dogs. Ver Radiol Ultrasound (2010), 51, 344-348
[3] Wavrille V et al. Correlation between histopathologic, arthroscopic and magnetic resonance imaging findings in dogs with medial coronoid disease. Veterinary surgery (2015), 44, 501-510
[4] McLarty E et al. Comparison of 18F-sodium fluoride positron emission tomography and CT: an exploratory study in 12 dogs with elbow pain. Vet Radiol Ultrasound (2021), 62, 498-506

PRESSURE SENSITIVE WALKWAY SYSTEM FOR EVALUATION OF LAMENESS IN DOGS AFFECTED BY UNILATERAL CRANIAL CRUCIATE LIGAMENT RUPTURE TREATED WITH POROUS TIBIAL TUBEROSITY ADVANCEMENT

M. Guadalupi (1), A. Monopoli (2), C. Vicenti (1), M. Stabile (1), L. Lacitignola (1), F. Staffieri (1), A.M. Crovace (3)

(1) DiMePRe-J, Università di Bari-Aldo Moro, Dottorato di Ricerca in "Trapianti di Tessuti e Organi e Terapie Cellulari"
(2) Istituto Tecnologico delle Canarie (ITC)
(3) Dipartimento di Scienze Veterinarie Università di Sassari

Corresponding Author: **Marta Guadalupi** - marta.guadalupi@uniba.it

The aim of this study is to evaluate objectively lameness in dogs affected by unilateral cranial cruciate ligament rupture treated with Porous Tibial Tuberosity Advancement before surgery and at three different timepoints after surgery, using the GAITRite® system, a pressure walkway combined with the original software developed for quadrupeds (GAITFour®Dog walkway), which is able to calculate several spatio-temporal gait parameters simultaneously for each limb.
Dogs presented for hindlimb lameness underwent a full orthopedic examination. CrCLR was diagnosed by performing the cranial drawer test, the tibial compression test, and the sitting test. Patients with other orthopedic, neurological, or neoplastic diseases were excluded from the study. Prior to surgery, the hindlimb to be treated was radiographically evaluated and gait analysis was performed. A minimum of three similar walks (same gait and velocity) have been collected for each dog. In addition, any intra-operative and post-operative complications, classified in minor (not requiring additional surgical or medical treatment to resolve) or major (requiring surgical or medical treatment to resolve), were considered. Dogs walked on the pressure sensitive walkway before (T0) and 30 (T1), 90 (T2), 120 (T3) days after surgery. Pressure measurements (Gait Lameness Score and Total Pressure Index %) were collected for T (treated with Porous TTA) at T0, T1, T2, T3 and statistically evaluated. The ANOVA test was performed to compare the data and a value of $p<0,05$ was considered significant. Twenty dogs (n=20) of various common breed and age with CrCLR were enrolled in the study.
The sample was homogeneously distributed in relation to weight and velocity, resulting in a bias-free evaluation. No intra- or post-operative complications were recorded.
The results showed that there was no statistically significant difference concerning GLS and TPI% between the T-T0 and T-T1 groups. Whereas a statistically significant difference was observed between the T-T0 and T-T2 groups ($p<0,05$) and the T-T0 and T-T3 groups ($p<0,05$) for both parameters considered. The data suggest that the objective evaluation of lameness show an effective increase in load of the hindlimb treated by performing the Porous TTA from ninety days after surgery with a positive outcome on patients' quality of life.

[1] Trisciuzzi, R. et al. 41 Cases of Treatment of Cranial Cruciate Ligament Rupture with Porous TTA: Three Years of Follow Up. Vet Sci 6 (2019). https://doi.org:10.3390/vetsci6010018
[2] Le Quang, T., Maitre, P., Roger, T. & Viguier, E. The GAITRite® system for evaluation of the spatial and temporal parameters of normal dogs at a walk. Computer Methods in Biomechanics and Biomedical Engineering 10, 109-110 (2007). https://doi.org:10.1080/10255840701479248
[3] Fahie, M. A., Cortez, J. C., Ledesma, M. & Su, Y. Pressure Mat Analysis of Walk and Trot Gait Characteristics in 66 Normal Small, Medium, Large, and Giant Breed Dogs. Front Vet Sci 5, 256 (2018). https://doi.org:10.3389/fvets.2018.00256
[4] Gordon-Evans, W. J. et al. Characterization of spatiotemporal gait characteristics in clinically normal dogs and dogs with spinal cord disease. Am J Vet Res 70, 1444-1449 (2009). https://doi.org:10.2460/ajvr.70.12.1444

USE OF PULSE-OXIMETRY TO DETECT THE INCIDENCE OF POSTOPERATIVE HYPOXEMIA IN DOGS RECOVERING FROM GENERAL ANESTHESIA

Piemontese C. (1), Stabile M. (1), Di Bella C. (1), Scardia A. (1), Vicenti C. (1), Acquafredda C. (1), Lacitignola L. (1), Crovace A. (1), Staffieri F. (1)

(1) Università degli Studi di Bari "Aldo Moro" Dipartimento di Medicina di Precisione e Rigenerativa e Area Jonica (DiMePRe-J) - Sezione Cliniche veterinarie e Produzioni animali

Corresponding Author: **Claudia Piemontese** - claudia.piemontese@uniba.it

The aims of this clinical study are 1) to validate the room-air SpO2 test (SpAT) in dogs, identifying a cut-off value able to discriminate hypoxemia (PaO2 < 80 mmHg) (phase 1), and 2) to use the SpAT to study the incidence of transitory pulmonary hypoxemia in healthy dogs recovering from general anesthesia (phase 2). The SpAT consisted in disconnecting the patients from oxygen supplementation for at least 3 minutes. [1][2] The SpO2 was continuously monitored with pulse oximetry probe (Masimo Rad- 87 Set Rainbow) and recorded after at least 1 minute of reading with an adequate prefusion index (≥ 1%). [3] Phase 1: dogs recovering from general anesthesia and having and arterial line in place were considered. Animals underwent SpAT within 10 minutes after extubation and at the same time an arterial blood sample was collected to determine the PaO2. A PaO2 < 80 mmHg was considered as hypoxemia. [4] Phase 2: dogs undergoing general anesthesia for different types of surgery and receiving different ventilatory managements were included. In these cases, the SpAT test was performed at 5 and 10 minutes after extubation. Based on the results of the phase 1 study, dogs were classified as hypoxemic when the SpO2 value detected at 5 and 10 minutes were lower than the cut-off value. A receiver operating curve (ROC) was utilized to determine the SpO2 cut-off value to optimize sensitivity and specificity to predict PaO2 < 80 mmHg. Multivariate binary regressions were conducted to see the existence of association between risk factors (BCS, dorsal recumbency, respiratory mode) and hypoxemia. Odd ratio, Confidence interval (95%) and P-value were reported for each factor. P-value <0.05 was considered as statistically significant. From the phase 1 study, 75 dogs were considered, and resulted that the cut-off value of SpO2 to detect PaO2 < 80 mmHg is < 95%, with 100% of sensitivity and 97.4% of specificity. The AUC is 0.996 (95% IC= 0.944-1; P<0.0001). For the phase 2 study were included 654 cases of which 54 were excluded. Hypoxemia was detected in 169 dogs (28.1 %). A BCS > 3/5, dorsal recumbency, FiO2 > 0.8, the absence of PEEP and the duration of anesthesia > 120 min had a significative odd ratio to induce postoperative hypoxemia of respectively 5.8, 1.9, 4.7, 1.7 and 1.9. The results of this study demonstrated that a SpO2 < 95% is indicative of a PaO2 < 80 mmHg in dogs and hypoxemia may be observed in up to 28% of cases and some risk factors have been identified.

[1] Ferrando, C., Romero, C., Tusman, G., et al. (2017) The accuracy of postoperative, non-invasive air-test to diagnose atelectasis in healthy patients after surgery: a prospective, diagnostic pilot study. BMJ Open 7, e015560
[2] Stabile M, Lacitignola L, Piemontese MR, et al. Comparison of CPAP and oxygen therapy for treatment of postoperative hypoxaemia in dogs. J Small Anim Pract. 2021;62(5):351-358. doi:10.1111/jsap.13295
[3] Hood, J. A. & Wilson, R. J. T. (2011) Pleth variability index to predict fluid responsiveness in colorectal surgery. Anesthesia and Analgesia 113, 1058-1063
[4] Sumner, C. & Rozanski, E. (2013) Management of respiratory emergencies in small animals. The Veterinary Clinics of North America. Small Animal Practice 2013; 43, 799-815

76° CONVEGNO SISVET
BARI, 21-22-23 GIUGNO 2023

INJECTABLE ANESTHESIA WITH KETAMINE, MEDETOMIDINE, AND BUTORPHANOL IN RATS

Matteo Serpieri (1), Anna Cerullo (2), Giuseppe Bonaffini (1), Giuseppe Quaranta (1), Mitzy Mauthe von Degerfeld (1)

(1) Centro Animali Non Convenzionali - Dipartimento di Scienze Veterinarie - Università di Torino; Grugliasco (TO), 10095, Italia
(2) Dipartimento di Scienze Veterinarie - Università di Torino; Grugliasco (TO), 10095, Italia

Corresponding Author: **Matteo Serpieri** - matteo.serpieri@unito.it

Ketamine, medetomidine, and butorphanol can be used to obtain sedation or anesthesia in rats, either as sole agents or in combination with other drugs [1]. However, the commonly used dosages in intraperitoneal administration are high and can produce cardiorespiratory depression and slow recovery [2]. The aim of this work is to evaluate the effects of the intramuscular administration of a cocktail of lower dosages of these drugs, followed by atipamezole, in rats. The trial included 28 Sprague-Dawley rats of 3 months of age and mean weight of 316.5 ± 27.6 g, undergoing laparotomy and, after 14 days, euthanasia as part of an experimental project. Rats received ketamine, medetomidine, and butorphanol (KMB: 30, 0.1, and 1 mg/kg, respectively) into the thigh muscles. Anesthesia was carried out twice in all subjects (laparotomy and euthanasia). For both procedures induction time, times of loss of palpebral and pedal reflexes, heart, and respiratory rate (HR, RR), peripheral saturation (SpO2) and need of isoflurane were evaluated. 5 minutes after the injection, depth of anesthesia was evaluated using scores (final score range: 0-16) reported for rats [3]. Each animal was given oxygen through a face mask, and isoflurane was delivered in case of need. After the surgical procedure, atipamezole (0.5 mg/kg, IM) and carprofen (5 mg/kg, SC) were administered and times of reappearance of palpebral and pedal reflexes, head lifting time, recovery time were evaluated. Results (mean ± SD): induction time was 3.1 ± 1.6 min, times of loss of palpebral and pedal reflexes were both 3.5 ± 2.0 min. Final score for depth of anesthesia was 15.1 ± 0.7. Times of reappearance of palpebral and pedal reflexes were 1.7 ± 0.7 min and 1.5 ± 0.6 min, respectively; head lifting, and recovery times were both 3.6 ± 1.0 min. All recoveries were smooth, with no complications. For all measures, mean HR, RR, and SpO2 were 272 ± 20 bpm, 63 ± 16 breaths/min, and 92 ± 9 %, respectively. All rats maintained spontaneous ventilation, and isoflurane was not needed. No mortality was recorded. The KMB combination allowed to rapidly obtain an adequate plan of anesthesia to perform laparotomy, without the need for assisted ventilation. The used dosages, lower than those reported for the same cocktail [2], allowed a faster recovery with no complications.

[1] Lofgren, J. L., Foley, P. L., & Golledge, H. D. (2020). Anesthesia, Analgesia, and Euthanasia. In The Laboratory Rat (pp. 699-745). Academic Press.
[2] Costea, R., Lastofka, D., & Mehedinţu, M. (2015). Comparison of Ketamine–Medetomidine-butorphanol and Ketamine–dexmedetomidine-butorphanol Anesthesia in Rats. Agriculture and Agricultural Science Procedia, 6, 305-308.
[3] Devor, M., & Zalkind, V. (2001). Reversible analgesia, atonia, and loss of consciousness on bilateral intracerebral microinjection of pentobarbital. Pain, 94(1), 101-112.

76° CONVEGNO SISVET
BARI, 21-22-23 GIUGNO 2023

CLINICAL COMPARISON BETWEEN INTRAVENOUS CONSTANT RATE INFUSION AND REPEATED SUBCUTANEOUS OR INTRAMUSCULAR INJECTION OF DEXMEDETOMIDINE IN ANAESTHETIZED HORSES: PRELIMINARY INVESTIGATION

Vanessa Rabbogliatti (1), Federica Alessandra Brioschi (1), Federica Di Cesare (1), Luigi Auletta (1), Davide Danilo Zani (1), Donatella De Zani (1), Giuliano Ravasio (1), Martina Amari (1)

(1) Department of Veterinary Medicine and Animal Sciences, Università Degli Studi Di Milano, Milan, Italy

Corresponding Author: **Vanessa Rabbogliatti** - vanessa.rabbogliatti@unimi.it

The study aims to compare the administration of dexmedetomidine (DEX) by intravenous (IV) constant rate infusion (CRI) and intramuscular (IM) or subcutaneous (SC) repeated injection in horses as adjuvant during general anaesthesia [1]. Thirty horses were sedated IV with acepromazine 0.03 mg/kg and detomidine 10 µg/kg. Anaesthesia was induced with IV ketamine 2.5 mg/kg and diazepam 0.05 mg/kg and maintained with steady state isofluorane (1.3%) in 60 % oxygen. Ringer's lactate was given at constant rate (2 ml/kg/h) and dobutamine was administered IV to maintain MAP ≥ 70 mmHg. All horses were mechanically ventilated: tidal volume of 15 ml/kg; peak inspiratory pressure close to 25 cmH2O; positive end-expiratory pressure 5 cmH2O; inspiratory time 2.0 seconds. To maintain normocapnia, expiratory time was set to adjust the respiratory rate. Horses randomly received either IV DEX at 2 µg/kg/h (CRI group) or DEX at 2 µg/kg administered by SC (SC group) or IM (IM group) injection repeated every 60 minutes. Physiological parameters, arterial blood gases, total dobutamine dose, ketamine rescue, urine production, time and attempts to attain sternal and standing position were noted. A blind anaesthesiologist assessed recovery quality with a standard scoring 5-point scale [2]. Data distribution was tested with Kolmogorov-Smirnoff test. Student's t, Mann-Whitney and ANOVA were applied ($p \leq 0.05$). There was no significant difference in anaesthesia duration (minutes; CRI 126 ± 9; SC 135 ± 24; IM 131 ± 15) ketamine rescue needed (CRI and IM 0 out of 10 horses; SC 1 out of 10 horses) and urinary output (ml/kg/hour; CRI 8 ± 3; SC 8 ± 3; IM 6± 2). A significant difference in dobutamine administration (µg/kg/minute; CRI 0.23 ± 0.13; SC 0.57 ± 0.21; IM 0.30 ± 0.15) was noted. Arterial blood gas parameters were within physiological ranges in all groups. There were significant differences in time to attain sternal recumbency (minutes; CRI 36 ± 17; SC 47 ± 10; IM 53 ± 12) and time (minutes; CRI 53 ± 10; SC 58 ± 10; IM 69 ± 17) and attempts (number; CRI 2 ± 1; SC 1 ± 1; IM 2 ± 2) to achieve standing position. However, no significant differences were detected on recovery scores. CRI group required a significant lower dobutamine dose and showed a faster recovery but a higher number of attempts to stand was observed. Dexmedetomidine administered either by IM or SC injection at indicated dosages, showed similar effect to CRI and proved to be useful in balanced isoflurane anaesthesia.

[1] Rabbogliatti V. et al. Use of dexmedetomidine repeated subcutaneous administration for balanced anaesthesia in horses. BMC Vet Res. 11, 18(1):269, 2022
[2] Young, S.S. et al. Factors Influencing the Outcome of Equine Anaesthesia: A Review of 1,314 Cases. Equine Vet. J. 25, 147–151, 1993.

EFFECTS OF *CISATRACURIUM* IN CATS UNDERGOING ORTHOPEDIC SURGERY ANAESTHETISED WITH SEVOFLURANE

Claudia Interlandi (1), Francesco Macrì (1), Vito Angileri (1), Simona Di Pietro (1), Filippo Spadola (1), Nicola Maria Iannelli (1), Giovanna Lucrezia Costa (1)

(1) Dipartimento di Scienze Veterinarie, Università degli studi di Messina

Corresponding Author: **Claudia Interlandi** - claudiadina.interlandi@unime.it

The clinical use and duration of action of nondepolarizing muscle relaxants in cats are still limited (1; 2). This study assessed the onset, duration recovery of the neuromuscular block (NMB) and sevofluorane requirement induced by a single bolus of cis-atracurium in 10 cats undergoing orthopedic surgery. Cats were 3.8±1.2 years old, living indoors/outdoors, weighed 3.9±0.6 kg and belonging to a ASA 2 class. Cats were premedicated with IM dexmedetomidine (3 mcgkg-1), induced with IM alfaxalone (3 mgkg-1) and maintained with sevoflurane in oxygen at variable dosage. After the induction, cis-atracurium (0.5 mgkg-1) were intravenously administered in all subjects All cats were mechanically ventilated with a median RR of 16 breaths per mins. Analgesia was performed using sciatic and femoral nerve block using levobupivacaine (2mgkg-1). The onset, duration and recovery of neuromuscular block and train of four (TOF) were evaluated. During anaesthesia, heart rate (HR), arterial pressure (SAP, MAP, DAP), end-tidal CO2 (EtCO2), oxygen saturation (SpO2), concentration in the inspiratory phases (Fiiso) and rectal temperature (T°) were continuously monitored. The measurements were collected at the following time points: premedication time (T0), induction time (Ti), after miorilaxant administration (Tm), and at 5 (T5), 10 (T10), 15 (T15), 20 (T20), 25 (T25), 30 (T30), 35 (T35) minutes. The results showed that HR, SAP, MAP, DAP, ETCO2 and T° decreased significantly at all time points (p= 0.005) compared with T0. It suggests a good patient adaptation to the ventilator and an adequate anesthesiological plan. The sevofluorane requirement was significantly reduced from Tm until T30 (Fiiso: 3% to 1%)(3). Mean time to suppression of 90% contraction was 2,6±0,8 minutes and mean time of maximum suppression of contraction was 3±0,7 minutes after IV administration of *cisatracurium*; the clinically effective duration of action was 23,3±4,4 minutes (time calculated from myorelaxant administration to 25% recovery). Mean time for the recovery (T1:T4) = 90% of TOF was at 27,3±8,6 minutes. No adverse effects were found. This study showed the effective and safe use of cisatracurium, at a dose of 0.5mgkg-1, as a part of a multimodal anesthetic protocol in feline patient undergoing to orthopedic surgery.

[1] Van Wijnsberghe AS, Ida KK, Dmitrovic P, Tutunaru A, Sandersen C. Neuromuscular blockade effects of cisatracurium in 11 cats undergoing ophthalmological surgery anaesthetised with isoflurane. J Feline Med Surg. 2022 Apr;24(4):402-406.
[2] Ida KK, Van-Wijnsberghe AS, Tutunaru A, Limpens V, Sauvage A, Serteyn D, Sandersen C. Onset and duration of cis-atracurium neuromuscular block during fentanyl and lidocaine infusions in isoflurane-anaesthetised dogs. Vet Rec. 2020 Sep 5;187(5): e33.
[3] Costa, G.L.; Leonardi, F.; Interlandi, C.; Spadola, F.; Fisichella, S.; Macrì, F.; Nastasi, B.; Macrì, D.; Ferrantelli, V.; Pietro, S.D. Levobupivacaine Combined with Cisatracurium in Peribulbar Anaesthesia in Cats Undergoing Corneal and Lens Surgery. Animals 2023, 13, 170.
[4] Interlandi, C.; Di Pietro, S.; Costa, G.L.; Spadola, F.; Iannelli, N.M.; Macrì, D.; Ferrantelli, V.; Macrì, F. (2022) Effects of Cisatracurium in Sevoflurane and Propofol Requirements in Dog-Undergoing-Mastectomy Surgery. Animals, 12, 3134.

76° CONVEGNO SISVET
BARI, 21-22-23 GIUGNO 2023

RETROSPECTIVE EVALUATION OF ALFAXALONE OR PROPOFOL USED FOR ANAESTHESIA INDUCTION IN CATS UNDERGOING EMERGENCY CAESAREAN SECTION

Lambertini Carlotta (1), Romagnoli Noemi (1), Ballotta Giulia (1), Iovine Ida Carlotta (1), Spaccini Francesca (1), Cunto Marco (1), Zambelli Daniele (1)

(1) Department of Veterinary Medical Sciences, University of Bologna, Italy

Corresponding Author: **Carlotta Lambertini** - carlotta.lambertini2@unibo.it

Propofol or alfaxalone administered for the induction of general anaesthesia provide similar anaesthetic effects in cats.1 The aim of the study was to evaluate the effects of propofol or alfaxalone on the female cats and on the newborns, when used for anaesthetic induction during emergency C-section.
The obstetric and anaesthetic records of female cats with dystocia and submitted to C-section between 2014 and 2022 were retrospectively evaluated. All the cases in which general anaesthesia was induced with propofol (P group) or alfaxalone (A group) and maintained with isoflurane were included. The following data of the queens were taken into consideration: heart rate (HR), respiratory rate (RR), fraction of expired CO2 (FECO2) and of isoflurane (FEISO %), haemoglobin oxygen saturation (SpO2) throughout pulse oximeter, and body temperature. Moreover, the following data of each kitten were recorded: survival at birth and 24 hours later and the values of HR, RR and mucous membranes appearance at birth. Data were compared between groups using a Student T-test or a Chi squared test, and among groups using a paired sample T-test. A $p<0.05$ was considered to be statistically significant.
Fifteen female cats submitted to emergency C-section were included in the study (P n=7; A n=8). The HR intraoperatively was lower compared with the baseline values at all time points in the P group and only at T20 and T30 in the A group. Hypotension (MAP < 60 mmHg) was recorded in both groups, but the average MAP was lower in the P group if compared with the A group throughout the procedure. The mean FEISO% was higher in the P group compared with the A group for most of the time points. The survival rate of the newborns was 96,8% in the A group and 69,2% in the P group, and the difference was statistically significant (p=0,013). The survival rate at 24 hours, as well as the HR, RR and mucous membranes appearance at birth did not differ significantly between the two groups.
In conclusion, contrarily on what previously reported in bitches2, the administration of alfaxalone for induction of general anaesthesia in cats undergoing emergency c-section, is associated with an higher newborns' survival rate when compared with propofol administration.

[1] Taboada FM, Murison PJ. Induction of anaesthesia with alfaxalone or propofol before isoflurane maintenance in cats. Vet Rec. 2010 17;167(3):85-9.
[2] Metcalfe S, Hulands-Nave A, Bell M, Kidd C, Pasloske K, O'Hagan B, Perkins N, Whittem T. Multicentre, randomised clinical trial evaluating the efficacy and safety of alfaxalone administered to bitches for induction of anaesthesia prior to caesarean section. Aust Vet J. 2014;92(9):333-338.

76° CONVEGNO SISVET
BARI, 21-22-23 GIUGNO 2023

SUTURELESS TECHNIQUE FOR SURGICAL CASTRATION IN ADULT BOARS: A FEASIBILITY STUDY

Stella Maria Teresa Romeo (1), Sarah Morrone (1), Toufic Akl (1), Alberto Maria Crovace (1), Antonio Scanu (1), Nicolò Columbano (1)

(1) Department of Veterinary Medicine, University of Sassari, Via Vienna 2, 07100 Sassari (SS), Italy

Corresponding Author: **Nicolò Columbano** - ncolumbano@uniss.it

The consumption of pork meat in the European Union (EU) is widespread, forming a major part of the traditional Italian culinary culture. However, androstenone, present in the meat of mature boars, makes it unfit for human consumption. While surgical castration of young piglets is a standard procedure, the castration of adult boars is more complex and involves the use of haemostatic measures, such as ligation of the spermatic cord, which increases the risk of complications. To address this issue, a novel sutureless castration technique was developed that does not require the use of foreign objects.
The study aimed to evaluate the feasibility of the sutureless swine orchiectomy procedure and determine the appropriate traction force needed to tie knots in the deferens duct of pigs of different ages and weights. Two groups were formed, with the first group undergoing orchiectomy by suture ligation, while the second group was castrated using the sutureless technique. The age of boars from group 1 ranged between 5 and 36 months, with an average of 18.22 months (sd.10.65), and the age of boars ranged between 4 and 60 months with an average of 17.97 months (sd. 12.80) in group 2. Deferens ducts of animals in the first group were collected, and their tensile strength was measured. Pearson's linear correlation was used to determine the relationship between the maximum traction force and weight and age groups. A correlation of 0.99 and 0.96 was shown between traction force and age and traction force and weight, respectively. The sutureless technique was performed on 20 animals, calibrating the pulling force needed according to their age and weight. The success rate was 100%, with no post-operative complications observed in either group, and animals resumed feeding 5 hours after complete recovery from anesthesia. In conclusion, the study showed that the sutureless orchiectomy technique is a feasible alternative to the traditional haemostatic measures used in the surgical castration of adult boars. The identification of the correlation between traction force and age and weight groups would allow for optimal nodal tightness without inducing vas deferens rupture, ensuring the success of the procedure. The validation of the sutureless technique would improve the welfare of animals and reduce the risk of complications associated with the use of foreign materials in the surgical site. The next step in the validation of sutureless orchiectomy in adult boars would be to perform short-, medium-, and long-term follow-ups of animals.

[1] Fubini, S.L.; Ducharme, N.G. Farm Animal Surgery; Saunders: Philadelphia, PA, USA, 2016
[2] Grimm, K.A.; Lamont, L.A.; Tranquilli, W.J.; Greene, S.A.; Robertson, S.A. Veterinary Anesthesia and Analgesia: The Fifth Edition of
[3] Lumb and Jones; Wiley-Blackwell: Hoboken, NJ, USA, 2015; pp. 941–943.
[4] Câmara, A.C.L.; Campebell, R.C.; Nogueira, K.; Junqueira, J.V.S.; Andrade, T.S.; Neto, A.R.T. Pre-Scrotal Castration Procedure in Boars. Res. Sq. (Posted) 2022.

76° CONVEGNO SISVET
BARI, 21-22-23 GIUGNO 2023

EVALUATION OF THE RADIOGRAPHIC BONE HEALING AFTER TIBIAL PLATEAU LEVELLING OSTEOTOMY WITH OR WITHOUT ELEVATION OF POPLITEAL MUSCLE FROM THE CAUDO-MEDIAL ASPECT OF THE TIBIA. A PRELIMINARY STUDY

Sassaroli S. (1), Salvaggio A. (1), Roggiolani F. (1), Di Bella C. (1), Pennasilico L. (1), Palumbo Piccionello A. (1)

(1) School of Biosciences and Veterinary Medicine, University of Camerino

Corresponding Author: **Sara Sassaroli** - sara.sassaroli@unicam.it

Objective. To evaluate the radiographic bone healing after performing the tibial plateau levelling osteotomy (TPLO) with or without elevation of popliteal muscle from the caudo-medial aspect of the tibia.
Study design: A blinded, prospective, randomized clinical study.
Methods. From 2020 to 2022, healthy dogs aged 1 to 5 years, affected by unilateral rupture of the cranial cruciate ligament (RCCL), were randomly underwent to TPLO with (C group) or without (M group) elevation of popliteal muscle and protection with gauze [1]. All dogs had the osteotomy stabilized with 3.5 Synthes plate and the post-operative care was identical among dogs of the two groups. A blinded radiologist evaluated orthogonal radiographs performed 45 days postoperatively with both a modified radiographic union scale for tibial fractures (mRUST) [2] and a 10-point bone healing scale (10-PBH) [3]. The surgical time, the postoperative tibial plateau angle (TPA) and the 45 days after surgery TPA were measured and the rockback (RB) [4] was calculated. All data were normally distributed for the Kolmogorov-Smirnov test and the two groups were compared using the One-Way ANOVA test. A p value < 0.05 was considered statistically significant.
Results. Thirty dogs (30 stifles) were included in the study, 15 stifles in C group and 15 stifles in M group. The median healing scores evaluated with both mRUST ($p = 0,010$) and 10-PBH scale ($p = 0,029$) were significantly higher at 45 days postoperatively for the M group compared to the C group. Age, body weight, body condition score, RB and surgical times were not statistically different between two study groups ($p < 0,05$). In all patients the cranial tibial artery was not injured.
Conclusion. Dogs underwent TPLO without soft tissue dissection healed rapidly. A minimally invasive access protects the biological environment that facilitates rapid bone healing by minimizing soft tissue damage.

[1] Pozzi A, Samii V, Horodyski MB. Evaluation of vascular trauma after tibial plateau levelling osteotomy with or without gauze protection. A cadaveric angiographic study. Vet Comp Orthop Traumatol 2011; 24: 266–271.
[2] Walker KJ, Litterine-Kaufman J, Barnes RF, French JM, Tsai SL. Evaluation of a modified radiographic union scale for tibial fractures scoring system in staged bilateral tibial plateau leveling osteotomy procedures and comparison of first and second side radiographic bone healing. Veterinary Surgery 2022; 51: 1231–1239.
[3] Kieves NR, MacKay CS, Adducci K, Rao S, Goh C, Palmer RH, Duerr FM. High energy focused shock wave therapy accelerates bone healing. A blinded, prospective, randomized canine clinical trial. Vet Comp Orthop Traumatol 2015; 28: 425–432.
[4] Siqueira De Souza E, Watanabe Minto B, Sales Luis JP, Nobile M, Testoni Lins B, Farias de Lucena DV, Portilho Gouveia MC, Gosuen Goncalves Dias LG. Rock back phenomenon in 32 dogs that underwent tibial plateau levelling osteotomy. Veterinarni Medicina 2021; 66 (02): 58–65.

76° CONVEGNO SISVET
BARI, 21-22-23 GIUGNO 2023

THE HEART TO SINGLE VERTEBRA RATIO (HSVR) IN CATS: PRELIMINARY RESULTS

Dario Costanza (1), Adelaide Greco (1), Erica Castiello (1), Diego Piantedosi (2), Pierpaolo Coluccia (1), Leonardo Meomartino (1)

(1) Centro Interdipartimentale di Radiologia Veterinaria, Università degli Studi di Napoli "Federico II"
(2) Dipartimento di Medicina Veterinaria e Produzioni Animali, Università degli Studi di Napoli "Federico II"

Corresponding Author: **Dario Costanza** - dario.costanza@unina.it

A key limitation of the Vertebral Heart Scale (VHS) [1] is represented by the presence of vertebral abnormalities such as spondylosis deformans, reduced intervertebral disc spaces, hemivertebrae, butterfly vertebrae or wedge vertebrae since these abnormalities could artifactually increase the VHS. To overcome this limitation, the hearth-to-single vertebra ratio (HSVR) was proposed in dogs [2]. The study found the HSVR to be a simple, quick, and reliable method with excellent agreement with the VHS and substantial intra- and inter-observer agreement. Another major limitation in cats is constituted by the lordosis of the thoracic spine that can artefactually increase the VHS [3]. The primary aim of this study was to investigate if the HSVR could also be reliable in cats. The electronic records of canine patients referred to the Interdepartmental Centre of Veterinary Radiology of the University of Napoli Federico II, between September 2017 and March 2023, were retrieved from the PACS, and the images were reviewed. All radiographic examinations included at least the right-lateral view, which was used to assess the heart dimensions. Exclusion criteria were as follows: abnormalities of the thoracic spine; inability to correctly visualize the cardiac silhouette; positioning or technical errors; skeletal immaturity. On each radiograph, the length of each single vertebra from T4 to T8, including the caudal intervertebral space, and the VHS were measured. The sum of the two cardiac axes was subsequently divided by the length of every single vertebra of the T4-T8 tract to obtain the HSVR for each vertebra. The two methods were compared using the concordance correlation coefficient (CCC), and the Bland-Altman plot. The error between the VHS and HSVR values was estimated using the mean absolute error (MAE). Fifty-one cats (4 intact females, 26 spayed females, 3 intact males, and 18 castrated males) were included in the final sample. The median age was 7.5 years (range 1−18 years), and the median body weight was 5 kg (range 2.5−7.7 kg). The breeds represented were domestic short-haired (n = 44), domestic long-haired (n = 5), Siamese (n = 1) and Maine Coon (n = 1). The data analysis showed a strong correlation between VHS and HSVR, particularly when considering T6 (CCC = 0.96; MAE = 0.21) and T7 (CCC = 0.95; MAE = 0.18). These preliminary results show that the HSVR could be a valuable alternative to the VHS in cats as in dogs.

[1] Buchanan, J. W., & Bücheler, J. (1995). Vertebral scale system to measure canine heart size in radiographs. Journal of the American Veterinary Medical Association, 206(2), 194–199.
[2] Costanza, D., Greco, A., Piantedosi, D., Bruzzese, D., Pasolini, M. P., Coluccia, P., Castiello, E., Baptista, C. S., & Meomartino, L. (2022). The heart to single vertebra ratio: A new objective method for radiographic assessment of cardiac silhouette size in dogs. Veterinary Radiology & Ultrasound. https://doi.org/10.1111/vru.13201
[3] Litster, A. L., & Buchanan, J. W. (2000). Vertebral scale system to measure heart size in radiographs of cats, Journal of the American Veterinary Medical Association, 216(2), 210-214. Retrieved Mar 19, 2023, https://doi.org/10.2460/javma.2000.216.210

76° CONVEGNO SISVET
BARI, 21-22-23 GIUGNO 2023

EVALUATION OF THE ANALGESIC EFFICACY OF INTRAOPERATIVE OVARIAN SPLASH BLOCK COMPARED TO PREOPERATIVE US-GUIDED INFILTRATION OF LIDOCAINE IN CATS UNDERGOING OVARIECTOMY

Galosi Margherita (1), Troisi Alessandro (1), Serino Federica (1), Angorini Alessio (1), Palumbo Piccionello Angela (1), Di Bella Caterina (1)

(1) University of Camerino, School of Biosciences and Veterinary Medicine

Correponding Author: **Margherita Galosi** - margherita.galosi@unicam.it

The aim of this study was to compare the analgesic effect of topical irrigation of the ovarian peduncle (splash block), compared to the pre-surgical ultrasound-guided periovarian infiltration with lidocaine, in cats undergoing elective ovariectomy. In this clinical, prospective, randomized study 20 female cats were enrolled. All patients were premedicated IM and general anesthesia was maintained by isoflurane in pure oxygen. The animals were randomly divided into two groups. The UB group (10 queens) received a periovarian ultrasound-guided infiltration of 0.1 ml/kg of lidocaine 2%, performed by a single operator who evaluated the level of accuracy in the execution of the infiltration through a specific score, from 0 (poor) to 5 (excellent). The SB group (10 queens) received an intraoperative splash block of lidocaine 2% (0.1 ml/kg), after correct visualization of both ovaries by the surgeon. The main hemodynamic and respiratory parameters were recorded for all patients 10 minutes before the start of surgery (BASELINE), during the skin incision (SKIN), during the traction of the first (TRACTION 1) and the second ovarian ligament (TRACTION 2) and during the application of the last skin suture (SUTURE). In the SB group, in addition to the study times listed above, the parameters were recorded also at the time of the intraoperative splash block of the two ovaries (SPLASH 1 and 2, respectively). The presence of nociceptive stimuli was considered upon the occurrence of an increase in heart rate (HR) and mean arterial pressure (MAP) of 20% compared to baseline. In this case, an intravenous opioid (fentanyl 1 mcg/kg) was administered promptly. Values of $p < 0,05$ were considered statistically significant. The results obtained showed that there are no statistically significant differences between groups at the different phases of the study. Our study agrees with Zilbestein et al., which have demonstrated the effectiveness of the ovarian splash block for feline ovariectomy in 2008. To the knowledge of the authors, there are no studies concerning the periovarian ultrasound-guided infiltration of lidocaine in dogs and cats.
However, the authors also considered that the correct visualization of the ovaries during surgery, in order to perform the splash block, requires a wider surgical access. This could be a factor to consider in choosing the analgesic technique.

[1] Zilbestein LF, Moens YP, Leterrier E. “The effect of local anesthesia on anaestetic requirements for feline ovariectomy”. Vet J. 2008 Nov;178(2):214-8.

76° CONVEGNO SISVET
BARI, 21-22-23 GIUGNO 2023

COMPARISON OF INDUCTION, MAINTENANCE AND RECOVERY OF DESFLURANE VERSUS SEVOFLURANE IN BALL PYTHONS (*PYTHON REGIUS*) UNDER DIAGNOSTIC PROCEDURES

S.Loi (1), F. Cubeddu (2), A. M. Crovace (2), S. M. T. Romeo (2), S. Morrone (2), G. M. Careddu (2), N. Columbano (2)

(1) Dipartimento di Medicina di Precisione e Rigenerativa e Area Jonica - Università degli studi di Bari Aldo Moro
(2) Dipartimento di Medicina Veterinaria - Università degli Studi di Sassari

Corresponding Author: **Nicolò Columbano** - ncolumbano@uniss.it

The aim of this study was to compare Sevoflurane (SEVO) and Desflurane (DES) in ball pythons. 10 healthy ball pythons, 5 male and 5 non-pregnant female snakes were anesthetized twice, separated by a washout period of 21 days, for diagnostic procedures. The animals were anesthetized using SEVO or DES, the loss and recovery of snake reflexes such as righting reflex, tongue flicking, head flinching reflex, pressure stimuli response and jaw reflex were evaluated by giving a score. For induction, Vaporizers were set at 18% for DES and 8% for SEVO delivered by face mask until it was possible to intubate. With animals connected to a a rotatory circuit, fresh gas flow was set at 1l/min (50% oxygen) with respiratory rates (RR) set at 3 acts per minute. During the procedures heart rate (HR), RR, end-tidal CO2, end-tidal inhalant anesthetic, and tidal volume were monitored and recorded at established times. The HR, RR, and reflexes were recorded at standard times before induction and after a 5 minute of equilibration period. Et DES and Et SEVO were changed every 5 minutes to standard predefined decreasing values, (DES Group 10%, 8%, 6.5%, 5%, 3.3%, SEVO Group 3%, 2.5%, 2%, 1.5%, 1%). At The end of procedures anesthetic administration was stopped and the times of recovery from anesthesia were recorded. The induction and recovery times were respectively 5,20 ± 1,55 and 25,20 ± 2,39 minutes for SEVO and 2,90 ± 0,74 and 17,60 ± 2,91 for DES ($p < 0,01$). The time of intubation (T4) and the time when the pythons resumed spontaneous ventilation (T10), the values of MAC multiples were significantly higher in SEVO compared to the DES group ($p<0,01$) 1.68 ± 0.07 and 0.30 ± 0.06 SEVO and 1.28 ± 0.05 and 0.20 ± 0.02 for DES respectively. The results showed that both SEVO and DES provided fast induction of anesthesia and a fast recovery at the end of the procedure. The vital signs were stable for the duration of the procedure, and no side effects of the drugs were reported. In addiction, measured reflex show more relaxation in SEVO group ($p>0,05$). In conclusion, inhalant anesthesia with SEVO or DES is a suitable alternative to injectable drugs for short anesthetic and minimally invasive procedures in healthy animals without any sign of respiratory disease. The results of this study suggest that SEVO and DES can be used safely and effectively in ball pythons during diagnostic procedures.

[1] Kane L. P., Chinnadurai S. K., Vivirito K., Strahl-Heldreth D., Allender M. C., 2020. " Comparison of isoflurane, sevoflurane, and desflurane as inhalant anesthetics in prairie rattlesnake (Crotalus viridis)": JAVA, vol 257, N° 9
[2] Maas A., Brunson D.2002, " Comparison of anesthetic potency and cardiopulmonary effects of isoflurane and sevoflurane in colubrid snakes". Proceedings. Annu Conf Am Assc Zoo Vet ;306-308
[3] Bertelsen MF, Buchanan R, Jensen HM, et al. 2015 "Assessing the influence of mechanical ventilation on blood gases and blood pressure in rattlesnakes". Vet Anaesth Analg ;42:386–393.
[4] Magnone W. et al. 2008. "Minimum Alveolar Concentration (MAC) of Sevoflurane in Ball Pythons (Python regius) and Burmese Pythons (Python molurus)" American Association of Zoo Veterinarians Conference 2008

76° CONVEGNO SISVET
BARI, 21-22-23 GIUGNO 2023

LED PHOTOTHERAPY IN THE MANAGEMENT OF SURGICAL WOUNDS HEALING BY SECONDARY INTENTION IN SEVEN HORSES

Lorenza Bandera (1), Paola Straticò (1), Laura Di Nunzio (1), Gianluca Celani (1), Giulia Guerri (1), Vincenzo Varasano (1), Lucio Petrizzi (1)

(1) Università degli Studi di Teramo, Dipartimento di Medicina Veterinaria

Corresponding Author: **Laura Di Nunzio** - dnz.laura@gmail.com

Wound healing can be divided into immediate (zero to 1 hour), early (1 to 24 hours), intermediate (1 to 7 days) and late (greater that 7 days) stages. Many physical and physiologic events occur simultaneously and sequentially during these stages to produce the final wound scar.1 Many factors influence the healing process, including defect size and shape, anatomic location and skin tension forces, systemic condition of the patient, blood supply, nutritional factors, wound infection, mobility, wound oxygen gradient, wound moisture and bandaging.1,4 Several peculiarities are recognized and characterize equine wound healing. In the horse fibroblastic reactivity to skin damage occurs remarkably early and is more pronounced than in other species. [1] This can lead to the formation of exuberant granulation tissue. For these reasons, the management of wound healing in horses can be challenging.

The aim of this study is to determine the effect of an innovative therapy based on photobiomodulation (PMB) for the management of surgical wounds healing by secondary intention in seven horses of different age, breed and sex: two affected by laparotomy surgical site infection, three treated for sarcoids with surgical excisions, one presenting chronic bacterial infection of the remnant of the right spermatic cord (scirrhous cord) and one with a vulvar squamous cell carcinoma. All these cases were managed with LED phototherapy, to improve wound healing. This technique involves the topical application of a photoconverter gel containing specific chromophores which are illuminated by a LED lamp.2,3 The horses were treated every other day for 5-15 minutes in relation to the size of the wound. The procedure was repeated variable times (4-18 applications), based on the quality and speed of wound healing.

Wounds were visually evaluated with a semiquantitative scoring system by taking into consideration wound appearance and clinical consequences of infections.4 No adverse reaction to the treatment was detected throughout the study and it was always reached a satisfying level of healing, even in the most complicated cases. The medium duration of the treatment was 16 days, with a mean wound score at T0 of 16,43 points and a mean wound score at the end of treatment of 4 points.

In conclusion, the authors could affirm that PMB is a safe and effective treatment to promote the healing of second-intention surgical wounds, although further standardization of the technique must be defined.

[1] Bertone A.L., Principles of wound healing. The Veterinary clinics of North America. Equine Practice. 1989. 5 (3) 449-63
[2] Marchegiani A., Spaterna A., Piccionello A.P., Meligrana M., Fruganti A., Tambella A.M., Fluorescence biomodulation in the management of acute traumatic wounds in two aged dogs. Veterinarni Medicina. 2020. 65 (05) 215-220
[3] Romanelli M., Piaggesi A., Scapagnini G., Dini V., Janowska A., Iacopi E., Scarpa C., Fauverghe S., Bassetto F., Evaluation of fluorescence biomodulation in the real-life management of chronic wounds: the EUREKA trial. Journal of wound care. 2018. 27 (11)
[4] Salvaggio A., Magi G.E., Rossi G., Tambella A.M., Vullo C., Marchegiani A., Botto R., Piccionello A.P., Effect of the topical Klox fluorescence biomodulation system on the healing of canine surgical wounds. Veterinary Surgery. 2018. 20 (20) 1-9

76° CONVEGNO SISVET
BARI, 21-22-23 GIUGNO 2023

HISTOMORPHOLOGICAL EVALUATION OF THERMAL INJURY FOLLOWING PALATOPLASTY PERFORMED WITH CO2 LASER OR LIGASURE DEVICE IN DOGS WITH BRACHYCEPHALIC AIRWAY OBSTRUCTIVE SYNDROME

Amanda Bianchi (1), Roberto Bussadori (2), Laura Bongiovanni (1), Francesco Collivignarelli (1), Melissa Teofani (1), Andrea Paolini (1), Roberto Tamburro (1)

(1) University of Teramo, Department of Veterinary Medicine
(2) Clinica Veterinaria Gran Sasso, Milan (Italy)

Corresponding Author: **Amanda Bianchi** - abianchi@unite.it

The elongated soft palate is an abnormality that characterises most brachycephalic dogs and contributes to the brachicephalic airway obstructive syndrome (BAOS). Palatoplasty is routinely performed after endoscopic staging of BAOS; several surgical techniques exist (1).The use of surgical instruments such as monopolar electrocoagulation, CO2 or diode laser, bipolar vessel sealing device and harmonic shears has become routine to reduce operating time, the intraoperative risk of bleeding and postoperative oedema (2). This prospective study aimed to compare the histomorphological effect of a CO2 laser and LigaSure device in palates of dogs undergoing palatoplasty. Twenty owned brachicepalic dogs were included, 10 palatoplasty were performed using CO2 laser and 10 using LigaSure. The dogs were positioned in sternal recumbency. A transoral apporach was performed: the enlonged soft-palate was grasped with Allis forceps and brought rostrally, the staphylectomy was performed using the tonsillar crypts as a point of repere. Surgical specimens were routinely fixed in 10% formalin. Two sections perpendicular to the surgical margins were trimmed from each sample, paraffin-embedded and stained with hematoxylin and eosin (H&E). Necrosis induced by the two types of surgical devices were graded (1-4, from minimal to severe) and the depth measured in μm on captured images (using an image analysis program - ImageJ). Mean values and standard deviations (SD) were calculated based on six measurements for each sample (3). The necrosis was graded 3.7±0.48 in group LigaSure and 2.8±1 in group Laser. The mean depth of termal necrosis was 874.94±184.92 μm in the LigaSure group and 451,76±137,86 μm in the Laser group. The comparison between the two groups showed significant lower grade and extension of thermal necrosis in the palate samples obtained with CO2 laser ($p<0.05$).
To the authors' knowledge, there are no other studies comparing the histopathological changes of samples with the two methods. Further studies comparing the clinical and histological effects of multiple devices are required to identify the most advantageous method to perform staphylectomy in brachycephalic dog.

[1] Conte A, Berlato D, Rasotto R, Bass J, Bussadori R, Nicoli S, Murgia D. Comparison of harmonic shears, diode laser, and scissor cutting and suturing for caudal palatoplasty in dogs with brachycephalic obstructive airway syndrome. Vet J. 2022 Feb; 280:105802. doi: 10.1016/j.tvjl.2022.105802. Epub 2022 Feb 10. PMID: 35151883.
[2] Dunié-Mérigot A, Bouvy B, Poncet C. Comparative use of CO_2 laser, diode laser and monopolar electrocautery for resection of the soft palate in dogs with brachycephalic airway obstructive syndrome. Vet Rec. 2010 Oct 30;167(18):700-4. doi: 10.1136/vr.c5107. PMID: 21257486.
[3] Tamburro R, Brunetti B, Muscatello LV, Mantovani C, De Lorenzi D. Short-term surgical outcomes and histomorphological evaluation of thermal injury following palatoplasty performed with diode laser or air plasma device in dogs with brachycephalic airway obstructive syndrome. Vet J. 2019 Nov; 253:105391. doi: 10.1016/j.tvjl.2019.105391. Epub 2019 Sep 25. PMID: 31685136.

76° CONVEGNO SISVET
BARI, 21-22-23 GIUGNO 2023

B-MODE AND STRAIN ELASTOGRAPHY CHARACTERISTICS OF BENIGN VS MALIGNANT MESENTERIC LYMPH NODES IN CATS: AN UPDATE

Francesca Del Signore (1), Elettra Febo (1), Nicola Bernabò (1), Andrea De Bonis (1), Martina Rosto (1), Andrea Paolini (1), Amanda Bianchi (1), Roberto Tamburro (1), Massimo Vignoli (1)

(1) Department of Veterinary Medicine, University of Teramo, Località Piano D'Accio, Teramo

Corresponding Author: **Francesca Del Signore** - fdelsignore@unite.it

Introduction. Strain elastography (SE) is an ultrasound-based technique able to non-invasively assess tissue elasticity, that varies based on underlying disease, with malignant tissues becoming stiffer (1).
The purpose of the study was to evaluate the SE diagnostic performance to differentiate feline mesenteric benign and malignant lymph nodes (LNs), providing scoring rubric based on several B-mode findings.
Materials and Methods. Feline abnormal mesenteric LNs detected during abdominal ultrasound were evaluated using B-mode, colour Doppler and SE with a LOGIQ S8 XD clear GE healthcare sonographic machine and a 11L probe. B-mode findings recorded with respective scores were Short-to-long axis (S/L) ratio (≤ 0.7 score 1 > 0.7, 2), borders (regular, 1; irregular, 2), echogenicity (homogenous, 1; heterogeneous, 2), hilum (present, 1; absent, 2), and colour Doppler vascular flow distribution (absent or hilar, 1; peripheral or mixed, 2) (2). SE was performed through a qualitative evaluation providing an elastographic pattern (EP) score from 1 to 5 based on colour distribution (red=soft, blue= hard) and through a semiquantitative analysis (strain ratio-SR) between the LN elasticity index of and the near field abdominal wall considered as reference tissue (3).
Cytologic and/or histologic diagnosis were used as reference standard to divide LNs in 3 groups, benign, malignant and sclerosing lymphadenitis, to avoid bias in elasticity analysis from the fibrosis on non-neoplastic LNs. B-mode score, EP and SR were used to provide a multivariate statistical analysis through a Principal Component Analysis (PCA) to explore the pattern of the data and assess their usefulness to distinguish the 3 groups of LNs.
Results. Eighty-eight LNs were included, 46 (52.3%) benign and 42 (47.7%) malignant; in the benign group, 40 LNs had a diagnosis of reactive hyperplasia (group 1) and 6 eosinophilic sclerosing lymphadenitis (group 2), in the malignant one 42 had a diagnosis of lymphoma (group 3). PCA approach allowed to evidence that combining B-mode based score, the 3 groups of LNs can be accurately distinguished, thus providing the evidence that the SE could be successfully applied to define LNs nature.
Conclusions. Our results evidence that a multivariate sonographic approach combining B-mode and SE can accurately distinguish benign from malignant LNs, thus potentially limiting the need for more invasive tests to achieve the definitive diagnosis.

[1] Lo WC, Cheng PW, Wang CT, Liao LJ. Real-time ultrasound elastography: an assessment of enlarged cervical lymph nodes. Eur Radiol. settembre 2013;23(9):2351–7.
[2] Kanagaraju V, Rakshith AVB, Devanand B, Rajakumar R. Utility of Ultrasound Elastography to Dif ferentiate Benign from Malignant Cervical Lymph Nodes. J Med Ultrasound. 10 dicembre 2019;28(2):92–8.
[3] Alam F, Naito K, Horiguchi J, Fukuda H, Tachikake T, Ito K. Accuracy of Sonographic Elastography in the Differential Diagnosis of Enlarged Cervical Lymph Nodes: Comparison with Conventional B-Mode Sonography. Am J Roentgenol. agosto 2008;191(2):604–10.

76° CONVEGNO SISVET
BARI, 21-22-23 GIUGNO 2023

USE OF QUADRATUS LOMBORUM BLOCK IN FEMALE CATS UNDERGOING OVARIECTOMY: A RANDOMIZED CONTROLLED TRIAL

Andrea Paolini (1), Francesco Santoro (2), Amanda Bianchi (1), Roberto Tamburro (1), Salvatore Parrillo (1), Alessandro Di Giosia (1), Augusto Carluccio (1), Francesco Collivignarelli (1), Claudia Ristori (1), Francesca Del Signore (1), Roberta Bucci (1), Giulia Costanzini (1), Massimo Vignoli (1)

(1) Faculty of Veterinary Medicine, University of Teramo, 64100 Teramo, Italy
(2) Department of Clinical Sciences and Services, The Royal Veterinary College, Hatfield AL9 7TA, UK

Corresponding Author: **Andrea Paolini** - apaolini@unite.it

Introduction. In veterinary medicine, loco-regional anesthesia is associated with lower peri-operative opioid consumption and less related side effects [1]. The quadratus lumborum (QL) block appears to provide adequate somatic and visceral analgesia of the caudal abdomen [2]. The QL block involves the release of local anesthetic in the fascial plane between the QL and psoas muscle [3]. The aim of the present study is to evaluate the peri- and post-operative efficacy of QL block in female cats undergoing laparotomic ovariectomy.
Materials and methods. Thirty-seven cats are involved in a randomized and blinded clinical trial. Cats were divided into two groups: control group (CG, n°19) and experimental group (QLBG, n°18). All cats received dexmedetomidine 5 mcg kg−1, alfaxalone 1 mg kg−1 and methadone 0.2 mg kg−1 intramuscularly. Ultrasound-guided QLB, using in-plane technique with needle inserted in a ventrodorsal direction, were performed bilaterally 0.4 ml kg−1 (1.5 mg kg−1 par side) of ropivacaine 0.4%. Time from premedication to skin incision, surgical procedures time, pre-incisional hear rate (HR), respiratory rate (fR), mean arterial pressure (MAP), temperature, total intraoperative fentanyl and total post-operative methadone consumption were noted. Rescue analgesia in the intra-operative period was set at the change of at least 30% of two vital parameters between HR, fR and MAP from baseline. Feline Grimace Scale [4] was applied to pain evaluation from premedication (T00) to T0, T2, T4, T6 and T8 hours post extubation. Methadone 0.1 mg kg−1 was administered as rescue analgesia for scores >4. Data distribution was assessed using the Shapiro-Wilk test. Normally distribuited data were analyzed with student's T test and presented as mean and SD; Mann-Whitney U-test was used to compare no normally distributed data (presented as median and range).
Results. Time from premedication to skin incision, surgical procedure and pre-incisional vital signs no statistically differences were found between groups. Amount of intraoperative rescce anal gesia was analyzed with Chi square test. Significance was set at $p \leq 0.05$. Demand of rescue analgesia was significantly lower in QLB than CG, $p < 0.001$. Assessment of post-operative pain does not present significative differences (p=0.425).
Conclusions. The QL block is an effective technique to manage intraoperative nociceptive stimuli and for post-operative analgesia in female cats undergoing laparotomic ovariectomy.

[1] Lascelles BDX, Kirkby Shaw K. An extended-release local anaesthetic: potential for future;
[2] Argus APV, Freitag FAV, Bassetto JE, Vilani RG. Quadratus lumbar block for intraoperative and postoperative analgesia in a cat. Vet Anaesth Analg. 2020 May;47(3):415-417. doi: 10.1016/j.vaa.2020.01.006;
[3] Dos-Santos JD, Ginja M, Alves-Pimenta S, Otero PE, Ribeiro L, Colaço B. A description of an ultrasound-guided technique for a quadratus lumborum block in the cat: a cadaver study. Vet Anaesth Analg. 2021 Sep;48(5):804-808. doi: 10.1016/j.vaa.2021.03.017;
[4] Evangelista MC, Watanabe R, Leung VSY, Monteiro BP, O'Toole E, Pang DSJ, Steagall PV. Facial expressions of pain in cats: the development and validation of a Feline Grimace Scale. Sci Rep. 2019 Dec 13;9(1):19128. doi: 10.1038/s41598-019-55693-8.

76° CONVEGNO SISVET
BARI, 21-22-23 GIUGNO 2023

EFFECTS OF PREMEDICATION WITH BUTORPHANOL OR DEXMEDETOMIDINE BUTORPHANOL ON EASE OF ENDOSCOPIC DUODENAL INTUBATION IN DOGS

Andrea Paolini (1), Amanda Bianchi (1), Paolo Crisi (1), Maria Veronica Giordano (1), Massimo Vignoli (1), Francesco Collivignarelli (1), Claudia Ristori (1), Sara Canal (1), Francesca Del Signore (1), Andrea De Bonis (1), Martina Rosto (1), Roberto Tamburro (1)

(1) Faculty of Veterinary Medicine, University of Teramo, 64100 Teramo, Italy

Corresponding Author: **Andrea Paolini** - apaolini@unite.it

Introduction. In veterinary medicine general anaethesia is required in order to perform gastroduodenoscopy [1]. However, anaesthetic drugs, such as opioid mu agonists can affect the gastrointestinal tract [2] Gastrointestinal complications are reported such as vomiting, reduction of gastrointestinal motility and the reduction patency of pyloric sphincter [3]. The use of alpha-2 agonist such as dexmedetomidine has never been investigated to ease duodenal intubation. The aim of this study is demonstrated that a low dose of dexmedetomidine does not affect intestinal motility and pyloric sphincter opening.

Material and Methods. A group of 18 dogs were enrolled and randomly assigned to one of the two premedication groups. An intravenous catheter was placed on the cephalic vein. Group B received 0.25 mg kg-1 butorphanol (Alvegesic; Dechra, UK) and group BD received 0.25 mg kg-1 butorphanol (Alvegesic; Dechra, UK) and 1 mcg kg-1 dexmedetomidine (Sedadex; Dechra, UK) intravenously.General anesthesia was induced with propofol (Propovet; Zoetis, Rome, Italy) and maintained with isoflurane (Isoflo; Zoetis, Rome, Italy) delivered in 100% oxygen (FE Iso = 1,2% at the beginning of the procedure for all dogs). Total amount of propofol in mg/kg was recorded.
At the 20th minute after administration of the premedication, the pyloric sphincter was examined for spontaneous opening (yes or no). The ease intubation of was also graded by the blinded observer using a 4-point scale. (Matz ME et al, 1991) as 1) immediate entry with minimal manoeuvring required; 2) rapid entry with moderate maneuvering; 3) difficult entry with multiple attempts required; and 4) no entry after 2 minutes. Normally distributed data were analyzed with the student's T test and presented as mean and SD; Mann-Whitney U-test was used to compare no normally distributed data (presented as median and range). Statistical significance was set as $p < 0.05$.

Results. Age, body weight and vital signs before procedures were not statistically significative between groups. Amount propofol consumption between BD and B was significative (3.7± 1.49 vs 6.5± 0.8 respectively, p=0.027). Intubation score was not significative B= 2 [1-3] and BD= 2 [1-4]; p=0.68. The patency was not significative (p=1.00).

Conclusion. Total amount of propofol is significantly lower in the experimental group. Low doses of dexmedetomidine do not appear to interfere with pyloric sphincter patency and ease of intubation of the duodenum.

[1] Zoran DL. Gastroduodenoscopy in the dog and cat. Vet Clin North Am Small Anim Pract. 2001 Jul;31(4):631-56, vii-viii. doi: 10.1016/s0195-5616(01)50063-7. PMID: 11487948.;
[2] McFadzean WJ, Hall EJ, van Oostrom H. Effect of premedication with butorphanol or methadone on ease of endoscopic duodenal intubation in dogs. Vet Anaesth Analg. 2017 Nov;44(6):1296-1302. doi: 10.1016/j.vaa.2017.05.004. Epub 2017 Jun 7. PMID: 29174961.
[3] Salla KM, Lepajoe J, Candido MV, Spillmann T, Casoni D. Comparison of the effects of methadone and butorphanol combined with acepromazine for canine gastroduodenoscopy. Vet Anaesth Analg. 2020 Nov;47(6):748-756. doi: 10.1016/j.vaa.2020.03.008. Epub 2020 Jul 27. PMID: 33041210.

HEMODYNAMIC, RESPIRATORY AND LUNG AERATION EFFECTS OF DEXMEDETOMIDINE INFUSION IN AN EXPERIMENTAL MODEL OF SEPTIC SHOCK

Scardia A. (1), Stabile M. (1), Lacitignola L. (1), Stasi A. (1), Franzin R. (1), Crovace A.M. (1), Staffieri F. (1)

(1) Università degli studi di Bari "Aldo Moro", Dipartimento di Medicina di Precisione e Rigenerativa e Area Jonica - (DiMePRe-J), sezione di Cliniche veterinarie e produzioni animali

Corresponding Author: **Annalaura Scardia** - annalaura.scardia@uniba.it

Dexmedetomidine in the septic patient requiring mechanical ventilation reduced 28-day mortality [1], inflammation [2-3], duration of mechanical ventilation [1,4] and length of hospital stay[4]. The aim of the study was to examine the effect of a low dose-infusion of dexmedetomidine on the cardiovascular stability, the respiratory mechanics and the lung aeration in a swine model of septic shock (approval number n° 1234/2020-PR). Fourteen pigs, body weight 50 - 70 kg, were premedicated with an intramuscular (IM) injection of tiletamine & zolazepam, dexmedetomidine, atropine and methadone. General anesthesia was induced using 5mg/kg propofol IV and maintained with a constant rate infusion (CRI) of propofol (0,1-0,3 mg/kg/min) IV. The animals were randomly assigned into two groups: DEX (n=7), septic pigs that received a CRI of dexmedetomidine (1 mcg/kg/h) IV, and NODEX (n=7), septic pigs that received a CRI of saline solution at the same rate. The induction of sepsis was performed in both groups using 300µg/kg of lipopolysaccharide (LPS) membrane of Escherichia coli IV 60 minutes after dexmedetomidine infusion start. The heart rate (HR, beats/minute), the mean arterial pressure (MAP, mmHg), the stroke volume index (SVI, ml/m2) and the cardiac index (CI, L/min/m2) were measured throughout a dedicated arterial line at three hours after LPS infusion. At the same time mechanical ventilation was instituted to measure the static compliance (Cs, mL/cmH20) and to examine the lung aeration through TC scan. Arterial blood gas analysis was performed to calculate the PaO2/FiO2 ratio (P/F) and the Shunt (%). The data were analyzed with the Kruskal-Wallis test ($p < 0.05$). In the DEX group the SVI and the MAP were significantly higher (7.0 □ 1.5 ml/m2; 81.4 □ 17.9 mmHg) as compared to the NODEX (4.9 □ 1.1 ml/m2; 62.0 □ 16.8 mmHg) group. The percentage of the poorly aerated lung area was lower in the DEX (23.6 □ 7.8 %) as compared to the NODEX (37.3 □ 8.7 %) group. The respiratory system compliance was higher in the DEX (47.5 □ 9.5 ml/cmH2O) as compared to the NODEX (34.8 □ 9.8 ml/cmH2O) group. The results of this study proved that the dexmedetomidine infusion in an animal model of septic shock may improve the hemodynamic, the respiratory system mechanics and the lung aeration.

[1] S. Aso, H. Matsui, K. Fushimi, e H. Yasunaga, «Dexmedetomidine and Mortality from Sepsis Requiring Mechanical Ventilation: A Japanese Nationwide Retrospective Cohort Study», J Intensive Care Med, vol. 36, fasc. 9, pp. 1036–1043, set. 2021, doi: 10.1177/0885066620942154.

[2] T. Zhang, Q. Mei, S. Dai, Y. Liu, e H. Zhu, «Use of dexmedetomidine in patients with sepsis: a systematic review and meta-analysis of randomized-controlled trials», Ann. Intensive Care, vol. 12, fasc. 1, p. 81, ago. 2022, doi: 10.1186/s13613-022-01052-2.

[3] Y. Ohta, K. Miyamoto, Y. Kawazoe, H. Yamamura, e T. Morimoto, «Effect of dexmedetomidine on inflammation in patients with sepsis requiring mechanical ventilation: a sub-analysis of a multicenter randomized clinical trial», Crit Care, vol. 24, fasc. 1, p. 493, dic. 2020, doi: 10.1186/s13054-020-03207-8.

[4] Z. Liu, Y. Zeng, B. Yang, e P. Liao, «Efficacy and safety of dexmedetomidine in sepsis patients requiring mechanical ventilation: a systematic review and meta-analysis», Clinical Pharmacy Therapeu, vol. 47, fasc. 3, pp. 298–305, mar. 2022, doi: 10.1111/jcpt.13548.

EFFECT OF THREE DIFFERENT TIDAL VOLUME ON LUNG AERATION, GAS EXCHANGE AND RESPIRATORY FUNCTION IN ANESTHETIZED AND MECHANICAL VENTILATED RABBITS

Di Bella Caterina (1), D'Urso Elisa Silvia (1), Selleri Paolo (1), Angorini Alessio (1), Bersanetti Giulia (1), Galosi Margherita (1), Martorelli Monia (1), Serino Federica (1), Palumbo Piccionello Angela (1)

(1) School of Bioscience and Veterinary Medicine, University of Camerino

Corresponding Author: **Caterina Di Bella** - caterina.dibella@unicam.it

The aim of this study was to describe the lung aeration and gas exchange in anesthetized rabbits ventilated mechanically with different tidal volumes. For this purpose, 24 rabbits (Oryctolagus cuniculus), underwent pre-operative CT control and subsequent ovariectomy, were selected. All patients were premedicated with dexmedetomidine (80 mcg/kg), ketamine (5 mg/kg) and methadone (0,2 mg/kg), intubated and maintained with isoflurane in pure oxygen. Rabbits were mechanically ventilated in volume-controlled mode with different tidal volumes: TV = 6 ml/kg (L group), TV = 10 ml/kg (M group) and TV = 12 ml/kg (H group). The ventilatory setting was: respiratory rate (RR) = 15-18 breath/min; inspiratory pause 25%; I:E ratio 1:2; PEEP 0 cmH2O. After 5 minutes of adaptation to mechanical ventilation (baseline), the main cardiovascular and respiratory parameters were monitored, and, in addition, an arterial blood sample and a CT scan were performed. The same procedure was done after 20 minutes (T20). Subsequently, intrapulmonary shunt (Fshunt), alveolar-arterial oxygen and carbon dioxide gradient, and indexed static and dynamic compliance (CstatIND and CdynIND, respectively) were calculated. Furthermore, lung aeration was analyzed by MicroDicom DICOM Viewer program considering hyperareate (-1000/-901 HUS), normoareate (-900/-501 HUS), hypoaerated (-500/-101 HUS) and non-aerated areas (-100/+100 HUS) [1]. Values of $p < 0.05$ were considered statistically significant. Analysis of the CT scans prove that group L showed several hypoaerated areas both at baseline and at T20. On the other hand, in group H, all areas analyzed were normoareated and
there were no regions of hyperaeration. CstatIND and CdynIND were significantly higher in group H than in the other 2 groups, both at Baseline and T20 phases. Furthermore, in group H, at T20, the Fshunt is significantly lower than baseline in the same group and compared to the other 2 groups at the same time of the study. In conclusion, our data show that a high TV (12 mL/kg) could be ideal for ventilation of rabbits in volume-controlled mode, similar to what was previously demonstrated in dogs [2]. However, there are currently no recent studies in the literature regarding the mechanical ventilation in healthy rabbits.

[1] De Monte V, Bufalari A, Grasso S, Ferrulli F, Crovace AM, Lacitignola L, et al. Respiratory effects of low versus high tidal volume with or without positive end-expiratory pressure in anesthetized dogs with healthy lungs. Am J Vet Res. (2018) 79:496 504. doi: 10.2460/ajvr.79.5.496

[2] Bumbachera S, Schramelb JP, Mosing M. Evaluation of three tidal volumes (10, 12 and 15 mL/kg) in dogs for controlled mechanical ventilation assessed by volumetric capnography: a randomized clinical trial. Vet Anaest Analg (2017) 44(4):775 -784. doi: 10.1016/j.vaa.2016.10.007

76° CONVEGNO SISVET
BARI, 21-22-23 GIUGNO 2023

EVALUATION OF THE EFFECTS OF AUTOLOGOUS LEUCOCYTE AND PLATELET-RICH FIBRIN MEMBRANES IN THE TREATMENT OF DELAYED HEALING WOUNDS

Federica Aragosa (1), Chiara Caterino (1), Giovanni Della Valle1 (1), Alfonso Piscitelli (2), Luigi Navas (1), Francesco Lamagna (1), Gerardo Fatone (1)

(1) Department of Veterinary Medicine and Animal Production, University of Naples "Federico II", Naples, Italy
(2) Department of Agricultural Sciences, University of Naples "Federico II", Portici, Italy

Corresponding Author: **Giovanni Della Valle** - giovanni.dellavalle@unina.it

Local and systemic factors may hinder wound healing, leading to a persistent non-healing condition (1). The use of platelet concentrates has recently been researched since growth factors and cytokines are crucial for optimal wound healing (2). Leukocyte-Platelet Rich Fibrin (L-PRF) is a second-generation of a platelet-based autologous haemostatic and wound-sealing agent used in surgical wound healing (3). This prospective study aimed to evaluate the clinical efficacy of L-PRF membranes, in treating delayed-healing wounds in dogs and cats. The study population included animals (n=18) with a history of delayed healing of skin lesions (plague, ulcer, fistula) referred to Veterinary Teaching Hospital of Naples from February 2022 to February 2023. Signalment and medical history data were collected. The physical examination, blood count, and serum biochemical analysis were performed. The shape and size of wounds were assessed from digital photographs, and wounds were classified at presentation and during follow-up using the Bates-Jensen Wound Assessment Tool (BWAT). Each wound was treated by application of L-PRF membranes, obtained by Caterino et al. production method, after cleaning and debridement. The dressings were changed every 2-3 days until healing. During follow-up, all wounds were assessed weekly for epithelialization, granulation tissue formation, wound healing rate, and contraction, still complete healing and the data were statistically analyzed. All patients have an history of trauma and lack or delayed healing after common treatments. The lesions were distributed fair evenly over the trunk, neck, and limbs. Mean total wound area was 19.2 (±17.8) cm2 at presentation, 13.3 (±13.7) cm2 at T1, 6.8 (±7.9) cm2 at T2, 3.8 (±4.4) cm2 at T3, and 2.5 (±2) cm2 at T4. All but one of the cases were treated with only one application of L-PRF for an average of 21 days after injury. All wounds healed after an average of 28 (±12) days. Over time, BWAT score, total wound area, and granulation tissue decreased significantly while wound contraction increased. No side effects were noted, and only one minor complication occurred (pocket wound). According to our results, L-PRF promotes the wound healing process and is a cost-effective treatment for delayed-healing wounds in dogs and cats.

[1] Pitt KA, Stanley BJ, Acvs D, Author C, Stanley B. Negative pressure wound therapy: experience in 45 dogs. Wiley Online Libr. 2014;43(4):380-387. doi:10.1111/j.1532-950X.2014. 12155.x
[2] Khalifa OA, elkasapy A, Sallam EA, Marei YM, Mohammed LS. Effect of Autologous platelet-rich plasma versus platelet-rich fibrin on the Second Intension Wound Healing in Dogs through higher regeneration capacity and modulation of inflammatory cytokines. Benha Vet Med J. 2021;41(1):1-7. doi:10.21608/bvmj.2021.92616.1465
[3] Caterino C, Della Valle G, Aragosa F, et al. Production Protocol Standardisation, Macroscopic and Histological Evaluation, and Growth Factor Quantification of Canine Leukocyte-and Platelet-Rich Fibrin Membranes. Front Vet Sci. 2022;9. doi:10.3389/fvets.2022.861255

76° CONVEGNO SISVET
BARI, 21-22-23 GIUGNO 2023

ERRORS AND CRITICAL INCIDENTS IN VETERINARY ANESTHESIA: ANALYSIS OF RISK FACTORS AT THE UNIVERSITY TEACHING HOSPITAL OF BERN

Di Palma C. (1), Micieli F. (1), Straziuso F. (2), Levionnois O. (3), Spadavecchia C. (3), Mirra A. (3)

(1) Department of Veterinary Medicine and Animal Productions, University of Napoli Federico II, Naples, Italy.
(2) Veterinary Practitioner
(3) Anaesthesiology and Pain Therapy Section, Department of Clinical Veterinary Medicine, Vetsuisse Faculty, University of Bern, Bern, Switzerland

Corresponding Author: **Cristina Di Palma** - cristina.dipalma@unina.it

Medical error is "the failure of a planned action to be completed as intended, or use of a wrong, inappropriate, or incorrect plan to achieve an aim" (1). In human medicine, anesthesia-related deaths are associated to human errors in 51–77% of the cases (2). Despite the relevance of this topic, only few studies have analyzed the critical incidents occurrence in veterinary anesthesia.
The aim of this retrospective study was to evaluate the critical incident reports of the Anesthesia Section of the University Teaching Hospital of Bern, and to identify the relative risk factors.
Data from 81 "significant event report" filled out between January 2017 and August 2021 were analyzed. The following data were evaluated: medical history; reason for anesthetic procedure; co-morbidities of the patient; date, time and place of the event; type of error; cause of error and outcome. The factors that contributed to the genesis of the error were classified as: related to the patient and to the communication with the patient's owner; technological; individual; collective; environmental; organizational.
The animals involved were: 51% dogs, 34% cats, 13% horses, 1% sheep, 1% goats. The majority of the cases occurred between June (16%) and July (14%). The 10% of the incidents resulted in death of the patient, 24% in severe complications, 66% in no complication. The 34% of the errors occurred between 8:00 and 13:00, the 52% between 13:00 and 18:00, and the 14% between 18:00 and 8:00. In 32% of the cases the incident was determined by factors related to the patient or to the communication with the owner, in 31% by technological factors, in 70% by individual factors, in 23% by collective factors, in 11% by environmental factors, and in 57% by organizational factors.
The present study shows that the "significant event report" is a useful tool to better understand the complex reality in which the veterinary anesthesiologists operate and to identify possible risk factors in order to prevent their future occurrence. Further studies on this field are desirable to improve the safety of the patients.

[1] WHO Conceptual Framework for the International Classification for Patient Safety - Final Technical Report January 2009. World Health Organization.
[3] G. Haller, T. Laroche, F. Clergue. Morbidity in anaesthesia: today and tomorrow. Best Pract Res Clin Anaesthesiol 2011 Jun;25(2):123-32.

76° CONVEGNO SISVET
BARI, 21-22-23 GIUGNO 2023

EVALUATION OF THE ROLE OF CLINICAL AND HISTOLOGICAL VARIABLES ON THE PREDICTABILITY OF RECTAL ECTASIA RESOLUTION AND RECURRENCE AFTER SURGICAL CORRECTION OF PERINEAL HERNIA IN DOGS

Ferrari Francesco (1), Auletta Luigi (1), Giudice Chiara (1), Grieco Valeria (1), Gariboldi Elisa Maria (1), Ubiali Alessandra (1), Rusconi Lorenzo (2), Romussi Stefano (1)

(1) Dipartimento di Medicina Veterinaria e Scienze Animali, Università di Milano (DIVAS UNIMI)
(2) Clinica Veterinaria Dott. Rusconi, Galbiate (LC)

Corresponding Author: **Francesco Ferrari** - francesco.ferrari@unimi.it

Perineal hernia (PH) is a common disease in intact male dogs amenable of surgical treatment1. Recurrence is associated to persistence of tenesmus and previous hernia repair2. Persisting Rectal ectasia (RE) represents a frequent complication of surgical reduction and may maintain persisting tenesmus. Aims of this study have been: to compare the histological features of the rectal wall in patients affected by PH with those from healthy dogs; to explore the possible correlation of RE and histological features with post-surgical RE persistence and PH recurrence.
A total of 15 male dogs with right or bilateral naïve PH were enrolled over a 2-years period (P group). RE was scored according to Brissot3 (+1, +2, +3) at the first consultation (T0), immediately after surgery (T1) and after 60 days (T2). All patients underwent surgical repair by internal obturator muscle transposition. Full thickness rectal biopsy was performed through the surgical access for PH repair at the site of maximum RE. The outcome was defined as "positive" when were recorded: complete resolution of clinical signs (tenesmus, dysuria) and RE; no signs of PH recurrence. Ten fresh canine cadavers, free from gastrointestinal pathologies and abnormalities at rectal exam, underwent a full thickness rectal biopsy (C group). Histological features reported as scores were: inflammation, fibrosis, elastic fiber appearance and orientation, number of ganglions and positivity to immunoperoxidase. The two groups (P vs. C) were compared for histological scores with the median test. RE and histopathological variables were tested for predictability on outcome and recurrence with nominal logistic regression. Significance was set at $p<0.05$.
The two groups were homogenous for age and bodyweight; they differed in inflammation ($p=0.01$) and fibrosis ($p=0.04$) scores. RE scores were: +2 in 3 and +3 in 12 at T0; +1 in 4, +2 in 8 and +3 in 3 at T1; +1 in 3, +2 in 7 and +3 in 5 at T2. Seven dogs had a negative outcome and 3 had recurrence within 12 months. +3 RE at T1 ($p=0.008$) and T2 ($p=0.002$) was predictive of negative outcome. Degree of fibrosis ($p=0.048$) and +3 RE at T1 ($p=0.0005$) and T2 ($p=0.02$) were predictive of PH recurrence.
In conclusion, the present study corroborates the use of full thickness rectal biopsy results to help the clinician in communicating with the owner on the probability of recurrence or uncomplete resolution of clinical signs, based on clinical and histological features of the rectal wall.

[1] Gill SS, Barstad RD. A Review of the Surgical Management of Perineal Hernias in Dogs. J Am Anim Hosp Assoc. 2018;54(4):179-87.
[2] Shaughnessy M, Monnet E. Internal obturator muscle transposition for treatment of perineal hernia in dogs: 34 cases (1998-2012). J Am Vet Med Assoc. 2015;246(3):321-6.
[3] Brissot HN, Dupre GP, Bouvy BM. Use of laparotomy in a staged approach for resolution of bilateral or complicated perineal hernia in 41 dogs. Vet Surg. 2004;33(4):412-21.

76° CONVEGNO SISVET
BARI, 21-22-23 GIUGNO 2023

PRELIMINARY EVALUATION OF HIND GUT MICROBIOTA IN HORSES OPERATED FOR COLIC

Spadari A. (1), Forni G. (1), Indio V. (1), Marzari F. (1), De Cesare A. (1), Rinnovati R. (1)

(1) Department of Veterinary Medical Sciences, University of Bologna, Bologna, Italy

Corresponding Author: **Giulia Forni** - giulia.forni6@unibo.it

Intestinal microbiota is reported to take part in maintenance of healthy status, while its alteration seems to be involved in the pathophysiology of multiple diseases in both humans and equids. Microbiotal composition can be influenced by several factors such as age, environment, and administration of antibiotics, moreover, a connection between its changes and the onset of colic has been recently hypothized. Aim of the present study was to evaluate the changes to large intestine microbiota in horses undergoing surgery for colic syndrome. Twelve horses were included in the study. During surgery, samples of intestinal content were collected both from the pelvic flexure and from the coecum. All the samples were immediately frozen in liquid nitrogen and stored at -80°C. Illumina 16S rRNA protocol (V3-V4 regions) was used and the taxonomic quantification was performed by applying the VSEARCH-based classifier implemented in QIIME adopting the Greengenes 13_8 97% OUT dataset as reference. An unpaired student t-test was used to compare relative abundances of families and phyla between discharged and non discharged horses, and between horses with stranulating and non strangulating disorders.
Our results showed an increased prevalence of *Protecobacteria* in the cecal content of horses that were discharged, although it is usually correlated to gastrointestinal disorders. Verrucomicrobia was significantly more abundant in the pelvic flexure of horses that survived, which is more in line with previous literature, given its role in maintenance of intestinal health. Other significant differences have been found regarding the abundances of phyla and families between survived and non-survived and strangulating and non-strangulating colics. Some of these alterations are congruent with literature whereas some are reported in the present work for the first time.
Research regarding the influence of microbiota in equine colics onset, course and recovery is still in the beginning and this preliminary study might add some useful informations to this topic.

[1] Lara, F., Castro, R., & Thomson, P. (2022). Changes in the gut microbiome and colic in horses: Are they causes or consequences? Open Veterinary Journal, 12(2), 242-249.
[2] Salem, S. E., Maddox, T. W., Antczak, P., Ketley, J. M., Williams, N. J., & Archer, D. C. (2019). Acute changes in the colonic microbiota are associated with large intestinal forms of surgical colic. BMC veterinary research, 15(1), 1-13.
[3] Weese, J. S., Holcombe, S. J., Embertson, R. M., Kurtz, K. A., Roessner, H. A., Jalali, M., & Wismer, S. E. (2015). Changes in the faecal microbiota of mares precede the development of post partum colic. Equine veterinary journal, 47(6), 641-649.

76° CONVEGNO SISVET
BARI, 21-22-23 GIUGNO 2023

LEUKOCYTES-PLATELETS RICH FIBRIN PREPARATION METHOD: PROTOCOL STANDARDIZATION, MACROSCOPIC AND HISTOLOGIC EVALUATIONS, AND GROWTH FACTORS ASSESSMENT IN DAIRY COWS

Giovanni Della Valle (1), Maria Chiara Alterisio (1), Jacopo Guccione (1), Chiara Caterino (1), Federica Aragosa (1), Gianmarco Ferrara (1), Davide De Biase (2), Paolo Ciaramella (1), Gerardo Fatone (1)

(1) Department of Veterinary Medicine and Animal Production, University of Naples "Federico II", Naples, Italy
(2) Department of Pharmacy/DIFARMA, University of Salerno, Fisciano, Italy

Corresponding Author: **Jacopo Guccione** - jacopo.guccione@unina.it

Leukocite-Platelets Rich Fibrin (L-PRF) is a platelet-based autologous hemostatic and wound-sealing agent used in surgical wound healing (1,2). This biological scaffold releases growth factors (GFs) and matrix proteins during the healing process after its application. Although some protocols have been described for L-PRF production in other species such as horses, dogs, and cats (1); to the Authors' best knowledge, however, a production protocol for L-PRF in the bovine species has not yet been described. This prospective study aimed to standardize the L-PRF production protocol in bovines using a modification of the L-PRF protocol proposed by Caterino et al. (1) in canine specie and evaluate macroscopically, histologically the L-PRF membranes obtained by using Wound Box (3). Moreover, the GFs release of the membranes was assessed at 1h (T0), 4h (T1), 24h (T2), and 168h (T3). Eighty cattle, divided into four groups, each consisting of 20 animals based on the lactation phase, were enrolled. During the routine clinical examination, a whole blood sample of 20 ml was taken and divided into two rates for L-PRF clot production. A dedicated device, L-PRF Wound Box (3), was then used to obtain L-PRF membranes, and the GFs analysis of L-PRF membranes was performed and statistically evaluated by Kruskal-Wallis test and One Way ANOVA. The reproducible protocol let us obtain 80 L-PRF clots and 80 membranes on 80 samples. Throughout the lactation phases, the Wound Box produced a membrane with a mean (± SD) of 34,4 (± 7,3), 12,5 (± 2,6), and 4,9 (± 0,17) cm in length, width, and weight, respectively.

Histological analysis showed a well-defined histoarchitecture consisting of a first layer of erythrocytes, a layer of leukocytes, erythrocytes, and platelets, a layer of leukocytes, a layer of platelets, and finally a layer of slightly eosinophilic fibrin. Elisa's analysis of GFs revealed a statistically significant increase with upward trends in all phases of lactation and in each time control. Our data support the efficiency of the suggested production methodology and the Wound Box in creating L-PRF membranes in cattle since the L-PRF characteristics discovered in this species are compatible with those present in the literature (4).

[1] Caterino C et al. Production Protocol Standardisation, Macroscopic and Histological Evaluation, and Growth Factor Quantification of Canine Leukocyte-and Platelet-Rich Fibrin Membranes. Front Vet Sci. 2022 Apr 15; 9:861255. doi: 10.3389/fvets.2022.861255.
[2] Dohan, D.M. et al. Platelet-rich fibrin (PRF): a second-generation platelet concentrate. Part I: technological concepts and evolution. Oral Surg. Oral Med. Oral Pathol. Oral Radiol. Endod., 2006, 101(3) e37-44.
[3] Crisci A et al. Standardized protocol proposed for clinical use of L-PRF and the use of L-PRF Wound Box®. J Unexplored Med Data. (2017) 2:77. 10.20517/2572-8180.2017.17
[4] Dohan Ehrenfest, Threedinsional architecture and cell composition of a choukroun's platelet-rich fibrin clot and membrane. J. Periodon- tol., 2010, 81(4) 546-555.

76° CONVEGNO SISVET
BARI, 21-22-23 GIUGNO 2023

RADIOGRAPHIC, ULTRASONOGRAPHIC AND ELASTOSONOGRAPHIC MEDIUM/LONG TERM EVALUATIONS OF PATELLAR LIGAMENT AFTER TIBIAL PLATEAU LEVELLING OSTEOTOMY IN DOGS

Luca Pennasilico (1), Antonella Volta (2), Caterina Di Bella (1), Sara Sassaroli (1) Valentina Riccio (1), Angela Palumbo Piccionello (1)

(1) Università degli studi di Camerino, Scuola di Bioscienze e Medicina Veterinaria
(2) Università degli studi di Parma, Dipartimento delle Scienze Mediche Veterinarie

Corresponding Author: **Luca Pennasilico** - luca.pennasilico@unicam.it

Objective. The aim of study was to evaluate the medium/long term thickness and elasticity changes of patellar ligament after TPLO.
Materials and methods. The patellar ligament was assessed preoperatevely (T0), at one (T1), two (T2) and six months (T3) after surgery, using radiographs, ultrasound and elastosonography. The knee radiographs were takenin mediolateral view with stifle and tarsus joints flexed at 90 degrees and femoral condyles superimposed over each other. The tickness of ptellar ligament was mesured one centimeter distal to the base of the patella, on centimeter proximal to the tibial tuberosity and halfway between the first two points.The patients were positioned in lateral recumbency and stifle in maximum passive flexion when examined with ultrasound. Percentage of tissue hardness (%HRD) and softness (%SFT) were calculated with a dedicated software. Radiographic and sonographic measurements of patellar ligament, % HRD, %SFT were compared between each time point and with the time elapsed from the onset of clinical signs (0-15 days, 16-60 days and >60 days).
Results. Twenty-two stifle joints in 20 dogs were evaluated.
At T0 the mean radiographic thickness (mm) of patellar ligament was 2,4+/-0,6 in the proximal portion, 2,2+/-0.5 in the middle portion and 2.3+/-0.5 in distal portion. At T3 mean radiographic thickness was 3.7+/-1.1 in the proximal portion, 4.5+/-1.8 in the middle portion and 5.2+/-2.2mm in distal portion. At T0, the mean sonographic thickness (mm) of patellar ligament was 2.3+/-0.2 in the proximal portion, 2.2+/-0.3 in the middle portion and 2.6+/-0.7 in distal portion. At T3, the mean sonographic thickness (mm) of patellar ligament was 2.7+/-0.9 in the proximal portion, 3.5+/-1.1 in the middle portion and 4.9+/-1.5 in distal portion.
Mean %HRD at T0 was 25.8+/-25.6, at T1 was 52.2+/-26, at T2 was 58.2+/-23.8, at T3 was 47.9+/-23.5. Mean %SFT at T0 was 74.4+/-25.8, at T1 45.9+/-26.1, at T2 was 44.1+/-23.6, at T3 was 52.1+/-24.3. Thickness was significantly increased between T0 and T1, T0 and T2 and T0 and T3, both for radiographic and sonographic measurements. %HRD was significantly increased and %SFT was significantly decreased between T0 and T1, T0 and T2, T0 and T3. Patellar ligament was significantly thicker in mid and distal portion in dogs that had onset of clinical signs >60 days only in T0. No other differences were found regarding the time elapsed from the onset of clinical signs.
Discussion. Radiographic and ultrasonographic findings highlighted a significant thickening of the patellar ligament especially in distal portion, which persists even 180 days after the TPLO. Elastosonography demonstrated a significantly increase of hardness in the patellar ligament post TPLO. The onset of patellar ligament alterations is probably related to biomechanical stifle changes that occurs following TPLO.

[1] Carey, K., et al. "Radiographic and clinical changes of the patellar tendon after tibial plateau leveling osteotomy." Veterinary and Comparative Orthopaedics and Traumatology 18.04 (2005): 235-242
[2] Mattern, Kara L., et al. "Radiographic and ultrasonographic evaluation of the patellar ligament following tibial plateau leveling osteotomy." Veterinary Radiology & Ultrasound 47.2 (2006): 185-191.
[3] Kuehn, Karolin, et al. "Radiographic and ultrasonographic evaluation of the patellar ligament following tibial tuberosity advancement." Veterinary Radiology & Ultrasound 52.4 (2011): 466-471.
[4] Piccionello, Angela P., et al. "Sonoelastographic features of the patellar ligament in clinically normal dogs." Veterinary and Comparative Orthopaedics and Traumatology 31.04 (2018): 279-284.

76° CONVEGNO SISVET
BARI, 21-22-23 GIUGNO 2023

RADIOGRAPHIC FINDINGS IN DOGS WITH PATENT DUCTUS ARTERIOSUS: LEFT-TO-RIGHT, RIGHT-TO-LEFT AND BIDIRECTIONAL SHUNT

Caterina Puccinelli (1), Tommaso Vezzosi (1), Camilla Pedrelli (1), Oriol Domenech (2), Edoardo Auriemma (2), Alessia Diana (3), Rosalba Tognetti (1), Simonetta Citi (1)

(1) Department of Veterinary Sciences, University of Pisa, Via Livornese lato monte, 56122, San Piero a Grado, Pisa, Italy
(2) Anicura Istituto Veterinario Novara, Novara, Italy; 3Department of Veterinary Medical Science, Alma Mater Studiorum, University of Bologna, Via Tolara di Sopra, 40064, Ozzano, Emilia, BO, Italy

Corresponding Author: **Caterina Puccinelli** – caterina.puccinelli@phd.unipi.it

Patent ductus arteriosus (PDA) is one of the most common congenital cardiac diseases in dogs. The ductus arteriosus is a vessel that develops from the embryonic left sixth aortic arch. Typically, ductal flow decreases dramatically within the first 12 hours of life in the neonatal dog, ceasing altogether within 7 days, but in some individuals the ductus can remain patent. The relative resistances of the pulmonary and systemic vascular beds determine the direction of blood flow through the PDA. Typically, PDA has left-to-right (L-R) shunt, from the aorta to the pulmonary artery. Chronic pulmonary overcirculation secondary to L-R shunt can induce severe pulmonary hypertension and pulmonary vascular resistance can exceed systemic vascular resistance. In this case, the shunt can become bidirectional (BID) or right-to-left (R-L). The aim of this study is to describe thoracic radiographic alterations in dogs with L-R, R-L, and BID PDA. This was a retrospective, multicenter, observational study. All the dogs included in the study had an echocardiographic diagnosis of PDA and had performed a radiographic study of the thorax. For the radiographic study, both quantitative and qualitative evaluations were performed. For the qualitative evaluation, the following variables were considered: presence of left heart enlargement (LHE), right heart enlargement (RHE), pulmonary artery dilation (PAD), and lung pattern (vascular, vascular-interstitial, vascular-alveolar). For the quantitative evaluation, vertebral heart score (VHS), vertebral left atrial size (VLAS), and radiographic left atrial dimension (RLAD) were measured. Twenty-two dogs with a diagnosis of PDA were included in the study (13 L-R, 6 R-L, and 3 BID). Dogs with R-L PDA had a higher prevalence of radiographic PAD compared to L-R PDA (100% of R-L patients and 50% of L-R patients; p=0.043), agreeing with the echocardiographic exam. The VHS and the VLAS were significantly higher in L-R PDA compared to R-L PDA (p=0.01 for VHS; p=0.03 for VLAS). No significant differences were highlighted for the remaining qualitative and quantitative parameters. All dogs with L-R PDA showed radiographic signs of RHE, not confirmed by echocardiography that only showed LHE. In conclusion, VLAS, VHS and the presence/absence of PAD on chest radiographs can be useful tools to differentiate between L-R and R-D PDA. Finally, the presence of radiographic signs of RHE can be secondary to severe LHE in dogs with L-R shunt.

[1] Greet V, Bode EF, Dukes-McEwan J, Oliveira P, Connolly DJ, Sargent J. Clinical features and outcome of dogs and cats with bidirectional and continuous right-to-left shunting patent ductus arteriosus. J Vet Intern Med. 2021 Mar;35(2):780-788.
[2] Buchanan JW. Patent ductus arteriousus morphology, pathogenesis, types and treatment. J Vet Cardiol. 2001 May;3(1):7-16.
[3]. Côté E, Ettinger SJ. Long-term clinical management of right-to-left ("reversed") patent ductus arteriosus in 3 dogs. J Vet Intern Med. 2001;15(1):39-42.

76° CONVEGNO SISVET
BARI, 21-22-23 GIUGNO 2023

PROGNOSTIC VALUE OF CONTRAST-ENHANCED ULTRASOUND (CEUS) IN DOGS WITH ACUTE RENAL INJURY (AKI) UNDERGOING HEMODIALYSIS THERAPY

Caterina Puccinelli (1), Ilaria Lippi (1), Tina Pelligra (1), Simonetta Citi (1)

(1) Department of Veterinary Sciences, University of Pisa, Via Livornese lato monte, 56122, San Piero a Grado, Pisa, Italy

Corresponding Author: **Caterina Puccinelli** – caterina.puccinelli@phd.unipi.it

Acute kidney injury (AKI) encompasses a spectrum of diseases associated with sudden parenchymal and functional renal damage, most typically characterized by the inability of the kidneys to meet the body's excretory, metabolic, and endocrine demands. In dogs, AKI is the most common indication for renal replacement therapies, including hemodialysis. Unfortunately, this therapy is expensive and may not be affordable for owners especially due to uncertain prognosis.
For this reason, prognostic tools are needed to predict outcomes in dogs with AKI when management by hemodialysis therapy is contemplated. Contrast-enhanced ultrasound is a noninvasive method used to study tissue perfusion, providing both qualitative and quantitative analysis.
Few clinical studies have been conducted to evaluate renal perfusion in dogs with AKI using the CEUS method, in which the authors were able to identify some significant changes in renal perfusion in pathological patients. The aim of the study was to evaluate the possible prognostic value of CEUS in dogs with AKI treated with hemodialysis therapy. Dogs with a diagnosis of AKI or AKI/CKD were included. In dogs included the CEUS examination was performed at patient admission prior to initiation of hemodialysis therapy (T0), after the first dialysis (T1), 7 days after patient admission (T7), and 30 days after patient admission (T30). Thirty dogs undergoing hemodialysis therapy were included in the study, of which 10 subjects survived at least 30 days (S), while 20 subjects survived less than 30 days (NS); there were no significant differences between CEUS values at patient admission (T0) between S and NS patients. In S patients, repeated CEUS renal examinations were performed in 6 subjects; in these patients only the cortical PI presented statistically significant differences in the subsequent controls; indeed, a significant difference of cortical PI values between T0, T1, T7, and T30, was highlighted and associated with the highest value of PI at T0, and a progressive reduction of its value in subsequent controls. In addition, this parameter was significantly reduced at T1 compared to T0 examination. In 12 subjects of NS patients who survived the first dialysis, no significant difference between cortical PI values of T0 and T1 was shown. In conclusion, the CEUS could be a useful tool to prognostically set dogs with AKI undergoing hemodialysis during hemodialysis therapy.

[1] Vaden, S. L., Levine, J., Breitschwerdt, E. B. (1997) A retrospective case-control of acute renal failure in 99 dogs. Journal of Veterinary Internal Medicine 11(2), 58-64.
[2] Cowgill, L. D., Langston, C. (2010) Acute kidney insufficiency. In: Nephrology and Urology of Small Animals. Bartges, J., Polzin, D. J. Wiley-Blackwell. pp 472-523.
[3] Mannucci T, Lippi I, Rota A, Citi S. Contrast enhancement ultrasound of renal perfusion in dogs with acute kidney injury. J Small Anim Pract. 2019 Aug;60(8):471-476.
[4] Gasser B, Uscategui RAR, Maronezi MC, Pavan L, Simões APR, Martinato F, Silva P,

EVALUATION OF SEDATION FOR STANDING MAGNETIC RESONANCE IMAGING (MRI) IN HORSES USING A ROMIFIDINE OR DETOMIDINE CONTINUOUS RATE INFUSION (CRI) COMBINED WITH MORPHINE: PRELIMINARY RESULTS

Cecilia Vullo (1), Vito Biondi (1), Marco Bifarella (1), Salvatore Monti (1), Giuseppe Catone (1)

(1) Veterinary Teaching Hospital, University of Messina

Corresponding Author: **Cecilia Vullo** - cvullo@unime.it

In equine patients, numerous diagnostic procedures, included magnetic resonance imaging (MRI), are performed under deep sedation because general anesthesia carries a substantial risk of mortality and morbidity in this species. Sedation protocols must be safe, provide a good quality of sedation without producing cardiorespiratory depression and severe ataxia. The aim of this prospective, blind, clinical study was to compare the cardiorespiratory effects, quality of sedation, and ataxia produced by administration of romifidine or detomidine in constant rate infusion (CRI) with morphine in standing horses submitted to MRI. Twelve client-owned adult horses ASA category I or II, referred to the Veterinary Teaching Hospital of University of Messina for standing MRI were included in this study. Baseline rectal temperature (RT), heart rate (HR), and respiratory rate (RR) were recorded. Horses were randomly assigned to one of two treatments group (DM or RM). All horses were premedicated with IM acepromazine 0.03 mg/kg, and 30 minutes later received an IV loading dose of detomidine (0.01 mg/kg) (Group DM) or romifidine (0.04 mg/kg) (Group RM), followed by a single IV bolus of morphine (0.05 mg/kg). Based on a 4-point sedation scale, additional sedation was administered if the horses were inadequately sedated. Once the exam was begun, an infusion of detomidine (0.01 mg/kg/h) or romifidine (0.03 mg/kg/h) was started. During the procedure, RT, HR, RR, depth of sedation and degree of ataxia were assessed every 10 min. Parametric data from each group were compared using ANOVA and non-parametric data using the Mann–Whitney test. A p value < 0.05 was considered statistically significant. No significant differences in HR, RR and RT were found between treatments ($p \geq 0.09$). In two horses, an additional dose of romifidine was required. The duration of the exam was longer in Group RM than Group DM ($p=0.04$) and ears partially responsive to surroundings during RM sedation were more evident than during DM sedation ($p=0.03$), suggesting there may be a subtle difference in the depth of sedation. In conclusion, both protocols produced satisfactory sedation and immobility without significant decrease of HR and RR or ataxia during MRI, but romifidine at this dose rate may produce less sedation than detomidine. Further studies are required to evaluate any clinical advantages to either drug, or whether a different CRI may be more appropriate during longer MRI exams.

[1] Porter EG, Werpy NM: New concepts in standing advcanced diagnostic equine imaging. Vet Clin Eq, 2014;
[2] Taylor P et al.: Evaluation of sedation for standing clinical procedures in horses using detomidine combined with buprenorphine. Vet Anaest Analg, 2014;
[3] Vigani A, Garcia-Pereira FL: Anesthesia and analgesia for standing equine surgery. Vet Clin Eq, 2013;
[4] Schauvliege S et al: How to score sedation and adjust the administration rates of sedatives in horses: a literature review and introduction of the Ghent Sedation Algorithm. Vet Anaesth Analg, 2018.

PLETHYSMOGRAPHIC VARIABILITY INDEX AS INTRAOPERATIVE FLUID THERAPY GUIDE IN DOGS

Vicenti C. (1), Romagnoli N. (1), Stabile M. (1), Scardia A. (1), Piemontese C. (1), Guadalupi M. (1), Lacitignola L. (1), Crovace A. (1), Staffieri F. (1)

(1) Dipartimento di Medicina di Precisione e Rigenerativa e Area Jonica - (DiMePRE-J), Piazza Giulio Cesare n.11-70124 Bari-Policlinico

Corresponding Author: **Caterina Vicenti** - caterina.vicenti@uniba.it

The aim of this prospective, randomised, clinical study was to compare the use of the Plethismographic Variability Index (PVI) to guide the rate of intra-operative fluid therapy with a traditional fixed fluid rate approach in ASA 1-2 dogs undergoing surgery.
Considering the inclusion and the exclusion criteria, 27 dogs undergoing general anesthesia for surgical procedures were randomly assigned to two different fluid-therapy management groups: classical (CFM group, n = 12) or PVI-guided (PVI group, n = 15). In the CFM group dogs received a fixed fluid rate of crystalloid solution of 5 ml/kg/h during the entire procedure. In the PVI group fluid rate was adjusted based on the PVI value according to the following scheme: PVI < 14% rate of 3 ml/kg/h; 14%, 14%< PVI>20%rate of 10 ml/kg/h; PVI > 20% rate of 15 ml/kg/h. In the CFM group, hypotension (MAP < 65 mmHg) was managed with a bolus of 5 ml/kg in 10 minutes repeated for maximum 2 times and in case of unresponsiveness with dobutamine 1-5 mcg/kg/min. In the PVI group hypotension was treated similarly to the CFM when PVI > 14% in case of PVI < 14% dobutamine was started directly. In any case dobutamine infusion was stopped when MAP was > 80 mmHg. For each case the total amount of fluids, the number of episodes of hypotension, the boluses of fluids required and the use of dobutamine were recorded. All dogs were anesthetized with different protocols according to the case needs and mechanically ventilated. The PVI was assessed with the Massimo rainbow SET® pulse oximeter with the clip type probe placed on the tongue. The PVI value was considered valid only in case of a perfusion index > 1%. Data were analyzed for normal distribution and were compared between groups with the Kruskal-Wallis test (P< 0.05). Body weight and age were similar between groups, as well as duration of anesthesia. The total amount of fluids was significantly lower in the PVI group (0.056 +- 0.27 ml/kg/min) as compared with the CFM group (0.132 +- 0.115 ml/kg/min). The incidence of hypotension was lower (p = 0.023) in the PVI group (0%) as compared to the CFM group (41 %). Mean arterial pressure was significantly higher during surgery in the PVI compared to the CFM group. These preliminary data proved that PVI could be a useful method to individually guide fluid therapy in dogs undergoing anesthesia. Future studies are required to confirm these data in a larger population and in cases of cardiovascular instability.

[1] Skouropoulou, D., Lacitignola, L., Di Bella, C., Stabile, M., Acquafredda, C., Brienza, N., Grasso, S., Crovace, A., Iarussi, F., Staffieri, F. (2021). Intraoperative Assessment of Fluid Responsiveness in Normotensive Dogs under Isoflurane Anaesthesia
[2] Sevim Cesur, Tülay Çardaközü, Alparslan Kuş, Neşe Türkyılmaz, Ömer Yavuz (2018) Comparison of conventional fluid management with PVI-based goal-directed fluid management in elective colorectal surgery

OXYGEN RESERVE INDEX TO EARLY DETECT DESATURATION IN APNEIC DOGS

Francesca Zanusso (1), Giulia Maria De Benedictis (1), Luca Bellini (1)

(1) Department of Animal Medicine, Productions and Health, University of Padova (PD), Italy

Corresponding Author: **Francesca Zanusso** - francesca.zanusso@studenti.unipd.it

Oxygen status can be monitored using pulse oximetry, that estimates the percentage of oxygensaturated hemoglobin (SpO2). However, SpO2 provides limited information in hyperoxemic patients, remaining >98% for arterial partial oxygen pressure (PaO2) >80mmHg [1]. The Oxygen Reserve index (ORi) is a new real-time continuous index measured with a multi-wavelength pulse co-oximetry technology, which ranges from 0 to 1, and estimates PaO2 between 100 and 200 mmHg in humans [2]. Also in dogs, ORi has been shown to be positively correlated with PaO2 and able to detect mild hyperoxemia [3]. Providing a non-invasive indication of PaO2>100mmHg, Ori might identify an impending desaturation earlier than SpO2 [4]. The aim of this study is to evaluate the ability of ORi to early detect desaturation in apneic dogs at recovery from anesthesia. This prospective observational study enrolled 71 non-obese owned-client anesthetized dogs. Animals were mechanically ventilated maintaining a fraction of inspired oxygen (FiO2) 0.50 for elective procedures. At the end of the procedure, ORi value was measured using a multiwavelength pulse co-oximeter with a probe applied to the dog's tongue. After detecting an ORi value of 1, dogs were maintained intubated, the administration of oxygen mixture was interrupted, and SpO2 and ORi simultaneously monitored. Time was recorded from ORi 0.9 to SpO2 95%, from ORi 0.5 to SpO2 95%, and from SpO2 98% to SpO2 95% and defined as "ORi0.9dec" "ORi0.5dec" and SpO2dec", respectively. Only dogs that remained apneic until SpO2 95% were included. When SpO2 reached 95%, animals were mechanically ventilated with pure oxygen for at least 2 minutes. Apneic dogs were 38 (19 males and 19 females), with median (min-max) weight of 14.1 (4.2-30.0) Kg and age of 75 (8-168) months. In all patients ORi was 1 at the end of the elective procedure. In each dog, ORi decreased from 1 to 0 in a median (min-max) time of 131.5 (8-456) sec, while SpO2 remained ³98%. Median (min-max) ORi0.9dec", "ORi05dec", and "SpO2dec" were 63 (10-586), 33 (5-290) sec and 7 (1-154) sec, respectively. Therefore, in apneic dogs, the decrease in oxygen blood content is detected earlier by ORi than by SpO2. ORi decrease time might provide added arterial desaturation warning in apneic dogs and could prevent a possible worsening oxygen status, allowing for safe management of recovery.

[1] Chan ED, Chan MM. Pulse oximetry: understanding its basic principles facilitates appreciation of its limitations. Respir Med, 107(6); 789-799, 2013
[2] Scheeren TWL et al. The oxygen reserve index (ORI): a new tool to monitor oxygen therapy. J Clin Monit Comput, 32(3); 379-389, 2018
[3] Zanusso F et al. The ability of oxygen reserve index to detect mild hyperoxemia in mechanically ventilated dogs: a preliminary study. European Veterinary Emergency and Critical Care Congress, 1-3 June 2023, Porto, Portugal
[4] Tsymbal E et al. Study of early warning for desaturation provided by Oxygen Reserve Index in obese patients. J Clin Monit Comput, 35(4); 749–756, 2021

76° CONVEGNO SIS VET
BARI, 21-22-23 GIUGNO 2023

ULTRASOUND-GUIDED SCIATIC AND FEMORAL NERVE BLOCKS IN COMMON KESTRELS (*FALCO TINNUNCULUS*): A CADAVERIC STUDY

Fabiana Micieli (1), Cristina Di Palma (1), Alessandro Mirra (2), Bruna Santangelo (3), Adriano Minichino (1), Pablo E. Otero (4)

(1) Department of Veterinary Medicine and Animal Productions, University of Napoli Federico II, Naples, Italy
(2) Anaesthesiology and Pain Therapy Section, Department of Clinical Veterinary Medicine, Vetsuisse Faculty, University of Bern, Bern, Switzerland
(3) Langford Vets, University of Bristol School of Veterinary Sciences, Langford, Bristol, United Kingdom
(4) Department of Anesthesiology and Pain Management, Facultad de Ciencias Veterinarias, Universidad de Buenos Aires, Buenos Aires, Argentina

Corresponding Author: **Cristina Di Palma** - cristina.dipalma@unina.it

Traumatic injuries account for 49.5% of the admission causes of birds of prey in wildlife rescue and rehabilitation centres (1). Locoregional anaesthesia can potentially improve these species' anaesthetic management and outcome; however, only a few techniques have been reported so far. The aims of this study were: 1) to describe a single-window ultrasound-guided lateral approach to the sciatic and femoral nerves, and 2) to investigate the staining of the correlated nerves. For this prospective experimental study (OPBA protocol number 2020/27536), six pelvic limbs from three common kestrels (*Falco tinnunculus*) that were either euthanized or died for reasons unrelated to the study were used. After the feathers were removed, animals were placed in lateral recumbency, and a small cushion was positioned between the legs to improve acoustic coupling. Ultrasound gel was applied to the area, and a portable ultrasound device (MyLab 30 gold; Esaote, Italy) with a high-frequency (8-13 MHz) linear-array transducer was used. The ultrasound probe was placed on the lateral aspect of the limb, perpendicular to the femoral long axis, and distal to the great trochanter. Then, the thigh was scanned in a proximal-to-distal direction to identify the target nerves in the same acoustic window. After identifying the anatomical landmarks, a 22- gauge 50 mm insulated needle was introduced in-plane and advanced in a caudolateral to craniomedial direction until the sciatic nerve was reached. Then, 0.5 mL kg-1 of a 3:1 solution of 2% lidocaine and methylene blue was injected. Afterward, the needle was redirected until the femoral nerve was reached, and 0.25 mL kg-1 of the same dye solution was injected. All the blocks were performed by the same operator. After anatomical dissection, longitudinal nerve staining was measured and considered adequate if >0.6 cm. The sciatic nerve was adequately stained in 6/6 and the femoral nerve in 5/6 cases. This study demonstrated the feasibility of a single-window approach to the sciatic and femoral nerve in common kestrel. Further in-vivo studies are necessary to confirm this finding in a clinical setting.

[1] R. A. Molina-Lopez, J. Casal, L. Darwich. Causes of Morbidity in Wild Raptor Populations Admitted at a Wildlife Rehabilitation Centre in Spain from 1995-2007: A Long-Term Retrospective Study. PLoS One, 2011;6(9): e24603.

76° CONVEGNO SISVET
BARI, 21-22-23 GIUGNO 2023

EFFECT OF THE MEDIUM PLATELET VOLUME (MPV) AND PLATELET COUNT (PC) *RATIO* ON SIZE AND CELL CONTENT OF THE ADVANCED PLATELET RICH FIBRIN (A-PRF) IN THE HORSE

Montano Chiara (1), Crisci Alessandro (2), de Chiara Mariaelena (1), De Biase Davide (3), Maurelli Maria Paola (4), Lamagna Barbara (1), Del Prete Chiara (5), Pasolini Maria Pia (1)

(1) Department of Veterinary Medicine and Animal Production, Unit of Surgery, University of Naples "Federico II", Via Federico Delpino 1, 80137, Naples, Italy
(2) Unit of Dermosurgery, Cutaneous Transplantation and Hard-To-Heal Wound, "Villa Fiorita" Private Hospital, Via Filippo Saporito, 24, 81031 Aversa, Italy
(3) Department of Pharmacology, University of Salerno,Via Giovanni Paolo II132, 84084, Fisciano (SA)
(4) Department of Veterinary Medicine and Animal Production, University of Naples Federico II, CREMOPAR, WHO Collaborating Centre ITA-116, Naples, Italy; 5) Department of Veterinary Medicine and Animal Production, Unit of Animal Reproduction, University of Naples "Federico II", Via Federico Delpino 1, 80137, Naples, Italy

Corresponding Author: **Chiara Montano** - chiara.montano@unina.it

The advanced-PRF (A-PRF) is a platelet-derived product showing a higher concentration of growth factors (GFs), an increased number of cells, a looser structure of the fibrin clot than leukocyte-PRF. A high variability in the size of PRF associated with patient, haematological features and centrifugation protocols was reported. The aims of this study were to establish: a protocol for production of A-PRF in the field (1); a correlation between macroscopic and microscopic features and medium platelet volume/platelet count (MPV/PC) ratio in equine A-PRF obtained with two centrifuges (2). Twenty healthy Standardbred sport horses (3-7 years) were sampled from the jugular vein in late Autumn, at an environmental temperature of 15-17°C. Glass tubes without anticoagulants were heated at 37°C for 15 minutes in a thermostat. A 2.5 ml blood sample in EDTA was sent to the laboratory for CBC. Two 9ml blood samples from each horse were centrifugated with a fixed-angle centrifuge (A-PRF+, 1300 rpm x 8') and a horizontal centrifuge (A-PRF, 1500 rpm x 14'). Clots were measured (weight, height, length, and width; mm) and placed on the Wound Box for a 2-minute compression. Membranes were measured as well and then fixed in 10% formalin. Sections were stained with haematoxylin and eosin. A-PRF clots and membranes measured respectively 482,3±107,8 and 294,7±81,6, while A-PRF+ measured 492,8±115,9 and 285,1±78,5 (mm2; means±ds). Clot and membrane surface did not differ for sex and centrifuge protocol. Correlation between both membrane surface and for A-PRF+ clots and the MPV/PC ratio was low (r=0,190) and moderate for A-PRF clots (r=0,399). Histological examination revealed a well-defined histoarchitecture characterized by 5 cellular layers and a colourless and low-cellular fibrin network. Successful production of L-PRF has shown to depend on protocols of blood sampling, centrifugation and compression temperatures (between 21-30°C) of the clot. In this study A-PRF was easily obtained in the field, therefore tubes' warming was effective to obtain the clots, regardless of the environmental temperature. Due to its size, a single membrane was enough to cover a small skin/corneal defect. In contrast with human literature, the equine A-PRF size was not related to the sex and age. The MPV/PC ratio has a negligible impact on the size of the A-PRF. Further studies are needed to evaluate the influence of patient parameters in young/elderly horses with particular haematological alterations.

[1] Dohan Ehrenfest, D.M., Sammartino, G., Shibli, J.A., Wang, H.-L., Zou, D.-R. and Bernard, J.-P. (2014) Classification of platelet concentrates Special Review: Consensus Conference Guidelines for the publication of articles related to platelet concentrates (Platelet-Rich Plasma-PRP, or Platelet-Rich Fibrin-PRF): the international classification of the POSEIDO.
[2] Crisci, A., Minniti, C.A., Conte, A., Crisci, M. and Cardillo, F. (2020) Second Generation Platelet Concentrates-L-PRF (Fibrin Rich in Platelets and Leukocytes) and Its Derivatives (A-PRF, i-PRF)-: Morphological Characteristics to be Used in Modern Regenerative Surgery. Experimental Research. J Clin Haematol 1, 90–102.
[3] Dohan Ehrenfest, D.M., Pinto, N.R., Pereda, A., Jiménez, P., Corso, M. Del, Kang, B.-S., Nally, M., Lanata, N., Wang, H.-L. and Quirynen, M. (2018) The impact of the centrifuge characteristics and centrifugation protocols on the cells, growth factors, and fibrin architecture of a leukocyte-and platelet-rich fibrin (L-PRF) clot and membrane. Platelets 29, 171–184.
[4] Miron, R.J., Dham, A., Dham, U., Zhang, Y., Pikos, M.A. and Sculean, A. (2019) The effect of age, gender, and time between blood draw and start of centrifugation on the size outcomes of platelet-rich fibrin (PRF) membranes. Clin Oral Investig 23, 2179–2185. https://doi.org/10.1007/s00784-018-2673-x.

76° CONVEGNO SISVET
BARI, 21-22-23 GIUGNO 2023

OCULAR ABNORMALITIES IN FRIESIAN CALVES FROM BIRTH TO FOUR WEEK OF AGE: PRELIMINARY RESULTS

Irene Nocera (1,2), Francesca Bonelli (2), Samanta Nardi (2,3), Grace Ann West (2), Giovanni Barsotti (2)

(1) Institute of Life Sciences, Sant'Anna School of Advanced Studies, Pisa, Italy
(2) Department of Veterinary Sciences, University of Pisa, Pisa, Italy
(3) Private Practitioner

Corresponding Author: **Irene Nocera** - irene.nocera@vet.unipi.it

Calves are prone to both congenital and acquired ocular abnormalities. Nevertheless, the true prevalence of these abnormalities are not known due to the infrequency with which these animals undergo a complete ophthalmic examination (1). Furthermore, in the veterinary literature differences in lacrimal secretion and intraocular pressure (IOP) were reported between neonatal and adult animals in various species. Thus, the aims of this study were: to evaluate ocular lesions in Friesian healthy neonate calves, and to examine the variation in Schirmer tear test I (STT I) readings and IOP values from birth to 4 weeks of age, comparing the results with those in adult cattles.
Fifty-four full-term Friesian calves (7/54 males and 47/54 females) were enrolled. Calves underwent both physical and APGAR evaluation, and a complete ophthalmic examination including the STT I and IOP assessment within 48 hours of birth (T0), and weekly for the following four weeks of age (T7, T14, T21, T28). Results were shown as frequency and percentage. Data concerning STT I and IOP were evaluated for normal distribution and results were summarized as mean and standard error. The STT I and IOP values were evaluated for differences according to time by ANOVA test ($p<0.05$). All the calves enrolled were healthy. Within 48h of birth, 42/54 (78%) calves did not show ocular abnormalities, while 12/54 (22%) calves had ocular abnormalities. Only one calf (1.8%) concurrently showed two different ocular lesions: bilateral cataract and unilateral subconjunctival haemorrhage. All the other affected calves had only one ocular lesion as follows: 6/54 (11.1%) subconjunctival haemorrhages, 3/54 (5.5%) retinal haemorrhages, and 2/54 (3.7%) bilateral cataract. In addition, all calves showed various degrees of remnants of hyaloid system and tunica vasculosa lentis during all the observation period. At birth, STT I and IOP mean and standard error values were 17.50±0.49 and 15.05±0.31, respectively. No statistical differences were found related to time.
Ocular haemorrhages were the mainly ocular lesions observed, and cataracts were the only congenital ocular abnormalities diagnosed in Friesian healthy calves of our study. At birth, calves showed lacrimal secretion and IOP values lower compared to those reported in the literature (2,3) for adult cattles, and tear secretion and IOP values did not change during the first month of life.

[1] Martins BC. Food and fiber animal ophthalmology. In: Gelatt KN, editor. Veterinary ophthalmology, 6th edition, Ames, IA: John Wiley and sons, USA, 2021. pp 1983-2054.
[2] Wieser B, et al. (2013). Correlation between corneal sensitivity and quantity of reflex tearing in cows, horses, goats, sheep, dogs, cats, rabbits, and guinea pigs. Veterinary ophthalmology, 16(4), 251-262.
[3] Peche N., et al. (2018). Intraocular pressure measurements in cattle, sheep, and goats with 2 different types of tonometers. Canadian Journal of Veterinary Research, 82(3), 208-215.

76° CONVEGNO SISVET
BARI, 21-22-23 GIUGNO 2023

SERRATUS CERVICIS VENTRALIS AND SERRATUS TORACIS VENTRALIS MUSCLES TEARS IN ENDURANCE HORSES: CLINICAL FEATURES, DIAGNOSTIC FINDINGS AND OUTCOME IN 11 CASES

Nicola Pilati (1), Angela Palumbo Piccionello (1), Caterina Di Bella (1), Lucrezia Accorroni (1), Massimo Puccetti (2), Francesca Beccati (3)

(1) School of Bioscience and Veterinary Medicine University of Camerino
(2) Dubai equine hospital, Dubai UAE
(3) Department of Veterinary Medicine, University of Perugia, Perugia, Italy

Corresponding Author: **Nicola Pilati** - nicola.pilati@unicam.it

The thoracic limb is connected to the axial skeleton by a synsarcosis in the horse, a junction made by muscles alone. It is made by several muscles acting during locomotion with different roles to allow important movements. The serratus cervicis ventralis (SCV) and serratus toracis ventralis (STV) are two of the most active muscles of horse's thoracic limb synsarcosis. The SCV improves the capacity of extension of the shoulder joint during retraction and also, moving cranially the proximal attachment of the triceps brachii, act as an extensor of the elbow during the caudal stance phase. The STV moves caudally the distal portion of scapula and has a role in the propulsion phase. Moreover, both muscles have a key role in shock adsorption and in the distribution of the ground reaction forces from the fore limbs to the trunk. The purpose of this retrospective case-series is to describe the clinical, diagnostic imaging findings and long term outcome of a group of 11 endurance horses affected by acute or chronic lesions of the SCV or STV muscles. The diagnosis was made after a thorough orthopedic examination and an ultrasound investigation. All of them had between 2 and 3 degrees out of five lameness and a typical clinical finding for the STV muscles tear was severe swelling on the shoulder region and a dorsocranial displacement of the apex of the scapula while clinical findings on SCV tears were more aspecific; a definitive diagnosis was made thorough an ultrasonographic examination of the shoulder region with a convex 3,5MhZ probe. Ten of them had a monolateral lesion and one had a bilateral lesion of SCV or STV. For all horses, the treatment consisted of 6-8 weeks of stall rest with a hand walking and then gradual reintroduction to physical activity for 3 months. Follow up informations were available for at least 18 months for any horse included in the study; After 6 months all the horses came back to full training and then 9 of them to international level competitions.
This retrospective study shows, for the first time, that lesions of the SCV and STV muscles must be considered in the differential diagnosis of lame endurance horses especially with an evident deformation of the shoulder region. An early and accurate diagnosis with a period of rest followed by a gradual resume of training allows a good functional recovery of the affected limb and return to competition.

[1] Payne, R. C., Veenman, P., & Wilson, A. M. (2005). The role of the extrinsic thoracic limb muscles in equine locomotion. Journal of Anatomy, 206(2), 193-204.
[2] Shokry, M. M. (2016). Unilateral rupture of the serratus ventralis muscle in a foal. Journal of Coastal Life Medicine, 4(4), 335-336.

ELECTROCHEMOTHERAPY AS MONOTHERAPY OR INTRAOPERATIVE ELECTROCHEMOTHERAPY COMPARED TO SURGERY TREATMENT IN CANINE SOFT TISSUE SARCOMA: A RETROSPECTIVE STUDY

Giulia Moretti (1), Alfredo Dentini (2), Francesca Beccati (1), Antonio Di Meo (1), Irene Di Matteo (2), Giuseppe Giovannini (2), Leonardo Amato (3), Antonello Bufalari (1)

(1) Department of Veterinary Medicine, University of Perugia, Perugia, Italy
(2) Tyrus Veterinary Clinic, Terni, Italy
(3) Animal Hospital Veterinary Clinic, Velletri, Italy

Corresponding Author: **Giulia Moretti** - giulia.moretti@unipg.it

To treat a soft tissue sarcoma (STS), the surgical options are marginal, wide, or radical resection. Radical surgery is the best treatment option, but when this is not achievable, it can be combined with radiation therapy, chemotherapy and electrochemotherapy (ECT). The use of ECT as a local ablative technique is both as a palliative treatment and as an alternative method to surgery when a wide excision is not possible because the tumour is located in an anatomic area of the body making it difficult to remove or when the owner refuses surgical therapy.
The purpose of this study was to compare the results of dogs treated with surgery or ECT as a treatment for STS, between January 2016 and July 2021. Sixty-eight clinical cases presenting distinct types of STS were analysed. Tumour localization, histotype, histological grade, stage, recurrence, the criteria of solid tumour response evaluation criteria (RECIST), disease free interval (DFI) and median overall survival time (OST) were evaluated. This study aimed to analyse the efficacy of repeated ECT applications coupled with Bleomycin compared with surgery in canine STS treatment.
Results showed that as sole procedures ECT was comparable to surgery, in terms of a median OST of 730 and 730 days, respectively, median DFI of 730 and 730 days, respectively, and a local recurrence rate (RR) of zero and 5%, respectively, while the combination of surgical debulking and ECT did not show any improvement of treatment efficacy with a median OST of 365 days, ranging from 60 to 1,095 days, a median DFI of 225 days, ranging from 30 to 730 days and a higher local recurrence rate of 33%. Further prospective case-control studies with randomized treatment groups are needed to limit the bias and analyse a homogeneous population, to better evaluate the effectiveness.

[1] Lowe R, Gavazza A, Impellizeri JA, Soden DM, Lubas G. The treatment of canine mast cell tumours with electrochemotherapy with or without surgical excision. Vet Comp Oncol. 2017 Sep;15(3):775-784. doi: 10.1111/vco.12217. Epub 2016 Mar 22. PMID: 27001443.
[2] Spugnini EP, Vincenzi B, Amadio B and Baldi A. "Adjuvant electrochemotherapy with bleomycin and cisplatin combination for canine soft tissue sarcomas: A study of 30 cases" Open Veterinary Journal, 2019; 9: 88-93
[3] Spugnini EP, Vincenzi B, Citro G, Santini D, Dotsinsky I, Mudrov N, Montesarchio V, Laieta MT, Esposito V, Baldi A. Adjuvant electrochemotherapy for the treatment of incompletely excised spontaneous canine sarcomas. In Vivo. 2007 Sep-Oct;21(5):819-22. PMID: 18019417
[4] Torrigiani F, Pierini A, Lowe R, Simčič P, Lubas G. Soft tissue sarcoma in dogs: A treatment review and a novel approach using electrochemotherapy in a case series. Vet Comp Oncol. 2019 Sep;17(3):234-241. doi: 10.1111/vco.12462. Epub 2019 Apr 8. PMID: 30688021.

SIFTVET

76° CONVEGNO SISVET
BARI, 21-22-23 GIUGNO 2023

ANTIMICROBIAL PRESCRIPTIONS IN NON-CRITICAL AND INTENSIVE CARE UNIT CATS AT THE VETERINARY TEACHING HOSPITAL OF THE UNIVERSITY OF PISA, ITALY

Vernaccini M. (1), Bacci S. (1), De Marchi L. (1), Briganti A. (1), Lippi I. (1), Marchetti V. (1), Intorre L. (1), Meucci V. (1)

(1) Department of Veterinary Sciences, University of Pisa, Pisa, Italy

Corresponding Author: **Matilde Vernaccini** - vernaccini.matilde@gmail.com

The inappropriate use of antimicrobials contributes to the emergence of antimicrobial resistance [1-2]. This study aimed to evaluate the antimicrobial prescribing behaviour between non-critical (NC) cats and intensive care unit (ICU) cats at the Veterinary Teaching Hospital (VTH) of the University of Pisa. Antimicrobial prescriptions were obtained by export from VTH management software. All statistical analyses were performed using Fisher's exact test, considering significant a p-value ≤ 0.05. Data were available from a total of 858 visits performed at the VTH (46% NC and 54% ICU) on a population of 606 non-surgical cats (207 NC and 399 ICU) that were referred to the VTH in the year 2022. A total of 220 antimicrobial prescriptions were written, of which 32% were NC and 68% ICU ($p<0.001$). In 194 cases (88%) antimicrobials were prescribed in monotherapy (34% NC and 66% ICU; $p<0.001$) and in 20 cases (9%) in empirical combinations (30% NC and 70% ICU; $p<0.001$). A total of 240 antimicrobials (32% NC and 68% ICU; $p<0.001$), belonging to eight different classes, were prescribed. Potentiated penicillins (66% vs 14%; $p<0.0001$) and penicillins (62% vs 0%; $p<0.0001$) were the most prescribed classes, followed by fluoroquinolones (26% vs 19%) and tetracyclines (3% vs 0%) in NC and ICU, respectively. A total of 57 (26%) of all antimicrobials were Highest Priority Critically Important Antimicrobials (HPCIAs), of which 39% were prescribed for NC and 61% for ICU. Fluoroquinolones represented the large majority (89%) of HPCIAs (39% NC vs 61% ICU; $p<0.0029$), followed by last-generation cephalosporins (9%). Only 18 antimicrobial prescriptions (17%) were supported by culture and susceptibility tests (CST), with no significant differences between NC (10%) and ICU (7%). CST supported 26% of all HPCIAs prescriptions (13% in both NC and ICU). The study shows differences in the prevalence of antimicrobial prescriptions and in the prescription patterns between NC and ICU units. Potentiated penicillins were the most prescribed antimicrobial classes in NC and penicillins in ICU. In addition, the study highlights a relevant use of HPCIAs that should be considered largely empirical in consideration of the scarce use of CTS.

[1] Robbins, S. N., et al. (2020). Antimicrobial prescribing practices in small animal emergency and critical care. Frontiers in Veterinary Science, 7, 110.
[2] Frey, E., et al. (2022). 2022 AAFP/AAHA Antimicrobial Stewardship Guidelines. Journal of the American Animal Hospital Association, 58(4), 1-5.

ANTIMICROBIAL USE IN CATS VISITED IN EMERGENCY ROOMS AND HOSPITALIZED AT THE VETERINARY TEACHING HOSPITALS OF THE UNIVERSITY OF PISA AND PARMA

De Marchi L. (1), Bacci S. (1), Bertini S. (2), Corsini A. (2), Fidanzio F. (2), Intorre L. (1), Meucci V. (1),0 Quintavalla C. (2), Rega M. (2), Vernaccini M. (1), Crosara S. (2)

(1) Department of Veterinary Sciences, University of Pisa, Pisa, Italy
(2) Department of Veterinary Sciences, University of Parma, Parma, Italy

Corresponding Author: **Lucia De Marchi** - lucia.demarchi@unipi.it

Assessment of antimicrobial prescribing practice represents a key component of antimicrobial stewardship [1-2]. Therefore, this study aimed to compare the antimicrobial use in cats visited in emergency rooms and hospitalized at the Veterinary Teaching Hospitals of the University of Pisa (PI-VTH) and Parma (PR-VTH), over one year (2022). Electronic medical records were retrospectively obtained excluding surgical populations. Statistical analyses were performed using Fisher's exact test, considering significant a p-value ≤ 0.05. A total of 1,165 visited cats were included (399 and 493 cats at PI-VTH and PR-VTH, respectively). Antimicrobials prescriptions represented 32% (n=149) and 37% (n=161) of total prescriptions at PI-VTH and PR-VTH, respectively. Antimicrobials were mainly in monotherapy (85% for the entire study), while 9.4% (PI-VTH) and 16.8% (PR-VTH) were in empirical associations. A total of 163 and 184 different antimicrobials, belonging to 6 and 9 different classes were prescribed at PI-VTH and PR-VTH, respectively. Significant differences were observed in terms of prescribed antimicrobial classes between PI and PR-VTHs: penicillins were most prescribed at PI-VTH (62% vs 0%; $p<0.0001$) while potentiated penicillins at PR-VTH (60% vs 13.5%; $p<0.0001$). The prescription of Highest-Priority Critically Important Antimicrobials (HPCIAs) represented 21% of total prescribed antimicrobials at PI-VTH, similar to 28% at PR-VTH. The antimicrobial prescriptions supported by culture and susceptibility tests (CSTs) were 7% at PI-VTH, significantly lower than 40% at PR-VTH ($p<0.001$). Similarly, CSTs supported only 13% of all HPCIAs at PI-VTH, significantly lower than 74 % at PR-VTH ($p<0.001$). These results highlight a similar antimicrobial prescription pattern between PI-VTH and PR-VTH, the latter characterized by greater use of CSTs, which makes the use of HPCIAs more justified in terms of the prudent use of antimicrobials [2].

[1] Chirollo, C., et al. (2021). Data on before and after the traceability system of Veterinary antimicrobial prescriptions in small animals at the University Veterinary Teaching Hospital of Naples. Animals, 11(3), 913.
[2] Frey, E., et al. (2022). 2022 AAFP/AAHA Antimicrobial Stewardship Guidelines. Journal of the American Animal Hospital Association, 58(4), 1-5.

PRESCRIPTIONS AND PRUDENT USE OF ANTIMICROBIALS IN HORSE PRACTICE FOR THE TREATMENT OF SKIN, RESPIRATORY AND OCULAR DISEASES AT THE VETERINARY TEACHING HOSPITAL OF THE UNIVERSITY OF PISA, ITALY

Bacci S. (1), Meucci V. (1), Sgorbini M. (1), De Marchi L. (1), Vernaccini M. (1), Intorre L. (1)

(1) Department of Veterinary Sciences, University of Pisa, Pisa, Italy

Corresponding Author: **Samanta Bacci** - samanta.bacci@phd.unipi.it

Documenting the use of antimicrobial drugs is considered a crucial step in achieving their appropriate use [1-2]. The aim of this study was to evaluate the antimicrobial prescribing patterns in equine practice for the treatment of skin, respiratory and ocular diseases at the Veterinary Teaching Hospital of University of Pisa (PI-VTH) over five years (2017-2021). The data were obtained via export from PI-VTH management software and inserted in a standardized database for subsequent analysis. Horses intended for human consumption and those undergoing surgery were excluded. Statistical analyses were performed using Fisher's exact test, considering significant a p-value ≤ 0.05. Data were available from a total of 120 antimicrobial prescriptions (48%, 32%, and 19% for the treatment of skin, respiratory and ocular diseases, respectively). A total of 9, 6, and 15 different antimicrobials, belonging to 7, 6, and 3 different classes were prescribed for the treatment of skin, respiratory and ocular diseases, respectively. Aminoglycosides were the most prescribed antimicrobial classes in all the conditions treated. Overall, empirical combinations of antimicrobials were used in 36% of cases. The prescription of Highest Priority Critically Important Antimicrobials (HPCIAs), which represented 30% of total prescribed antimicrobials, was lower for the treatment of skin (4%) compared to respiratory (59%) and ocular (33%) diseases ($p<0.0001$). Culture and susceptibility tests (CST) supported only 7% and 8% of all antimicrobial prescriptions and HPCIAs, respectively. The results showed that HPCIAs prescriptions and limited CST use are the two most disregarded rules of prudent use of antimicrobials in equine practice. Moreover, results highlight the need to improve the use of clinical diagnostics approach and to ensure the appropriate use of antimicrobials, particularly when HPCIAs are prescribed [3].

Acknowledgements: Research supported by PRA_2022_80 (University of Pisa)

[1] Raidal, S. L. (2019). Antimicrobial stewardship in equine practice. Australian Veterinary Journal, 97(7), 238-242.
[2] BEVA Protect-Me: https://www.beva.org.uk/Protect-Me
[3] Dunkel, B. (2021). Responsible antimicrobial use in critically ill adult horses. Equine Veterinary Education, 33(12), 653-658.

76° CONVEGNO SISVET
BARI, 21-22-23 GIUGNO 2023

RAW COW MILK EVALUATION OF ANTIMICROBIAL RESISTANCE PROFILES AND POSSIBLE GREEN VETERINARY PHARMACOLOGY (GVP) INTERVENTIONS

Cristian Piras (1), Rosario De Fazio (1), Francesca Oppedisano (1,2), Enrico Gugliandolo (4), Rosalia Crupi (4), Ernesto Palma (1,2,3), Rainer Cramer (5), Domenico Britti (1)

(1) Department of Health Sciences-University of Catanzaro Magna Græcia, CISVetSUA
(2) Institute of Research for Food Safety & Health (IRC-FSH), Department of Health Sciences-University of Catanzaro Magna Græcia, Catanzaro, Italy
(3) Nutramed S.c.a.r.l. Complesso Ninì Barbieri, Roccelletta di Borgia, Catanzaro, Italy
(4) Department of Chemical, Biological, Pharmaceutical and Environmental Science, University of Messina, Italy
(5) Department of Chemistry, University of Reading, Whiteknights, Reading, UK

Corresponding Author: **Cristian Piras** - c.piras@unicz.it

Introduction. Natural and human-related ecological pressure promotes the selection and expression of genes related to antimicrobial resistance (AMR). These genes might be present in a bacterial consortium but might not be expressed. Their expression could be induced by the presence of antimicrobial compounds that could originate from a given ecological niche or from human activity. The early detection of the antimicrobial-resistance profiles may facilitate a possible timely intervention via GVP approaches e.g. through the use of plants with antimicrobial properties.
Methods. Penicillinase activity of raw cow milk bacterial consortia was obtained by calculating the LAP-MALDI MS signal intensity ratio of the protonated decarboxylated hydrolysed ampicillin and the protonated ampicillin after an incubation time of 2h. For the resistome, the LC-MS/MS proteomics datasets were searched against The Comprehensive AMR Database (CARD, https://card.mcmaster.ca/) as FASTA files.
Annotated plants active against *S.aureus* (Piras et al. 2022) were searched using their scientific name and "*Staphylococcus aureus*" (e.g. *Cinnamomum camphora* and *Staphylococcus aureus*) in pubmed, web of science and scopus. After the duplicates removal, the file was uploaded in rayyan (https://www.rayyan.ai/) for the keywords search of the relevant experimental works indicating the MICs values to be enrolled in the meta-analysis.
Results and discussion. Lactamase-based AMR was detected after a short incubation period (2 hours or less), acquiring the mass spectral data within seconds. This workflow substantially cuts the time to detect AMR compared to classical bacteriological AMR testing, which typically involves the growth of individual bacterial colonies.
Meta-proteomics and resistome analysis revealed the presence of 29 proteins/proteoforms linked to AMR. These proteins were mainly beta-lactamases from *Klebsiella pneumoniae*, *Escherichia coli*, or *Acinetobacter baumannii*.
GVP approach is progressing by analysing the recorded MICs of the annotated plants active against S. aureus. It was chosen *S.aureus* because of its frequent involvement recorded in bovine mastitis and other relevant pathologies. The meta-analysis aiming at the detection of the plant extracts showing lowest MICs against the growth of *S.aureus* is still ongoing with the aim to select a shortlist of 5 plant extracts to be tested for further GVP approaches.

[1] Piras C, Tilocca B, Castagna F, Roncada P, Britti D, Palma E. Plants with Antimicrobial Activity Growing in Italy: A Pathogen-Driven Systematic Review for Green Veterinary Pharmacology Applications. Antibiotics (Basel). 2022 Jul 8;11(7):919. doi: 10.3390/antibiotics11070919.

RESISTANCE TO FLUOROQUINOLONES IN BACTERIA COLONIZING THE GUT OF THE LOGGERHEAD SEA TURTLE CARETTA CARETTA

Nicola Pugliese (1), Olimpia Lai (1), Simona Soloperto (2), Claudia Zizzadoro (1), Rossella Samarelli (1), Antonio Camarda (1), Giuseppe Crescenzo (1)

(1) Department of Veterinary Medicine, University of Bari Aldo Moro, Valenzano BA, Italy
(2) Sea Turtles Rescue Center "Luigi Cantoro" Torre Guaceto Protected Area, Carovigno BR, Italy

Corresponding Author: **Nicola Pugliese** - nicola.pugliese@uniba.it

Antimicrobial resistance (AMR) is considered a priority challenge by all the major national and international health agencies, both in human and veterinary medicine [1]. It impairs the efficacy of antimicrobial drugs, with a significant increase in morbidity and mortality. Within that framework, the environment is a critical factor, which contributes to the diffusion of resistant strains even in absence of selective pressure [2]. Among the compounds, fluoroquinolones are considered critical but their large use in human and veterinary practice has caused a rise in resistant strains [3].
This study investigated the diffusion of enrofloxacin-resistant bacterial strains in the gut of 15 sea turtles *Caretta caretta*. Two cloacal swabs were collected from each animal and incubated into TSB and then on TSA at 37/27 °C. When growth was observed, a single, well-isolated colony was streaked onto TSA+ENR to obtain a pure culture. The species were identified by MALDI-TOF. Susceptibility to ampicillin (AMP), cefotaxime (CTX), cefepime (FEP), imipenem (IMP), streptomycin (STR), gentamycin (GEN), amikacin (AMK), chloramphenicol (CHL), colistin (COL), co-trimoxazole (SXT) and tetracycline (TET) was assessed, and the enrofloxacin MIC was determined.
Fourteen ENR-resistant strains were isolated from 10 out of 15 turtles (66.7%), with MIC ranging from 16 to 256 □g/mL. Twelve were multidrug-resistant, mostly AMP (78,57%), SXT (57,14%), STR (50%), and TET (50%) resistant. Three *Escherichia coli* strains were resistant to the third-generation cephalosporin CTX. No resistance was found to FEP, IMP, GEN, or AMK.
The study reveals the wide circulation of ENR-resistant strains in the marine environment, despite fluoroquinolones being scarcely used in fishery, and almost insoluble in water. It is possible that resistant strains were selected in other contexts, among terrestrial animals or even humans, and then they have been spread into the sea. It may be the case of *E. coli*, colonizing both aquatic and terrestrial animals. Once in the sea, the genetic determinants of resistance could have been transferred to marine species, such as *V. fluvialis*. Most strains were resistant to the oldest compounds, such as AMP; TET, and SXT, confirming the strict link between antibiotic use and resistance spread. Therefore, it is crucial that newer drugs (FEP or IMP) should be limited as possible to avoid a further loss of efficacy due to the circulation of pathogenic and commensal resistant strains.

[1] Velazquez-Meza ME, Galarde-López M, Carrillo-Quiróz B, Alpuche-Aranda CM. Antimicrobial resistance: One Health approach. Vet World. 2022, 15:743-749. doi: 10.14202/vetworld.2022.743-749
[2] Larsson DGJ, Flach CF. Antibiotic resistance in the environment. Nat Rev Microbiol. 2022, 20:257-269. doi: 10.1038/s41579-021-00649-x
[3] Committee for Medicinal Products for Veterinary use (CVMP), Committee for Medicinal Products for Human Use (CHMP). Categorisation of antibiotics in the European Union. European medicines Agency, 2019. Available online at https://www.ema.europa.eu/documents/report/categorisation-antibiotics-european-union-answer-request-european-commission-updating-scientific_en.pdf

TRANSCRIPTOMIC BACKGROUND OF ACARICIDE RESISTANCE IN DERMANYSSUS GALLINAE

Nicola Pugliese (1) Antonella Schiavone (1) Daniel R.G. Price (2), Stewart T.G. Burgess (2), Rossella Samarelli (1), Elena Circella (1), Claudia Zizzadoro (1), Giuseppe Crescenzo (1), Alasdair J. Nisbet (2), Antonio Camarda (1)

(1) Department of Veterinary Medicine, University of Bari Aldo Moro, Valenzano BA, Italy
(2) Moredun Research Institute, Pentlands Science Park, Edinburgh, United Kingdom

Corresponding Author: **Nicola Pugliese** - nicola.pugliese@uniba.it

Dermanyssus gallinae, the poultry red mite (PRM), is a major concern for the poultry industry worldwide, for both the direct effects on animals and its vectorial role for pathogens. The chemical control of the PRM mostly relies on synthetic pyrethroids (SPs), organophosphates (OPs), spinosad, and the isoxazoline fluralaner [1]. However, the frequent abuse/misuse of the few available compounds has led to the development of PRM populations resistant to acaricides [2]. Pesticide resistance in arthropods has been correlated to target-site insensitivity and enhanced detoxification, which have been previously reported also in *D. gallinae*, suggesting the role of metabolic enzymes such as glutathione-S-transferases (GST) [3] and cytochrome P450 monooxygenases (P450) [4]. Here, we evaluated both mechanisms in PRM populations from Italy resistant to the OP phoxim and the SP cypermethrin. First, we analyzed the voltage-gated sodium channel (vgsc) and acetylcholinesterase (AChE), targets of cypermethrin and phoxim, respectively, observing point mutations in both sites, including those widely known for their role in pesticide resistance, specifically M827I and M918L/T in the vgsc and G119S in the AChE. In addition, RNA-seq analysis was performed from pairwise comparisons between five groups from three selected populations: fully susceptible; phoxim resistant exposed to phoxim and unexposed; cypermethrin resistant exposed to cypermethrin and unexposed to cypermethrin. Detoxification enzymes such as P450s and GSTs, ABC transporters, and cuticular proteins were overexpressed in both cypermethrin and phoxim-resistant mites, while esterases and transcription factors were constitutively overexpressed only in cypermethrin-resistant mites. Furthermore, heat shock proteins were both inductively and constitutively up-regulated in phoxim-resistant mites. Those findings strongly suggest that acaricide resistance in *D. gallinae* is due to both target-site insensitivity and enhanced expression of defense-related genes, which seem to be constitutive and not influenced by exposure to acaricides. Such evidence could be helpful to set up novel molecular diagnostic tests for detecting resistance and to verify the potential correlation between the repeated use of acaricides and the selection of resistant populations.

[1] Schiavone A, Pugliese N, Otranto D, Samarelli R, Circella E, De Virgilio C, Camarda A. Dermanyssus gallinae: the long journey of the poultry red mite to become a vector. Parasit Vectors. 2022, 15:29. doi: 10.1186/s13071-021-05142-1
[2] Pugliese N, Circella E, Cocciolo G, Giangaspero A, Horvatek Tomic D, Kika TS, Caroli A, Camarda A. Efficacy of λ-cyhalothrin, amitraz, and phoxim against the poultry red mite Dermanyssus gallinae De Geer, 1778 (Mesostigmata: Dermanyssidae): an eight-year survey. Avian Pathol. 2019. 48:S35-S43. doi: 10.1080/03079457.2019.1645295
[3] Bartley K, Wright HW, Bull RS, Huntley JF, Nisbet AJ. Characterisation of Dermanyssus gallinae glutathione S-transferases and their potential as acaricide detoxification proteins. Parasit Vectors. 2015, 8:350. doi: 10.1186/s13071-015-0960-9
[4] Wang C, Xu X, Huang Y, Yu H, Li H, Wan Q, Pan B. Transcription profiling and characterization of Dermanyssus gallinae cytochrome P450 genes involved in beta-cypermethrin resistance. Vet Parasitol. 2020, 283:109155. doi: 10.1016/j.vetpar.2020.109155

ASSOCIATION OF MICRONIZED N-PALMITOYL-D-GLUCOSAMINE WITH HESPERIDIN TO PREVENT THE INFLAMMATORY DEVELOPMENT OF INTERSTITIAL CYSTITIS AND BLADDER PAIN SYNDROME (IC/BPS)

Fabio Bruno (1), Laura Messina (1), Patrizia Licata (1), Domenico Britti (2), Enrico Gugliandolo (1), Rosalia Crupi (1)

(1) Department of Veterinary Science, University of Messina, Viale Annunziata, 98168, Messina, Italy.
(2) Department of Health Sciences, University of Catanzaro Magna Græcia, 88100 Catanzaro, Italy.

Corresponding Author: **Fabio Bruno** - fabio.bruno@studenti.unime.it

Interstitial cystitis/Bladder Pain Syndrome (IC/BPS) are chronic inflammatory diseases characterized by visceral pain. A recent developed and validated rat model of chronic cyclophosphamide (CYP) has shown great similarities with IC/BPS. Some matches concern the persistent inflammatory response, which causes in the long-term chronic condition, focal urothelial damage and bladder oedema associated with the painful behavioural aspects. To these purpose, the application of Aliamides, natural anti-inflammatory lipid compounds, is helpful for pain relieving action. Actually much attention has been paid so far to palmitoylethanolamide (PEA), but some remarkable indication on the benefits of the aliamide N-palmitoyl-D-glucosamine (PGA) is currently being picked up.
The aim of this study was to improve the combined effects of antalgic and anti-inflammatory action of micronized PGA (PGAm) with the antioxidant effect of hesperidin into chronic IC/BPS, CYP-induced rats. The Cystitis induction was developed by repetitive intraperitoneal injections of CYP 40mg/kg from day 0 to 6, every three days. Daily oral administration of PGAm-hesperidin (3:1 ratio) was started 3 days before CYP and maintained to the end of the experiment (day 10).
CYP instillation caused macroscopic and histological bladder inflammatory changes, increased lipid peroxidation and lowered the pain threshold. PGAm-hesperidin reduced CYP-induced IC/BPS and oxidative stress measured by myeloperoxidase and malondialdehyde levels, decreased the mast cells number into bladder tissue and relieved visceral pain.
PGAm-hesperidin may be a useful addition in the management of animal IC/BPS in order to predict possible applications in human idiopathic cystitis.

[1] Augé C, Gamé X, Vergnolle N, Lluel P, Chabot S. Characterization and Validation of a Chronic Model of Cyclophosphamide-Induced Interstitial Cystitis/Bladder Pain Syndrome in Rats. Front Pharmacol. 2020 Aug 28;11:1305. doi: 10.3389/fphar.2020.01305. PMID: 32982733; PMCID: PMC7485435.

A UHPLC-MS/MS METHOD FOR THE DETERMINATION OF THE PHARMACOKINETICS OF FLORFENICOL AND FLORFENICOL AMINE IN BULL SERUM AND SEMINAL PLASMA

A. Bardhi (1), J.E. Romano (2,3), G. Pagliuca (1,4), A. Caneschi (1), A. Barbarossa (1,4)

(1) Department of Veterinary Medical Sciences, University of Bologna, Ozzano Emilia (BO), Italy
(2) 3R Ranch, Somerville, TX, United States and the Cooperative Agriculture Research Center
(3) College of Agriculture and Human Sciences of Prairie View A&M University, Prairie View (TX), USA
(4) Health Sciences and Technologies-Interdepartmental Centre for Industrial Research (CIRI-SDV) - University of Bologna, Ozzano dell'Emilia (BO), Italy

Corresponding Author: **Anisa Bardhi** - anisa.bardhi@unibo.it

Florfenicol is a broad-spectrum antibiotic belonging to the amphenicols class that acts inhibiting protein synthesis by binding to bacteria ribosomal subunits. This drug is commonly used in veterinary medicine to treat bacterial infectious diseases in cattle, swine, poultry, and fish [1]. Florfenicol is mainly metabolized into florfenicol amine, which is considered the marker residue of this antibiotic, and Maximum Residual Limits (MRLs) have been established for both compounds in all food producing animals. While several analytical methods based on traditional liquid or gas chromatography (LC, GC) coupled to mass spectrometry (MS or MS/MS) have been reported for the quantification of florfenicol and florfenicol amine in water, feed, and animal-derived food, the analysis of animal biological fluids has been less frequent, mainly involving serum and plasma.
To date, no study was focused on the pharmacokinetics of florfenicol and florfenicol amine in seminal plasma, and a single method their quantification in both serum and seminal plasma by liquid chromatography coupled to tandem mass spectrometry (LC-MS/MS) has never been proposed. Therefore, our research aimed to develop and validate a simple and quick approach to be applied to samples collected during a pharmacokinetic study in bulls.
The proposed method uses a fast and simple sample preparation, followed by analysis in UHPLC-MS/MS in a chromatographic run of just 3.5 min under programmed conditions. The technique was fully validated [2] in each matrix over ranges suitable for field concentrations of florfenicol and florfenicol amine. The results showed good linearity during each day of testing (R2 always >0.99), and excellent accuracy and precision, with calculated bias always within ±15% and CV% always below 15% at all QC levels tested. For both analytes, the strength of the method was also confirmed by the satisfactory results obtained during recovery, matrix effect and process efficiency investigations in serum and seminal plasma.
The present approach was successfully applied for the determination of florfenicol and its main metabolite concentration-time profiles in serum and seminal plasma of a healthy bull, providing a first insight into their pharmacokinetics.

[1] Papich MG. Florfenicol. In: Papich MG, ed. *Saunders Handbook of Veterinary Drugs.* 4th ed. St. Louis, MO: Elsevier, Inc; 2016:327-329.
[2] European Medicines Agency (EMA). ICH guideline M10 on bioanalytical method validation and study sample analysis, London, U.K. July 2022 (accessed online 10.1.23). EMA/CHMP/ICH/172948/2019. https://www.ema.europa.eu/en/documents/scientific-guideline/ich-guideline-m10-bioanalytical-method-validation-step-5_en.pdf

76° CONVEGNO SISVET
BARI, 21-22-23 GIUGNO 2023

IN VITRO EFFICACY OF NITAZOXANIDE AND MILTEFOSINE AGAINST FELINE HERPESVIRUS TYPE-1

Consiglia Longobardi (1), Gianmarco Ferrara (1), Sara Damiano (1), Serena Montagnaro (1), Ugo Pagnini (1), Roberto Ciarcia(1)

(1) Department of Veterinary Medicine and Animals Productions, University of Naples "Federico II", Naples, IT

Corresponding Author**: Consiglia Longobardi** - consiglia.longobardi94@gmail.com

Feline *herpesvirus* type 1 (FeHV-1) is the main pathogen that causes rhinotracheitis and ocular symptoms in cats. Although numerous antiviral drugs are proposed for the treatment of this kind of infection, no effective molecule with low overall toxicity has been identified yet [1].
This study aimed to evaluate the in vitro efficacy of two drugs for the treatment of FeHV-1 infection: nitazoxanide (NTZ), an antiprotozoal drug currently studied for its broad-spectrum antiviral efficacy *in vitro* [2], and miltefosine (MLT), a licensed drug for the treatment of leishmaniosis [3].
Crandell-Reese feline kidney (CRFK) cells were treated with different doses of MLT (0.1-1.5 µM) and NTZ (5-20 µM). The MTT (3-[4,5-dimethylthiazol-2-yl]-2,5-diphenyl tetrazolium bromide) assay was performed to determine the lowest dose capable of not affecting cell viability while also lowering viral titer (TCID50 assay). Once the experimental conditions were set, supernatants, pellets, and cryolysates were collected 24 hours post-infection to evaluate the effects of the treatments on viral proliferation using the TCID50 assay, real-time PCR (using specific primers for thymidine-kinase and β-globulin genes), and Western blot assay.
NTZ showed to be effective in reducing the viral titer at a dose of 20 µM. Moreover, the western blot assay showed a reduction in the expression of viral glycoproteins (i.e., gB and gI) when a monoclonal anti-FeHV-1 was used. MLT (1.5 µM), induced not only a reduction in viral proliferation but also a reduction in the phosphorylation of Akt protein, generally involved in viral entry and phosphorylated during FeHV-1 infection. Viral glycoproteins were less expressed in this experimental condition as well.
In conclusion, we have confirmed the antiviral activity of NTZ and MLT against FeHV-1 *in vitro*. Future studies should focus on their possible synergistic effects (including other antiviral drugs), possible mechanisms of action (currently only hypothesized for Nitazoxanide), and potential in vivo use.

[1] Thomasy et al. "A review of antiviral drugs and other compounds with activity against feline herpesvirus type 1." Veterinary Ophthalmology. 19 Suppl 1(Suppl 1):119-130, 2016.
[2] Wang et al. "Antiviral activities of niclosamide and nitazoxanide against chikungunya virus entry and transmission." Antiviral research. vol. 135: 81-90, 2016.
[3] Cheshenko et al. "HSV activates Akt to trigger calcium release and promote viral entry: novel candidate target for treatment and suppression." FASEB journal: official publication of the Federation of American Societies for Experimental Biology, 27(7), 2584–2599, 2013.

EVALUATION OF ENVIRONMENTAL RESIDUES OF IVERMECTIN PRODUCED BY SWINE FARMS

Carrillo Heredero Alicia Maria (1), Segato Giulia (2), Menotta Simonetta (2), Butovskaya Elena (2), Giovanetti Alice (2), Bertini Simone (1)

(1) Dept. of Veterinary Sciences, University of Parma, Parma, Italy
(2) Food and Feed Chemical Dept., Experimental Zooprophylactic Institute of Lombardy and Emilia-Romagna, Brescia, Italy

Corresponding Author: **Alicia Maria Carrillo Heredero** - aliciamaria.carrilloheredero@unipr.it

In recent years, interest in ecotoxicology has increased, especially regarding pharmaceutical residues. The toxic effect of environmental residues of ivermectin is proven in several species. Those residues may represent a possible threat to non-target organisms, among which fish and some arthropods, who suffer serious effects when in contact with the lowest doses of the drug (33.3 PPB in dung beetle 1). In this study, our aim was to follow the route of ivermectin from the animal (feces) to the sewage, ending in the soil, and to understand if the concentrations reached in the soil were capable of causing the toxic effects reported in the literature. The same number of samples were collected from two swine farms in Emilia Romagna, Italy: an intensive and an extensive organic farm, which served as the negative control. We analyzed fecal samples from the rectal ampoule of sows one day after subcutaneous treatment (300 µg per kilogram of body weight), and samples of sewage from collection tanks and soil at the time of fertilization. An analytical method recently published was used for the extraction and purification of samples 2. The method was then adapted and validated for LC/MS/MS analysis to reach PPT concentrations. The limit of detection (LOD) was 150 PPT and the limit of quantification (LOQ) was 300 PPT in the extract. Doramectin, a macrocyclic lactone like ivermectin, was used as the process standard. The samples were concentrated to the fourth or eighth power to measure the analyte in feces, sewage, and soil. The results were interpreted using a calibration curve, with intercept values, indicating excellent linearity ($R^2>0.999$). The measured levels in the intensive farm samples were 156-589 PPB in feces, 327-674 PPT in sewage, and 150-500 PPT in soil. Even if ivermectin in feces reaches concentrations measurable in PPB, the dilution provoked by sewage stocking and distribution on the fields for fertilization may considerably reduce the environmental risk. Ivermectin was not detected in samples from the organic farm, confirming that the antiparasitic drug was not in use at the farm. These preliminary results suggest that ivermectin residues could be irrelevant to cause toxic effects on non-target species. Moreover, further studies and analysis will be necessary to deepen the knowledge of the impact of ivermectin on the swine farm.

[1] Verdú et al., 2015, Scientific Reports;
[2] Carrillo Heredero et al., 2023, Journal of Analytical Methods in Chemistry

ENVIRONMENTAL CONTAMINANTS AND CARDIOTOXICITY: EFFECTS OF CHRONIC EXPOSURE TO VINCLOZOLIN PESTICIDE

Alessio F. Peritore (1), Enrico Gugliandolo (1), Rosalia Crupi (1), Domenico Britti (1)

(1) University of Messina

Corresponding Author: **Rosalia Crupi** – rnglss92r55f251l@studenti.unime.it

Vinclozolin is one of the most used fungicides in the control of fungi in fruits, vege-tables, and ornamental plants [1-3]. New research links exposure to high levels of VZN to health damage; however, little is known about the effect of VZN on cardiovascular disease. The purpose of the present study was to evaluate, for the first time, biochemical and cardiovascular changes in male rats resulting from chronic VZN exposure. The animals were divided into four groups: group 1 served as the control, group 2 received 1 mg/kg of VZN by gavage, group 3 re-ceived 50 mg/kg of VZN by gavage, and group 4 received 100 mg/kg of VZN by gavage for 28 days. The University of Messina Review Board for the care of animals approved this research (n°409\2022-PR). Animal care was in accord with Italian (DM 116192) and European Economic Community regulations (OJ of EC L 358/1 12/18/1986) for the protection of experimental animals.For multiple comparisons, a two-way/one-way ANOVA was used, followed by a Bonferroni post-hoc test. Graphpad Prism 8 was used for statistical analysis. Statistically significant differences in the mean are indicated by astericks. Results showed that VZN 100mg/kg markedly increased plasma concentrations of cardiac markers (CK-MB, cTnT, ANP, BNP). Moreover, compared to the control group, VZN treatment elevated cardiac oxidative stress, as shown by increased MDA level and decreased ac-tivity of SOD, CAT, and GPx. Furthermore, collagen deposition was amplified owing to VZN 100 mg/kg toxicity. This harmful effect was confirmed by a histological study using H-E and Sirius Red staining. Overall, our results clearly proved the cardiotoxicity caused by chronic exposure of VZN.

[1] Zubrod, J.P.; Bundschuh, M.; Arts, G.; Brühl, C.A.; Imfeld, G.; Knäbel, A.; Payraudeau, S.; Rasmussen, J.J.; Rohr, J.; Scharmüller, A. Fungicides: an overlooked pesticide class? Environmental science & technology 2019, 53, 3347-3365.
[2] Mann, R.M.; Hyne, R.V.; Choung, C.B.; Wilson, S.P. Amphibians and agricultural chemicals: review of the risks in a complex environment. Environmental pollution 2009, 157, 2903-2927.
[3] Giambò, F.; Teodoro, M.; Costa, C.; Fenga, C. Toxicology and microbiota: How do pesticides influence gut microbiota? A review. International Journal of Environmental Research and Public Health 2021, 18, 5510.

76° CONVEGNO SISVET
BARI, 21-22-23 GIUGNO 2023

COMPARISON BETWEEN PER/POLY-FLUORINATED ALKYLATED SUBSTANCES CONTENT IN TISSUES FROM WILD BOARS AND SWINES BELONGING FROM THE SAME GEOGRAPHIC AREA

Susanna Draghi (1), Federica Di Cesare (1), Giulio Curone (1), Federica Riva (1), Francesco Arioli (1), Daniele Vigo (1), Roberto E. Villa (1), Carolina Fontanarosa (2), Michele Spinelli (2), Gennaro Battaglia (2), Angela Amoresano (2), Petra P. Cagnardi (1)

(1) Dept. of Veterinary Medicine and Animal Sciences, University of Milan, Lodi, Italy
(2) Dept. of Chemical Sciences, University of Naples, Napoli, Italy and consorzium I.N.B.B., Rome, Italy

Corresponding Author: **Susanna Draghi** - susanna.draghi@unimi.it

Wild boars can be exposed to persistent organic contaminants mainly through the oral route and hence can be considered bioindicators of environmental pollution. Swines and wild boars belong to the same *Sus scrofa* species and share the same exposure route and accumulation patterns for contaminants. Exposure to Per and Poly-Fluorinated Alkylated Substances (PFASs) can lead to disruption of endocrine functions. This study aimed to quantify PFASs concentrations in muscle and liver from wild boars and swines bred in a semi-extensive way in a specfic area of Northern Italy. Muscle and liver samples from 20 wild boars, killed during the hunting activity, and from 20 slaughtered swines were collected. Among PFASs, Perfluorooctanoic acid (PFOA), Perfluorooctanesulfonic acid (PFOS), Perfluorobutanesulfonic acid (PFBS) and N-Ethylperfluorooctanesulfonamido acetic acid (NEtFOSAA) were detected using validated LCMSMS method. For statistical comparison, animals were grouped according to species. All compounds were detected in all samples, except NEtFOSAA which resulted in traces in all swine liver samples. Higher concentration in liver were reported for all compounds in the two species, but statistically significant only for PFOS ($p<0.05$). In addition, NEtFOSAA resulted significantly higher in wild boar liver with mean concentrations 14.61 ± 23.34 µg/kg. PFOA content resulted statistically higher in wild boar liver than in swine with a mean concentration of 18.85 ± 5.41 µg/kg and 12.7 ± 6.34 µg/kg respectively. No differences in muscle concentration for all PFASs were highlighted but a trend of higher content in wild boar for all substances was observed. The higher content of PFASs in liver and muscle from wild boars could be attributable to different attitudes of these animals, stronger bond to the natural environment and the agricultural activity and with a longer lifespan. Wild boars resulted more contaminated by these emerging environmental pollutants and could be confirmed as bioindicator tools to assess their presence in an area and in case avoiding its use as pasture.

[1] Death C, Bell C, Champness D, Milne C, Reichman S, Hagen T. Per- and polyfluoroalkyl substances (PFAS) in livestock and game species: A review. Sci Total Environ. 2021 Jun 20;774:144795. doi: 10.1016/j.scitotenv.2020.144795. Epub 2021 Jan 26. PMID: 33609849.
[2] Stahl T, Falk S, Failing K, Berger J, Georgii S, Brunn H. Perfluorooctanoic acid and perfluorooctane sulfonate in liver and muscle tissue from wild boar in Hesse, Germany. Arch Environ Contam Toxicol. 2012 May;62(4):696-703. doi: 10.1007/s00244-011-9726-3. Epub 2011 Nov 23. PMID: 22108952.

MITIGATING EFFECT OF QUERCETIN NANOPARTICLES IN NILE TILAPIA FISH EXPOSED TO SILVER NANOPARTICLES

Alessandro Di Cerbo (1), Stefano Rea (1), Mahmoud Alagawany (2)

(1) School of Biosciences and Veterinary Medicine, University of Camerino, Italy
(2) Poultry Department, Faculty of Agriculture, Zagazig University, Zagazig 44519, Egypt

Corresponding Author: **Alessandro Di Cerbo** - alessandro.dicerbo@unicam.it

Nanotechnology has become an important research field with an involvement in aquaculture. In this context, silver nanoparticles (AgNPs) raised great interest as antibacterial, antiviral, and antifungal agents but also a great concern due to natural water pollution and hazardous effects on aquatic organisms. In this study, we investigated the role of quercetin nanoparticles (QNPs) against AgNPs-induced toxicity in Nile tilapia (n = 240, 2-month age, unsexed). Fish were equally divided into 4 groups: control, exposed to 1.98 mg L-1 AgNPs, fed with 400 mg L-1 QNPs, and exposed to AgNPs and fed with QNPs over a 60-day period. AgNPs significantly increased serum levels of ALT, AST, total cholesterol, and triglycerides and decreased glycogen and growth hormone. The intestinal (□-amylase, protease, and lipase) enzymes' activities were significantly inhibited, while the oxidative damage liver enzymes (catalase, superoxide dismutase, glutathione, malondialdehyde, and protein carbonyl), intestinal bacterial and Aeromonas counts, and Ag residues in the liver were significantly increased. AgNPs also significantly upregulated the expression of hepatic Hsp70, caspase3, and p53 genes. These findings indicate the oxidative and hepatotoxic effects of AgNPs. An overall significant restoration of physiological parameters and health status was observed regarding the fish fed 400 mg L-1 QNPs and exposed to AgNPs.

[1] Farag et al. "Effect of Quercetin Nanoparticles on Hepatic and Intestinal Enzymes and Stress-Related Genes in Nile Tilapia Fish Exposed to Silver Nanoparticles" Biomedicines 2023, 11, 663; doi: 10.3390/biomedicines11030663
[2] Mawed et al. "Dunaliella salina Microalga Restores the Metabolic Equilibrium and Ameliorates the Hepatic Inflammatory Response Induced by Zinc Oxide Nanoparticles (ZnO-NPs) in Male Zebrafish". Biology 2022, 11, 1447; doi: 10.3390/biology11101447
[3] Mawed et al. "Zinc Oxide Nanoparticles (ZnO-NPs) Suppress Fertility by Activating Autophagy, Apoptosis, and Oxidative Stress in the Developing Oocytes of Female Zebrafish". Antioxidants 2022, 11, 1567; doi:10.3390/antiox11081567

IMMUNOMODULATORY AND TOXIC EFFECTS OF ENDOCRINE DISRUPTOR EXPOSURE ON COLLAGEN-INDUCED ARTHRITIS

Livia Interdonato (1), Enrico Gugliandolo (1), Salvatore Cuzzocrea (1), Domenico Britti (1)

(1) University of Messina

Corresponding Author: **Rosalia Crupi** – rnglss92r55f251l@studenti.unime.it

Endocrine disruptors (EDs) are chemical substances capable of affecting endocrine system functioning and interfering with organ morphogenesis and physiological functions [1, 2]. The development and regeneration of bone tissues have a complex hormonal regulation, and therefore, bone tissue cells can be considered potential targets for endocrine disruptors [3]. In that regard, the aim of this research was to investigate the impact of ED exsposure on immunologiocal profoile and on the inflammatory response and oxidative stress after an immunological challenge. In particular, we employed an *in vitro* model (PBMC) and an *in vivo* model of collagen-induced arthritis (CIA). ED exposure caused worsening of clinical signs (erythema and edema in the hind paws), histological and radiographic changes, as well as behavioral deficits, induced by CII injections. Furthermore, ED exposure significantly increased the degree of inflammation and oxidative damage induced by arthritis [4]; this upregulation wasmore evident after exposure to ATR than to other EDs. The results fromour study suggest that exposure to EDs may play a deleterious role in the progression of RA; therefore, exposure to EDs should be limited.

[1] D'Amico, Ramona, et al. "Toxic Exposure to Endocrine Disruptors Worsens Parkinson's Disease Progression through NRF2/HO-1 Alteration." *Biomedicines* 10.5 (2022): 1073.
[2] Interdonato, Livia, et al. "Endocrine Disruptor Compounds in Environment: Focus on Women's Reproductive Health and Endometriosis." *International Journal of Molecular Sciences* 24.6 (2023): 5682.
[3] Di Paola, Davide, et al. "Chronic exposure to Vinclozolin induced fibrosis, mitochondrial dysfunction, oxidative stress, and apoptosis in mice kidney." *International Journal of Molecular Sciences* 23.19 (2022): 11296.
[4] Impellizzeri, Daniela, et al. "Blocking prokineticin receptors attenuates synovitis and joint destruction in collageninduced arthritis." *Journal of Molecular Medicine* (2023): 1-12.

WORSENING EFFECT OF BISPHENOL A ON OBESITY-INDUCED MOOD DISORDERS IN MICE: FOCUS ON ANXIETY-LIKE BEHAVIOR AND NEUROINFLAMMATION

Filomena Del Piano (1), Adriano Lama (2), Nicola Opallo (2), Federica Comella (2), Stefania Melini (2), Rosaria Meli (2), Maria Carmela Ferrante (1)

(1) Department of Veterinary Medicine and Animal Productions, University of Naples Federico II
(2) Department of Pharmacy, University of Naples Federico II

Corresponding Author: **Filomena Del Piano** - filomena.delpiano@unina.it

Bisphenol A (BPA) is a synthetic organic compound mainly used in the manufacturing of polycarbonates and epoxy resins with several applications and is frequently detected in biotic/abiotic matrices and foodstuffs. BPA is considered one of the most widespread endocrine-disrupting chemicals and it is associated with adverse consequences for both animal and human health. It also affects the nervous system and metabolic homeostasis [1, 2]. Obesity is the main risk factor for metabolic alterations as well as mood disorders [3], and exposure to environmental chemical stressors may contribute to the exacerbation of anxiety and depression behaviors. Here, we aimed to investigate the effects of BPA exposure on anxiety-like behavior and neuroinflammation induced by high-fat diet (HFD). Male C57Bl/6J mice were divided into 4 groups: control group (STD) receiving chow diet and BPA vehicle; STD group treated with BPA (50 ug/kg/die); HFD group receiving BPA vehicle; HFD group treated with BPA. The treatment started 12 weeks after HFD feeding and lasted 3 weeks. At the end of the experimental time, anxiety-like behavior was evaluated through open field (OF) and elevated plus maze (EPM) tests. Then, animals were sacrificed, and prefrontal cortex (PFC), hypothalamus, and amygdala were collected for molecular and biochemical analysis. OF and EPM tests revealed the worsening of anxiety-like behavior in HFD+BPA group. Indeed, the OF test showed a reduction of the thigmotaxis evaluated through the total distance travelled and the number of entries into the center of arena. This result was confirmed by EPM test, during which BPA amplified the reduction of time spent in open arms caused by HFD, with respect to the STD groups. The anxiogenic effect of BPA also emerged from hyperactivation of the hypothalamic-pituitary-adrenal (HPA) axis in PFC, highlighted by the increased transcription of Crh and its receptor. This effect was also accompanied by the activation of NLRP3 inflammasome and exacerbation of neuroinflammation in PFC of obese mice, as evidenced by the increased gene expression of TNF-a, IL-1b, and MCP-1. Contextually, BPA increased TNF-a levels in the hypothalamus and amygdala, and triggered mastocytosis in the hypothalamus. Overall, obtained data show that BPA exposure represents an additional risk factor for pathological features of obesity-induced mood disorders associated with HPA axis alterations, neuroinflammation, and immune activation.

[1] Pirozzi et al., 2020. Oral Bisphenol A Worsens Liver Immune-Metabolic and Mitochondrial Dysfunction Induced by High-Fat Diet in Adult Mice: Cross-Talk between Oxidative Stress and Inflammasome Pathway. Antioxidants (Basel).
[2] Mesnil et al., 2020. Brain Disorders and Chemical Pollutants: A Gap Junction Link? Biomolecules.
[3] Fulton et al., 2022. The menace of obesity to depression and anxiety prevalence. Trends Endocrinol. Metab.

SIRA

COMPARISON OF ADULT BUFFALO USE OF OVSYNCH AND P4 PROGRAM DURING DIFFERENT MONTHS OF THE YEAR

Massimo Capuano (1)

(1) Freelance Veterinarian, 71022 Ascoli Satriano, Foggia, Italy

Corresponding Author: **Massimo Capuano** - massimocapuano.vet@gmail.com

The infectious pressure of Brucellosis on buffalo farms and the market needs of buffalo milk, opposed to the natural reproductive performance of the species, suggest to prefer a reproductive management based on artificial insemination rather than natural mating.
Given the low tendency to the behavioural manifestations of the buffalo in oestrus, in the practical management of breeding it is essential to implement programs of synchronization of heats with fixed-time insemination (TAI). The two most widely used programs are called Ovsynch and Program P4. Following this logic, at a farm in the province of Foggia (average annual consistency of the adult herd 1031 heads), in the years from 2018 to 2021 I carried out a total of 6285 IAs in adult buffaloes, constantly guaranteeing an intercalving value of the herd of about 440 days, in the absence of natural mating. I applied the following synchronization protocols: Ovsynch – G0 GnRH / G7 PGF2α / G10 GnRH + IA; Program P4 – G0 GnRH+ insertion Prid / G7 PGF2α + extraction Prid / G10 GnRH + IA. The pregnancy rate obtained was 48% overall (maximum value 71% in January 2019, minimum value 26% in May 2020).
In this study I compared the effectiveness in the different months of the year of the synchronization programs implemented to determine which was the best solution in terms of result (pregnancy rate) and economic sustainability. In fact, the costs of the two programs are significantly different: €8/head for Ovsynch and €26/head for P4.
The pregnancy rates referring to the AIs carried out in the 4 years aggregated by months were as follows: with Ovsynch – December 55%, January 56%, February 51%, March 50%, April 47%, May 30%, June 32%, July 32%, August 52%, September 44%; with Program P4 – December 0, January 69%, February 0, March 49%, April 49%, May 44%, June 45%, July 42%, August 44%, September 50%. The trend was similar in each year.
The results obtained suggest that the P4 program allows significantly better reproductive performance in the months of May, June and July that justify the greater expense to be faced. In other months, it is preferable to use the cheaper Ovsynch.

[1] Giorgio A. Presicce, Domenico Vistocco, Massimo Capuano, Luigi Navas, Angela Salzano,Giovanna Bifulco, Giuseppe Campanile and Gianluca Neglia. Pregnancies following Protocols for Repetitive Synchronization of Ovulation in Primiparous Buffaloes in Different Seasons. . Vet. Sci. 2022, 9, 616.
[2] Neglia, G.; De Nicola, D.; Esposito, L.; D'Occhio, M.J.; Fatone, G. Reproductive management in buffalo by artificial insemination. Theriogenology 2020, 150, 166–172.
[2] Neglia, G.; Capuano, M.; Balestrieri, A.; Cimmino, R.; Iannaccone, F.; Palumbo, F.; Presicce, G.A.; Campanile, G. Effect of consecutive re-synchronization protocols for ovulation on pregnancy rate in buffalo (Bubalus bubalis) heifers out of the breeding season. Theriogenology 2018, 113, 120–126

76° CONVEGNO SISVET
BARI, 21-22-23 GIUGNO 2023

INTRAUTERINE USE OF OZONE AT CALVING IN DAIRY COW: PRELIMINARY INVESTIGATIONS

Carbonari A. (1), Burgio M. (1), Cicirelli V. (1), Aiudi G.G. (1), Catella C. (1), Lucente S. (1), Manginelli T. (2), Camero M. (1), Rizzo A. (1)

(1) Department Of Veterinary Medicine - University of Bari
(2) ASL BA

Corresponding Author: **Alice Carbonari** - alice.carbonari@uniba.it

Ozone (O3) is a triatomic form of oxygen. As O3 rapidly dissociates into water and releases a reactive form of oxygen that may oxidize cells, the gas mixture of O3/O2 is used in medicine. It was first used as a microbicidal molecule, owing to its oxidant properties. During the last century, several studies revealed other important activities, such as its antioxidant and anti-inflammatory functions and its immuno-stimulatory effect.
Considering the high incidence of complications caused by bacterial colonization of the uterus in postpartum (PP) cows, the aim of the present study was to evaluate the effects of intrauterine administration of an Oxygen/Ozone gaseous mixture, 24 hours after delivery considering clinical and reproductive parameters, uterine bacterial contamination and inflammatory status. To evaluate O3 anti-inflammatory and immune-stimulatory effects, interleukin 1β and 10 concentrations were assessed.
The experiment was conducted on 9 Holstein cows, randomly assigned to two groups: Treated group (T-group) including 6 cows, treated with an intrauterine administration of 200 ml of an Ozone/Oxygen (O3/O2) gas mixture at 20 µg of O3/ml, within 24 hours PP; Control group (C-group) including 3 cows, treated with an intrauterine administration of 200 ml of saline solution, within 24 hours PP. A clinical evaluation and the sampling of cervico-vaginal mucus (CVM) were performed before treatment, and at 7, 14, 21, and 28 days PP. The cervico-vaginal mucus samples were used to assess interleukin 1β and 10 concentrations and to perform microbiological investigations.
The clinical evaluation of the animals after intrauterine treatment with the Oxygen/Ozone gas mixture did not reveal any adverse? systemic or local side effects. As to CVM IL-1β concentrations, statistically significant differences between the T-group and C-group at T7 and T28 were observed while no differences were observed for IL-10 concentrations. No significant differences in microbiological investigations were reported between the T-group and C-group.
These preliminary results showed that the intrauterine insufflation of O3 has no adverse effects clinically valuable and that O3 could interact in the inflammatory process of the uterine involution. It could be interesting to evaluate whether a single or repeated O3 treatments may be able to reduce the uterine bacterial load and/or shorten the uterine involution process.

[1] Duričić, D., Valpotić, H., Samardžija, M., 2015. Prophylaxis and therapeutic potential of ozone in buiatrics: Current kno wledge. Anim. Reprod. Sci. 159, 1–7. https://doi.org/10.1016/j.anireprosci.2015.05.017
[2] Sciorsci, R.L., Lillo, E., Occhiogrosso, L., Rizzo, A., 2020. Ozone therapy in veterinary medicine: A review. Res. Vet. Sci. 130, 240–246. https://doi.org/10.1016/j.rvsc.2020.03.026
[3] Lillo, E., Cordisco, M., Trotta, A., Greco, G., Carbonari, A., Rizzo, A., Sciorsci, R.L., Corrente, M., 2023. Evaluation of antibacterial oxygen/ozone mixture in vitro activity on bacteria isolated from cervico-vaginal mucus of cows with acute metritis. Theriogenology 196, 25–30. https://doi.org/10.1016/j.theriogenology.2022.10.031

INNOVATIVE CLINICAL EXPERIENCES IN THE TREATMENT OF EQUINE HYPOFERTILITY

Alessandra Diaz (1), Bianca Rollin (1), Alberto Crovace (2), Enrica Potop (1), Sylvia Versace (2), Eraldo Sanna Passino (2,3)

(1) NFC - New Fertility Center, Sassari, Italia
(2) ODVU - Ospedale Didattico Veterinario Universitario, Dipartimento di Medicina Veterinaria, Università degli Studi di Sassari, Italia
(3) Centro di Chirurgia Comparata, Università degli Studi di Sassari, Italia

Corresponding Author: **Eraldo Sanna Passino** - esp@uniss.it

Endometrial disease is a frequent cause of infertility in mares and has a significant economic impact on the sport horse breeding industry. Reduced fertility is often the result of various causes: among these, the advanced age of the mare and the state of the endometrium play a central role, sometimes with a synergistic effect between these two factors.
The purpose of this work was to use innovative and unconventional treatments to improve the quality of the endometrium and ensure a recovery of the mare's fertility since the objective of the breeder is to obtain the highest possible pregnancy rate, especially in prestigious genealogies. For this purpose, intrauterine infusion of PRP (platelet-rich plasma) and Ozone have been compared as innovative treatments for infertility, evaluating their efficacy from a clinical point of view, both by ultrasound monitoring of the uterus, than in the final result with the diagnosis of pregnancy.
In our study, among all the mares treated during the last two breeding seasons, we selected 22 S.I. mares, aged between 10 and 23 years and weighing between 450 and 600 kg, with a history of hypofertility. The PRP, prepared according to the method described by prof. M. Del Bue and modified at the NFC, was placed in the uterus as it is 24 hours before the expected ovulation. The O3 therapy protocol used involves intrauterine insufflation of 240 ml of O3 gas at 20 ug/ml three times 24 hours apart in mid-estrus and at least 24 hours before ovulation. The mares were divided into three groups (A- acute endometritis; B- chronic endometritis; C- post breeding endometritis). In all the mares treated, the results were very encouraging with an excellent percentage of problems solved and pregnancies completed.
The clinical study has made it possible to standardize specific protocols for each pathology. An important aspect is linked to the decrease, in our case non-use, of antibiotic therapy, a highly topical issue and confirms the importance and validity of regenerative medicine and new therapeutic protocols for the treatment of these pathologies with excellent management of difficult and complicated situations and improvement of reproductive efficiency.

[1] Carluccio A, Veronesi M C, Plenteda D, Mazzatenta A. Platelet-rich plasma uterine infusion and pregnancy rate in barren mares with chronic degenerative endometritis. Pol. J Vet Sci. 2020 Sep;23(3):431-438.
[2] Katila T., Ferreira-Dias (2022). Evolution of the concepts of endometriosis, post breeding endometritis, and susceptibility of mares. Animals 12(6)779.
[3] Nascente, E., de, P Chagas, S.R., Pessoa, A. V. C., Matos, M. P. C. Andrade, M. A., & Pascoal, L. M. (2019). Potencial antimicrobiano do ozônio: aplicações e perspectivas em medicina veterinária. Pubvet, 13, 130.
[4] Reghini MFS, Ramires Neto C, Segabinazzi LG, Castro Chaves MMB, Dell'aqua CPF, Bussiere MCC, et al. (2016) Inflammatory response in chronic degenerative endometritis mares treated with platelet-rich plasma. Theriogenology 86:516, 22.

THE *IN VITRO* EFFECT OF DIFFERENT OZONE PREPARATIONS AGAINST BACTERIA RESPONSIBLE FOR EQUINE ENDOMETRITIS.

Gian Guido Donato (1), Tiziana Nervo (1), Maria Cristina Stella (1), Patrizia Nebbia (1), Giovanna Romano(2), Denis Necchi (3), Mariagrazia Lucia Poletto (1), Daniela Scalas (1), Patrizia Robino (1).

(1) Department of Veterinary Sciences, University of Turin, Grugliasco, Italy
(2) Centro Equino Arcadia, Villafranca Piemonte, Italy
(3) Keros Embryo Transfer Center, Passendale, Belgium

Corresponding Author: **Gian Guido Donato** - gianguido.donato@unito.it

Equine endometritis represents a complex challenge for the researchers working in equine breeding that may lead to substantial economic losses. Infectious endometritis is observed in up to 60% of barren mares and requires intrauterine or systemic antibiotics therapy [1]. Within this framework, ozone could play an important role due to its antimicrobial effect, thus supporting its use in equine reproduction practice. The goal of the present study was to characterize the in vitro antimicrobial properties of two different ozone preparations against the most common bacteria linked to endometritis. Accordingly, we tested strains of Streptococcus equi subsp. zooepidemicus, Escherichia coli, Staphylococcus aureus, Pseudomonas aeruginosa and Klebsiella pneumoniae isolated from the uterus of mares diagnosed with infectious endometritis. Briefly, bacterial suspensions were prepared and adjusted to 0.5 McFarland turbidity in sterile saline solution and cultured on Tryptic Soy Agar or Columbia Blood Agar dishes at 37°C for 24h. For each bacterium, the cultures were divided in different groups: untreated positive control, distilled water (H2O), ozonated distilled water (H2O-O3, 40 µg/ml for 10 minutes), gaseous O3 at a concentration of 15 µg/ml and 40 µg/ml for 1, 3 and 5 minutes. Plates from H2O and H2O-O3, groups were covered, respectively, with 2 mL of ozonated and nonozonated water for 10 minutes, while plates from gaseous O3 groups were placed uncovered inside of a glass hermetic box coupled to an O3 generator. Gaseous ozone drastically reduced the growth of bacteria in all concentrations and times tested, resulting in a minimal residual bacterial growth (less than 10 CFU/plate) after 5 minutes of continuous gas flow at 40 µg/ml. On the contrary, ozonated water didn't reduce bacterial growth since bacterial density was similar to the one observed in the control groups (>2x107CFU/plate). In conclusion, gaseous ozone, even at low concentrations, represents a promising therapy for infectious endometritis, while ozonated water at the concentration and time used in this study doesn't seem to be effective. Thus, it can be speculated that gaseous ozone could contribute to scale down or even prevent the use of antibiotics, which are still commonly applied in broodmares, even in absence of clinical signs of endometritis, bacterial culture and antimicrobial sensitivity test [2], a dangerous habit that may lead to an increase of antibiotic-resistant bacterial strains.

[1] Leblanc MM, Causey R. Clinical and subclinical endometritis in the mare: both threats to fertility. Reprod Domest Anim 2009; 44: 10–22.
[2] Mouncey R, Arango-Sabogal JC, de Mestre A, Verheyen K. Descriptive Study of Medication Usage and Occurrence of Disease and Injury During Gestation in Thoroughbred Broodmares. J Equine Vet Sci 2022; 118: 104104.

76° CONVEGNO SISVET
BARI, 21-22-23 GIUGNO 2023

ALPHA-FETOPROTEIN IN FOALS AFFECTED BY PREMATURITY, SEPSIS AND NEONATAL ENCEPHALOPATHY

Nicola Ellero (1), Aliai Lanci (1), Carolina Castagnetti (1,2), Francesca Perina (1), Francesco Dondi (1), Jole Mariella (1)

(1) Department of Veterinary Medical Sciences, University of Bologna, Via Tolara di Sopra 50, Ozzano dell'Emilia, 40064 Bologna, Italy
(2) Health Sciences and Technologies Interdepartmental Center for Industrial Research, University of Bologna, 40100 Bologna, Italy

Corresponding Author: **Nicola Ellero** - nicola.ellero3@unibo.it

Alpha-fetoprotein concentration (AFPc) has recently been reported in a population of healthy foals [1], and proposed as a biomarker of sepsis in foals born from mares with experimentally induced ascending placentitis [2]. This study aimed to describe the diagnostic and prognostic value of plasma AFPc in foals spontaneously affected by different pathological conditions. All foals less than 72h old that met the following diagnostic criteria were included in the study: prematurity (PRE), when born prior to 320 days of gestation with immature physical characteristics; sepsis (SEP), in the presence of both positive blood culture and SIRS; neonatal encephalopathy (NE), with evidence of hypoxic-ischemic insult. They received a complete physical and haematobiochemical evaluation and blood culture collection at hospital admission. Forty-six foals with an average age of 16h were enrolled in the study and divided into: PRE group (n=7); SEP group (n=14); NE group (n=25). AFPc was measured in plasma collected at admission using a commercially available immunoassay previously validated for horses (Immulite® 2000, Siemens). Differences between groups were analyzed with Kruskal-Wallis test, and Spearman's rank correlation coefficient was evaluated among different variables. AFPc was higher in foals in the PRE, SEP and NE groups than in healthy ones ($P<0.001$), but was not able to discriminate between different pathological conditions and outcomes. Overall, AFPc was negatively correlated with SAA levels ($P=0.011$; $r=-0.4$). AFP acts as a growth regulator, immunoregulator and antioxidant [3]. Although AFP has been suggested as a positive acute phase protein, it appears to be a useful but non-specific indicator of neonatal disease, since it upregulates not only in the presence of SIRS and bacteremia, but also during prematurity and hypoxic-ischemic damage. The relationship between AFP and SAA should be further investigated in light of the suppressive role of AFP on macrophages, which through IL-6 synthesis are the main promoters of hepatic SAA production [4].

[1] Lanci et al. High-risk pregnancy is associated with increased alpha-fetoprotein concentrations in the amniotic fluid and foal plasma. J Equine Vet Sci 2022, 119, 104124.
[2] Borba et al. Peripheral blood markers of sepsis in foals born from mares with experimentally induced ascending placentitis. Vet Rec 2020, 187, 29.
[3] Rizzo et al. Alpha-fetoprotein in animal reproduction. Res Vet Sci 2019, 123, 281-5.
[4] Witkowska-Piłaszewicz et al. Serum amyloid A in equine health and disease. Equine Vet J 2019, 51, 293-8.

76° CONVEGNO SISVET
BARI, 21-22-23 GIUGNO 2023

NUTRIENT RESTRICTION DURING EARLY GESTATION IN DAIRY CATTLE IMPAIRS OVARIAN DEVELOPMENT IN THE OFFSPRING

Frau A. (1), Edache D. (2), Sale S. (3), Gallo A. (4), Franciosi F. (5), Miragliotta V. (6), Succu S. (1), Bebbere D. (1), Atzori A.S. (2), Mossa F. (1)

(1) Department of Veterinary Medicine, University of Sassari, Sassari, Italy
(2) Department of Agriculture, University of Sassari, Sassari, Italy
(3) Veterinary gynecologist, Dorgali (NU), Italy
(4) Department of Animal Science, University Cattolica del Sacro Cuore, Piacenza, Italy
(5) Department of Veterinary Medicine and Animal Sciences, University of Milan, Italy
(6) Department of Veterinary Medicine, University of Pisa, Italy

Corresponding Author: **Adele Frau** - a.frau4@studenti.uniss.it

Female beef calves born to mothers exposed to a nutritionally restricted diet in early gestation had a reduced total number of ovarian follicles (ovarian reserve; Mossa et al., 2013).
The aim of this work was to investigate the impact of maternal nutrient restriction from shortly before conception to early gestation on the development of the reproductive tract in female progeny in dairy cattle.
Holstein-Friesian heifers (n=42) homogenous for age (14-17 mo.) and weight (366.2□41.1 kg) were randomly assigned to three experimental groups and, starting 10 d (days) before artificial insemination (AI), were individually fed at: (i) 0.6 of their maintenance energy requirements (M) up to 80 (Nutrient Restricted,NR80;n=16) or (ii) 120 DG (Days of gestation;NR120,n=16), and (iii) 1.8M until 120 DG (Control, C; n=10). Estrus cycles were synchronized, and heifers were inseminated with sex-sorted semen from a single sire. Pregnancy was diagnosed and confirmed via ultrasound (MyLab Omega, Esaote, with 4-10MHz sectorial probe) 28 and 55 d after AI, respectively. After the end of the differential diet, all heifers were group fed ad libitum until calving.
Twenty-two single female calves were born (NR80=8; NR120=9; C=5); body weight (BW) and height at withers (H) were measured regularly until slaughter at 4.5 mo. Ovaries were measured, weighed, visible antral follicles were counted and cumulus oocytes complexes (COCs) were collected. Data were analyzed with R software with One-way ANOVA and mean contrast separated with Tukey post-hoc test. Results are expressed as mean±SEM.
BW at birth was lower in NR80 than C calves ($p<0.05$) and similar between NR120 and NR80 (C=41.4±1.1; NR80=36.7±0.6; NR120=38.3±1.2 kg), while BW at slaughter and H were similar among groups. Ovarian volume was similar among groups. Ovarian weight was lower ($p<0.05$) in NR120 compared to C and similar between NR120 and NR80 (C=10.4□1.3; NR80=7.4±0.9; NR120=6.7□0.5 g). NR120 heifers had less ($p<0.05$) visible antral follicles than C while no difference was detected between NR80 and C (C=197.2□36.5; NR80=150.1±20.9; NR120=104.2□10.7). Fewer COCs were retrieved ($p<0.05$) from NR120 and NR80 compared to C ovaries (C=75.8±12.57; NR80=48±3.5; NR120=48.2±6.68).
Maternal exposure to undernutrition from preconception to 120 DG resulted in a reduction of ovarian weight, visible antral follicles and retrieved COCs in their female offspring indicating a potential impairment of the size of the ovarian reserve.

[1] Mossa et al. 2013, Maternal undernutrition in cows impairs ovarian and cardiovascular systems in their offspring. Biology of Reproduction, 88(4), 1–9.

MICRORNA CARGO EVALUATION IN SPERMATOZOA FROM LOW-FERTILITY BULLS BEFORE AND AFTER INCORPORATION OF EXTRACELLULAR VESICLES ISOLATED FROM PROVEN FERTILTY BULLS' SEMINAL PLASMA

Giulia Gaspari (1), Emanuele Capra (2), Marina Cretich (3), Roberto Frigerio (3), Fausto Cremonesi (1) Anna Lange Consiglio (1)

(1) Università degli Studi di Milano, Dipartimento di Medicina Veterinaria e Scienze Animali (DIVAS)
(2) Istituto di Biologia e Biotecnologia Agraria, Consiglio Nazionale delle Ricerche IBBA CNR, Lodi, Italy
(3) Istituto di Scienze e Tecnologie Chimiche "Giulio Natta", Consiglio Nazionale delle Ricerche SCITEC-CNR, Milan, Italy

Corresponding Author: **Anna Lange Consiglio** - anna.langeconsiglio@unimi.it

Seminal plasma (SP) contains extracellular vesicles (EVs) that vehicle RNA, microRNA (miRNA), proteins, and other molecules able to influence the biological function of spermatozoa. In a study on semen provided by a bull center with respective progeny data, the incorporation of EVs derived from SP (SP-EVs) of proven fertility bulls into low-fertility bulls' spermatozoa was found to improve motility and rate of in vitro embryo production [1].
Assuming that this change is due to the transfer of miRNAs contained in EVs, this study investigated i) the miRNA cargo of low-fertility spermatozoa before and after treatment with SP-EVs of proven fertility bulls; ii) the differences in miRNA cargo between SP-EVs of proven and low-fertility bulls. At first, EVs isolated from SP of 3 proven fertility bulls (by ultracentrifugation at 100.000xg for 1h at +4°C) were labeled and co-incubated with low fertility spermatozoa (400x106 EVs in 1 ml with 5x106 sperm) and their presence in the middle tract was detected by confocal and transmission electron microscope after 3h. RNA extraction was carried out on spermatozoa of three low-fertility bulls, before and after EV-treatment to prepare small RNA libraries, which were sequenced by Illumina Hiseq 2500 System.
In the second step, SP-EVs of 3 proven and 3 low-fertility bulls were isolated, and NanoSight analysis did not detect any differences in terms of concentration. Then, RNA from SP-EVs of proven and low-fertility bulls was extracted to create libraries.
From the sequencing analysis, 48 differently expressed (DE) miRNAs in low-fertility spermatozoa before and after their treatment with EVs were identified. Among them, miR-2284x, miR-2284y and miR-101 were found to be more present in spermatozoa incubated with EVs than in no treated ones. In SP-EVs, 82 DE miRNAs between proven and low-fertility bulls were detected, with miR-2284x most present in the SP-EVs of proven fertility bulls. These results show that miR-2284x present in the EVs of proven fertility bulls could be transferred into the spermatozoon of low-fertility ones improving their fertility in vitro [1]. Literature searches revealed that this miRNA targets TFB2M gene, more highly expressed in embryos derived in vitro from higher fertility bulls [2]. Our data support the hypothesis that alterations of EV molecule delivery in vivo, such as miRNAs, could influence mechanisms regulating sperm functioning, and thus the fertility.

[1] Lange-Consiglio et al. Extracellular vesicles from seminal plasma to improve fertilizing capacity of bulls. REPRODUCTION AND FERTILITY 3:313–327, 2022.
[2] Kropp et al. Male fertility status is associated with DNA methylation signatures in sperm and transcriptomic profiles of bovine preimplantation embryos. BMC GENOMICS 18:280, 2017.

NEONATAL EVALUATION OF DROMEDARY CAMEL CALVES AT BIRTH: PRELIMINARY OBSERVATIONS

Davide Monaco (1), Zeeshan Ansar (2), Aliai Lanci (3), Maria Cristina Veronesi (4)

1 Department of Veterinary Medicine, University of Bari Aldo Moro, Italy
2 Department of Advanced Biotechnology and Research, Salam Veterinary Group, Buraidah, Saudi Arabia
3 Department of Veterinary Medical Sciences, Università degli Studi di Bologna, Italy
4 Department of Veterinary Medicine and Animal Sciences, Università degli Studi di Milano, Italy

Corresponding Author: **Davide Monaco** - davide.monaco@uniba.it

The transition from the intrauterine to the extrauterine environment represents a critical phase in the newbornlife, as many maturation and adaptation/adaptive processes have to take place. Caring for newborn is particularly challenging in veterinary medicine, where species-related differences require specific knowledges; the employment of a routine evaluation method immediately after birth is hence fundamental to detect any developmental abnormalities or maladjustment to extrauterine life, and to promptly identify newborns requiring for medical intervention. To date, very few information are available regarding the physiological features of the newborn dromedary calf at birth (1); therefore, the aim of the present study was to describe the main physiological parameters in healthy dromedary newborn calves immediately after birth. Ten healthy dromedary calves born at term after a normal gestation and spontaneous calvings were enrolled. Parturitions were monitored in order to detect any alteration during birth process and the following parameters were evaluated for each newborn at birth: gender, weight (Kg), rectal temperature (°C), presence of erupted teeth, attachment of the epidermal membrane, heart rate per minute (BPM), respiration rate per minute (RPM), mucous membrane colour (eye and mouth), ear irritability reflex (lateral and bilateral), body muscle tone, suckling reflex (SR), time of head shaking (THS, min), time to raise neck (TRN, min), time to sternal recumbency (TSR, min), and time to first vocalizations (TFV, min). Among the 10 newborns, 6 were males and 4 were females. Median (min-max) values for each parameter were calculated as follow. Weight: 42 Kg (36.4-47.4); rectal temperature: 37°C (36.6-37.6); BPM: 121 (86-160); RPM: 32 (24-36); THS: 2 min (1-12); TRN: 3 min (2-14); TSR: 10 min (4-19); TFV: 14 min (4-36). Teeth were not erupted in 7/10 newborns, and epidermal membrane was attached in 3/10 newborns. Eye mucous membranes were for 20% cases pink, 40% reddish, and 40% pigmented; mouth mucous membranes were 40% pink, 10% reddish, and 50% pigmented. Lateral and bilateral ear irritability reflex were 100% vigorous, body muscle tone was with some movements in 40% and active/sternal in 60% cases, while SR was weak in 40% newborns and vigorous in 60% newborns. These preliminary observations may be implemented in order to establish normal ranges for the main physiological parameters in the newborn dromedary calf at birth.

[1] Tibary A, Rodriguez J, Walker P, Anouassi A. Neonatal Care and Neonatal Emergencies in Camelids. In: Robert A. Smith (Ed) Proceedings of the Forty-First Annual Conference. American Association of Bovine Practitioners. September 25-27, 2008. Charlotte, North Carolina. Frontier Printers, Inc.
[2] I. Hammadi, M. Chniter, M. Atigui, M. Brahmi, M.M. Seddik, W.B. Salem, F. Lévy, R. Nowak, M. Hammadi, Dam parity and calf sex affect maternal and neonatal behaviors during the first week postpartum in stabled Maghrebi dairy camels, Animal, Volume 15, Issue 3, 2021, 100149
[3] A. Tibary, A. Anouassi Obstetrics and neonatal careA. Tibary (Ed.), Theriogenology in camelidae: anatomy, physiology, BSE, pathology and artificial breeding (Actes ed.), Institut Agronomique et Veterinaire Hassan II (1997), pp. 391-409.

76° CONVEGNO SISVET
BARI, 21-22-23 GIUGNO 2023

HEMATOLOGICAL AND BIOCHEMICAL PROFILE IN HEALTHY NEWBORN DROMEDARY CAMEL CALVES FROM BIRTH TO 7 DAYS OF AGE

Monica Probo (1), Ahmed Abd Elraouf (2), Francesca Freccero (3), Davide Monaco (4)

(1) Department of Veterinary Medicine and Animal Sciences, Università degli Studi di Milano, Italy
(2) Department of Advanced Biotechnology and Research, Salam Veterinary Group, Saudi Arabia
(3) Department of Veterinary Medical Sciences, Università degli Studi di Bologna, Italy
(4) Department of Veterinary Medicine, University of Bari Aldo Moro, Italy

Corresponding Author: **Monica Probo-** monica.probo@unimi.it

The knowledge of hematologic and biochemical reference values in healthy animals is a fundamental tool in the early detection and monitoring of disturbances, as many blood parameters have been shown to vary in relation to species, breed and age (1-3). To date, there is a dearth in the literature regarding the reference ranges of the newborn dromedary calf, particularly during the first days after birth (4). The aim of this study was to evaluate the age-related changes of hematologic and biochemical parameters in healthy newborn dromedary camel calves during the first week of life. The experimental protocol was approved by the ethic committee of the Department of Veterinary Medicine, University of Bari Aldo Moro (approval number 15/2022). Ten healthy dromedary camel calves were blood sampled from the jugular vein immediately after birth before colostrum intake (T0), and at 24 hours (T24), 72 hours (T72) and 7 days (T7) after birth. For all the newborn dromedary calves, the following data were recorded at birth: sex, weight, maturity and absence of malformations. Health status was assessed on each sampling day through a complete physical examination performed by veterinarians. The complete blood cell count (ABX MICROS ES 60®, HORIBA, Japan) included: RBC count, hemoglobin (Hb), hematocrit (Ht), MCV, MCH, MCHC, RDV, platelets (PLT), MPV, WBC count and lymphocyte, neutrophil, monocyte, esosinophil and basofil % and absolute numbers (#). The biochemical panel (AU480® automated clinical chemistry analyzer, Beckman Coulter, USA) included: albumin, AST, ALT, ALP, creatinine, glucose, urea, total protein, total (tBIL) and direct (dBIL) bilirubin, Cl-, Na+ and K+ concentrations. Statistical analysis detected many significant differences ($p<0.05$) among sampling times. In details, total WBC, neutrophil# and creatinine were lower at T0 compared to all subsequent samples; lymphocyte#, eosinophil#, Ht and MCV decreased from T0 to T72-T7; Hb decreased from T0 to T72; PLT increased from T24-T72 to T7; AST increased from T0 to T7, Na+ decreased at T7 compared to all previous, and dBIL decreased from T0-T24 to T7; glucose and ALT increased from T0 to T24-T72; tBIL decreased from T24 to T7, and ALP decreased from T0-T24 to T72-T7. Although preliminary, the present results showed that age-specific values should be considered for a precise interpretation of hematologic and biochemical values in newborn dromedary calves, as already demonstrated for newborns of other species.

[1] Mohri M, Sharifi K, Eidi S. Hematology and serum biochemistry of Holstein dairy calves: Age related changes and comparison with blood composition in adults. Res. Vet. Sci. 2007, 83:30–39.
[2] Faramarzi B, Rich L. Haematological profile in foals during the first year of life. Vet. Rec. 2019, 184:503.
[3] Veronesi MC, Gloria A, Panzani S, Sfirro MP, Carluccio A, Contri A. Blood analysis in newborn donkeys: hematology, biochemistry, and blood gases analysis. Theriogenology 2014, 15:294-303.
[4] Tharwat M, Al-Sobayil F, EL-Deeb W. Haematology and serum biochemistry profiles in dromedary camel calves during the first month of age. J. Camel Pract. Res. 2015, 22:107-122

76° CONVEGNO SISVET
BARI, 21-22-23 GIUGNO 2023

OXYGEN/OZONE GASEOUS MIXTURE ACTIVITY IN VITRO ON CAPRINE HERPESVIRUS TYPE 1 (CPHV-1), ISOLATED FROM GOATS WITH VULVO-VAGINITIS

Lillo E. (1), Pellegrini F. (1), Carbonari A. (1), Burgio M. (1), Cicirelli V. (1), Aiudi G.G. (1), Mnerosky D.(1), Losurdo M. (2), Tempesta M. (1), Rizzo A. (1)

(1) Department of Veterinary Medicine - University of Bari
(2) ASL Matera

Corresponding Author: **Edoardo Lillo** - edoardolillo@gmail.com

Alphaherpesviruses cause genital lesions and reproductive failure in both humans and animals. Some of them infect the genital tract establishing a lifelong latent infection in the lumbosacral sensory ganglia which could be recurrently reactivated by immunosuppression or hormonal changes. Caprine herpesvirus type -1 (CpHV-1) is a widespread virus in goat herds which could affect the reproductive apparatus causing vulvo-vaginitis, abortions and stillbirth in female, and balanoposthitis in bucks. As there are currently no commercially available vaccines and because of the emergence of antiviral drugs resistance, the control of CpHV-1 is based on prevention and eradication. Ozone (O3) is an oxidating gas showing a strong microbicidal activity on bacteria, fungi, viruses and protozoa. The in vitro virucidal action of an Ozone/Oxygen (O3/O2) gas mixture on CpHV-1 was assessed in this study.
A CpHV-1 strain isolated from the vaginal swabs, collected in female goats with vulvo-vaginitis, was used. A medical O3 generator was used to produce an O3/O2 gas mixture containing 20 µg of O3/ml. Petri dishes containing 1 ml of CpHV-1 were put into an hermetic box and exposed to the gas mixture for 30 seconds, 1, 3 and 5 minutes. After the exposition to the gas, the virus was titrated in 96-well plates containing Madin Darby Bovine Kidney (MDBK) cells incubated for 72 hours at 37 °C in 5% CO2. The titre was calculated on the basis of the cytopathic effect on MDBK cells.
CpHV-1 exposed for 30 seconds, 1, 3, and 5 minutes, showed a significant viral titre reduction of 1.25, 1.25, 1.5 and 2 log10 TCID50/50 µl, respectively, compared with control group.
The virucidal activity of O3 is likely accounted for by lipid and/or protein peroxidation of envelope and/or capsid; in addition, it could damage nucleic acids. Although these results are interesting from a microbiological point of view, further studies are necessary to evaluate the long-term effects on vulvar and vaginal epithelial cells in vitro and then the clinical efficacy of the in vivo treatment of herpetic genital lesions caused by CpHV-1 in goats. Moreover, the similar association between CpHV-1 and Herpes simplex virus type -2 (HSV-2) also open therapeutic perspectives for HSV-2 infection in humans.

[1] Camero M, Marinaro M, Losurdo M, Larocca V, Bodnar L, Patruno G, et al. Caprine herpesvirus 1 (CpHV-1) vaginal infection of goats: Clinical efficacy of fig latex. Nat Prod Res 2016;30:605–7. https://doi.org/10.1080/14786419.2015.1028061.
[2] Sciorsci RL, Lillo E, Occhiogrosso L, Rizzo A. Ozone therapy in veterinary medicine: A review. Res Vet Sci 2020;130:240–6. https://doi.org/10.1016/j.rvsc.2020.03.026.

76° CONVEGNO SISVET
BARI, 21-22-23 GIUGNO 2023

THE EFFECTS OF UTERINE OZONE INSUFFLATION IN MARES AFFECTED BY ENDOMETRITIS: PRELIMINARY RESULTS

Rebecca Moroni (1), Paola Marmorini (2), Diana Fanelli (1), Pierre Melanie (1), Carlo Cantile (1), Fabrizio Passamonti (3), Francesco Camillo (1), Alessandra Rota (1), Duccio Panzani (1)

(1) Dipartimento di Scienze Veterinarie, Università di Pisa. Via Livornese (Lato Monte), 1289, 56122, San Piero a Grado, Pisa (PI), Italia
(2) Veterinario Libero Professionista, Cenaia, Pisa (PI), Italia
(3) Dipartimento Medicina Veterinaria, Unità di Malattie Infettive, Via S. Costanzo 4, 06126, Perugia (PG)

Corresponding Author: **Rebecca Moroni** - rebecca.moroni@phd.unipi.it

Endometritis represents one of the main causes of subfertility in mares. Recently, alternative therapies such as ozone uterine insufflation have been proposed [1] [2]. The present study aimed to describe uterine ozone (O3) effects on mares affected by endometritis in terms of pregnancy rate (PR), cytological, microbiological, and histopathological findings (altogether: diagnostic procedures = DP). In Study 1, five former embryo recipient mares, aged 10-21 years, were artificially inseminated (AI) for 2 control cycles (N=10) with fresh pooled semen from two fertile stallions and flushed for embryo recovery. In another 2 cycles, the same mares underwent DP and O3 treatment, in the first cycle, and DP, AI, with the same stallions, and uterine flushing for embryo recovery, in the second cycle. In Study 2, five barren commercial mares, aged 16-20 years, underwent DP followed by O3 treatment, in the first cycle, and DP, AI, with different commercial stallions and semen, and pregnancy diagnosis, in the second cycle. In both studies, treatment consisted of intrauterine O3 insufflation (volume: 300 ml; [O3]: 40 µg/ml), for 3 consecutive days. Samples for cytological and microbiological evaluation were collected by uterine lavage with 100 ml of Sterile Ringer Lactate, while samples for histopathology by endometrial biopsy. Fisher exact test was used to compare results pre and post O3 treatment, when indicated. In Study 1, 9/10 cycles (90%) before and 4/5 (80%) after O3 treatment resulted in an embryo recovery (NS), while, in Study 2, 0/5 mares before and 4/5 (80%) after O3 treatment were pregnant at 14 and 60 days (P=0.0476). Considering Study 1 and 2 altogether, only two mares resulted positive at cytological examination pre O3 treatment and both were negative after; only one mare was cultured positive, before and after treatment, and histopathology score improved post O3 treatment in 6/10 mares (NS). In conclusion, intrauterine ozone treatment for equine endometritis, appeared not harmful for fertility and able to improve pregnancy rates in barren mares. Further studies in a larger number of animals are warranted to confirm these results and to better describe the effects of such treatment on uterine quality.

[1] BM Escandón, JS Espinoza, FP Perea, F Quito, R Ochoa, GE López, DA Galarza, JP. Garzón; Intrauterine therapy with ozone reduces subclinical endometritis and improves reproductive performance in postpartum dairy cows managed in pasture-based systems; Trop Anim Health Prod, 52 (2020), pp. 2523-2528, 10.1007/s11250-020-02298-3
[2] JC Ferreira, RS Amaral, LM Cardoso, SP Marchió, MA Rodrigues, CEF Alves, C. Meira; Endometrial angiogenesis induced by uterine insufflation with an oxygen–ozone gas mixture in mares; Reprod Domest Anim, 56 (2021), pp. 1176-1183, 10.1111/rda.13958

RETROSPECTIVE ANALYSIS ABOUT FACTORS ASSOCIATED WITH UMBILICAL DISEASES IN NEONATAL FOALS

Francesca Perina (1), Jole Mariella (1), Nicola Ellero (1), Sara Velluti (3) Francesca Freccero (1), Carolina Castagnetti (1,2), Aliai Lanci (1)

(1) Department of Veterinary Medical Sciences, University of Bologna, Via Tolara di Sopra 50, Ozzano dell'Emilia, 40064 Bologna, Italy
(2) Health Sciences and Technologies Interdepartmental Center for Industrial Research (CIRI-SDV), University of Bologna, 40100 Bologna, Italy
(3) Practitioner, Reggio Emilia, Italy

Corresponding Author: **Francesca Perina** - francesca.perina2@unibo.it

In neonatal foals, umbilical remnants (UR) can be affected by infectious and non-infectious diseases. The aim of this study was to retrospectively evaluate history, management and clinical factors that may be related to the occurrence of UR diseases. All the clinical reports of foals born or hospitalized within 24 h of age at a Veterinary Teaching Hospital from 2017 to 2021 were reviewed. Umbilical Remnant Pathologies (URP) considered were: patent urachus, omphalitis, umbilical abscess, umbilical hematoma, umbilical hernia. Forty/183 (21.9%) foals developed URP, while 143/183 (78.1%) had Normal Umbilical Remnant (NUR). Twenty-four/40 (60%) foals had patent urachus, 16/40 (40%) omphalo-arteritis, 10/40 (25%) urachitis, 9/40 (22.5%) umbilical abscess, 4/40 (10%) omphalo-phlebitis, 3/40 (7.5%) umbilical hematoma, 12/40 (30%) had more than one URP. Thirteen/40 (32.5%) had hyperthermia. The frequency of URP was higher in foals hospitalized after birth (17/46 vs 23/137; p=0.0068), was lower in foals which had access to the paddock before 3rd day of life (p=0.0426) and higher in recumbent foals (p=0.0001). URP occurred more frequently after dystocic delivery (p=0.0068), prolonged stage II parturition (19±20.51 vs 13±6.41 in NUR; p=0.0279), when manual traction was applied at foaling (p=0.0005), and in foals with lower APGAR score (8±1.72 vs 9±0.86 in NUR; p=0.0063). Sepsis (p=0.0245), Neonatal Encephalopathy (p=0.0014), meconium retention (p=0.0241) and congenital flexural limb deformities (p=0.0049) were the most common associated diseases. Furthermore, UC coiling, abnormal UC rupture, umbilical haemorrhage and increased UR volume were significantly higher in URP group than in NUR group (p=0.0329; p=0.0191; p=0.0007; p<0.00001 respectively). Medical treatment was successful for complete resolution in 29/40 (72.5%), while surgical remnant removal was necessary in 11/40 (27.5%). Some conditions/circumstances and intrinsic UC characteristics, such as umbilical cord coiling, cord haemorrhage, dystocia and related diseases may be considered as predisposing factors although they cannot be predicted. However, factors such as manual traction, paddock access, and manual UC rupture, which have never been evaluated before, should be considered for proper management of the foal during parturition and in the first days of life to decrease the risk of umbilical disease.

76° CONVEGNO SISVET
BARI, 21-22-23 GIUGNO 2023

A RARE CASE OF SEMI-PLACENTA DIFFUSA IN A JERSEY COW

Roberta Bucci (1), Ippolito De Amicis (1), Salvatore Parrillo (1), Giuseppe Marruchella (1), Monica Probo (2), Domenico Robbe (1), Augusto Carluccio (1)

(1) Dept. of Veterinary Medicine, Veterinary Teaching Hospital, University of Teramo
(2) Dept. of Veterinary Medicine, Veterinary Teaching Hospital, University of Milano

Corresponding Author: **Salvatore Parrillo** - sparrillo@unite.it

Placental development and functioning are crucial for successful pregnancy and parturition. Abnormalities (e.g., placenta anulata, enlarged placentomes, microplacentomes) more frequently occur in the pregnancy of somatic cell clones and may lead to pregnancy loss or dystocia (1).
Adventitial placenta, or semi-placenta diffusa, is a pathological condition determined by the development of areas of accessory placentation between the cotyledons (inter-cotyledonary placentation) due to the abnormal growth of placentomes (2).
A 3-year-old Jersey Cow, artificially mated with frozen/thawed semen, was referred for dystocia after a full-term pregnancy, which resulted in the delivery of a dead calf. On clinical evaluation, the cause of dystocia was found to be foetal malposition (flexed shoulders and wrists). Once the dystocia resolved, the placenta was spontaneously expelled.
Grossly, cotyledons appeared reduced in size and number in one placental horn, while the surface of the other horn was covered with microplacentomes. Tissue samples were collected and routinely processed for histopathology. Numerous villous structures, without trophoblastic coating, were highlighted. The dominant feature was represented by an intense inflammatory reaction, with a mixed cellular infiltrate, mainly consisting of mononuclear cells. Necrotic foci were identified on the villous surface. The findings are consistent with inter-cotyledonal placentitis, which likely led to adventitial placentation.
Based on gross and microscopic findings, adventitial placentation (diffuse semi-placenta) was diagnosed. Subsequent serological investigations for BVD, IBR, Neospora, and Toxoplasma ruled out the suspicion of a previous infectious disease.
To the best of our knowledge, adventitial placentation is rarely reported in naturally or artificially mated cows. Semi-placenta diffusa likely compensates for the inadequate development of placentomes and may occur as a congenital or acquired defect. The outcome of semi-placenta diffusa depends on the severity (i.e., the extension) of the process; in the worst scenario, pregnancy may not proceed beyond midterm and may be complicated by hydrallantois. In the case under examination, the dimensions of the cotyledons (2 to 10 cm) seem to have favored the natural course of pregnancy, differently from the cases reported in the literature (3, 4), complicated by hydroallantois and fetal death, in which these placental structures had dimensions of less than 5 cm.

[1] HILL, J. et al., (2000). Evidence for placental abnormality as the major cause of mortality in first-trimester somatic cell cloned bovine fetuses. Biology of reproduction, 63(6), 1787-1794.
[2] MAXIE, G. (2015). Jubb, Kennedy & Palmer's pathology of domestic animals: vol. 2. Elsevier health sciences.
[3] KUMARESAN, A., et al., (2013). Effect of estradiol and cloprostenol combination therapy on expulsion of mummified fetus and subsequent fertility in four crossbred cows. In Veterinary Research Forum (Vol. 4, No. 2, p. 85). Faculty of Veterinary Medicine, Urmia University, Urmia, Iran.
[4] RAJA, S., et al., (2019). Prepubic Desmorrhexis in Hydrallantois Due to Adventitious Placentation in a Crossbred Heifer. Indian Vet. J, 96(04), 63-64.

76° CONVEGNO SISVET
BARI, 21-22-23 GIUGNO 2023

REVIEW OF OBSTETRIC GYNECOLOGICAL OBSERVATIONS IN THE CAREER OF A BUIATRIC VETERINARY IN APULIAN

Tinelli Sebastiano

(1) DMV, Specialist in Physiopathology of the Domestic Animal Reproduction, Mottola (TA)

Corresponding Author: **Sebastiano Tinelli** - tinelli.s@tiscali.it

In recent decades, the Apulian livestock reality has undergone a transformation with a reduction in the number of medium-small farms and a concentration of the same number of cows in industrial farms. Cattle breeding is no longer identified as an appendix of agricultural activity but has become an entrepreneurial activity (1). So that, the technical and professional adaptation to this different zootechnical reality has sometimes not adapted to the market demands. By now, the clinic of the individual cow can no longer ignore the clinic of the herd (data collection, computerized monitoring of the animals, synchronization plans) in intensive farms (2).
In these decades of clinical activity, some cases of prolonged pregnancy, hydroallantois, fetal monstrosities, birth canal lesions, cervical adhesions and caesarean delivery with macerated and mummified fetus have mainly been highlighted, all of which have been resolved therapeutically.
The fertility clinic is the main object of the health and productive management of the herd (3). A more careful management of zootechnical evaluation in relation to animal welfare and the protection of the landscape must be aimed at increasing the production of food of animal origin and at the breeding of increasingly functional and productive dairy cows.

[1] Noakes D.E., Parkinson T.J., England G.C.W. Arthur's Veterinary Reproduction & Obstetrics, 10th Ed. W.B. Saunders (2018)
[2] Youngquist R.S. Current Therapy in Large Animal Theriogenology, 2nd Ed. W.B. Saunders (2006)
[3] Sali G. "Manuale di Teriogenologia bovina" Ed. Edagricole (1996).

76° CONVEGNO SISVET
BARI, 21-22-23 GIUGNO 2023

PREVALENCE OF MYCOPLASMA CANIS AND CYNOS IN DOGS AFFECTED WITH REPRODUCTIVE FAILURES, ITALY

Yusuf M.SH M. (1), Burgio M. (1), Cicirelli V. (1), Mrenoshki D. (1), Tempesta M. (1), Buonavoglia D. (1), Aiudi G.G. (1), Greco G. (1)

(1) Department Of Veterinary Medicine - University of Bari

Corresponding Author: **Giulio Guido Aiudi** - giulioguido.aiudi@uniba.it

The genus Mycoplasma includes cell wall less bacteria that affect different animal species including carnivores (Razin et al., 1983, Chalker 2005). In dogs, Mycoplasma spp. infections are associated, either exclusively or in co-infection, with viral or bacterial agents, with multiform clinical signs such as complex respiratory disease (CIRD), anaemia, arthritis. (Chalker 2005). Previous studies described the presence of Mycoplasma spp. in the genital tract of both male and female dogs including healthy or unhealthy dogs (Doig et al., 1981). In particular, several Mycoplasma species have been isolated from the prostate, epididymis and bladder in dogs with urogenital diseases and/or hypofertility (LAbee-Lund et al., 2003). Currently, in Italy there are no studies describing the presence and impact of Mycoplasma species on the reproductive sphere. Our study aims to investigate the occurrence and the risk factor analyses of Mycoplasma spp. in canine populations affected with low reproductive efficiency.

Sixty-eight dogs, 25 males and 43 females, were included in the analyses. For each animal genital samples were screened for Mycoplasma canis and Mycoplasma cynos by using molecular methods (cPCR). Samples were submitted for the DNA extraction and analysed by using two specific cPCR assays. Out of 68 tested animals, Mycoplasma spp. DNA was detected in 29 dogs (42.65%, CI 95%: 30.89-54.40) being 13 (19.12%, CI 95%: 9.77-28.46) males and 16 (23.53%, CI 95%: 13.45-33.61) females. When considering Mycoplasma species, M. cynos (18/68, 26.47%, CI 95%: 8.59-26.71) was more frequently detected compared to M. canis (11/68, 16.18%, CI 95%: 7.42-24.93) with males more at risk of M. cynos infection (OR: 2.916). Furthermore, animals affected with reproductive impairment were four times more likelihood infected than healthy animals (OR: 4.34).

The present study describes for the first time M. canis and M. cynos in dogs in Italy describing a possible link between the presence of Mycoplasma spp. infection and the impairment of canine reproductive efficiency, thus supporting the need to adopt appropriate prevention measures

[1] Chalker, Vicki J. (2005). Canine Mycoplasmas. In Research in Veterinary Science (Vol. 79, Issue 1, pp. 1–8). https://doi.org/10.1016/j.rvsc.2004.10.

[2] Doig, P. A., Ruhnke, H. L., & Bosu, W. T. K. (1981). The genital Mycoplasma and ureaplasma flora of healthy and diseased dogs. Canadian Journal of Comparative Medicine, 45(3).

[3] L'Abee-Lund, T. M., Heiene, R., Friis, N. F., Ahrens, P., & Sørum, H. (2003). Mycoplasma canis and urogenital disease in dogs in Norway. Veterinary Record, 153(8), 231–235. https://doi.org/10.1136/vr.153.8.231

[4] Razin, S., Yogev, D., & Naot, Y. (1998). Molecular Biology and Pathogenicity of Mycoplasmas. Microbiology and Molecular Biology Reviews, 62(4). https://doi.org/10.1128/mmbr.62.4.1094-1156.1998

76° CONVEGNO SISVET
BARI, 21-22-23 GIUGNO 2023

ACCURACY OF PARTURITION DATE PREDICTION USING BREED/SIZE SPECIFIC PUBLISHED EQUATIONS IN CHIHUAHUA BITCHES

Alessia Bertero (1), Ada Rota (1), Luca Pappadia (1), Penelope Banchi (1), Patrizia Ponzio (1)

(1) Department of Veterinary Science, University of Turin

Corresponding author: **Alessia Bertero** - alessia.bertero@unito.it

Because of the peculiar physiology, prediction of parturition date may be challenging in the bitch. Different methods are available, but, among them, B-mode ultrasonography is particularly convenient due to its practicality, non-invasiveness, and the possibility to be used even if the day of ovulation is unknown. Despite these benefits, determining the gestational age through embryo-fetal biometry has limitations mainly depending on the equations that are applied, for they are greatly influenced not only by the size of the dog, but also by the body conformation traits characteristics of the different breeds. To address this problem, different equations, especially for breeds having peculiar morphological tracts, as the Chihuahua, have been developed.
The purpose of this study was to assess the accuracy of parturition date prediction based on embryo-fetal biometry parameters obtained through serial ultrasonographic examinations in the Chihuahua breed, using published equations.
Twenty pregnant Chihuahua bitches were enrolled in the study, and three different equations developed for small size [1], miniature [2] bitches and Chihuahuas [3] were evaluated. The embryo-fetal ultrasonographic parameters measured were the inner chorionic cavity diameter (ICC), in the first half of pregnancy, calculated as the mean of the perpendicular distances between the inner walls of the chorionic cavity, and the biparietal diameter (BP), in the second half, obtained considering the external limit of the greater distance between the parietal bones of the fetus' skull.
For ICC, applying the equations for small and miniature breeds, the prediction accuracy (calculated as percentage of correct parturition dates, at ± 1 to ±7 days) ranged between 26.67 to 40.00% at ±1 day respectively, reaching 80.00% only when considering a deviation of ±4 days; slightly worse results were obtained using the specific equation for chihuahuas (±1 day: 6.67%; ±4 days: 66.67%).
For BP, applying the small breed formula, an accuracy of 42.86% was reached at ±1 day and of 85.71% at ±4 days, whilst miniature and Chihuahua equations showed a better prediction precision (±1 day: 50.00%, ±3 days: 78,57%, both).
The accuracy in prediction resulted too low to be useful for planning an elective caesarean section. It is necessary to develop more formulas for the Chihuahua breed, based on measurements of higher numbers of foetuses, and possibly taking into account litter size.

[1] Beccaglia et al. Determination of gestational time and prediction of parturition in dogs and cats: an update. Reprod Domest Anim. 2016;51:12-17.
[2] Socha & Janowski. Specific fetometric formulas of ICC and BP for calculating the parturition date in the miniature breeds of canine. Reprod Domest Anim. 2018;53:545-549
[3] Vieira et al. Estimated date of delivery in Chihuahua breed bitches, based on embryo- fetal biometry, assessed by ultrasonography. Anim Reprod. 2020;17:e20200037

INFLUENCE OF CROCIN SUPPLEMENTATION TO THE EXTENDER ON CANINE COOLED SEMEN QUALITY

Calabria Alfonso (1), Spada Stefano (1), Del Prete Chiara (1), Alfano Mariateresa (1), Longobardi Consiglia (1), Kosior Michal Andrzej (1), Cocchia Natascia (1), Gasparrini Bianca (1)

(1) Department of Veterinary Medicine and Animal Production, University of Naples, Federico II

Corresponding Author: **Alfonso Calabria** - calabria_123@hotmail.it

The use of chilled semen for canine artificial insemination allows semen from genetically superior dogs to be transported across countries, avoiding animal transport related issues and improving genetic variability. Reduced sperm longevity represents a crucial limiting factor, which is associated to oxidative stress, resulting in increased production of reactive oxygen species (ROS) and reduced antioxidants. Crocin is one of the carotenoids responsible for the antioxidant capacity of saffron and is known to be able to protect sperm of various species from oxidative damages by scavenging ROS. The aim of the present study was to evaluate the effects of crocin on sperm quality during prolonged storage at 4°C. Ejaculates collected from 10 dogs were diluted to 100 × 10^6 sperm/mL in either egg-yolk TRIS-citrate glucose (EYT-G), i.e., the control group, or in YET-G supplemented with 0.5 mM crocin (Crocin) and stored at 4 °C for 4 days. Control and treated semen samples were evaluated for: sperm membrane integrity, by HOS test; sperm motility and kinetic parameters using Sperm Class Analyzer system, and lipid peroxidation by malondialdehyde (MDA) concentration, after 4 days of storage. Data, analyzed by Friedman test, and Wilcoxon's signed-rank test for post hoc, are expressed as median and interquartile ranges.
After 4 days storage at 4°C sperm membrane integrity was higher ($P \leq 0.05$) in crocin-treated group compared to the control [86.5 (43.0-94.0) %; 81 (40.5-88.8) %, respectively]. No differences were No differences in lipid peroxidation, indicated by the MDA concentrations, were detected between the control and crocin-treated groups after 4 days [5.7 (4.1-5.8) vs 5.8 (3.9-5.8) mM, respectively]. In conclusion, the obtained results showed that the enrichment of the extender with 0.5 mM crocin improves sperm membrane integrity and in part sperm kinetics after 4 days storage at 4°C, without affecting though lipid peroxidation.

[1] Longobardi V, Della Valle G, Iannaccone F, Calabria A, Di Vuolo G, Damiano S, Ciarcia R, Gasparrini B. Effects of the antioxidant crocin on frozen-thawed buffalo (Bubalus bubalis) sperm. Italian J Anim Sci 2021;20(1):2095-2101
[2] Longobardi V, Zullo G, Cotticelli A, Salzano A, Albero G, Navas L, Rufrano D, Claps S, Neglia G. Crocin Improves the Quality of Cryopreserved Goat Semen in Different Breeds. Animals 2020, 10, 1101; doi:10.3390/ani10061101
[3] Del Prete C, Calabria A, Longobardi V, Palumbo V, Merlo B, Iacono E, Tafuri S , Carotenuto D, Ciani F , Damiano S , Ciarcia R, Cocchia N. Effect of Aqueous Extract of Maca Addition to an Extender for Chilled Canine Semen. Animals 2022, 12, 1638. https://doi.org/10.3390/ani12131638
[4] Aitken, R.J., 2017. Reactive oxygen species as mediators of sperm capacitation and pathological damage. Mol. Reprod. Dev. 84, 1039–1052

PERIOPERATIVE INFLAMMATORY RESPONSE IN DOGS FOLLOWING CLASSICAL AND LAPAROSCOPIC ELECTIVE OVARIECTOMY: PRELIMINARY STUDY

Cocchia Natascia (1), Calabria Alfonso (1), Della Valle Giovanni (1), Micieli Fabiana (1), Palumbo Veronica (1), Del Prete Chiara (1), Gasparrini Bianca (1)

(1) Department of Veterinary Medicine and Animal Production, University of Naples Federico II, Italy

Corresponding Author: **Natascia Cocchia** - ncocchia@unina.it

Over the past decade, the development and implementation of laparoscopy in veterinary surgery have accelerated, particularly for one of the most common procedures performed in veterinary practice, and for which owners are seeking a less invasive option: elective canine ovariectomy (OVE). Advantages of minimally invasive compared with the traditional open techniques in small animals have been reported. Previous studies have investigated the effects of laparoscopy on cardiovascular, respiratory and metabolic parameters, as well as on perioperative complications, pain and morbidity, and inflammation response. The aim of this study is to evaluate the inflammatory response, measuring the potential systemic changes of serum amyloid A (SAA) and C-Reactive Protein (CRP) as inflammatory acute phase proteins in laparoscopic OVE (LOVE), by comparing them with those determined in conventional open surgery (OOVE) in dog. Ten entire healthy bitches aged between 18 and 48 months and weighed between 6 and 40 kg were included. Only the animals completely healthy and in anaestrus reproductive status were enlisted in this study. Animals were randomly divided into two groups: 5 animals underwent a two cannulas LOVE (LOVE group) and in the other 5 the OOVE was conducted through laparotomic surgery (OOVE group). Blood samples were taken by jugular venipuncture just before ovariectomy (T0), at the end of the surgery (T1), at 2 h (T2), 48 h (T3), and 7 days (T4) post-surgery. At each time point, serum concentrations of SAA and CRP were measured and compared between groups by Mann-Whitney test. CRP levels tended to increase in OOVE and LOVE groups compared to baseline (?) at T2 (media ± standard deviation; 3.3 ± 0.2 mg/L and 3.9 ± 0.3 mg/L, respectively) and T3 (4.6 ± 0.3 mg/L and 5.4 ± 0.3 mg/L, respectively); then CPR decreased at T4 only in the LOVE group (2.0 ± 0.3 mg/L). CRP levels were higher in the OOVE group than in the LOVE group at T3 (5.4 ± 0.3 mg/L vs 4.6 ± 0.3 mg/L) and T4 (4.2 ± 0.6 mg/L vs 2.0 ± 0.3 mg/L). SAA tended to increase in both groups immediately after surgery (T1) compared to T0. However, a decrease of SAA concentration was observed in OOVE between T1 and T2 (0.8 ± 0.3µg/dl vs 0.5 ± 0.1µg/dl), and in LOVE group between T2 and T3 (0.7 ± 0.2 µg/dl vs 0.5 ± 0.1µg/dl). These preliminary results suggest a decreased post-surgery inflammation when LOVE is carried out versus conventional open approach in dogs.

[1] Advances in laparoscopic spay techniques for dogs: the past, present and future Mathieu Manassero, Véronique Viateau VET RECORD 22/29 December 2018.
[2] Laparoscopic versus Open Ovariectomy in Bitches: Changes in Cardiorespiratory Values, Blood Parameters, and Sevoflurane Requirements Associated with the Surgical Technique
Silvia Fernández-Martín, Victoria Valiño-Cultelli 2 and Antonio González-Cantalapiedra Animals 1438 december 2022.

76° CONVEGNO SISVET
BARI, 21-22-23 GIUGNO 2023

LONG TERM EFFECT OF GONADECTOMY ON THE ULTRASONOGRAPHIC APPEARANCE OF CANINE PROSTATE GLAND: A PRELIMINARY STUDY

Stefano Spada (1), Daniela De Felice (1), Chiara Del Prete (1), Alfonso Calabria (1), Marco Russo (1)

(1) Department of Veterinary Medicine and Animal Production, University of Naples, Federico II

Corresponding Author: **Daniela De Felice** - daniela.defelice2@unina.it

Ultrasound (US) is the method of choice to detect prostate disorders in both intact and neutered dogs. However, besides recent studies suggesting that gonadectomy may play a role in the development of prostatic neoplasia, little is known about the characteristics of normal and abnormal prostate in neutered dogs, and the effects of castration on prostatic parenchyma and vascularization. The present study aimed to acquire preliminary data on ultrasonographic and contrast-ultrasound (CEUS) appearance of the canine prostate gland. Ten adult neutered dogs underwent B-mode US and CEUS of the prostate twice: at least six months after castration (T0) and six years later (T1). The prostate gland was evaluated on B-mode US and the prostatic volume was calculated using Atalan's formula to assess the regression rate. For CEUS examination, an intravenous contrast agent (SonoVue) was injected into the cephalic vein and prostatic contrast enhancement was subjectively assessed. Videoclips were acquired and analysed, and time-intensity curves were obtained to determine contrast parameters such as peak enhancement (PPI) and time to peak (TTP). Volumetric and perfusion results were then compared between T0 and T1 using Wilcoxon's signed rank test. The prostate gland appeared similar between T0 and T1 on B-mode US in terms of shape, margins and echotexture and minimally decreased in volume in all dogs ($p < 0,05$) with a mean regression rate of 0,23 cm3. Prostate perfusion significantly reduced in all dogs between T0 and T1, with a PPI decrease from 54,92 % ± 9,54 to 29,59 % ± 10,8 ($p < 0,05$) and an increase in TTP from 26,3 s ± 8,86 to 47,02 s ± 12,38 ($p < 0,05$). The preliminary data suggest that the prostatic involution is a non-ending process and the ultrasonographic features appear to be unique.

[1] Spada, S.; England, G.C.W.; Vignoli, M.; Carluccio, A.; Russo, M. Contrast-Enhanced Ultrasound Imaging of Prostate Gland in Neutered Dogs. Animals 2021, 11, 559. https://doi.org/10.3390/ani11020559

[2] Cazzuli G, Damián JP, Molina E, Pessina P. Post-castration prostatic involution: A morphometric and endocrine study of healthy canines and those with benign prostatic hyperplasia. Reprod Domest Anim. 2022 Feb;57(2):157-164. doi: 10.1111/rda.14036. Epub 2021 Nov 9. PMID: 34724270.

[3] Vignoli, M.; Russo, M.; Catone, G.; Rossi, F.; Attanasi, G.; Terragni, R.; Saunders, J.H.; England, G.C. Assessment of Vascular Perfusion Kinetics Using Contrast-enhanced Ultrasound for the Diagnosis of Prostatic Disease in Dogs. Reprod. Domest. Anim. 2011, 46, 209–213.

[4] Schrank M, Romagnoli S. Prostatic Neoplasia in the Intact and Castrated Dog: How Dangerous is Castration? Animals (Basel). 2020 Jan 5;10(1):85. doi: 10.3390/ani10010085. PMID: 31948021; PMCID: PMC7022700.

76° CONVEGNO SISVET
BARI, 21-22-23 GIUGNO 2023

MATERNAL SHAPING OF PUPPIES' FAECAL MICROBIOTA

Angela Del Carro (1), Alessia Bertero (1), Barbara Colitti (1), Luigi Bertolotti (1), Ada Rota (1)

(1) Department of Veterinary Sciences, University of Turin

Corresponding Author: **Angela Del Carro** - angela.delcarro@unito.it

Many factors affect the faecal microbiota composition of an adult dog, but little is known about the initial seeding of a puppy's gut microbiota and about the role of the dam in it [1,2,3].
The objective of this study was to describe the eubiotic faecal microbiota of puppies from birth to around-weaning, and to assess the maternal effect on it, using a metagenomic approach. Four dams of two medium-size breeds, housed in the same kennel, and their litters (21 puppies), naturally delivered after healthy pregnancies, were studied over a 17- month period. A dry commercial food (MONGE Medium Puppy & Junior Rich in Chicken®) was fed to the dams in the last two weeks of pregnancy and for weaning their puppies.Colostrum (day 0, 2), vaginal (day 0, 2) and rectal (day 0, 2 and 30) samples were collected from the dams; rectal samples were collected from the puppies at the 3 time points, for a total number of 28 samples from dams and 63 from their puppies. Environmental air samples (day 0) were taken as control. Microbiota was investigated with 16S rRNA sequencing, adopting RNeasy Power Microbiome kit (Qiagen). Illumina libraries were prepared and data were analysed with Qiime2 (standard pipeline) and R (cluster analysis). Results of the taxonomic identification were investigated (Silva database). The pattern distribution depicted a very heterogeneous situation. Jaccard plot reveals a quite clear separation given by the collection time. At day 2, the puppies' rectal microbiota has a characteristic pattern without any similarity with other sampling sites and times (beta diversity permanova q value <0.05 in all the comparisons). At day 30, it is similar to the dams' rectal microbiota, with an increase in the number of bacteria but a decrease in bacteria diversity (beta diversity permanova q value >0.05). Moreover the environmental samples showed a significant difference with all the samples except the sample at time 0. This result suggests that the initial microbiome is a quasi-sterile environment that change its composition through time. A strong maternal effect was detected, since a dam and her litter showed more similarity than different dam-litter units, confirming previous observations obtained with cultural methods [2]. A previous study differs because up to 8 weeks the puppy's microbiome was different from the dam [3].
The knowledge of the healthy gut microbiota of newborns and of its origin can lay the groundwork for future studies of intestinal dysbiosis [4].

[1] Garrigues et al. Gut microbiota development in the growing dog: a dynamic process influenced by maternal, environmental and host factors. Front. Vet. Sci. 2022;9:964649.
[2] Del Carro et al. The evolution of dam-litter microbial flora from birth to 60 days of age. BMC Vet Res. 2022;18:95.
[3] Guard et al. Characterization of the fecal microbiome during neonatal and early pediatric development in puppies. PLoS One 2017;12:e0175718.
[4] Grellet et al. Risk factors of weaning diarrhea in puppies housed in breeding kennels. Prev Vet Med. 2014;117:260-5.

ANY DIFFERENCE IN TOBACCO SMOKE UPTAKE BETWEEN PREGNANT AND NON-PREGNANT BITCHES?

Pecile A. (1), Pizzi G. (1), Mazzola S. (1), Bronzo V. (1), Giussani E. (1), Groppetti D. (1)

(1) Department of Veterinary Medicine and Animal Sciences, Università degli Studi di Milano, via dell'Università, 6 - 26900 Lodi, Italy

Corresponding author: **Debora Groppetti** - debora.groppetti@unimi.it

Pregnancy is a particular moment in a life characterized by many physiologic transformations that lead to changes in the absorption and metabolism of many drugs, including cotinine [1]. Cotinine is a specific marker of nicotine intake due to both active smoking and environmental exposure to cigarette smoke [2]. Pregnant women metabolize nicotine and cotinine more quickly than non-pregnant ones [3]. Recently, cotinine has been detected in the serum and hair of dogs living with smoking owners [4], but there are no data in pregnant bitches.

The hypothesis of different sensitivity to smoke exposure due to pregnancy status was explored in this study by comparing the concentration of cotinine in serum and hair of pregnant and non-pregnant bitches exposed to tobacco smoke.

For this purpose, twelve bitches exposed to the owner's smoke, six pregnant and six non-pregnant, were enrolled. Dogs were considered exposed to tobacco smoke in case of indoor consumption by the owner of at least one cigarette per day in the last two months. Cotinine in serum and hair was measured using a specific commercial ELISA immunoassay.

Contrary to humans, serum and hair cotinine concentrations were higher in pregnant (15.2 □8.1 ng/mL and 23.6□11.7 ng/mg, respectively) compared to non-pregnant bitches (7.8□3.4 ng/mL and 18.4□13 ng/mg, respectively), even though without statistical significance. The different metabolic mechanisms and placental structure between the human and canine species can explain this aspect.

Although preliminary, our findings suggest a different susceptibility to tobacco smoke exposure during gestation. The role of pregnancy status on cotinine uptake in dogs certainly deserves further investigation, especially since prenatal exposure to tobacco smoke can adversely affect the development of fetal endocrine, reproductive, respiratory, cardiovascular and neurological systems [3].

[1] Isoherranen, N., Thummel, K.E. Drug metabolism and transport during pregnancy: how does drug disposition change during pregnancy and what are the mechanisms that cause such changes? Drug Metab Dispos 2013, 41(2): 256-62.
[2] Benowitz, N.L. Epidemiol Rev 1996, 18(2): 188-204.
[3] Arbuckle, T.E., Liang, C.L., Fisher, M. et al. Exposure to tobacco smoke and validation of smoking status during pregnancy in the MIREC study. J Expo Sci Environ Epidemiol 2018, 28: 461–469.
[4] Groppetti, D., Pizzi, G., Pecile, A., Bronzo, V., Mazzola, S.M. Cotinine as a sentinel of canine exposure to tobacco smoke. Animals (Basel) 2023, 13(4): 693.

76° CONVEGNO SISVET
BARI, 21-22-23 GIUGNO 2023

EFFECT OF AQUEOUS EXTRACT OF MACA ON THE QUALITY OF FROZEN-THAWED CANINE SEMEN

Del Prete Chiara (1), Merlo Barbara (2), Calabria Alfonso (1), Spada Stefano (1), Iacono Eleonora (2), Longobardi Consiglia (1), Alfano Maria Teresa (1), Gasparrini Bianca (1), Cocchia Natascia (1)

(1) Department of Veterinary Medicine and Animal Production, University of Naples Federico
(2) Department of Veterinary Medical Sciences, University of Bologna, Italy

Corresponding Author: **Chiara Del Prete** - chiara.delprete@unina.it

The addition of antioxidants to semen extender has been demonstrated to improve the quality of frozen-thawed semen, by counteracting cryopreservation-induced oxidative stress. Maca is an Andean plant with antioxidant properties, that has been recently proposed to protect spermatozoa during storage.
The aim of this study was to evaluate the effect of Maca in the freezing extender on the post-thaw quality of dog semen.
Ten canine ejaculates were frozen following a two-step protocol using a tris-glucose-citrate egg yolk extender with or without the addition of 10 µl/mL of aqueous extract of Maca (Maca and Ctrl groups, respectively). In each group, sperm concentration of malondialdehyde (MDA), as an indicator of lipid peroxidation, was measured using the thiobarbituric acid before and after freezing-thawing (T0); sperm motility and kinetic parameters using Sperm Class Analyzer system were evaluated before freezing (fresh semen) and after 1 h of incubation at 37°C after thawing (T1). Due to non-normal distribution of data, data are expressed as median and interquartile ranges and Wilcoxon test was used to compare groups at each time point and to compare time points within each group.
MDA levels did not differ between fresh and frozen-thawed (T0) semen in both groups; however, the MDA concentration at T0 was lower in Maca-treated semen than in the control ($p<0.05$). As expected, a reduction ($p< 0.05$) in motility and kinetic parameters was observed in both groups at T1 compared to fresh semen. However, total motility (TM), the percentage of sperm with medium velocity (medium) and WOB were higher ($p< 0.05$) in Maca-treated than in the control at T1 [TM: 33,1 (18-40,1) vs 23,8 (15,9-36,3)%; medium: 2.3 (0.7-4) vs 1.4 (0.4- 2.8)%; WOB 48.4 (44.6 -53.1) vs 43.4 (36.9-51.4)%]. Moreover, progressive motility decreased ($p <0.05$) at T1 in the control group, whereas it was not affected in Maca group. In addition, the percentage of hyperactivated spermatozoa remained constant at T1 in the control, while in the Maca group an increase ($p< 0.05$) of this parameter was recorded.
The addition of Maca before freezing was able to increase the sperm cryotolerance, reducing lipid peroxidation during freezing-thawing process and activating canine sperm motility and hyperactivation after thawing. These findings can indicate a protective role of Maca on the quality of frozen-thawed canine spermatozoa suggesting further studies to investigate the effect on fertilization outcome.

[1] Leiva-Revilla J, Rolón M, Siyadatpanah A, de Lourdes Pereira M, Nissapatorn V. (2022) First study of in vitro protective effect of Lepidium meyenii (Maca) on frozen–thawed bovine spermatozoa. Vet World 15:1481-1488. https://doi.org/10.14202%2Fvetworld.2022.1481-1488
[2] Del Prete C, Calabria A, Longobardi V, Palumbo V, Merlo B, Iacono E, Tafuri S, Carotenuto D, Ciani F, Damiano S, Ciarcia R. (2022) Effect of Aqueous Extract of Maca Addition to an Extender for Chilled Canine Semen. Animals 12:1638. https://doi.org/10.3390/ani12131638

VASECTOMY IN CAPTIVE RED DEER (*CERVUS ELAPHUS*) AND ROE DEER (*CAPREOLUS CAPREOLUS*) AS MANAGEMENT TOOL OF NON-RELEASABLE WILDLIFE

Burgio M. (1), Carbonari A. (1), Angelucci S. (2), Di Tana F. (2), Cicirelli V. (1), Aiudi G.G. (1), Sciorsci R.L.(1), Rizzo A. (1)

(1) Department Of Veterinary Medicine - University of Bari
(2) Maiella National Park, Wildlife Research Center, Caramanico Terme, Teramo, Italy

Corresponding Author: **Matteo Burgio** - matteo.burgio@uniba.it

The aim of the study was to describe the technique of vasectomy in Red deers (*Cervus elaphus*) and Roe deers (*Capreolus capreolus*) in the Maiella National Park (Central-Eastern Italy) to reduce overcrowding conditions in enclosures that host animals (with permanent disabilities or poorly imprinted animals that have lost fear of people and/or cannot recognize their own species). Orchiectomy is not a practical technique in Red deers and Roe deers as antler abnormalities defined in different cervid species as perukes, cactus antlers, wig antlers or "antleroma" (as tumorlike fibrocartilaginous outgrowths) will develop by altering hormone secretions. The outcome is continuously growing antlers that never mineralize, caused by excessively rapid ossification, so the bone is more porous than normal. Therefore, castration, especially for animals housed in enclosures, would certainly lead to both social and welfare problems of the affected individual. Three 7-month-old Red deers and three 6-8 month-old Roe deers were subjected to teleanesthesia using Dexmedetomidine (20 mcg/kg IM), Tiletamine and Zolazepam (5 mg/kg IM), Butorphanol (0.2 mg/kg IV) and Ketamine (3 mg/kg IM). Before surgery, antibiotic treatment with Amoxicillin and Clavulanic Acid (20 mg/kg IM) was performed. At the level of the scrotal neck an anterior approach was done with a 3 cm incision of median raphe of skin and dartos, using a proximal-distal approach. Then the tunica vaginalis proper of each testis was incised and the deferens ductus was isolated, separating the deferential vessels. Two ligatures, approximately 1 cm apart, were made on the duct with polyglycolic acid suture thread USP 2 (Surgicryl-Stainerberg-Belgium) and the tract of the deferens vas between the two ligatures was removed. Subsequently, the skin was sutured with detached U-shaped stitches. At the end of surgery, long-acting Cefovecin (8 mg/kg) and Dexamethasone (0.03 mg/kg) were administered. The animals were released into an enclosure for awakening and were administered Atipemazole (0.14 mg/kg) and were counted at a distance until they reached quadrupedal station (total duration of anaesthesia 65±5 min) The surgical technique was quick (26 ± 1 min) and easy to perform. There were no complications either during surgery or during the postoperative period. A month after the vasectomy, the same animals were anesthetized again to be transferred to another area of the park; at the same time the surgical wound had perfectly healed and did not present abnormality. In conclusion, vasectomy in Red and Roe deers appears to be a minimally invasive surgery which could be proposed for birth control in non-releasable cervids hosted in enclosures, preventing antlers abnormalities, keeping unaltered hormone-dependent sexual characters and, consequently, hierarchical and social status.

[1] Boulanger, J. R., & Curtis, P. D. (2016). Efficacy of surgical sterilization for managing overabundant suburban white-tailed deer. Wildlife Society Bulletin, 40(4), 727–735. https://doi.org/10.1002/wsb.706

76° CONVEGNO SISVET
BARI, 21-22-23 GIUGNO 2023

OVARIECTOMY IN MOUFLONS (OVIS ARIES) IN THE FIELD: APPLICATION OF INNOVATIVE SURGICAL TECHNIQUE IN MIDLINE VERSUS FLANK APPROACH

Vincenzo Cicirelli (1), Alice Carbonari (1), Matteo Burgio (1), Giulio Guido Aiudi (1), Francesca Giannini (2), Annalisa Rizzo (1)

(1) Department of Veterinary Medicine - University Of Bari
(2) Natural Park of the Tuscan Archipelago

Corresponding Author**: Vincenzo Cicirelli** - vincenzo.cicirelli@uniba.it

The aim of this study was to describe an innovative surgical technique of ovariectomy in female mouflons in field conditions. Two surgical approaches, flank, and midline were used, and the AESCULAP CAIMAN® Seal and Cut device, was employed for clamping the ovary, stapling the vessels, and cutting in one stroke. Twenty female mouflons of reproductive age were ovariectomized, 10 via midline (M group) and 10 via the left flank (F group), using the CAIMAN®. Anesthesia was performed using xylazine and an association of tiletamine and zolazepam. After 10 minutes, propofol was administered intravenously and anesthetic maintenance was performed with isoflurane. The animals were monitored through multiparametric monitor. Animals of the F group were gonadectomized using the left flank as a surgical access. A vertical skin incision was performed on the left flank on the paralumbar fossa close to the iliac wing. All muscular layers were punctured with the scalpel and muscle fibers were separated down to the peritoneum, which was held with the forceps, punctured with the scalpel, and cut with scissors. The surgeon grasped and exteriorized the uterus, locating the ovaries. The CAIMAN® clamp was affixed to the base of the ovary, and ovariectomy was performed. All procedures were repeated on the other ovary. After ovarian removal peritoneum, muscle and skin were closed with absorbable suture. Ovariectomy of the M group was carried out similarly but using a midline access. The incision was performed cranial to the udder, at about 10 cm from the umbilical scar. The surgical procedures were similar to those described above. Surgical operation times, intraoperative nociceptive response, postoperative pain, and the frequency of complications were compared in two groups using T student. Duration of surgery was 21.3 min (± 5.35) and 19 min (± 1.32) in the flank and midline approach, respectively. For all patients, no intra or post-operative complications were reported, and all animals were gonadectomized without side effects. In conclusion the mouflons ovariectomy duration in 2 group was similar, but the postoperative control of the healing of animals is easier in animals neutered from the flank.

[1] Evans, C.S.; DeNicola, A.J.; Warren, R.J. Comparison of treatment effort for immunocontraceptive vaccines and surgical sterilization in deer. Wildl. Soc. Bull. 2016, 40, 593–598, doi:10.1002/wsb.684.
[2] Cicirelli, V.; Carbonari, A.; Burgio, M.; Giannini, F.; Rizzo, A. Ovariectomy in Mouflons (Ovis aries) in the Field: Application of Innovative Surgical and Anaesthesiological Techniques. Animals 2023, 13, 491. https://doi.org/10.3390/ani13030491

76° CONVEGNO SISVET
BARI, 21-22-23 GIUGNO 2023

PRELIMINARY RESULTS ON THE SEMEN CHARACTERIZATION OF ITALIAN RABBIT BREEDS

Fabrizio Lauriola (1), Michele Di Iorio (1), Giusy Rusco (1), Letizia Lerza (1), Michele Schiavitto (2), Nicola Iaffaldano (1)

(1) Dipartimento Agricoltura, Ambiente e Alimenti. Università degli Studi del Molise. Campobasso
(2) Associazione Nazionale Coniglicoltori Italiani (ANCI-AIA), Volturara Appula (FG)

Corresponding Author: **Nicola Iaffaldano** - nicolaia@unimol.it

In Italy, over the years, there has been a progressive decrease in population of Italian rabbit breeds due to the massive use of high-performing commercial hybrids [1]. The "Cun-Fu 2" project aimed at promoting and supporting the conservation of the Italian rabbit genetic resources and in the meantime improving the productive and reproductive performances by actions for phenotypic, genetic, characterization of different rabbit breeds. In order to provide knowledge on reproductive performances among the breeds the fresh semen quality from different rabbit breeds was assessed. It resulted useful for both the in vivo and in vitro conservation program.
In total 21 animals from 4 rabbit breeds were used: Argentata di Champagne (A.R.), Fulva di Borgogna (F.), Blu di Vienna (B.), Leprino di Viterbo (L.V.) were used.
Ejaculates were collected by artificial vagina and the following semen parameters were evaluated: volume, concentration (Spectrophotometric analysis), viability (Cytofluorometric technique) and motility (CASA system) [2]. Sperm variables were compared among the breeds using the one-way method ANOVA. No significant differences among the breeds for volume, concentration and total motility were found, whilst significantly higher values of progressive motility were observed in B. (56.5%) and F. (57.9%) compared to L.V. (35.0%).
Higher values of motility kinetic parameters (curvilinear velocity and average path velocity) were found in F. (53.0 and 29.7 µm/s) than L.V. (38.8 and 18.2 µm/s) respectively ($p<0.05$). Lastly, better sperm viability (87.9%) in the F. breed in respect to that in the A.R. breed (75.6%) was shown ($p<0.05$).
The characterization of semen production in Italian recognised rabbit breeds provides a useful tool in predicting the fertilizing ability of each donor. Moreover, the quality of fresh semen is an important prerequisite for successful semen cryopreservation, which is a safe and effective biotechnology for ex situ in vitro program conservation, throughout the establishment of a semen cryobank. Cryopreservation of semen allows long-term storage of male genetic material, which is of strategic importance when in situ conservation methods are ineffective in maintaining genetic variability and preventing breed extinction.
The characterization of fresh semen quality in other rabbit breeds and the knowledge of the biological mechanisms that lead to the sperm differences among breed will be our next goals.

[1] Di Iorio et al. Improving the rabbit semen cryopreservation protocol: comparison between two extenders and inseminating doses. Annals of Animal Science, 20:887–898, 2020.
[2] Rusco et al. Proteomic analysis of rabbit fresh and cryopreserved semen provides an important insight into molecular mechanisms of cryoinjuries to spermatozoa. Theriogenology, 191:77–95, 2022.

USE OF ALFAXALONE AND DEXMEDETOMIDINE FOR LAPAROSCOPIC SPAYING OF RED-EARED SLIDERS (*TRACHEMYS SCRIPTA ELEGANS*) A PILOT STUDY

Pelizzone Igor (1), Vetere Alessandro (1), Bigliardi Enrico (1), Bresciani Carla (1), Di Ianni Francesco (1)

(1) Università degli Studi di Parma, Dipartimento di Scienze Medico-Veterinarie

Corresponding Author: **Igor Pelizzone** - igor.pelizzone@unipr.it

Pond sliders (Trachemys spp.) have recently been included in the list of invasive animals in Europe. So far, castration is considered a reliable method for birth control in these species instead of suppression. A less invasive method for spaying Trachemys sp. is endoscopic assisted ovariectomy/orchiectomy through the prefemoral fossa [1]. The goal of this pilot study was to evaluate efficacy of alfaxalone and dexmedetomidine as anaesthetic agents for mininvasive procedures like laparoscopic ovariectomy and orchiectomy of pond sliders [2-3]. 10 adults (6 females and 4 males) Trachemys scripta elegans weighing between 0.3 and 1.3Kg. underwent gonadectomy with laparoscopic assisted technique and bilateral access. Animals were acclimatised at 26°C for 20 minutes before surgery and Alfaxalone (5 mg/kg) and Dexmedetomidine (50 mcg/kg) were delivered intramuscularly (IM) in the right forelimb. After loss of reflexes each animal was intubated and ventilated manually at 4 bpm [4] with a mixture of oxygen and isoflurane (2%). At the end of surgery atipamezole (0.5 mg/kg) was administered intramuscularly (IM) as reversal agent in left forelimb. Time of loss of reflexes, time of intubation, heart rate, end tidal CO2, duration of surgery and time of recovery were 16 ±2.5 min, 19.7 ±3.5 min, 30.3 ±8.6 bpm, 11.3 ±2.9, 78.8 ±11.4 min and 85.9 ±12.4 min, respectively. Results of this study suggest that effects of alfaxalone in combination with dexmedetomidine for IM anaesthesia in Trachemys spp. are rapid in onset and good in duration and allow for a rapid intubation. A combination of alfaxalone and dexmedetomidine associated with 2% isoflurane provided an adequate surgical plane for laparoscopic assisted ovariectomy and orchiectomy of pond sliders.

[1] Bardi E et al. Comparison of Lateral and Dorsal Recumbency during Endoscope-Assisted Oophorectomy in Mature Pond Sliders (Trachemys scripta). Animals (Basel) 2020 Aug 19;10(9):1451
[2] Hansen LL et al. Assessment of the effects of intramuscular administration of alfaxalone with and without medetomidine in Horsfield's tortoises (Agrionemys horsfieldi).Vet Anaesth Analg 2013 Nov;40(6): 68-75
[3] Kischinovsky M, et al. Intramuscular administration of alfaxalone in red-eared sliders (Trachemys scripta elegans) effects of dose and body temperature.Vet Anaesth Analg 2013 Jan;40(1):13-20
[4] Williams CJA et al. Influence of assisted ventilation and recumbency on cardiorespiratory physiology in anesthetized freshwater turtle Trachemys scripta scripta.Comp Biochem P

76° CONVEGNO SISVET
BARI, 21-22-23 GIUGNO 2023

ENDOSCOPIC EGG REMOVAL IN FOUR DYSTOCIC LEOPARD GECKOS (*EUBLEPHARIS MACULARIUS*)

Alessandro Vetere (1), Igor Pellizzone (1), Enrico Bigliardi (1), Marco Masi (2), Matteo Rizzi (1), Elisa Leandrin (3), Francesco Di ianni (1)

(1) Department of Veterinary Science, via del Taglio 10, 43126, Parma, Italy
(2) Centro Veterinario Specialistico, Via Sandro Giovannini 51/53, 00137, Roma, Italy
(3) Clinica Veterinaria Madonna di Rosa, Via Rosa 1, 33078, San Vito al Tagliamento, Italy

Corresponding Author: **Alessandro Vetere** - alessandro.vetere@unipr.it

Dystocia is a multifactorial, life-threatening condition commonly affecting pet reptiles. Treatment for dystocia can be either medical or surgical. Medical treatment usually involves the administration of oxytocin, but in some species or, in some cases, this treatment does not work as expected. Surgical treatments such as ovariectomy or ovariosalpingectomy are resolutive, but invasive in small-sized reptiles. In this paper, we describe four cases of post ovulatory egg retention in four leopard geckos (Eublepharis macularius) successfully treated through a cloacoscopic removal of the retained eggs, after a non resolutive medical treatment. A cloacal endoscopic examination was performed on all four animals. A voluminous egg protruding through the urogenital papilla to the cloaca was visible. All the eggs were removed easily using endoscopic forceps. In two geckos, the eggshell was torn, and the content was aspirated to reduce the egg volume. The intervention was fast, non-invasive, and no procedure-related adverse effects were noted. Cloacoscopy should be considered a valuable, non-invasive tool for egg removal in dystocic leopard geckos when the egg is accessible to manipulation. Recrudescence or complications such as adhesions, oviductal rupture, or the presence of ectopic eggs should recommend surgical intervention.

[1] Hedley, J. Reproductive diseases of reptiles. In Practice,2016, 38, 457–462; DOI:10.1136/inp.i4772.
[2] Stahl, S.J. Reptile production medicine. Semin. Avian Exot. Pet Med. 2001, 10, 140–150; DOI:10.1053/saep.2001.24256.

76° CONVEGNO SISVET
BARI, 21-22-23 GIUGNO 2023

MID AND TERM PREGNANCY IGF-I AND NEFA CONCENTRATIONS IN FETAL FLUIDS OF QUEENS: PRELIMINARY RESULTS

Fusi J. (1), Bucci R. (2), Pividori I. (3), Faustini M. (1), Prandi A. (3), Veronesi M.C. (1)

(1) Università degli Studi di Milano, Department of Veterinary Medicine and Animal Sciences, Lodi, Italy
(2) Università degli Studi di Teramo, Department of Veterinary Medicine, Teramo, Italy
(3) Università degli Studi di Udine, Department of Agricultural, Food, Environmental and Animal Sciences, Udine, Italy

Corresponding Authos: **Jasmise Fusi** - jasmine.fusi@unimi.it

Insulin-like growth factor-I (IGF-I) and non-esterified fatty acids (NEFA) are involved in fetal growth and development. Whilst IGF-I has been considered as a possible indicator of potential growth, NEFA is recognized as a marker of fat mobilization, because of energy request [1]. A study on canine foetal fluids collected at term [1] reported the absence of significant differences in NEFA concentrations, but higher IGF-I in amniotic than allantoic fluids, and in large breeds than small and medium breeds in dog, providing the first evidence that amniotic IGF-I could be used as an indicator of potential growth in dogs. However, differently to the dog, the study of foetal fluids composition received little scientific interest in cats. Although a couple of studies [2,3], described the biochemical composition of feline foetal fluids at different time of pregnancy, the authors are not aware of studies about IGF-I and NEFA concentrations in feline foetal fluids. Therefore, this study aimed to report preliminary results about the IGF-I and NEFA concentrations in foetal fluids of cats at mid and term of pregnancy. The study was performed on 8 queens submitted to mid pregnancy ovariohysterectomy for unwanted pregnancy and on 3 queens submitted to Caesarean section because of dystocia occurred at term of pregnancy. Amniotic and allantoic fluids were separately collected from 37 mid pregnancy foetuses and from 10 term pregnancy, normally developed and viable foetuses, and immediately stored at -20° C until analysis for IGF-I and of NEFA concentrations by RIA. The results showed no significant differences in NEFA concentrations neither between the two foetal fluids at both mid and term pregnancy nor between the two times of sampling within each fluid. On the opposite, a significant ($p<0.05$) higher IGF-I concentration was found in amniotic and allantoic fluids at term in comparison to the same fluids collected at mid pregnancy, whilst no differences were found between the two fluids at both sampling times. These results suggest that, in cats, higher amniotic and allantoic fluid IGF-I concentrations at term could be related to the final growth of the foetus at term, and, likely to what reported for the dog, could be considered as an indicator of foetal potential growth.

[1] Meloni et al. IGF-I and NEFA concentrations in fetal fluids of term pregnancy dogs. Theriogenology, 81(9):1307-11, 2014.
[2] Fresno et al. Modulation of the biochemical composition of amniotic and allantoic fluids as a control mechanism of feline foetal development. Placenta, 33(6):522-7, 2012.
[3] Bigliardi et al. Evaluation of Biochemical Composition of Amniotic and Allantoic Fluids at Different Stages of Pregnancy in Queens. Animals (Basel), 30;12(11):1414, 2022.

ELASTOGRAPHY AS A TOOL TO PREDICT THE TESTICULAR FUNCTION

Claudia Bracco (1), Alessia Gloria (1), Alberto Contri (1)

(1) University Of Teramo, Department of Veterinary Medicine, Località Piano D'Accio, 64100 Teramo

Corresponding Author: **Alessia Gloria** - agloria@unite.it

In andrology, the B-mode ultrasound of the testis represents a supportive tool to complete the breeding soundness evaluation, enabling visualization of the echotexture (Pugh et al., 1990). B-mode ultrasound is, however, subjective requiring the interpretation of the clinician. Ultrasound elastography is a modern ultrasound-based technique that evaluates tissue elasticity (Ophir et al., 1991). In veterinary, the research conducted for the standardization of elastography in tissue evaluation is recent and the information on this new technique for studying the stiffness of testes is limited. Thus, the present study aimed to verify if the strain elastography will reflect the testicular function based on epididymal sperm attributes. Preliminary, the appropriate procedure for the strain elastography of canine testis was defined. Strain elastography was performed during the routine andrological examination of 22 dogs presented for elective orchiectomy at the Veterinary Medicine Hospital, University of Teramo, Italy. Three elastographic images from each of the three different clips were collected by the same operator. Testicular elastographic semi-quantitative index (SEI) and ratio (the proportion between the SEI of the testis and of the mediastinum as a reference – SER) were recorded in different regions (proximal – between the albuginea and the mediastinum; distal – between the mediastinum and the distal albuginea) and scan sections (longitudinal and transversal). After routine orchiectomy, tail epididymal spermatozoa were analyzed and used to classify testis with normal or abnormal function, based on sperm primary abnormalities. The SEI and SER were compared in testes classified with normal and abnormal functions.
Both SEI and SER were reliable in both longitudinal and transversal sections, but values recorded in the distal region were more variable (intraclass correlation coefficient: 0.773 and 0.529), suggesting the proximal region (intraclass correlation coefficient: 0.917 and 0.952) is more representative of the testicular stiffness. Testes with normal function were softer (SEI 1.7 ± 0.3 and SER 0.55, respectively) compared with abnormal testes (SEI 3.7 ± 0.7 and SER 1.5, respectively; $P < 0.05$). The results in the dog supported the findings in humans, in which the testes affected by non-obstructive azoospermia were stiffner compared with testes with normal spermatogenesis or obstructive azoospermia (Li et al., 2012).

[1] Li, M., Du, J., Wang, Z., & Li, F. (2012). The Value of Sonoelastography Scores and the Strain Ratio in Differential Diagnosis of Azoospermia. Journal of Urology, 188(5), 1861–1866. https://doi.org/10.1016/j.juro.2012.07.031
[2] Ophir, J., Céspedes, I., Ponnekanti, H., Yazdi, Y., & Li, X. (1991). Elastography: A Quantitative Method for Imaging the Elasticity of Biological Tissues. Ultrasonic Imaging, 13(2), 111–134. https://doi.org/10.1177/016173469101300201
[3] Pugh, C. R., Konde, L. J., & Park, R. D. (1990). TESTICULAR ULTRASOUND IN THE NORMAL DOG. Veterinary Radiology, 31(4), 195–199. https://doi.org/10.1111/j.1740-8261.1990.tb01810.x

PREDICTION OF WHELPING DATE IN JACK RUSSELL TERRIER

Petra Lascialfari (1), Cristiana Manetti (1), Matteo Tesi (1), Diana Fanelli (1), Alessandra Rota (1)

(1) Università di Pisa, Dipartimento di Scienze Veterinarie

Corresponding Author: **Petra Lascialfari** - petra.lascialfari@phd.unipi.it

Predicting whelping date is essential to be ready to provide help in case of dystocia, and Jack Russell Terrier have been identified as the fourth breed most frequently presented in emergency for dystocia in the UK [1]. The main purposes of this scientific paper were: 1) to develop breed-specific formulas to predict the days before parturition (dbp) in Jack Russell Terrier bitches based on inner chorionic cavity (ICC) diameters and biparietal (BP) diameters; 2) to evaluate the accuracy of these formulas and the accuracy of estimated ovulation date in predicting parturition date. Ultrasound examinations of 22 pregnancies of 15 bitches were included in this retrospective study: 19 pregnancies for ICC group and 17 for BP group. Only physiological pregnancies were included, single puppy pregnancies were excluded and multiple images for each conceptus were taken. The ultrasound examinations were performed between September 2018 and December 2022. ICC diameters were measured from 21 to 27 days post-ovulation, while BP diameters were measured from 39 to 47 days post-ovulation. Ovulation date was estimated through serum progesterone concentration (considering 4.01–8.0 ng/ml as the day of ovulation) [2] and confirmed by ovarian ultrasonography [3]. The parturition date and the number of puppies delivered were recorded. For ICC group, the correlation showed a weak relationship with litter size (rho=-0.17) and this was not included in the simple linear regression. The latter (dbp=44.983-0.6352*ICC) was statistically significant (P<0.001, r2=77.58). For BP group, the correlation of dbp with litter size was moderate (rho=-0.49) and this was included in the regression (dbp=49.40-1.228*BP-0.546*number of fetuses; P<0.001, r2=90.85). Effect of BP (P<0.001) and number of fetuses (P=0.006) was significant. Accuracy for single parameters of ovulation date, ICC and BP was 71.4%, 78.6% and 85.7% with an error of ±1 day, and 85.7%, 100% and 100% with an error of ±2 days, respectively. When considering all these parameters together, the accuracy was not increased compared to that of the BP formula (85.7% at ±1 days and 100% at ±2 days). BP diameter taken within the sixth week of pregnancy was the best morphometric parameter in predicting whelping date, when corrected by the number of fetuses, as already described. In conclusion, to improve the prediction of the parturition date in Jack Russell Terriers, a re-evaluation of pregnancy at the 6th-7th week of pregnancy is indicated.

[1] O'Neill DG, O'Sullivan AM, Manson EA, Church DB, McGreevy PD, Boag AK, Brodbelt DC. Canine dystocia in 50 UK first-opinion emergency care veterinary practices: clinical management and outcomes. Vet Rec. 2019 Mar 30;184(13):409
[2] Rota A, Vannozzi I, Marianelli S, Gavazza A, Lubas G. Laboratory and Clinical Evaluation of a Feia Method for Canine Serum Progesterone Assay. Reprod Domest Anim. 2016 Feb;51(1):69-74
[3] England GC, Russo M, Freeman SL. Follicular dynamics, ovulation and conception rates in bitches. Reprod Domest Anim. 2009 Jul;44 Suppl 2:53-8

76° CONVEGNO SISVET
BARI, 21-22-23 GIUGNO 2023

IMMUNOEDITING OF CANINE TRANSMISSIBLE VENEREAL TUMOUR DURING REGRESSION

Marino G. (1), Di Giorgio S. (1), Giambrone G. (1), Mazzullo G. (1), Fassati A.(2), Sfacteria A. (1)

(1) Department of Veterinary Sciences, University of Messina, Via G. Palatucci, 98168 Messina, Italy
(2) UCL Institute of Immunity & Transplantation, The Pears Building Pond Street, NW3 2PP London, UK

Corresponding Author**: Gabriele Marino** - marinog@unime.it

The canine transmissible venereal tumour (CTVT) is a tumour of dogs that mainly affects the external genitalia and is transmitted through sexual contact, bites, sniffing or licking (1). CTVT is a spontaneous transmissible tumour, in which the mutated tumour cell is the causative agent and is perpetuated as a parasitic allograft in the host. This tumour can colonise a new host and cross histocompatibility barriers; to avoid the host's immune response, CTVT utilizes several immune escape strategies including loss of MHC and expression of immunosuppressive cytokines (2). Erythropoietin (EPO) is a cytokine involved not only in the regulation of erythropoiesis but also of the immune system. It is hypothesized that there may be a paraneoplastic syndrome due to erythropoietin production by the tumour since, despite the blood loss from the neoplasm, the haematocrit does not show any alterations except, rarely, a slight leukocytosis (3). Therefore, the aim of this study was first to investigate the immunohistochemical expression and tissue localization of EPO and its receptor EPOR in 4 CTVTs biopsied during the progression and regression phases. The immune cell infiltrate was investigated for the following markers: mast cell tryptase, macrophage marker, CD3 and IgG. The results showed a marked expression of EPO/EPOR in the CTVT cells during the progression phase. During the regression phase, the positivity decreased significantly in CTVT cells but became strong in the mucosal epithelial cells and infiltrating immune cells. Cells expressing macrophage marker, CD3 and IgG increased in regressive phases, while those expressing mast cell tryptase decreased. EPO exerts anti-inflammatory and angiogenic action and may stimulate the proliferation and migration of keratinocytes. In progressive CTVTs, the first two actions seem prevalent. In the regressive phase, mucosal epithelial cells are involved in the epithelization of the tumour wound, but our findings are suggestive of a more complex role in the modulation of the immune response. In conclusion, CTVT proves to be an excellent candidate for studying the involvement of the EPO/EPOR complex in tumour biology. Furthermore, its complete regression after chemotherapeutic treatment makes it an ideal candidate for studying the mechanisms involved in immunoediting during neoplastic progression and regression.

[1] Abeka YT. Review on Canine Transmissible Venereal Tumor (CTVT). Canc Therapy & Oncol Int J. 2019;14:555895.
[2] Siddle HV, Kaufman J. Immunology of naturally transmissible tumours. Immunology. 2015;144:11-20.
[3] Cohen D. The canine transmissible venereal tumor: a unique result of tumor progression. Adv Cancer Res. 1985;43:75-112.

PRENATAL DIAGNOSIS OF TWINNING IN DOGS AND CATS

Marino G. (1), Pecchia F. (2), Monti S. (1), Di Giorgio S. (1), Catone G.(1)

(1) Department of Veterinary Sciences, University of Messina, Via G. Palatucci, 98168 Messina, Italy
(2) Polivet, Via Salaria 1317, 00138 Roma, Italy

Corresponding Author**: Gabriele Marino** - marinog@unime.it

Prenatal diagnosis is a set of investigations, both instrumental and laboratory, which aim to monitor some aspects of the health of the foetus during pregnancy, from the early stages of embryonic development to the moments preceding delivery. Maternal blood analyses and ultrasound are the main non-invasive and safe techniques, while amniocentesis and chorionic villus sampling carry a minimal risk of miscarriage, in the human species (1,2). In companion animals, a growing interest is emerging for the preterm ultrasound morphological screening of embryos and foetuses, aimed to assess the integrity and vitality of the conceptus as well as the early diagnosis of anomalies, which can cause complications in parturition. Among these, twinning has been poorly studied in dogs and cats, probably for the fact that most of the cases are not more recognizable at delivery. This study is a retrospective analysis of the ultrasonographic findings of twinning in the author's clinical activity from 2016 to 2022. Dichorionic dizygotic twins are the normality in the multiple offspring of dogs and cats, we considered twins only foetuses sharing the same allantoic sac (monochorionic twins). Only six cases of twinning were recorded, 2 in female cats and 4 in female dogs, out of the whole number of evaluations performed on 3120 foetuses (2640 canines and 480 felines), with an incidence of 0.4% and 0.2% in feline and canine foetuses (1.2% and 0.9% considering the mothers). All the twins had their amniotic sac and umbilical cord but presented a single placenta and a unique allantoic sac (monochorionic diamniotic twins). No monoamniotic twins were found. Considering that no genetic assay was performed, the finding of opposite-sex foetuses in 2/6 cases. Twinning may have an impact on the success of pregnancy, for the risk of dystocia, foetal or neonatal death, as observed in the reported cases. Prenatal ultrasound allows early recognition of twinning in dogs and cats. Planned C-section is the main indication for twinning in this species.

[1] Cheng WL, Hsiao CH, Tseng HW, Lee TP (2015). Non-invasive prenatal diagnosis. Taiwan J Obstet Gynecol 54:343-9.
[2] Tabor A, Vestergaard CH, Lidegaard O (2009). Fetal loss rate after chorionic villus sampling and amniocentesis: an 11-year national registry study. Ultrasound Obstet Gynecol 34:19-24.

ULTRASONOGRAPHIC MONITORING OF CANINE FETAL GASTROINTESTINAL MOTILITY DURING THE LAST TEN DAYS OF PREGNANCY

Giulia Siena (1), Stefano Romagnoli (1), Michele Drigo (1), Barbara Contiero (1), Francesca di Nardo (1), Chiara Milani (1)

(1) Department of Animal Medicine, Production and Health, via dell'Università 16, 35020 Legnaro (PD)- Italy

Corresponding Author: **Chiara Milani** - chiara.milani@unipd.it

A recent study report that fetal gastrointestinal motility (FGM) is correlated with fetal maturity [1]. A preliminary assessment showed an increase in the percentage of fetuses showing FGM (FGM%) visualized by ultrasound (US) in the peripartum period [2]. Our study aimed to quantify the amount of FGM in relation to days before parturition (DBP), maternal size, and sex ratio of pups during the last 10 DBP. Twenty-three clinically healthy pregnant bitches of 16 different breeds, 2–9-year-old and 3.5–56.8 kg bodyweight were monitored using an 8–5 MHz convex transducer connected to a US unit (Philips Affiniti 50G, Italy). US was performed in dorsal or lateral recumbency, after hair shaving and application of contact gel on the abdominal region. Fetal intestine was observed for at least 30 seconds in a longitudinal and transversal scan on three of the most caudal fetuses in both uterine horns. Gestational age was counted backward from the parturition day (day 0). The number of fetuses showing FGM was recorded in time I (−11/−5 DBP) and II (−4/0 DBP). Mann-Whitney test was performed to analyse the variations of FGM% observed in relation to time intervals and parity of dams (primiparous vs pluriparous). Kruskal-Wallis test was performed to identify variations of FGM% in relation to maternal size (small ≤10 kg, medium 11–25 kg and large ≥26 kg) and sex ratio of pups (percentage of females ≤40%, 41–60%, >60%). Significance was set as $P < 0.05$. A total of 147 FGM observations on 50 US monitoring were performed. The FGM% was higher during time II compared to time I (median: 33%, range 0–100% vs. 100%, range 33–100%; $P < 0.0001$). FGM% was higher in small compared to large size bitches (median: 100%, range 67–100% vs. 67%, range 0–100%; $P = 0.01$). FGM% was not affected by parity and sex ratio of pups. A significant increase in FGM% was observed in the last five DBP. FGM observation may be influenced by the maternal size, with easier evaluation in small size bitches compared to large size bitches. The US equipment and positioning of the dam may influence the observations of the operator.

[1] E.M.U. Gil et al., In utero development of the fetal intestine: Sonographic evaluation and correlation with gestational age and fetal maturity in dogs. Theriogenology 84,5,681–686, 2015.
[2] C. Milani et al., Ultrasonographic analysis of fetal gastrointestinal motility during the peripartum period in the dog. Ani Reprod Sci 219, 2019,106514, 2020

76° CONVEGNO SISVET
BARI, 21-22-23 GIUGNO 2023

POST-SURGICAL ULTRASOUND SUTURE ASSESSMENT FOLLOWING SMALL ANIMAL REPRODUCTIVE SURGERY

Giulia Contato (1), Maria Carlos Pereira (1), Magdalena Schrank (1), Alessia Franzoso (1), Alessandra Sarnicola (1), Stefano Romagnoli (1)

(1) Department of Animal Medicine, Production and Health (MAPS), University of Padova, Italy

Corresponding Author: **Maria Carlos Pereira** - decarvalhogoncalvespereira@phd.unipd.it

Ovariectomy and ovariohysterectomy are the most frequently performed surgical procedures in small animal practice (1). Postoperative monitoring of these procedures is conducted mainly by visual and digital inspection of the suture by the veterinary surgeon, and visually by the owners. However, potential internal complications of surgical sutures might go unnoticed to owners and only become visible at advanced stages. With this observational study we propose an additional method of suture assessment using ultrasound (US). The primary aim of this study is to describe the normal ultrasonographic aspect of the surgical incision on the day of the surgery and 1 week after.
A total 10 bitches and 5 queens presented to the Veterinary Teaching Hospital of the University of Padova for one of the following reproductive surgical procedures were included in the study: ovariectomy (n = 12), ovariohysterectomy (n = 3). US assessment of the surgical wound was conducted without sedation, 4-6 hours and 7-10 days after surgery.
The ultrasonographic aspect of the surgical wound on the day of surgery shows a slight inflammatory reaction at the subcutaneous level, where hypoechogenic areas can be found. Seven to 10 days post-surgery, the inflammatory reaction is still evident and seroma is present. Hyperechogenic lines or aggregates may be observed on the inside, which correspond to fibrin deposits and seroma reorganization. Seroma was present in 57.1% of the animals with an average extent of 1.75 ± 0.37 mm and in 100% of the animals with an average extent of 5.2 ± 3.9 mm, 4-6 hours and 7-10 days post-surgery, respectively.
This is the first report of US description of reproductive surgical wounds during the first 10 days after surgery. This study represents an additional method of post-surgical wound inspection which does not require experienced imaging skills. In our limited experience post-surgical US assessment allowed to confirm proper abdominal wall closure, which may be difficult to assess solely from visual and digital inspection. Although no case of pathological suture was observed in our study, this method is likely to allow for a precocious detection of internal suture dehiscence, herniation and excessive inflammation. Additional studies, that are already under way, are warranted to estimate the effect of animal (age and bodyweight) and surgery (duration of the surgical procedure and length of skin incision) factors on emergence of complications detectable on US.

[1] Greenfield CL, Johnson AL, Schaeffer DJ., Frequency of use of various procedures, skills, and areas of knowledge among veterinarians in private small animal exclusive or predominant practice and proficiency expected of new veterinary school gra duates. J Am Vet Med Assoc. 2004 Jun 1;224(11):1780–7.

76° CONVEGNO SISVET
BARI, 21-22-23 GIUGNO 2023

CANINE FETAL KIDNEY PARAMETERS TO MONITOR FETAL DEVELOPMENT DURING THE LAST 10 DAYS OF PREGNANCY

Giulia Siena (1), Francesca di Nardo (1), Stefano Romagnoli (1), Barbara Contiero (1), Tommaso Banzato (1), Antonio Mollo (1), Chiara Milani (1)

(1) Department of Animal Medicine, Production and Health, University of Padova, Italy

Corresponding Author: **Giulia Siena** - giulia.siena@phd.unipd.it

Our study aimed to evaluate different canine fetal kidney parameters during the last 10 days of pregnancy. Ten clinically healthy pregnant bitches of 2-8-year-old and 8.8-40.3 kg bodyweight were monitored using ultrasound (8-5 MHz convex, Philips Affiniti 50G, Italy) at least twice from -10 to 0 days before parturition (dbp, day 0=delivery). Kidney length (L), cortical (CT) and medullary thickness (MT), and their ratio (CT/MT) were measured in a longitudinal scan on three most caudal fetuses. Sonograms were analysed using Fiji Image J software (National Institute of Health, USA). Mean gray level (MGL) and standard deviation (SD) of renal cortex (C) and medulla (M) were measured. Linear mixed model considering maternal size as fixed effect, dbp and litter size as covariate, and the bitch as random and repeated effect was performed. Least-square means and standard errors were calculated. Significance: $P<0.05$. L (n=123), CT (n=119) and MT (n=119) were directly related to dbp (b= 0.68±0.14, 0.04±0.01, 0.12±0.02 mm, respectively, $P<0.01$). CT/MT decreased as parturition approached (b=0.01±0.004 mm, $P<0.01$). L showed lower values in small compared to large size bitches (17±1 vs 24±2 mm, P=0.02) and CT showed lowest values in small size and higher values in large size bitches (1.57±0.04 vs 1.77±0.04 vs 1.99±0.05 mm, $P<0.001$). C-SD (n=63) and C/M-SD (n=63) decreased as delivery approached (b=0.23±0.06, $P<0.001$; b=0.05±0.02, P=0.038). C/M-MGL was affected by maternal size, being different in large (1.95±0.13) compared to small (1.41±0.10, p=0.027) and medium size bitches (1.51 ± 0.09, p=0.016). C/M-MGL decreased as litter size increased (b=-0.08±0.03, P=0.018). Our results on fetal kidney measurements agree with what reported in human and canine medicine [1,2]. L, CT, MT and CT/MT could be useful to monitor canine kidney structures development. C-SD and C/M-SD were affected by dbp, and not by maternal and litter size, however they do not seem to be useful for parturition timing, being time consuming and not straightforward. Further studies are needed to investigate the correlation between these parameters and parturition timing as well as canine fetal renal conditions.

[1] Devriendt A, Cassart M, Massez A, Donner C, Avni FE. Fetal kidneys: additional sonographic criteria of normal development. Prenat Diagn. 2013 Dec;33(13):1248-52. doi: 10.1002/pd.4240. Epub 2013 Oct 7. PMID: 24105773.
[2] Gil EMU, Garcia DAA, Giannico AT, Froes TR. Early results on canine fetal kidney development: Ultrasonographic evaluation and value in prediction of delivery time. Theriogenology. 2018 Feb;107:180-187. doi: 10.1016/j.theriogenology.2017.11.015. Epub 2017 Nov 16. PMID: 29169145.

76° CONVEGNO SISVET
BARI, 21-22-23 GIUGNO 2023

EXPRESSION OF HEAT SHOCK PROTEINS 60, 70, 90 IN EQUINE GRANULOSA CELLS.

Maria Albrizio (1), Salvatore Desantis (1), Antonio Ciro Guaricci (1), Mario Cinone (1)

(1) Department of Precision and Regenerative Medicine and Ionian Area (DiMePre-J), University of Bari Aldo Moro, Italy

Corresponding Author: **Maria Albrizio** - maria.albrizio@uniba.it

It is well known that heat shock proteins (HSPs) are not only mediators of heat stress but also regulators of cell proliferation (1), differentiation (2) and apoptosis (3). During the equine reproductive cycle, the ovary experiences all these events. The aim of our study was to investigate on the quantitative presence of HSPs (60, 70, 90) in granulosa cells from follicles of small (6-10mm), medium (11-20mm) and large (>20mm) size (4). Granulosa cells were recovered from ovaries obtained from slaughtered females. Proteins were extracted and run on polyacrylamide gel, transferred to PVDF membranes and hybridized to monoclonal mouse primary antibodies (anti-HSP 60 cat. n. SC-13115, Santa Cruz Biotechnology; anti-HSP 70 cat. n. SC-7298, Santa Cruz Biotechnology and anti-HSP 90 cat. n. SMC-107, StressMarq Biosciences). For normalization anti-actin primary antibody was utilized (cat. n. A2103, SIGMA-Aldrich). A negative control was obtained omitting each primary antibody. Revelation was performed by the Vectastain elite system (Vector-Laboratories) and positive signals quantified by Quantity one software (BioRad). The values, expressed as arbitrary units, were calculated as the ratio of the specific protein to the corresponding beta-actin optical density. Results evidenced that granulosa cells from all different kind of follicles do not express HSP60. On the contrary, the expression level of the HSP70 is about six times higher than that of HSP90 despite the size of the follicle. Granulosa cells from large follicles show the highest HSP70 expression level compared to medium and small follicles ($P<0.05$), while HSP90 is more expressed in granulosa cells from medium follicles than that from small and large respectively ($P<0.05$). This work evidenced for the first time in the equine species the expression of HSP70 and 90 in follicular granulosa cells. Investigations on the relation about HSPs expression and the follicular size will allow establishing the involvement of these proteins in all phases of the mare reproductive cycle.

[1] G. D.Lianos et al., Cancer letters 360 (2): 114-118, 2015.
[2] A. Curci et al., Developmental Biology 144: 362–368, 1991.
[3] H.M. Beere, Journal of Cell Science 117: 2641–2651, 2004.
[4] C. Hinrichs, Biology of Reproduction 62:1402-1408, 2000.

EFFECTS OF COMBINED EXPOSURE TO HEAT AND CADMIUM ON IN VITRO MATURATION OF OVINE OOCYTES

Podda A. (1), Nieddu S. (1), Maltana A. (1), Leoni G.G. (1), Satta V (1). Martino N.A, (2), Dell'Aquila M.E (2), Bogliolo L. (1)

(1) Department of Veterinary Medicine, University of Sassari, Sassari, Italy
(2) Department of Biosciences, Biotechnologies & Environment, University of Bari Aldo Moro, Bari, Italy

Corresponding Author: **Luisa Bogliolo** - luis@uniss.it

The impact of a broad spectrum of environmental stressors, such as heavy metals, on female reproductive health and fertility in mammals is an alarming problem. Cadmium (Cd) is one of the most toxic heavy metals and it is known to adversely affect oocyte quality even at low concentrations (Martino et al., 2017; doi 10.1016/j.reprotox.2017.02.005). Information on the interaction of stressors, such as Cd toxicity and global warming on the mammalian female gametes, is still lacking. This study aimed at exploring the effects of combined exposure to heat stress (HS) and low Cd concentration on in vitro maturation (IVM) of ovine oocytes.

Oocytes collected from ovaries of slaughtered juvenile ewes were submitted to IVM for 24h at 38.5°C without (n=114, control group, CTR) or with 100 nM CdCl2 (n=107, Cd group) and at 41.5°C without (n=109, HS group) or with CdCl2 (n=126, HS-Cd group). Following IVM, (i) cumulus cells (CCs) expansion and prevalence of apoptosis (Tunel labelling); (ii) oocyte meiotic maturation (Hoechst-33342 staining) and early apoptosis (Annexin-V staining); (iii) oocyte intracellular reactive oxygen species (ROS) and glutathione (GSH) levels were measured. Data on CCs, oocyte nuclear maturation and apoptosis, were analyzed by Chi-square test; ROS and GSH levels by parametric analysis of variance (ANOVA) using Stata/IC 11.2.

The percentage of oocytes showing CCs full expansion was lower ($P<0.05$) in HS-Cd group (11.4%) compared to HS (22.3%), Cd (44.4%) and CTR (53.5%) groups. The CCs apoptotic rate was higher ($P<0.05$) in HS (74.4%) and HS-Cd (73.5%) groups than CTR (85.5%) and Cd (81.9%) oocytes. Nuclear maturation rate decreased ($P<0.05$) in HS (79.8%) and HS-Cd (73.8%) oocytes respect to CTR ones (91.2%). There was a significant increase ($P<0.05$) in Annexin-V (+) oocytes in HS-Cd group (49.2%) compared to the other groups. HS-Cd group also showed higher levels of ROS ($P<0.05$) respect to the other groups, whereas GSH levels increased in both HS and HS-Cd groups compared to Cd and CTR ones.

Our findings indicated that warmer temperature potentiated the negative impact of low Cd concentration on CCs and oocytes. Further studies are ongoing to better understand joint toxicity mechanisms of HS and Cd exposure on the quality of female mammalian gametes.

Funded by EU, (PNRR–D.D.1032 17/06/2022,CN00000022), Agritech National Research Center and Fondo di Ateneo per la ricerca 2020- Bogliolo.

[1] Martino NA, Marzano G, Mangiacotti M, Miedico O, Sardanelli AM, Gnoni A, et al. Exposure to cadmium during in vitro maturation at environmental nanomolar levels impairs oocyte fertilization through oxidative damage: A large animal model study. Reprod Toxicol. 2017;69:132–145. doi: 10.1016/j.reprotox.2017.02.005.

MELATONIN PROMOTES THE *IN VITRO* MATURATION OF CAT VITRIFIED OOCYTES

Martina Colombo (1), Alessandra Mascaro (1), Jasmine Fusi (1), Gaia Cecilia Luvoni (1)

(1) Dip. Medicina Veterinaria e Scienze Animali (DIVAS), Università degli Studi di Milano

Corresponding Author: **Martina Colombo** - martina.colombo@unimi.it

The combination of immature oocyte cryopreservation and in vitro maturation (IVM) allows exploiting the genetic material of valuable individuals, domestic or wild, after spaying or death, but results still need improvements. Preserved oocytes mature to lower rates than fresh ones and oxidative stress and apoptosis hinder survival and development of cryopreserved oocytes. Melatonin (MLTN) was suggested to exert antioxidant and antiapoptotic effects on in vitro matured oocytes of different mammalian species, but it remains to be investigated on cat oocytes. Thus, the aim of this study was to test if MLTN could be beneficial for the IVM of cat oocytes vitrified by Cryotop. Fresh oocytes were firstly used to determine the best concentration of MLTN.

Ovaries were obtained at spaying and cumulus oocytes complexes (COCs) were matured as fresh for 24 h in a controlled atmosphere (38.5°C and 5% CO2 in air) in Medium 199 with 3 mg/mL bovine serum albumin, 10 ng/mL epidermal growth factor (EGF), 0.6 mM cysteine and 0.5 IU/mL follicle-stimulating hormone (FSH) + 0.5 IU/mL luteinizing hormone (LH), supplemented with four concentrations (10-7 M, 10-9 M, 10-11 M, 0 M as control) of MLTN. The best concentration was added during oocyte vitrification-warming and/or IVM of vitrified oocytes. Chromatin configurations were assessed by bis-benzimide (Hoechst 33342) staining after IVM. Data were analyzed by Fisher's exact test (significance at $p<0.05$).

Fresh control COCs reached full maturation (telophase I-metaphase II, TI-MII) at 51.48% (18/35 COCs), 10-11 M MLTN lowered it to 38.71% (12/31), and the addition of melatonin 10-7 and 10-9 M brought it to 58.06% (18/31; $p=0.63$ vs fresh COCs). The lowest concentration with beneficial effects (10-9 M) was used for vitrified oocytes. While TI-MII rate of control vitrified COCs (no MLTN) was 12.12% (4/33) and the addition of MLTN only during vitrification-warming or IVM slightly increased the rate to 22.58% (7/31; $p=0.33$), its addition both during cryopreservation and IVM significantly improved it, giving 48.39% (15/31) mature COCs ($p<0.01$).

In summary, MLTN 10-9 M improved the IVM of cat vitrified oocytes. The use of MLTN during cat oocyte vitrification and culture could contribute to improve the developmental competence of biobanked oocytes, bringing it closer to fresh gametes and allowing the achievement of better outcomes from the genetic material of valuable subjects.

Supported by UNIMI "PSR Linea 2" 2020 and 2021.

76° CONVEGNO SISVET
BARI, 21-22-23 GIUGNO 2023

ASSESSMENT OF POLYSTYRENE NANO- AND MICROPLASTIC (NMPS) UPTAKE IN IN VITRO EXPOSED CUMULUS-OOCYTE COMPLEXES

Podda A. (1), Mongelli I. (2), Manchisi M. (2), Leoni G. (1), Bogliolo L. (1), Dell'Aquila M.E. (2), Martino N.A. (2)

(1) Department of Veterinary Medicine, University of Sassari, Via Vienna n. 2, 07100 Sassari, Italy
(2) Department of Biosciences, Biotechnologies & Environment, University of Bari Aldo Moro, Via Edoardo Orabona, 70125 Bari, Italy

Corresponding Author: **Nicola Martino** - nicola.martino@uniba.it

One of the main concerns related to plastic pollution regards the impact that nano-microplastics (NMPs) can have on human and animal health. They have been shown to accumulate in the gastrointestinal tract of different animal species. Recently, NMPs have been detected in human placenta (1) and ovarian tissue, affecting mice fertility (2). To date, there are no experimental evidences demonstrating the detection of NMPs at cumulus-oocyte complex (COC) level. Therefore, the aim of the present study was to verify the uptake and the bioaccumulation of fluorescent NMPs in ovine COCs exposed in vitro.
Cumulus-oocyte complexes from the ovaries of slaughtered prepubertal lambs (4/6-months old) underwent in vitro maturation (IVM) in a TCM199-based medium under 5% CO2 in air at 38.5°C for 24h (3) in presence of fluorescent polystyrene NMPs beads (PS-NMPs) of different size (50 or 200 nm in diameter) and concentration (5 or 100 ug/ml). Oocytes cultured in absence of beads were used as controls. Following IVM, COCs were fixed in paraformaldehyde, mounted on glass slide, stained with Hoechst 33258 for nuclear chromatin evaluation and observed under Confocal Laser Scanning Microscopy (CLSM) for NMP detection.
A total of 110 oocytes were cultured for IVM. After 24 hours, COCs exposed to 100 ug/ml PS-NMPs showed intense fluorescence detection of beads in cumulus cells regardless of particle size. In fact, for both 50 nm PS-NMPs (20/20) and 200 nm PS-NMPs (20/20), COCs showed a consistent intracellular bead uptake. There was no evidence of their uptake when COCs were exposed to 5 ug/ml PS-NMPs compared to controls, for both NMP sizes (0/20 for 50 nm PS-NMP; 0/20 for 200 nm PS-NMP). Samples cultured under control conditions did not show any nonspecific fluorescence signal (0/30). In our preliminary study, NMPs do not enter the oocyte which could be protected by the zona pellucida and/or by the structure of the COC (eg. by the size of the intercellular communications of the CCs and CC-oocyte).
In conclusion, our results demonstrate that different PS-NMP sizes are taken up by cumulus cells during IVM. This was clearly visible when 100 ug/ml PS-NMP were used. The lack of fluorescence signals with at 5 ug/ml PS-NMPs could be related to their low concentration or to CLSM sensitivity. Further studies are needed to define the subcellular localization of NMPs and to assess their toxicity.

[1] Ragusa et al., Environment International 146 (2021) 106274
[2] Wei et al., Toxicology 465 (2022) 153059
[3] Martino et al., Reproductive Toxicology 65 (2016) 204–211

76° CONVEGNO SISVET
BARI, 21-22-23 GIUGNO 2023

GENE EXPRESSION ANALYSIS IN CUMULUS CELLS OF IVM OVINE OOCYTES: PREPUBERTAL VERSUS ADULT COMPARISON

Martino N.A. (1), Mongelli I. (1), Bogliolo L. (2), Temerario L. (1), Mastrorocco A. (1), Lacalandra G.M. (3), Dell'Aquila M.E. (1)

(1) Department of Biosciences, Biotechnologies & Environment, University of Bari Aldo Moro, Via Edoardo Orabona, 70125 Bari, Italy
(2) Department of Veterinary Medicine, University of Sassari, Via Vienna n. 2, 07100 Sassari, Italy
(3) Department of Veterinary Medicine, University of Bari Aldo Moro, Str. Prov. Casamassima Km. 3, 70010 Valenzano, Italy

Corresponding Author: **Nicola Martino** - nicola.martino@uniba.it

Cumulus cells (CCs) play important roles during cumulus-oocyte complex (COC) growth, maturation and fertilization [1]. Recently, the transcriptomic profiles of CCs isolated from mature ovine oocytes has been analysed and correlated with functional aspects of oocyte development [2]. The aim of the study was to evaluate the expression levels of genes related to oocyte competence in CCs of COCs retrieved from the ovaries of slaughtered prepubertal (p, <6 months) and adult (a, 2-8 years) sheep. COCs were individually in vitro cultured in maturation medium for 24h at 38.5°C under 5% CO2 [2]. After IVM, CCs of both groups were removed and only those isolated from matured oocytes, showing the first polar body, were pooled (groups of CCs from 10-20 COCs/sample) and stored at -80°C until analysis. Each CCs sample was processed for RNA extraction, retrotranscription to cDNA and Real-Time PCR, to evaluate differentially expressed genes. Oocyte nuclear maturation rates were analysed by the Chi-square test whereas gene expression data were compared by the Student's t-test. Differences were considered to be significant when $P<0.05$.
Five and three replicates were performed for p- and a- specimens, respectively. A total of 80 p- and 33 a-COCs were cultured, respectively. No significant differences were obtained in oocytes nuclear maturation rates between groups (p, 43/80, 53.7%; a, 17/33, 51.5%; $P>0.05$). Genes involved in oocyte growth processes (competence and viability acquisition) such as IGF2, IGF2R, VEGFA and STAR were statistically downregulated ($P<0.001$) in adult sheep, as a sign of their reduced proliferation and differentiation requirements compared to prepubertal ones. On the contrary, genes involved in folliculogenesis (BMP15), cellular metabolism (PFKL) and transport of metal ions and small molecules (SLC17A5 and SLC40A1) were upregulated ($P<0.001$) in prepubertal to adult development. Metallothionein 1A was downregulated ($P<0.001$) meaning increased protection against oxidative stress. HYAL2 did not show different expression ($P>0.05$) but had a rising trend in adults, probably related to the preservation of hyaluronidase functionality in COC expansion.
In conclusion, our results on genes identified as differentially expressed in CC of p- and a- sheep improve the knowledge on molecular mechanisms underlying oocyte maturation and development processes. Furthermore, these genes could be considered as non-invasive and predictive biomarkers of oocyte quality.

[1] Dhali et a., J Assist Reprod Genet, 34:1493–1500, 2017
[2] Martino et al., Biology, 12:249, 2023

76° CONVEGNO SISVET
BARI, 21-22-23 GIUGNO 2023

EFFECTS OF DI-(2-ETHYLHEXYL) PHTHALATE AND CADMIUM MIXTURE DURING IVM ON PREPUBERTAL SHEEP OOCYTE NUCLEAR MATURATION AND BIOENERGETIC/OXIDATIVE PARAMETERS

Antonella Mastrorocco (1), Valeria Vurchio (1), Letizia Temerario (1), Nicola Antonio Martino (1), Giovanni Michele Lacalandra (2), Luisa Bogliolo (3), Maria Elena Dell'Aquila (1)

(1) Department of Biosciences, Biotechnologies & Environment, University of Bari Aldo Moro, Bari, Italy
(2) Department of Veterinary Medicine, University of Bari Aldo Moro, Valenzano, Italy
(3) Department of Veterinary Medicine, University of Sassari, Sassari, Italy

Corresponding Author: **Antonella Mastrorocco** - antonella.mastrorocco@uniba.it

Di-(2-ethylhexyl) phthalate (DEHP) and Cadmium (Cd) are ubiquitous environmental contaminants [1]. In vitro studies, performed to date, separately analyzed their damaging effects on female germ cells [2-4]. The aim of the present study was to analyze the effects of a DEHP/Cd mixture on nuclear maturation and bioenergetic/oxidative status of prepubertal sheep oocytes compared with those of each single compound. Cumulus-oocyte complexes (COCs) recovered from slaughterhouses ovaries underwent in vitro maturation (IVM) [4] in presence of DEHP, Cd or DEHP/Cd mixture. Cd and DEHP concentrations were chosen based on a previous study on Cd (0.1µM) [4] and preliminary trials with DEHP (0.5 µM). COCs cultured in IVM medium with MeOH, as DEHP vehicle, were used as control (CTR). After IVM, nuclear maturation and bioenergetic/oxidative parameters were evaluated [4]. Data were analyzed by Chi-square test and one-way ANOVA (significance at $p<0.05$). To select an effective DEHP concentration (0.1 or 0.5µM), 301 oocytes were analyzed in 3 replicates. The maturation rate was not affected at both tested concentrations. However, 0.5µM DEHP reduced the percentage of oocytes showing healthy perinuclear/pericortical (pp) mitochondria (mt) distribution pattern (10/41, 24% vs 17/34, 50%, $p<0.05$), mt activity (275±94 vs 368±147, $p<0.05$), intracellular ROS levels (164±104 vs 323±169, $p<0.001$) and mt/ROS colocalization (0.4±0.1 vs 0.7±0.1, $p<0.001$) compared to CTR. For the mixture test, 265 oocytes were analyzed in 3 replicates. No effects were noticed on oocyte maturation rate at any tested conditions (DEHP/Cd mixture, Cd, DEHP, CTR). However, the mixture and both compounds reduced the rate of mature oocytes with pp mt pattern (2/33, 6%; 4/29, 14%; 8/34, 24% vs 15/27, 56% for mix, DEHP, Cd and CTR respectively, $p<0.05$), mt activity (316±235, 180±158, 298±244 vs 607±455 for mix, DEHP, Cd, mix and CTR respectively, $p<0.01$) and mt/ROS colocalization (0.4±0.3, 0.3±0.2, 0.4±0.2 vs 0.6±0.2 for mix, DEHP, Cd and CTR respectively, $p<0.01$) compared to CTR. As well, intracellular ROS levels were reduced at any tested condition, but they attained statistical significance only in MII oocytes exposed to DEHP (260±185, 140±133, 239±169 vs 476±325 for mix, DEHP, Cd, and CTR respectively, $p<0.05$, ns,ns). In conclusion, the DEHP/Cd mixture altered bioenergetic/oxidative status of prepubertal sheep oocytes, similarly to what observed for DEHP and Cd separetely.

[1] Lauretta et al. 2019 Front Endocrinol
[2] Ambruosi et al. 2011 PLoS One
[3] Marzano et al. 2019 Mol Reprod Dev
[4] Martino et al. 2017 Reprod Toxicol

76° CONVEGNO SISVET
BARI, 21-22-23 GIUGNO 2023

THREE-DIMENSIONAL FEMALE REPRODUCTIVE IN VITRO TOXICITY TEST: EFFECTS OF THE MYCOTOXIN BEAUVERICIN.

Antonella Mastrorocco (1), Ludovica Cacopardo (2), Giovanni Michele Lacalandra (3), Fiorenza Minervini (4), Maria Elena Dell'Aquila (1)

(1) Department of Biosciences, Biotechnologies & Environment, University of Bari Aldo Moro, Via Edoardo Orabona, 70125 Bari, Italy
(2) Research Centre E. Piaggio, University of Pisa, Largo Lucio Lazzarino 1, 56122 Pisa, Italy
(3) Department of Veterinary Medicine, University of Bari Aldo Moro, Str. Prov. Casamassima Km. 3, 70010 Valenzano, Italy
(4) Institute of Sciences of Food Production (ISPA), National Research Council of Italy (CNR), Via G. Amendola 122/O, 70125, Bari, (BA), Italy

Corresponding Author: **Antonella Mastrorocco** - antonella.mastrorocco@uniba.it

In vitro exposure of cumulus-oocyte complexes (COCs) to the mycotoxin Beauvericin (BEA) in conventional 2D in vitro maturation (IVM) systems induced structural and functional COC damage [1]. The use of 3D-IVM systems, better mimicking the in vivo microenvironment, could provide more affordable data to predict the effects of toxic compounds. We recently produced a 3D-IVM protocol in which COCs were encapsulated in alginate microbeads. This method improved oocyte viability and competence compared to 2D-IVM [2,3]. The aim of this study was to analyze the effects of BEA on nuclear maturation of COCs cultured under 3D versus 2D-IVM. Increasing micromolar BEA concentrations were added to IVM medium of prepubertal ovine COCs [1,2]. After IVM, oocytes were stained with Hoechst 33258, fixed in 3.8% formaldehyde solution in PBS and observed under epifluorescence microscopy to assess their meiotic stage (Chi-square test: significance at $P<0.05$). A total of 1277 oocytes was analyzed in 5 replicates. Data showed greater sensitivity of COCs to BEA when cultured under 3D compared to the 2D system. Infact, after 3D-IVM, BEA significantly reduced oocyte maturation rate at any tested concentration (5, 3 and 1 µM; 57/156, 37%, 72/143, 50%, 74/152, 49% vs 83/129, 64% for 5, 3, 1 µM BEA and controls, respectively; $p<0.001$, $p<0.05$, $p<0.01$). Moreover, after 3D-IVM, 5 µM BEA significantly increased the rate of oocytes remained at the GV stage (44/156, 28% vs 22/129, 17% for 5µM BEA and controls, respectively, $p<0.05$) and showing abnormal chromatin configurations (27/156, 17% vs 9/129, 7% for 5µM BEA and controls, respectively, $p<0.01$). Instead, after 2D-IVM, BEA reduced the maturation rate only at 5 µM (62/132, 47% vs 70/110, 64%, for exposed and controls, respectively; $P<0.01$). So, it can be hypothesized that, under 3D conditions, BEA ionophoric properties could have been better explicated, thus causing greater COC damage. In conclusion, these results complement and reinforce previous data on BEA effects on oocyte maturation. Moreover, the importance of performing female reproductive in vitro toxicity tests by using COCs cultured under 3D more physiological conditions is highlighted.

[1] Mastrorocco et al., Mol Reprod Dev. 2019;
[2] Mastrorocco et al., PLOS ONE. 2020;
[3] Mastrorocco et al., Cells. 2021

CULTIVATION AND IDENTIFICATION OF MICROBIOTA IN SHEEP FOLLICULAR FLUID

Slavcho Mrenoshki (1), Grazia Visci (2), Elisabetta Notario (3), Marinella Marzano (2), Nicola Antonio Martino (3), Antonella Mastrorocco (4), Letizia Temerario (3), Marialaura Corrente (5), Daniela Mrenoshki (5), Giovanni Michele Lacalandra (5), Graziano Pesole (3), Maria Elena Dell'Aquila (3)

(1) Ss. Cyril and Methodius University in Skopje, Faculty of Veterinary Medicine / Visiting professor at University of Bari A ldo Moro, Department of Biosciences, Biotechnologies and Environment
(2) Institute of Biomembranes, Bioenergetics and Molecular Biotechnologies, National Research Council, Bari
(3) University of Bari Aldo Moro, Department of Biosciences, Biotechnologies and Environment
(4) University of Teramo, Faculty of Veterinary Medicine
(5) University of Bari Aldo Moro, Department of Veterinary Medicine

Corresponding Author: **Slavcho Mrenoshki** - mrenoski@fvm.ukim.edu.mk

In the microbiome research, the distant organs such as fallopian tubes and ovaries including follicular fluid (FF) are the very last investigated area of the reproductive tract. Though low in number, almost all of the reports concerning the FF microbiota are related to the humans. Additionally, there are still doubts about the microbiota findings in FF - are they genuine colonizers or contaminants introduced during the sampling procedure. Motivated by the previous, we performed this research aiming to demonstrate for the first time microbiota colonizers of sheep FF and expand the microbiome knowledge of the upper reproductive tract, particularly in non-human subjects. Sheep reproductive tracts and later separated ovaries, obtained from commercially slaughtered healthy animals, were first decontaminated by sterile saline/PBS washings and FFs (15 in total) were aspirated by syringe from a dominant or 2-3 largest follicles. To control the applied procedure and possible samples contamination, we also swabbed 6 prepubertal sheep ovary surfaces after the decontamination procedure and before the FF sampling. FF samples and swabs were cultivated in nutrient broth which allows multiplication of wide range of fastidious anaerobic and aerobic microorganisms. The inoculated tubes (including uninoculated broth as negative control and tubes inoculated with laboratory strains of Escherichia coli and Staphylococcus aureus as positive controls) were incubated at 37oC in aerobic atmosphere. After 7 days of incubation, the tubes with visible bacterial growth (increased turbidity, 10 in total), as well as the control tubes, were centrifuged and bacterial pellets harvested for further investigation. The enriched bacterial genera were identified by the amplicon based metagenomic approach, adopting as sequencing target the V4 hypervariable region of the 16S rDNA. Some of the identified genera were described in previous FF microbiome studies (Escherichia-Shigella group, Streptococcus and Corynebacterium), but for Burkholderia-Caballeronia-Paraburkholderia group, Oligella, Brevibacterium and Dietzia this is the first report of its presence in FF, either animal or human. Since the possible contamination during the sampling procedure was excluded by comparing results from the paired surface swabs and FFs of the same ovary, we can conclude that the sheep follicular fluid can be colonized by bacteria that includes previously not reported genera in this niche of the reproductive tract.

[1] Pelzer ES, Allan JA, Cunningham K, Mengersen K, Allan JM, et al. (2011) Microbial colonization of follicular fluid: alterations in cytokine expression and adverse assisted reproduction technology outcomes. Hum Reprod 26: 1799-1812.
[2] Pelzer ES, Allan JA, Theodoropoulos C, Ross T, Beagley KW, Knox CL. Hormone-dependent bacterial growth, persistence and biofilm formation--a pilot study investigating human follicular fluid collected during IVF cycles. PLoS One. 2012;7(12):e49965. doi: 10.1371/journal.pone.0049965. Epub 2012 Dec 4. PMID: 23226503; PMCID: PMC3514270.
[2] Noor SO, Alosaimi EH, Najjar AA, et al. (2020) Detection of Human Follicular Fluid Microorganisms at the Time of Oocyte Retrieval and its Impact on in-vitro Fertilization Outcomes. Prensa Med Argent, S2:004. DOI: https://doi.org/10.47275/0032-745X-S2-004.
[4] Kim SM, Won KH, Hong YH, Kim SK, Lee JR, Jee BC, Suh CS. Microbiology of Human Follicular Fluid and the Vagina and Its Impact on in Vitro Fertilization Outcomes. Yonsei Med J. 2022 Oct;63(10):941-947. doi: 10.3349/ymj.2022.0190. PMID: 36168247; PMCID: PMC9520042.

DEVELOPING A GENTILE DI PUGLIA LOCAL SHEEP BREED GERMPLASM CRYOBANK: VITRIFICATION OF PREPUBERTAL IMMATURE CUMULUS-OOCYTE COMPLEXES

Temerario L. (1), Mastrorocco A. (1), Martino N.A. (1), Cseh S. (2), Lacalandra G.M. (3), Ciani E. (1), Dell'Aquila M.E. (1)

(1) Department of Biosciences, Biotechnologies and Environment, University of Bari "Aldo Moro", Italy
(2) University of Veterinary Medicine, Budapest, Hungary
(3) Department of Veterinary Medicine, University of Bari "Aldo Moro", Italy

Corresponding Author: **Letizia Temerario** - letizia.temerario@uniba.it

Gentile di Puglia (GdP) is a local sheep breed, typical of Southern Italy, with an aptitude for meat, wool and milk production. The wool industry crisis and indiscriminate crossbreedings have greatly reduced the number of individuals of this breed with high productive, historical and cultural value [1]. The cryopreservation of immature cumulus-oocyte complexes (COCs) from prepubertal animals represents a chance to rescue germplasm in farms located in marginal areas, far from equipped laboratories. The aim of the present study was to evaluate the in vitro maturation (IVM) rate of vitrified/warmed (V/W) immature COCs, from GdP prepubertal lambs and the bioenergetic/oxidative status of matured oocytes.
GdP prepubertal lamb COCs underwent vitrification [2]. V/W and fresh COCs (n.107 and 121, respectively) underwent IVM and nuclear chromatin evaluation ($\chi 2$ test) in 5 independent runs. Oocytes at the metaphase II (MII) stage were assessed for mitochondrial distribution pattern ($\chi 2$ test) and activity, intracellular reactive oxygen species (ROS) levels and mitochondria/ROS overlap coefficient (Unpaired Student's t test) [3]. Statistical significance was set at $p<0.05$.
After IVM, V/W COCs showed significantly reduced maturation rate when compared to fresh ones (14%, 14/97 vs 52%, 58/112, respectively; $p<0.00001$). Concerning the bioenergetic-oxidative status, the rate of MII oocytes showing perinuclear/subcortical mitochondrial distribution patterns did not differ between groups (50%, 7/14 vs 66%, 38/58). Moreover, no differences were observed for mitochondria activity (666,5 ± 264, 5 vs 687,2 ± 445,6), ROS levels (115,4 ± 79,4 vs 204,8 ± 226,4) and overlap coefficient (0,4 ± 0,1 vs 0,4 ± 0,2) between groups, thus indicating healthy cytoplasmic conditions.
Our results are in line with those of a previous study in which vitrification significantly reduced the maturation rate of prepubertal lamb immature COCs of other breeds (13% vs 80%) [4]. Moreover, our study highlighted that vitrification preserves oocyte cytoplasmic maturity, expressed as bioenergetic-oxidative status, important for oocyte developmental competence. Further studies are needed to test the potential embryonic development of V/W immature COCs and to improve vitrification procedure and/or IVM conditions with the aim of developing a cryobank of GdP sheep breed.

Funded by PhD Program 2020 FSC - Piano Stralcio "R&I 2015-2017", University of Bari, Italy

[1] Sardaro & La Sala, 2021. doi: 10.3390/ani11030731
[2] dos Santos-Neto et al., 2020. doi: 10.1016/j.cryobiol.2020.01.002
[3] Mastrorocco et al., 2021. doi: 10.3390/cells10061458
[4] Silvestre et al., 2006. doi: 10.1016/j.anireprosci.2005.08.006

SOFIVET

CORTISOL AND DHEA IN RAINBOW TROUT (ONCHORHYNCUS MYKISS) EXPOSED TO ACUTE STRESS: INFLUENCE OF SEX AND SEXUAL MATURITY

Martina Bortoletti (1), Andrea Meloni (1), Elisa Fonsatti (1), Gianfranco Gabai (1), Giuseppe Radaelli (1), Daniela Bertotto (1)

(1) Department of Comparative Biomedicine and Food Science (BCA), University of Padova

Corresponding Author: **Martina Bortoletti** - martina.bortoletti@unipd.it

Fish welfare is traditionally assessed measuring cortisol levels, the ultimate stress hormone in these animals. However, relying only on this biomarker might be limiting, especially when describing long-term stress. Among the physiological indicators, dehydroepiandrosterone (DHEA), a precursory androgen with anti-stress effects, has already been used in diagnosing chronic stress, but only in humans [1]. Although DHEA has recently been demonstrated to increase in fish exposed to a chronic stress [2], still very little is known about the role of this hormone in fish physiology. For this reason, we aimed to study how the levels of this steroid are affected not only by stress, but also by sex and degree of sexual maturity. To this purpose, four groups of rainbow trout were sampled at a commercial farm: mature female and males, immature females and males. Half fish of each group were subjected to 30 minutes confinement stress and the other half were controls. For short- and long-term stress assessment, blood, lateral muscle and caudal fin were collected to quantify both cortisol and DHEA through Radioimmunoassay analysis. As expected, stressed fish had higher serum cortisol levels than controls ($p<0.0001$). Mature fish had higher muscle ($p<0.01$) and fin ($p<0.001$) cortisol levels than the immature. However, we did not find sex-related differences in cortisol levels in any of the matrices. Conversely, we found that DHEA levels were lower in males than in females in all the matrices ($p<0.05$). Along the same line, mature fish had lower fin DHEA levels than the immature ($p<0.001$). As expected, we found no differences in serum DHEA since, in this study, fish were exposed to an acute stress. Nevertheless, we found stressed fish had higher muscle DHEA levels than controls and the opposite trend in the fin ($p<0.05$), which clearly warrant further investigation. We are currently seeking the meaning of the interactions between the three factors studied, using also other less-invasive matrices, such as skin mucus and scales. In conclusion, we successfully quantified cortisol and DHEA in all the matrices and, to the best of our knowledge, this is the first time DHEA has been evaluated with respect to sex and degree of sexual maturity in fish. In the future, we plan to implement the present study assessing a longer-term stress to gain insights into the physiological meaning of DHEA and its suitability as a complementary physiological indicator of chronic stress in fish.

[1] Whitham JC, Bryant JL, Miller LJ. Beyond Glucocorticoids: Integrating Dehydroepiandrosterone (DHEA) into Animal Welfare Research. Animals (Basel). 2020; 10:1381. https://doi.org/10.3390/ani10081381
[2] Kennedy EKC, Janz DM. Chronic stress causes cortisol, cortisone and DHEA elevations in scales but not serum in rainbow trout. Comp Biochem Physiol A Mol Integr Physiol. 2023; 276:111352. https://doi.org/10.1016/j.cbpa.2022.111352

GENE EXPRESSION AND ENZYMATIC ACTIVITY OF CHORIOLYSIN IN EGGS OF EUROPEAN EEL (*A. ANGUILLA*) FROM SPAWNING TO HATCHING

Alberto Elmi (1), Augusta Zannoni (1), Nadia Govoni (1), Antonio Casalini (1), Monica Forni (1), Domenico Ventrella (1), Albamaria Parmeggiani (1), Oliviero Mordenti (1), Maria Laura Bacci (1)

(1) Department of Veterinary Medical Sciences, Alma Mater Studiorum - Università di Bologna

Corresponding Author: **Alberto Elmi** - alberto.elmi2@unibo.it

Hatching of fish embryos is a cascade event involving enzymatic digestion of the chorion. Choriolysin, or hatching enzyme, is a proteolytic enzyme secreted by the hatching gland of the embryo, belonging to the astacin zinc-depent metalloproteinases family. Its molecular structure and functions were mainly characterized in Medaka, whose hatching enzyme system consists of two zinc-proteases: a high and a low choriolytic enzyme [1-2]. In the Japanese eel (A. japonica), but never in the European one (A. anguilla), only one eel hatching enzyme (EHE) was identified. The EHE cleavage sites on the chorion proteins are located in the N-terminal repeat regions, and proteolytic activity induces swelling of the egg envelope, prodromal to hatching [3-4]. Since the European eel is listed as critically endangered under the IUCN Red List and complete life cycle in captivity was never achieved, deepening knowledge regarding its reproductive physiology is critical. Aim of this study was to evaluate the EHE in A. anguilla embryos at different developmental stages. Six females (n=6) were included in the experiment. Embryos/eggs were collected at spawning (T0, artificial fertilization), upon separation between fertilized (F) and unfertilized (UF), and after 50h of incubation (T50, embryonated eggs before hatching), divided in viable (V) and non-viable (NV). EHE gene expression was assessed by qRT-PCR; collagenase activity upon activation was quantified using a fluorometric Collagen degradation/Zimography kit, with modified protocol adapted to fish. Paired T tests and ANOVAs were performed. Collagenase activity and EHE gene expression were quantifiable in all samples. EHE gene was already expressed at T0, with statistically relevant higher levels in F eggs ($p= 0.0103$). At T50, EHE was more expressed ($p= 0.0009$), but without differences between groups (V vs NV). Collagenase activity also increased at T50 ($p= 0.0368$), again without differences between V and NV. Results show how EHE gene mRNAs are already present at spawning, most likely of maternal derivation, and fertilized eggs may show higher levels due to better cytoplasmic maturation. The increase in EHE gene expression and collagenase activity at T50 is a reflection of functional development of the hatching gland in preparation to hatching. This study represents a first report regarding the presence and activity of EHE in European eel and may support further studies aimed at captive breeding of this endangered species.

[1] Yamagami K., "Studies on the Hatching Enzyme (Choriolysin) and Its Substrate, Egg Envelope, Constructed of the Precursors (Choriogenins) in Oryzias latipes: A Sequel to the Information in 1991/1992", Zoological Science, 13(3) : 331-340 (1996).
[2] Miftari M. H., Jan-Inge B. Chun J. R., Frank N., Bernt T. W.. «Choriolytic and Non-Choriolytic Proteases during Hatching of Atlantic Salmon Embryos». Comparative Biochemistry and Physiology Part B: Biochemistry and Molecular Biology 262 (2022): 110776.
[3] Sano, K., Mari K., Masayuki Y., Toyoji K., Toshiomi T., Ichiro I., Shigeki Y.. «Hatching Enzyme of Japanese Eel Anguilla Japonica and the Possible Evolution of the Egg Envelope Digestion Mechanism». The FEBS Journal 278, n. 19 (2011): 3711–23.
[4] Hiroi J., Kouichi M., Kouji K., Toyoji K., Ritsuko O., Shigeki Y. «Structure and Developmental Expression of Hatching Enzyme Genes of the Japanese Eel Anguilla Japonica: An Aspect of the Evolution of Fish Hatching Enzyme Gene». Development Genes and Evolution 214, n. 4 (2004): 176–84.

PUPPIES RAISED DURING THE COVID-19 LOCKDOWN SHOWED FEARFUL AND AGGRESSIVE BEHAVIORS IN ADULTHOOD: AN ITALIAN SURVEY

Luigi Sacchettino (1), Claudia Gatta (1), Luigi Avallone (1), Francesco Napolitano (1,2), Danila d'Angelo (1)

(1) Department of Veterinary Medicine and Animal Production, University of Naples Federico II, 80137 Naples, Italy
(2) CEINGE-Biotecnologie Avanzate Franco Salvatore-Via G. Salvatore, 486-80145 Naples, Italy

Corresponding Author: **Luigi Sacchettino** - sacchettinoluigi@gmail.com

During the COVID-19 pandemic, the Prime Minister's decree issued by the Italian government on 9 March 2020, known as "#Iorestoacasa" (I stay at home), required everyone to stay indoors, with a few exceptions, from 11 March to 3 May 2020. This decree had a substantial impact on the mental health of both people and dogs (1). We carried out a national survey to compare the personalities of adult dogs (2) who were puppies in lockdown (from March to May 2020) with those of adult dogs born after that time (from June 2020 to February 2021). Our results highlighted a significant increase in personality traits related to fear and aggression in dogs who experienced lockdown restrictions during their socialization period (3), further confirming that the COVID-19 pandemic strongly impacted the behavioral development of dogs. Therefore, it may be advantageous for these dogs to be closely monitored by veterinary behaviorists and receive specialized behavioral rehabilitation therapy to lower the risk of episodes of aggression and fearfulness as well as to increase the wellbeing of dogs raised under social restrictions.

[1] d' Angelo, D.; Chirico, A.; Sacchettino, L.; Manunta, F.; Martucci, M.; Cestaro, A.; Avallone, L.; Giordano, A.; Ciani, F. Human-Dog Relationship during the First COVID-19 Lockdown in Italy. Animals 2021, 11, 2335.
[2] Jones, A.C. Development and Validation of a Dog Personality Questionnaire. Ph.D. Thesis, University of Texas, Austin, TX, USA, 2009.
[3] Tiffani J Howell, Tammie King & Pauleen C Bennett (2015) Puppy parties and beyond: the role of early age socialization practices on adult dog behavior, Veterinary Medicine: Research and Reports, 6:, 143-153.

76° CONVEGNO SISVET
BARI, 21-22-23 GIUGNO 2023

ANIMAL HOARDING DISORDER AND ANIMAL WELFARE: A PRELIMINARY RETROSPECTIVE STUDY IN ITALY

Gatta C. (1), Sacchettino L. (1), Avallone L. (1), Napolitano F. (1-2), Bellini F. (3), Riverini A. (4), d'Angelo D. (1)

(1) Department of Veterinary Medicine and Animal Productions, University of Naples Federico II, Naples, Italy
(2) CEINGE-Biotecnologie Avanzate, Naples, Italy
(3) Local health authority Roma 1
(4) Local health authority Roma 4

Corresponding Author: **Claudia Gatta** - gattaclaudia@gmail.com

The "hoarding of animals" or "compulsive hoarding of animals" is a psychiatric disease that has important social implications and a profound influence on animal welfare [1]. It is characterised by the collection of animals which has a significant impact on both the rules of personal hygiene and the care of animals [2]. Bad living conditions are common. These include the presence of excessive dirt, waste, mould, pests and even animal carcasses [3]. It is a disease that includes social and psychopathological problems related to mental health, animal welfare and public safety [4]. It is considered a form of cruelty to animals nowadays, and is punished by Italian law (Article 544a of the Penal Code) "Crimes against animal sentiment". Like other forms of cruelty to animals, hoarding can sometimes be a revelation of a serious state of neglect. Several cases of animal hoarding described in the United States, Australia, Brazil, Canada and Spain have highlighted social, legal and bioethical aspects and considered this psychiatric pathology as an underrated emerging phenomenon. In Italy there are no reports and exchanges of information on the phenomenon and the animals involved. Therefore, in the present work we have analyzed the data collected from specific areas of the Lazio Region, trying to emphasize the problems related to animal welfare. We focused on 29 cases of animal hoarding in the period 2019-2022 and took into account some parameters, such as general living conditions, age and gender. We collected information from all the animals reported by the local health authority, namely 532 dogs, 460 cats, 21 birds, 52 horses and 15 rabbits, and we evaluated their state of health. We have drawn a preliminary profile of hoarders, represented by 15 females and 14 males. Most of them were over 50 years old and lived in a family context, characterized by a serious accumulation of one or more categories of objects (for example, newspapers, food and remaining waste). Finally, some of them (39%) justify such behavior to save animals. These are the first data of a retrospective analysis ever carried out in Italy. The results obtained in this study can be useful to identify the profile of the hoarder and the subjects at risk and to prevent and intervene promptly. Animal hoarding concerns health, legal and veterinary issues and we believe that a multidisciplinary approach and further research is needed to find efficient protocols to solve and prevent this problem.

[1] Patronek, G. Animal Hoarding: A Third Dimension of Animal Abuse. In The International Handbook of Animal Abuse and Cruelty: Theory, Research, and Application; Ascione, F.R., Ed.; Purdue University Press: West Lafayette, Indiana, 2008; pp. 221–240.
[2] Calvo, P.; Duarte, S.; Bowen, J.; Bulbena, A.; Fatjó, Y.J. Characteristics of 24 cases of animal hoarding in Spain. Anim. Welf. 2014, 23, 199–208.
[3] Andrews-McClymont, J.G.; Lilienfeld, S.O.; Duke, M.P. Evaluating an animal model of compulsive hoarding in humans. Rev. Gen. Psychol. 2013, 17, 399–419.
[4] D'Angelo, D.; Ciano, F.; Zaccherini, A.; Tafuri, T.; Avallone, L.; d'Ingeo, S.; and Quaranta, A. Human-Animal Relationship Dysfunction: A Case Study of Animal Hoarding in Italy. Animals. 2020, 10, 1501.

76° CONVEGNO SISVET
BARI, 21-22-23 GIUGNO 2023

FELINE COGNITIVE DECLINE PREDICTS THE COGNITIVE PERFORMANCE IN THE UNSOLVABLE TASK IN SENIOR CATS

Patrizia Piotti (1), Holly Memoli (2a), Irit Grader (2), Paola Scarpa (1), Eleonora Biffi (3), Mariangela Albertini (1), Carlo Siracusa (2b), Federica Pirrone (1b)

(1) University of Milan, Department of Veterinary Medicine and Animal Sciences, Lodi
(2) University of Pennsylvania, School of Veterinary Medicine, Department of Clinical Sciences and Advanced Medicine, Philadelphia
(3) Independent researcher

Corresponding Author: **Patrizia Piotti** - patrizia.piotti1@unimi.it

Feline cognitive dysfunction syndrome (CDS) is a neurodegenerative condition associated with disorientation, altered interactions, altered sleep-wake cycle, house-soiling, and altered activity levels and anxiety (DISHA [1]). We hypothesised that the severity of DISHA signs affected the performance in a cognitive "unsolvable task" test [2,3] in a population of senior cats.
A sample of 23 cats above the age of 7 years (MdnAge=8 years, range=7-13 years; Females=9, all neutered) attending two veterinary centres for routine health exams performed an "unsolvable task" test at home with their owners. During the test, after the cats learned that they could retrieve some food from a container, the food was made unretrievable and the gazes between the owner and the container were measured. The cats were also assessed through semi-structured interviews, where a higher score in each DISHA domain indicated more severe and frequent signs, that had emerged late in life [1].
Results from linear models indicated that the cats looked at the container for periods 30 times shorter for each increasing point in the disorientation score, and 15 times longer for each increasing point in the changes of activity levels score (GLM duration: AIC=112.64, p=0.002; disorientation estimate=-29.71+/-10.02, p=0.008; activity estimate=15.07+/-4.54, p=0.003), while more severe house-soiling predicted more frequent looks at the container (GzLM frequency: AIC=93.73, p=0.044; house soiling estimate=0.10+/-0.05, p=0.035). Conversely, the cats performed more and longer gaze-alternations between the owner and the container as disorientation increased (GLM duration: AIC=-73.46, p<0.001; disorientation estimate=0.55+/-0.14, p<0.001; GzLM frequency: AIC=92.71, p=0.01; disorientation estimate=1.17+/-0.40, p=0.004).
This is the first evidence of a relationship between persistence in a cognitive test involving cat-human interaction and age-related behaviour changes, reported by the owners, in senior cats. It appeared that disorientation increased the owner-directed referential communication in senior cats.
The study received the approval of the IACUC protocol 807030. Funding Morris Animal Foundation (Mark L Morris Jr Investigator Award, D21FE-508).
All authors declare no conflict of interest.

[1] Bellows, Center, Daristotle, Estrada, Flickinger, Horwitz, Lascelles, et al.. "Evaluating aging in cats: How to determine what is healthy and what is disease." JFMS 18, no. 7 (2016): 551-570
[2] Miklósi, Pongrácz, Lakatos, Topál, Csányi. "A comparative study of dog-human and cat-human interactions in communicative contexts." JCP 119, no. 2 (2005): 179-186.
[3] Piotti, Albertini, Trabucco, Ripari, Karagiannis, Bandi, Pirrone. "Personality and Cognitive Profiles of Animal-Assisted Intervention Dogs and Pet Dogs in an Unsolvable Task." Animals 11, no. 7 (2021): 2144.

HOW CAN WE MONITOR THE IMPACT OF TRANSPORT STRESS ON ANIMALS?

Aloma Zoratti (1), Isabella Pividori (1), Edi Piasentier (1), Antonella Comin (1), Tanja Peric (1), Alberto Prandi (1)

(1) Università degli Studi Udine, Dipartimento di Scienze Agroalimentari, Ambientali e Animali

Corresponding Author: **Isabella Pividori** - isabella.pividori@uniud.it

Non-invasive measurements of stress levels are getting in importance. The sampling of matrices such as hair and faeces allows a minimal disturbance both to animals and farm activities. The evaluation of cortisol in cattle's hair is a useful tool to determine long-term HPA activity (with a lag time of approximatively two weeks) (Probo et al., 2021) while faecal biomarkers, such as 11,17-dioxoandrostanes, are widely used as helpful indicators of adrenal activation in a shorter period (with a lag time of 8 to 16 hours) (Palme & Möstl, 1997; Palme et al., 2000). The aim of this study was to obtain an easy and standardized way to have a comprehensive view of the HPA axis activity of cattle transported over a long (more than eight-hours) road journey. This preliminary study included 29 Salers (Bos taurus taurus) bulls (aged 11 ± 2 months old; mean ± SD) which were transported on truck for 10 hours. Hair and faeces were collected at the farm on the day after arrival. Hair samples were stored in paper envelope prior extraction with methanol and subsequent in-house ELISA analysis for cortisol quantification. Faecal samples were immediately frozen until lab extraction with methanol as described by Palme et al. (2013). The concentrations of faecal cortisol metabolites were determined with a commercially available competitive ELISA kit (11-oxoetiocholanolone ELISA kit, Cayman chemical, No. 501420, Ann Arbor, MI, USA) validated for ungulates and used for the first time on cattle. All the animals showed similar or slightly higher hair cortisol concentrations than those reported in the literature (4.87 ± 2.16 pg/mg; mean ± SD). The 11-oxoethiocholanolone concentrations were higher than the baseline values reported in the literature but they were comparable to those recorded after a transport (147.8 ± 72.4 ng/g; mean ± SD). This study allowed us to observe that travel impacted differently on animals that were reared under similar husbandry conditions; it would be interesting in further studies to understand the reasons for this different reaction to the transport operations. Moreover, the tested sampling technique seems be a straightforward and standardized tool to monitor/evaluate the stress levels triggered by road transport; it could be used by government agencies or animal traders both at the farm level and in slaughter facilities.

[1] Palme, R. & Möstl, E. (1997). Measurement of cortisol in faeces of sheep as a parameter of cortisol concentration in blood. Zeitschrift fuer Saeugetierkunde, 62(2), 192-197.
[2] Palme, R., Robia, C., Baumgartner, W., & Möstl, E. (2000). Transport stress in cattle as reflected by an increase in faecal cortisol metabolite concentrations. Veterinary Record, 146(4), 108–109. https://doi.org/10.1136/vr.146.4.108.
[3] Palme, R., Touma, C., Arias, N., Dominchin, M. F., & Lepschy, M. (2013). Steroid extraction: Get the best out of faecal samples. Wiener Tierarztliche Monatsschrift- Veterinary Medicine Austria, 100, 238–246.
[4] Probo, M., Peric, T., Fusi, J., Prandi, A., Faustini, M., & Veronesi, M. C. (2021). Hair cortisol and dehydroepiandrosterone sulfate concentrations in healthy beef calves from birth to 6 months of age. Theriogenology, 175, 89–94. https://doi.org/10.1016/j.theriogenology.2021.08.037.

ASSESSMENT OF THE ANTIOXIDANT AND INTESTINAL IMMUNOMODULATORY EFFECTS OF BOVINE COLOSTRUM SUPPLEMENTATION IN RABBITS' DIETS

Curone G. (1), Draghi S. (1), Agradi S. (1), Fehri N.E. (1), Mencchetti L. (2), Vigo D. (1), Serra V. (1), Barbato O. (3), Catrica M. (4), Riva F. (1), Brecchia G. (1)

(1) Department of Veterinary Medicine and Animal Sciences (DIVAS), University of Milan, Lodi, Italy
(2) School of Biosciences and Veterinary Medicine, University of Camerino, Camerino, Italy
(3) Department of Veterinary Medicine, University of Perugia, Perugia, Italy
(4) Department of Comparative Biomedicine and Food Science, University of Padua, 35020 Legnaro, PD, Italy

Corresponding Author: **Giulio Curone** - giulio.curone@unimi.it

Rabbits play an important role as both EU livestock and experimental animal, but their sensitivity to bacterial infections presents challenges. Although antibiotics are commonly used to treat these infections, alternative solutions are being sought to prevent the development of antibiotic resistance. Bovine colostrum (BC), with its immunoregulatory compounds, antimicrobial peptides, and growth factors, has been investigated for its potential in treating and preventing various diseases. The aim of the study is to assess effects of the BC diet supplementation on the rabbit intestinal tract evaluating the gene expression of IL-8, IL-10, TGF-b, PLVAP, CTNNb1, SOD. Thirty female New Zealand White rabbits were randomly divided into three groups according to the diet: control group (n=10) was fed with commercial feed, while the other two groups were given the same diet with lyophilized BC added at 2.5% (n=10) and 5% (n=10) rates. The rabbits were weaning at 35 day and were fed with the different diets from 35 to 90 days of age. Before weaning the young rabbits were suckled by mothers who had the same diet. Digestive tracts were removed at slaughter, tissue samples from jejunum, cecum, and colon were collected with sterile scalpel, and placed in RNAlater. Total RNA was isolated, retrotranscribed to cDNA, and gene expression was evaluated via qRT-PCR. At jejunum level IL-8, TGFb, CTNNb1 resulted upregulated in group fed with 5% BC ($p<0,05$), while PLVAP in 2.5% BC ($p<0,05$); IL-10 and SOD1 showed difference differences among the groups. In caecum and colon, only SOD showed significantly higher expression in BC 5% ($p<0,05$). The results indicate that adding 5% BC to the diet results in the upregulation of IL-8 in the jejunum, which not only stimulates inflammation but also facilitates the migration of immune cells. Additionally, the use of BC promotes the expression of genes responsible for the negative regulation of inflammatory responses (TGF-B) and gut-vascular barrier integrity (CTNNb1). Furthermore, the inclusion of BC at 5% enhances antioxidant activity in the cecum and colon. These results suggest that BC has significant effects on the rabbit gastrointestinal tract's inflammatory and antioxidant response, but further research is required to fully understand its histological and physiological impact.

[1] Dudhwala, Z.M., Drew, P.A., Howarth, G.S., Moore, D. and Cummins, A.G., 2019. Active β-catenin signaling in the small intestine of humans during infancy. Digestive Diseases and Sciences, 64, pp.76-83.
[2] Beck, P.L., Rosenberg, I.M., Xavier, R.J., Koh, T., Wong, J.F. and Podolsky, D.K., 2003. Transforming growth factor-β mediates intestinal healing and susceptibility to injury in vitro and in vivo through epithelial cells. The American journal of pathology, 162(2), pp.597-608.
[3] Menchetti, L., Curone, G., Filipescu, I.E., Barbato, O., Leonardi, L., Guelfi, G., Traina, G., Casagrande-Proietti, P., Riva, F., Casano, A.B. and Piro, F., 2020. The prophylactic use of bovine colostrum in a murine model of TNBS-induced colitis. Animals, 10(3), p.492.
[4] Playford, R.J. and Weiser, M.J., 2021. Bovine colostrum: Its constituents and uses. Nutrients, 13(1), p.265.

76° CONVEGNO SISVET
BARI, 21-22-23 GIUGNO 2023

PRELIMINARY RESULTS ON THE CHARACTERIZATION OF THE GUT AND MILK MICROBIOTA IN GÖTTINGEN MINIPIG SOWS TREATED WITH AMOXICILLIN DURING LACTATION

Silvia Bencivenni (1), Patrizia Brigidi (2), Augusta Zannoni (1), Domenico Ventrella (1), Alberto Elmi (1), Maria Laura Bacci (1), Federica D'Amico (2,3), Silvia Turroni (3), Monica Forni (1)

(1) Università di Bologna – Dipartimento di Scienze Mediche Veterinarie
(2) Università di Bologna – Dipartimento di Scienze Mediche e Chirurgiche
(3) Università di Bologna – Dipartimento di Farmacia e Biotecnologie

Corresponding Author: **Silvia Bencivenni** - silvia.bencivenni2@unibo.it

The alteration in the composition and function of the gut microbiota (GM) is called dysbiosis and can contribute to the onset and progression of pathologies in the host [1]. Dysbiosis in the first years of life can have long-term consequences and it's associated with an increased risk of developing immune pathologies [2]. The GM composition is influenced by the diet which, for newborns, generally consists of breast milk [2]. Recent studies suggest that breast milk has its own distinctive microbiota [3]; therefore, its dysbiosis could directly affect the development of the newborn's GM. It is still unknown if medications commonly prescribed during lactation, as the antibiotic amoxicillin, influence the milk microbiota. The animal model chosen to investigate this matter is the Göttingen Minipig due to its metabolic and physiological similarities with humans, in addition to its small size and microbiologic standardization [4].
The aim of this research is to test the amoxicillin effect on the GM and the milk microbiota of lactating Göttingen sows, and to evaluate the validity of Göttingen minipigs as a model for studies on milk microbiota alteration after drug administration.
Amoxicillin was administered intramuscularly (7 mg/kg/day) to 3 Göttingen Minipig sows from the second week of lactation until piglet weaning (day 28). Milk was sampled at different timepoints throughout the trial and stored at -80°C, while stool samples were collected before the treatment beginning and at the end of the trial. DNA was extracted from faecal samples using the QIAamp DNA Stool Minikit (Qiagen). V3-V4 regions of the bacterial 16S rRNA gene were sequenced on a MiSeq Illumina platform. Bioinformatics analysis was performed with R software. DNA was extracted from pig milk using the DNeasy Blood & Tissue kit (Qiagen). After PCR with primers for the 16S rRNA gene, these DNA samples included a high quantity of aspecific products. Attempts to remove the aspecific products are still ongoing.
Sequencing of bacterial DNA extracted from faeces showed that treatment with amoxicillin tended to decrease the alpha diversity and proportions of Spirochaetes. There were also inter-individual differences in baseline GM that persisted throughout the trial, despite antibiotic treatment. These results should be confirmed by increasing the number of samples.

[1] Candela et al. Intestinal microbiota is a plastic factor responding to environmental changes, Trends Microbiol, 20(8):385-91, 2012
[2] Tamburini et al. The microbiome in early life: implications for health outcomes, Nat Med, 22(7):713-22, 2016
[3] Fernández et al. The human milk microbiota: origin and potential roles in health and disease, Pharmacol Res, 69(1):1-10, 2013
[4] Colleton et al. The Use of Minipigs for Preclinical Safety Assessment by the Pharmaceutical Industry: Results of an IQ DruSafe Minipig Survey, Toxicol Pathl, 44(3):458-66, 2016

76° CONVEGNO SISVET
BARI, 21-22-23 GIUGNO 2023

EFFECTS OF INSIDE-OUT SUBMITOCHONDRIAL PARTICLES (IO-SMPS) ON MITOCHONDRIA ISOLATED FROM A PORCINE EX VIVO MODEL OF DONATION AFTER CARDIOCIRCULATORY DEATH (DCD) HEART

Ilaria Troisio (1), Salvatore Nesci (1), Anna Maria Tolomeo (2), Alberto Elmi (1), Cristina Algieri (1), Assunta Fabozzo (3), Domenico Ventrella (1), Gino Gerosa (2,3), Maria Laura Bacci (1)

(1) Department of Veterinary Medical Sciences, Alma Mater Studiorum - Univeristà di Bologna
(2) Department of Cardiac, Thoracic, Vascular Sciences and Public Health, Università di Padova
(3) Cardiac Surgery Division, Azienda Ospedaliera di Padova

Corresponding Author: **Ilaria Troisio** - ilaria.troisio2@unibo.it

When it comes to heart transplantation, donation after brain death (DBD) has become the standard of care as this method is supported by many advantages, mainly due to the possibility to easily assess donor heart function before retrieval and sensibly shorten the detrimental period of warm ischemia. Lately, however, the ever-increasing number of patients in waitlist for heart transplantation has pushed the interest of researchers to find new methods to increase the donor pool [1], including heart donation after cardiocirculatory death (DCD). The latter, nonetheless, shows several disadvantages including a longer period of warm ischemia, long-term outcomes uncertainty, and lack of standardized reliable biomarkers and physiological parameters predictive of function and transplantability of DCD hearts [2]. One of the earliest cardiomyocytes degenerations starting after cardiac death is borne by mitochondria that are involved in some distinct types of cell death. Therefore, studying such events may help better understanding the physiological processes underlying DCD heart transplantability, eventually leading to definition of useful biomarkers. To achieve this, the focus of this work was the F_1F_o-ATPase complex, considered as the leading candidate responsible for the opening of a channel though the inner membrane called mitochondrial permeability transition pore (mPTP) when the natural cofactor (Mg^{2+}) is replaced with the physio-pathological cofactor (Ca^{2+}) [3]. Specifically, trying to preserve mitochondrial bioenergetics and integrity, we assessed the efficacy of IO-SMPs (inside-out submitochondrial particles) obtained by swine hearts isolated by stepwise centrifugation from mitochondria [4], on an ex-vivo porcine model of DCD heart (20 min of no touch after cardiac arrest followed by 2h of warm ischemia). We evaluated the effect of IO-SMPs on Oxidative Phosphorylation and their influence on mitochondria calcium retention capacity (CRC), an indirect index of mPTP opening, as already highlighted in other studies. Studies are currently ongoing, but the preliminary results show that IO-SMPs are capable of delaying the mPTP opening when swine heart mitochondria are exposed to Ca^{2+}. Overall, this study represents a first step towards an in-depth characterization of the physiological process activated by a prolonged warm ischemia within mitochondria, and investigates the potential beneficial effects of IO-SMPs, that may be applicable to all DCD solid organs.

1] Alamouti-fard E, Garg P, Wadiwala IJ, Yazji JH, Alomari M, Hussain MWA, et al. Normothermic Regional Perfusion is an Emerging Cost-Effective Alternative in Donation After Circulatory Death (DCD) in Heart Transplantation. Cureus [Internet]. 29 giugno 2022 [citato 11 novembre 2022]; Disponibile su: https://www.cureus.com/articles/101906-normothermic-regional-perfusion-is-an-emerging-cost-effective-alternative-in-donation-after-circulatory-death-dcd-in-heart-transplantation
[2] Pettit SJ, Petrie MC. Transplantation of Hearts Donated After Circulatory-Determined Death: A Song of Fire and Ice. Circ Heart Fail. aprile 2019;12(4):e005991.
[3] Algieri C, Trombetti F, Pagliarani A, Ventrella V, Bernardini C, Fabbri M, et al. Mitochondrial Ca2+-activated F1FO-ATPase hydrolyzes ATP and promotes the permeability transition pore. Ann N Y Acad Sci. 2019;1457(1):142–57.
[4] Nesci S, Ventrella V, Trombetti F, Pirini M, Pagliarani A. Mussel and mammalian ATP synthase share the same bioenergetic cost of ATP. J Bioenerg Biomembr. giugno 2013;45(3):289–300.

THE REDUCED EMBRYONIC DEVELOPMENT OF SHEEP OOCYTES FERTILIZED BY ICSI DEPENDS ON AN ABNORMAL CALCIUM PATTERN

Luisa Gioia (1), Luca Palazzese (2), Marta Czernik (3), Domenico Iuso (3), Pasqualino Loi (3)

(1) Università degli Studi di Teramo, Facoltà di Bioscienze e Tecnologie Agro-Alimentari e Ambientali.
(2) Institute of Genetics and Animal Biotechnology of the Polish Academy of Sciences, Poland.
(3) Università degli Studi di Teramo, Facoltà di Medicina Veterinaria.

Corresponding Author: **Luisa Gioia** - lgioia@unite.it

In mammals, Ca2+ is the universal activator of development at fertilization, playing a central role in early events associated with egg activation and the egg-to-embryo transition. In details, oocyte activation depends on a rise in intracellular free calcium ions (Ca2+) that is triggered by the sperm-specific phospholipase C zeta (PLCζ) and consists in a series of characteristic oscillations [1]. Intracytoplasmic sperm injection (ICSI), which bypasses fusion of the gametes, has been widely used in several species, with variable efficiency. While a great progress has been achieved in humans, the success of this technique remains limited in ruminants [2], particularly in sheep where the first cleavage is very low (35%) due to failure of pronuclear (PN) apposition and fusion, despite a high rate (80%) of activation and PN formation [3]. Given that PN migration and fusion are Ca-dependent processes, we have recently hypothesized that an abnormal Ca2+ response could be the limiting factor in sheep ICSI oocytes. So, we compared the oscillation patterns of in vitro matured (IVM) oocytes fertilized by ICSI or in vitro fertilization (IVF), microinjected with the Ca-sensitive dye Fluo-4, and analysed by time lapse microscopy. Our results clearly demonstrated that an oscillatory response occurs in IVF oocytes. In particular, all responding oocytes (44%, n=140) showed repetitive Ca2+ oscillations from the time of gamete fusion until 2PN stage (frequency: 1 spike every 14±6 min). Interestingly, all ICSI oocytes (n=120) did not evoke the normal calcium response, showing (29%) a single spike or only a few (2-4) abnormal Ca2+ increments with low frequency (1 spike every 50-65 min). Hoechst staining at the end of the Ca2+ analysis revealed that all IVF oocytes responding with Ca2+ oscillations reached the 2PN stage, while all ICSI oocytes that showed a Ca2+ response formed only a single PN (78%) or 2PN without apposition (22%). In conclusion, our results confirm our hypothesis demonstrating for the first time that in sheep the failure of cleavage of ICSI oocytes depends on an abnormal Ca2+ response after sperm microinjection, due to lacking or prematurely ending Ca2+ oscillations. This pattern only sustains the early events of activation, such as the end of meiosis and formation of female PN, whereas other Ca-dependent events that could be more strictly related the sperm contribution appear deficient and fail, so compromising the development to blastocyst stage.

[1] Saunders et al. PLCζ: a sperm-specific trigger of Ca2+ oscillations in eggs and embryo development, DEVELOPMENT, 129:3533-3544, 2002.
[2] Shirazi et al. Male pronuclear formation and embryo development following intracytoplasmic injection of ovine pretreated sperm. AVICENNA J MED BIOTECHNOL (AJMB), 10:41-48, 2018.
[3] Ressaissi et al. The impaired development of sheep ICSI derived embryos is not related to centriole dysfunction, THERIOGENOLOGY, 159:7-12, 2021.

RECOMBINANT LATE EMBRYOGENESIS ABUNDANT PROTEINS (LEAP) PROTECT EARLY SHEEP EMBRYOS FROM ACUTE COLD STRESS

M. Lo Sterzo (1), M. Moncada (1), F. Boffa (1), L. Palazzese (1), D. Iuso (1), P. Loi (1), M. Czernik (1)

(1) Laboratory of Embryology, Faculty of Veterinary Medicine, University of Teramo

Corresponding Author: **Martina Lo Sterzo** - mlosterzo@unite.it

Late Embryogenesis Abundant proteins (LEAp) are found in plants and many invertebrates where they preserve biological tissue from desiccation injury. The LEAs acquire an adaptive protein conformation during drying and, by interacting with trehalose and other proteins, form a vitrified state which stabilizes and protects the cells. Our group demonstrated that the overexpression of three LEA genes (pTag-RAB17-GFP-N, pTag-WCOR410-RFP, pTag-LEA-BFP) in sheep fibroblast cell lines confer significant protection against desiccation. Recently, these results were also confirmed in a human hepatoma cell line. In this work we investigate the effect of LEA proteins on mammalian embryos, aiming to direct future drying experiments with the final objective of establishing dry biobanks. By an E.Coli expression system, we produced three different recombinants LEA proteins (cytosolic/nuclear RAB17-GFP, membranal WCOR410-RFP, mitochondrial LEA-BFP) and injected them into sheep zygotes 4 hours post in-vitro fertilization. Mainly in this work, we injected 70 embryos with RAB17-GFP protein and 62 embryos with the opti-MEM medium as control (CTR). Embryo development rates did not differ between the groups in terms of cleavage (51.4% (36/70) vs 45.2% (28/62), RAB17-GFP vs CTR respectively) and blastocyst rate (37.1% (26/70) vs 33.9% (21/62), RAB17-GFP vs CTR respectively). Surprisingly, RAB17 protein tagged with GFP was maintained and visible by fluorescence until the blastocyst stage, without any apparent degradation. Together these results demonstrate no adverse effects of RAB17-GFP protein on embryo development. To test the eventual protection of recombinant RAB17-GFP in sheep embryos, we have injected 45 embryos with control medium (CTR) and 50 with RAB17-GFP. Both groups of injected embryos are kept for 24 hours at 4°C at the 2-cells stage, then put back in culture. After one week in culture, 60% (27/45) of the CTR group were lysed, while 40% (18/45) arrest their development. In contrast, no one lysed for those injected with RAB17-GFP, 74% (37/50) did not cleave further, but remarkably 26% (13/50) developed into expanded blastocysts. Based on the results, we selected RAB17 as a promising protectant LEA for future experiments on embryo desiccation. In conclusion, we have demonstrated that plant RAB17 protein does not affect embryo development in sheep and can be explored in the next future as a molecule to protect mammalian embryos during desiccation.

[1] Loi P., Iuso D., Czernik M., Zacchini F., Ptak G. (2013). Towards storage of cells and gametes in dry form. Trends Biotechnol 31(12), 688-95.
[2] Czernik M., Fidanza A., Luongo F.P., Valbonetti L., Scapolo P.A., Patrizio P., Loi P. (2020). Late Embryogenesis Abundant (LEA) proteins confer water stress tolerance to mammalian somatic cells. Cryobiology. Feb1; 92:189-196.
[3] Li S., Chakraborty N., Borcar A., Menze M.A., Toner M., Hand S.C. (2012). Late embryogenesis abundant proteins protect human hepatoma cells during acute desiccation. Proc Natl Acad Sci U S A. Dec 18;109(51), 20859-64.

76° CONVEGNO SISVET
BARI, 21-22-23 GIUGNO 2023

NERVE GROWTH FACTOR (NGF) SEASONAL VARIATION IN SEMINAL PLASMA AND PRESENCE AND EXPRESSION OF NGF/COGNATE RECEPTOR SYSTEM ON THE GENITAL TRACT OF RAMS (*OVIS ARIES*)

Mercati F. (1), Dall'Aglio C. (1), Marti J.I. (2), Marenzoni M.L. (1), Caivano D. (1), Anipchenko P. (1), Palmioli E. (3), Palermo F.A. (4), Cocci P. (4), Guelfi G. (1), Capaccia C. (1), Zerani M. (1), Maranesi M. (1)

(1) Dipartimento di Medicina Veterinaria, Università di Perugia, Italy
(2) Department of Animal Pathology, University of Zaragoza, Spain
(3) Dipartimento di Filosofia, Scienze Sociale e Educazione, Università di Perugia, Italy
(4) Scuola di Bioscienze e Medicina Veterinaria, Università di Camerino, Italy

Corresponding Author: **Margherita Maranesi** - margherita.maranesi@unipg.it

Several studies testified the role of nerve growth factor (NGF) in animal reproduction (Adams et al., 2016). NGF has long been known as the main ovulation-inducing factor in species with induced ovulation, but recent studies suggested the NGF role also in those with spontaneous ovulation (Maranesi et al., 2021), expanding its function for reproductive success in these species as well.
In order to evaluate the possible relationship between the NGF production variations related to the ram reproductive seasonality, this study investigated the seminal plasma values of NGF, during the whole year. The second aim was to evaluate presence and gene expression of NGF and its cognate receptor (NGFR) in the ram genital tract. Semen was collected during the whole year weekly, from 5 adult rams, with an artificial vagina. NGF seminal plasma values were assessed by ELISA method. Genital tract samples were collected from 5 adult rams regularly slaughtered at a local abattoir. NGF and NGFR presence and expression were evaluated in the testis, epididymis, prostate, seminal vesicles, bulbourethral gland, and deferens ampullae by immunohistochemistry and real-time PCR, respectively. Data were analyzed by one-way ANOVA followed by the Student-Newman-Keuls t-test.
NGF seminal plasma concentration was greater from January to May ($p<0.01$). NGF and NGFR immunoreactivity was detected in all male organs examined. NGF positive immunostaining was observed in the spermatozoa of the germinal epithelium and in the cells of the secretory epithelium of annexed glands, whereas NGFR immunopositivity was localized in the nerve fibers.
NGF and NGFR gene transcripts were highest ($p<0.01$) in the seminal vesicles and lowest ($p<0.01$) in the testis than in the other tissues.
This study evidenced that the NGF/NGFR system is expressed in the reproductive tissues of all the different genital tract examined, confirming the role of NGF in the ram reproduction. Sheep are short-day breeders, with an anestrus that corresponds to the highest seminal plasma NGF levels, thus suggesting the intriguing idea that this factor could participate in an inhibition mechanism of male reproductive activity, activated during the female anestrus. A deeper knowledge of these complex cellular mechanisms could be useful to improve the reproductive performance of farm animals and humans too.

[1] Adams GP et al. Ovulation-inducing factor (OIF/NGF) in seminal plasma: a review and update. Reprod Domest Anim. 2016, 51 Suppl 2:4-17.
[2] Maranesi M et al. Nerve Growth Factor (NGF) and Animal Reproduction. Adv Exp Med Biol. 2021; 1331:277-287.

ATP-CITRATE LYASE, A KEY METABOLIC PLAYER IN SHEEP BLASTOCYST DEVELOPMENT

Margherita Moncada (1), Luca Palazzese (2), Martina Lo Sterzo (1), Francesca Boffa (1), Marta Czernik (1), Pasqualino Loi (1), Domenico Iuso (1)

(1) Laboratory of Embryology, Faculty of Veterinary Medicine, University of Teramo, Teramo, Abruzzo, Italy
(2) Institute of Genetics and Animal Biotechnology of the Polish Academy of Sciences, Warsaw, Jastrzebiec, Poland

Corresponding Author: **Martina Moncada** - mmoncada@unite.it

During the first stages of life, dynamic metabolism-epigenetic crosstalk events are crucial to design a gene expression profile that drives cell differentiation and sustains development (1). Cytosolic/Nuclear Acetyl-Coenzyme A (Acetyl-CoA), produced from citrate by the ATP Citrate Lyase (ACLY), was described as a primary acetyl donor source for histone acetylation as well as for de novo fatty acid synthesis (FASN)(2). Our study aims to deeply understand the balance between citrate metabolism, FASN and histone acetylation during blastocyst formation to build targeted metabolic interventions and improve in vitro blastocyst development.
In the present work, we investigated the ACLY's role during the preimplantation development of in vitro sheep embryos. Following the in vitro development of zygotes injected by a siRNA targeting for ACLY (siACLY) and a non-targeting siRNA as CTR (siCTR), we found that the cleavage rate to 2 cells stage did not differ between the groups (60,8 % (31/51) vs 59,9% (33/56), 2-cells stage/zygotes, siACLY vs siCTR respectively), while the blastocyst percentage had a lower tendency in siACLY group than CTR (siCTR 35,3% (18/51) vs siACLY 25% (14/56), blastocysts/zygotes, P=0,2454, chi-square test), and particularly siACLY blastocysts showed a clear abnormal morphology. The results were confirmed by adding an ACLY inhibitor (SB204990-ACLYi) in embryo culture media instead of using the siACLY; blastocysts developed in medium enriched by ACLYi (1µM) were less than control (CTR 43,40% (23/53) vs ACLYi 17,02% (8/47) blastocysts/zygotes, P=0,0051, chi-square test) and showed a significantly lower number of cells than CTR ones (CTR 168 +/- 59 vs ACLYi 90 +/- 28, average cell number +/- SD, P=0,0303, t-test). Together these results demonstrate the essential ACLY's role in sheep preimplantation embryo development and indicate the metabolic citrate as a fundamental Acetyl-CoA source for blastocyst development. Future investigations will question the effect of ACLY on histone acetylation and FASN of blastocysts.

[1] Shi, Lingjun, and Ji Wu. "Epigenetic regulation in mammalian preimplantation embryo development." Reproductive Biology and Endocrinology 7 (2009): 1-11.
[2] Wellen, Kathryn E., et al. "ATP-citrate lyase links cellular metabolism to histone acetylation." Science 324.5930 (2009): 1076-1080.

DOES AUTOPHAGY PLAY A ROLE DURING FOLLICULAR DEVELOPMENT IN SHEEP'S OVARY?

Aurora Scudieri (1), Luisa Gioia (1), Domenico Iuso (2), Pasqualino Loi (2)

(1) Dipartimento di Bioscienze e Tecnologie Agro-Alimentari e Ambientali, Università degli Studi di Teramo
(2) Dipartimento di Medicina Veterinaria, Università degli Studi di Teramo

Corresponding Author: **Luisa Gioia** - lgioia@unite.it

Autophagy is an intracellular lysosome-mediated degradation process that involves the sequestration of cytoplasmic organelles and macromolecules within autophagosomes. The products of degradation are recycled, so they become available substrates for anabolic pathways and energy production. In addition to a pro-survival role, increasing evidence demonstrated that autophagy also acts as a pro-death mechanism under different conditions [1]. In several mammalian species, it has been demonstrated that autophagy is also involved in processes related to reproduction such as gametogenesis, and embryo and placenta development, while in sheep little is known about the role of autophagy in reproductive processes. It has been also demonstrated in several species including rodents and pigs that autophagy is involved in the regulation of follicle destiny in the ovary, inducing a programmed cell death in granulosa cells (GCs) through a cooperative role with apoptosis [2]. As in ovine species it has never been investigated, the aim of the present study was to evaluate whether there is a correlation between autophagy and follicular development by analyzing the expression of the autophagosomal marker, LC3 protein, in GCs of sheep antral follicles. For this purpose, sheep ovaries were collected from local slaughterhouses and antral follicles were isolated and classified into three groups (healthy, early atretic, advanced atretic), based on their health status according to validated morphological parameters. Then, GCs were collected and pooled from each group, and then were divided in two parts to carry out assays for both apoptosis (TUNEL) and autophagy. For the latter, the amount of LC3-I protein converted to LC3-II, which is an index of autophagic induction, was assessed in GCs by western blotting, and the subcellular localization of the LC3 protein was evaluated by immunofluorescence and confocal microscope analysis. Our preliminary results confirmed the activation of apoptosis in GCs of atretic antral follicles. Moreover, we demonstrated for the first time a different abundance of the autophagic marker LC3 in sheep GCs of healthy vs atretic antral follicles, indicating a correlation between follicular health status and autophagy enhancement and suggesting that also in sheep, like in other species, the process of autophagy might play a role in follicular fate.

[1] Baehrecke, Eric H. "Autophagy: dual roles in life and death?." Nature reviews Molecular cell biology 6.6 (2005): 505-510. https://doi.org/10.1038/nrm1666
[2] Escobar, M. L., O. M. Echeverría, and G. H. Vázquez-Nin. "Role of autophagy in the ovary cell death in mammals." Autophagy-a double-edged sword-cell survival or death (2013): 423-441. http://dx.doi.org/10.5772/54777

76° CONVEGNO SISVET
BARI, 21-22-23 GIUGNO 2023

LONG *IN VITRO* CULTURE OF CUMULUS-OOCYTE COMPLEXES COLLECTED FROM EARLY ANTRAL FOLLICLES IN SHEEP: A PROMISING STRATEGY FOR PRODUCING COMPETENT OOCYTES

Mohammadreza Ebrahimi (1), Maria Dattena (2), Laura Mara (2), Valeria Pasciu (1), Fabrizio Chessa (2), Alberto Maria Luciano (3), Fiammetta Berlinguer (1)

(1) Department of Veterinary Medicine, University of Sassari, Via Vienna 2, Sassari, Italy
(2) Department of Animal Science, Agricultural Research Agency of Sardinia, 07100 Sassari, Italy
(3) Reproductive and Developmental Biology Laboratory, Department of Veterinary Medicine and Animal Sciences, University of Milan, Via dell'Università 6, 26900 Lodi, Italy

Corresponding Author: **Mohammadreza Ebrahimi** - mre962@gmail.com

In vitro culture of cumulus-oocyte complexes (COCs) derived from early antral follicles (EAFs) has shown promising results in cattle (1,2). In this study, we investigated whether this technology can be transferred to sheep. Sheep ovaries were collected during the breeding (October–November) and non-breeding season (March–May). COCs were isolated from EAFs (350-450 µm) and individually cultured for five days in TCM199 supplemented with 0.15µg/mL Zn sulfate, 10-4IU/mL FSH, 10ng/mL estradiol, 50ng/mL testosterone, 50ng/mL progesterone and 5µM Cilostamide (1). After long in vitro culture (LIVC), they were in vitro matured under standard conditions used for this species (Mara et al., 2014). The diameter of the oocytes collected from EAFs did not differ between the breeding and non-breeding seasons (109.4 ± 0.2 µm vs 108.8 ± 0.2 µm). After LIVC the diameter increased in both seasons (GLM, $p<0.000$), but oocytes grew significantly more in the breeding season (116.3 ± 0.2 µm) compared to the non-breeding season (114.9 ± 0.2 µm). However, they were significantly smaller than fully grown oocytes collected from antral follicles (133.6 ± 4.5 µm). Oocyte viability decreased significantly after LIVC only during the non-breeding season (from 76.5 ± 3.7% to 64.7 ± 5.1%; GLM, $p<0.000$). After LIVC, both groups showed a decrease in gap junction communication and a shift in chromatin configuration from the non-surrounded nucleolus (NSN) to surrounded nuclear envelope (SNE) ($p<0.000$). Global transcription activity exhibited a reduction in both groups after LIVC, with a greater decline observed during the breeding season ($p<0.05$). Oocytes collected from EAFs showed no meiotic competence before LIVC. After LIVC, oocyte meiotic competence significantly increased in both seasons (17.7 ± 4.4 and 10.3 ± 3.8% in the breeding compared to the non-breeding season; $p<0.000$). However, MII rates were lower compared to those obtained from COCs collected from antral follicles ($p<0.000$). In conclusion, our study suggests that the breeding season is associated with improved efficiency of the LIVC technology consequent to a higher quality of the COCs isolated from EAFs, which may have important implications for optimizing the LIVC system in mammals with seasonal reproduction.

[1] Barros, R. G., Lodde, V., Franciosi, F., & Luciano, A. M. (2020). In vitro culture strategy for oocytes from early antral follicle in cattle. JoVE (Journal of Visualized Experiments), (161), e61625.
[2] Barros, R. G., Lodde, V., Franciosi, F., & Luciano, A. M. (2023). A refined culture system of oocytes from early antral follicles promotes oocyte maturation and embryo development in cattle. Reproduction, 165(2), 221-233.
[3] Mara, L., Sanna, D., Casu, S., Dattena, M., & Muñoz, I. M. (2014). Blastocyst rate of in vitro embryo production in sheep is affected by season. Zygote, 22(3), 366-371.

76° CONVEGNO SISVET
BARI, 21-22-23 GIUGNO 2023

REPROTEN for *in vitro* folliculogenesis: new perspectives for biotechnologies applied to animal reproduction

Alessia Peserico (1), Chiara Di Berardino (1), Giulia Capacchietti (1), Chiara Camerano Spelta Rapini (1), Romasco Aurora (1), Elena Pia Vitale (1), Liliana Liverani (2,3), Aldo Roberto Boccaccini (2), Barbara Barboni (1)

(1) Department of Bioscience and Technology for Food, Agriculture and Environment, University of Teramo, 64100 Teramo, Italy
(2) Institute of Biomaterials, Department of Materials Science and Engineering, Friedrich-Alexander University of Erlangen-Nuremberg, 91054 Erlangen, Germany
(3) DGS S.p.A., 00142 Rome, Italy

Corresponding Author: **Alessia Peserico** - apeserico@unite.it

Exploiting novel strategies aimed at boosting the reproductive performance of domestic animals or preserving biodiversity by counteracting the loss of mammalian endangered species remains a challenging goal of assisted reproductive technology (ART)(Herrick, 2019). Among ART techniques, in vitro recapitulation of early phases of folliculogenesis (ivF) represents an efficient way to recruit the reserve of immature and low competent female gametes towards assisted reproductive technologies (ART). Importantly, the reproductive research area has been developed in the last decade to leading-edge advances based on the use biocompatible materials designed for engineering reproductive systems. The new field of REPROductive Tissue Engineering have been proposed to generate innovative solution for infertility (Liverani et al, 2019).
To this aim, the present research has been designed to assess the possibility to exploit a reproductive biomimetic material for innovating ivF protocols applied to prepubertal sheep. The advancement of ivF protocol has been designed by comparing the culture of single ovine preantral follicles on a previously validated methods (3D-oil method) (Di Berardino et al, 2021) with a new one carried out on biomimetic electrospun poly(epsilon-caprolactone) (PCL) fibers made with different topology (random vs. patterned fibers)(Di Berardino et al, 2022). Perfromances of both ivF systems (3D-oil vs PCL) were assessed by analyzing the morphological and functional outcomes of in vitro growth early antral (EA) follicles and oocyte.
Gathered data showed how only PCL scaffold with patterned topology was able to support follicle growth under a constant eCG stimulation. In addition, PCL patterned system boosts ivF performances by increasing the rate of follicle growth (75% vs. 70% patterned PCL vs 3D-oil: $p>0.05$), the incidence of antrum formation (88% vs. 63%: $p<0.01$), the estradiol production, and, the rate of oocyte maturation rate (80% vs. 68%: $p<0.01$).
Overall, the present research has demonstrated for the first time that REPROTEN can be applied to ivF by generating concrete solutions for broadening the use of ART to the large source of immature gametes, a key step toward the improvement of reproduction performances in domestic animal and the preservation of endangered species.

[1] Herrick JR. Assisted reproductive technologies for endangered species conservation: developing sophisticated protocols with limited access to animals with unique reproductive mechanisms. Biol Reprod. 2019 May 1;100(5):1158-1170. doi: 10.1093/biolre/ioz025. PMID: 30770538.
[2] Liverani L, Raffel N, Fattahi A, Preis A, Hoffmann I, Boccaccini AR, Beckmann MW, Dittrich R. Electrospun patterned porous scaffolds for the support of ovarian follicles growth: a feasibility study. Sci Rep. 2019 Feb 4;9(1):1150. doi: 10.1038/s41598-018-37640-1. PMID: 30718584; PMCID: PMC6362199.
[3] Di Berardino C, Peserico A, Capacchietti G, Crociati M, Monaci M, Tosi U, Mauro A, Russo V, Bernabò N, Barboni B. Equine Chorionic Gonadotropin as an Effective FSH Replacement for In Vitro Ovine Follicle and Oocyte Development. Int J Mol Sci. 2021 Nov 17;22(22):12422. doi: 10.3390/ijms222212422. PMID: 34830304; PMCID: PMC8619287.
[4] Di Berardino C, Liverani L, Peserico A, Capacchietti G, Russo V, Bernabò N, Tosi U, Boccaccini AR, Barboni B. When Electrospun Fiber Support Matters: In Vitro Ovine Long-Term Folliculogenesis on Poly (Epsilon Caprolactone) (PCL)-Patterned Fibers. Cells. 2022 Jun 19;11(12):1968. doi: 10.3390/cells11121968. PMID: 35741097; PMCID: PMC9222101.

USE OF TRANSFORMING GROWTH FACTOR-B (TGF-B) TO IMPROVE BLASTOCYST QUALITY IN SHEEP

F. Boffa (1), M. Moncada (1), M. Lo Sterzo (1), D.Iuso (1), P. Loi (1), M. Czernik (1)

(1) Università degli Studi di Teramo

Corresponding Author: **Francesca Boffa** - fboffa@unite.it

In vitro fertilization (IVF) is a widely used assisted reproductive technology (ART) technique not only in human medicine but also in veterinary science and practice. Unfortunately, the quality of embryos produced in vitro, compared to those in vivo, is still disappointedly low. This study aims to investigate the impact of transforming growth factor-β (TGF-β) regulatory pathways on sheep embryo development. Specifically, the research examines a membrane-crossing member of the TGF-β family, which governs various cellular activities crucial for embryonic growth, adhesion, migration, and differentiation. Early morula stage sheep embryos were cultured with 2.5 μM TGF-β until reaching the blastocyst stage, while untreated embryos served as the control (CTR). Preliminary results indicate a positive effect of TGF-β treatment, as evidenced by improved developmental rates in sheep embryos, resulting in a higher number of blastocysts on day 7 of culture compared to the CTR group [blastocysts/oocytes, TGF-β: 46.6% (134/288) vs untreated: 26.7% (74/275), $p<0.001$]. Furthermore, the quality of TGFβ-treated blastocysts was enhanced. The inner cell mass (ICM) cells in TGF-β embryos exhibited greater compactness and definition, while the trophoblastic cells (TE) demonstrated regular and hexagonal shapes, contrasting with the CTR group where the cells were ill-defined, less compact, and irregular. Moreover, the total cell count in TGF-β blastocysts was higher, particularly in the TE compartment (average ± SD, TGF-β: 167 ± 10 vs untreated: 103 ± 26 cells, $p=0.01$), while no significant differences were observed in the ICM cell count (48 vs 46, respectively). These preliminary findings show that TGFβ significantly improves blastocysts' quality in sheep.

[1] Park, K. S. (2011). Tgf-Beta family signaling in embryonic stem cells. International journal of stem cells, 4(1), 18.
[2] Dobbs, Kyle B., et al. "Regulation of pluripotency of inner cell mass and growth and differentiation of trophectoderm of the bovine embryo by colony stimulating factor 2." Biology of Reproduction 89.6 (2013): 141-1.

WHOLE GENOME, LIPIDOMIC AND FUNCTIONAL PRESERVATION OF VACUUM-DRIED RAM SPERMATOZOA FOLLOWING TWO YEARS OF STORAGE AT ROOM TEMPERATURE

Luca Palazzese (1), Federica Turri (2), Debora Agata Anzalone (3), Joseph Saragusty (3), Jacques Bonnet (4, 5), Marthe Colotte (6), Sophie Tuffet (6), Flavia Pizzi (2), Andrea Boari (3), Marta Czernik (1,3), Pasqualino Loi (3)

(1) Institute of Genetics and Animal Biotechnology of the Polish Academy of Sciences, Jastrzebiec, 05-552, Warsaw, Poland
(2) Institute of Agricultural Biology and Biotechnology (IBBA), National Research Council (CNR), Lodi, 26900, Italy
(3) Dipartimento di Medicina Veterinaria, Università degli Studi di Teramo, 64100, Teramo
(4) Laboratoire de Recherche et développement, Imagene Company, Pessac, France
(5) Université de Bordeaux, Institut Bergonié, INSERM, Bordeaux, France
(6) Imagene, plateforme de production, Genopole, Evry, France

Corresponding Author: **Luca Palazzese** - lpalazzese@unite.it

The first offspring produced from lyophilized mouse epididymal spermatozoa in 1998 [1] triggered extensive work to develop alternative storage methods for cryopreservation. However, most of the work has been conducted in the laboratory mouse, while little information has been gathered on large animals that could also benefit from this kind of storage. This work adapted a technique known as vacuum-drying encapsulation (VDE), originally developed for nucleic acid conservation in the anhydrous state, to ram spermatozoa, and compared it to canonical lyophilization (FD). Long-term storage (two years) at room temperature (RT) and 4ºC (4C) was also tested. The results demonstrated better structural stability, namely lipid composition and DNA integrity, in vacuum-dried spermatozoa than in the lyophilized ones. Sperm freeze-dried with both techniques (FD and VDE) were subjected to quality analyzes such as: global lipidomics, cytofluorimetric analysis for acrosome and DNA integrity (SCSA) and finally the functionality was tested by in vitro fertilizing mature sheep oocytes through ICSI (Intracytoplasmic Sperm Injection).
Likewise, embryonic development following intracytoplasmic sperm injection was higher when using RT stored spermatozoa upon both VDE and FD (blastocyst formation: 12.8 vs. 5.8%, $P < 0.001$, VDE_RT and VDE_4C respectively; 8.7% vs 3.2%; $P < 0.001$, FD_RT and FD_4C respectively). Unexpectedly, genomic and membrane stability was better preserved at room temperature than at 4ºC. This was the first work to investigate the global lipid profile of dried spermatozoa. The results demonstrated that fatty acid conformation was closely related to the drying and storage (room temperature or 4ºC) methods. Our findings indicate that it is important to consider dehydration-related changes in sperm polyunsaturated fatty acids in addition to DNA alterations, given their crucial role in embryonic development.

[1] T Wakayama, R Yanagimachi. Development of normal mice from oocytes injected with freeze-dried spermatozoa. Nat Biotechnol. 1998 Jul;16(7):639-41. doi: 10.1038/nbt0798-639.

76° CONVEGNO SISVET
BARI, 21-22-23 GIUGNO 2023

CALVES GROWTH PERFORMANCES: ANY IDHEA(S)?

Matilde Giombolini (1), Alessio Cotticelli (1,2), Aloma Zoratti (1), Alberto Prandi (1), Tanja Peric (1)

(1) Università degli Studi di Udine, Dipartimento di Scienze Agroalimentari, Ambientali e Animali
(2) Università degli Studi di Napoli Federico II, Dipartimento di Medicina Veterinaria e Produzioni Animali

Corresponding Authors: **Aloma Zoratti** - zoratti.aloma@spes.uniud.it

The perinatal period is a sensitive phase for dairy calves development and their future zootechnical performances. After birth, calves are subjected to several new stimuli and stressors [1]. The effects of these stressors on the HPA axis activity can be investigated by measuring cortisol (C) and dehydroepiandrosterone (sulfate) (DHEA(S)) concentrations. Because of the opposite effects of C and DHEA(S), a common measure employed to test the impact of both hormones simultaneously is their ratio [2]. Daily weight gain (DWG) in dairy calves is one of the most important factors affecting their lifetime productivity [4]. We studied hair DHEA(S) and C/DHEA(S) ratio in calves during the perinatal period in relation to DWG. The experiment had approval from the Ethical Animal Care and Use Committee of the University of Naples Federico II (protocol no. PG/2021/0130478). The research was conducted in a dairy farm in Friuli Venezia Giulia and involved 30 healthy Holstein Friesian calves. Steroids concentrations were measured in hair, a non-invasive matrix that allows a retrospective measurement of hormones. Hair samples were taken at 15 (T1) and 45 (T2) days of life, reflecting the pre- and perinatal period (T1) and the following 30 days (T2). Hormone concentrations were determined by an in-house RIA method.

DHEA(S), C and the C/DHEA(S) ratio were higher at T1 than T2 ($p<0.01$). DHEA(S) and C were positively correlated (0.33, $P<0.08$ at T1 and 0.74, $P<0.0001$ at T2). DWG (1-60 days) and weight at 60 days of life (W) were positively correlated to DHEA(S) at T1 (0.51 and 0.47, $p<0.01$) and no correlation was found with T2. On the other hand, the C/DHEA(S) ratio at T1 and T2 was negatively correlated to DWG and W (-0.49 and -0.52, $P<0.01$; -0.31 and -0.36, $p<0.05$, respectively). The positive correlation between DHEA(S) at T1 and DWG/W underlines a possible link between DHEA(S) and growth performances, as described for children [3]. The negative correlation between C/DHEA(S) ratio at T1 and DWG/W needs further investigation as it may be influenced by the high concentrations of physiological C required by calving mechanism and calf maturation. During the postnatal period C concentrations are expected to decrease. If C concentrations remain high, it could be due to postnatal stressors with DHEA(S) unable to counterbalance its effects. The negative correlation between the C/DHEA(S) ratio at T2 and DWG/ W seems to highlight that if C remains high during the postnatal period, the weight gain is negatively affected. In conclusion, better growth performances would comply with high DHEA(S) in both the pre- and perinatal periods, and with lower C/DHEA(S) ratio during the first months of calf life.

[1] Hulbert, Lindsey E., and Sonia J. Moisá. 2016. “Stress, Immunity, and the Management of Calves.” Journal of Dairy Science 99 (4): 3199–3216.
[2] Qiao, Shan, Xiaoming Li, Samuele Zilioli, Zheng Chen, Huihua Deng, Juxian Pan, and Weigui Guo. 2017. “Hair Measurements of Cortisol, DHEA, and DHEA to Cortisol Ratio as Biomarkers of Chronic Stress among People Living with HIV in China: Known-Group Validation.” PloS One 12 (1): e0169827.
[3] Santos-Silva, Rita, Manuel Fontoura, João T. Guimarães, Milton Severo, Henrique Barros, and Ana Cristina Santos. 2022. “Persistent Weight Gain between 0 and 4 Years of Age Is Associated with Higher Dehydroepiandrosterone Sulphate Levels at 7 Years Old: Data from the Generation XXI Birth Cohort.” Clinical Endocrinology 97 (5): 588–95.
[4] Soberon, F., and M. E. Van Amburgh. 2013. “Lactation Biology Symposium: The Effect of Nutrient Intake from Milk or Milk Replacer of Preweaned Dairy Calves on Lactation Milk Yield as Adults: A Meta-Analysis of Current Data.” Journal of Animal Science 91 (2): 706–12.

76° CONVEGNO SISVET
BARI, 21-22-23 GIUGNO 2023

SYSTEMIC AND INTRAFOLLICULAR THYROID HORMONE LEVELS OF BEEF COWS: EFFECTS OF FOLLICLE SIZE AND CORPUS LUTEUM PRESENCE OR ABSENCE

Deborah La Fauci (1), Vincenzo Aronica (2), Pietro Medica (1), Katiuska Satué Ambrojo (3), Cristina Cravana (1)

(1) Department of Veterinary Sciences, Messina University, Via Palatucci 13, 98168, Messina, Italy
(2) Istituto Zooprofilattico Sperimentale della Sicilia, Area Barcellona, Via Sant'Andrea, 96, 98051, Barcellona Pozzo di Gotto, Messina, Italy
(3) Department of Animal Medicine and Surgery, Faculty of Veterinary Medicine, CEU-Cardenal Herrera University, 46115 Valencia, Spain

Corresponding Author: **Deborah La Fauci** - deborah.lafauci@unime.it

Ovarian follicular fluid (FF) fills the cavity of the antral follicles and originates from both the secretions of different types of follicular cells (granulosa and thecal layers) and the blood plasma filtration. It has been reported that Thyroid Hormones (THs) are required for bovine ovarian follicular function (Błaszczyk et al., 2006). The aim of this study is to measure intrafollicular and serum total tri-iodothyronine (TT3) and thyroxine (TT4) concentrations, in order to evaluate whether the corpus luteum (CL) could modify the FF hormonal content from the neighboring different-sized follicles in the cows. Ovaries were taken from 28 clinically healthy and cyclic beef cows (Bruna breed) of different ages (range 12-24 months) after slaughtering. Blood samples were collected from the jugular vein before the slaughter. The stage of the cycle was determined postmortem. The ovaries were classified in two groups according to the presence (CL+) or absence (CL-) of CL. The follicles were classified into three different categories, in relation to their size: small (<6mm), medium (7-9mm) and large follicles (10-20mm). Serum and intrafollicular fluid samples were evaluated for THs with the automated chemiluminescence immunoassay analyser. A paired sample t-test was performed to compare concentrations found in the blood serum and the FF. In addition, correlation coefficients between intrafollicular and serum levels of THs parameter were calculated.
Results demonstrated that the values for intrafollicular THs concentrations were in the same range as those found in serum. The FF concentrations of THs from follicles of different size categories were lower when compared to the serum. Intrafollicular TT4 concentrations increased more in larger and medium follicles than small ones. FF concentration of TT4 increased with increasing follicular size, both in Cl- and CL+ ovaries. For the same follicle size category, THs concentrations in CL- ovaries were higher than those in CL+ ovaries.
Our results indicated that differences in THs levels between follicle goups may be related to follicle growth and maturation in cattle. A considerable part of the THs in FF probably originates from serum, increasing with the increase of blood circulation in the growing follicles. Furthermore, the different concentrations of THs between the CL- and CL+ ovaries could indicate a possible different local action of the CL, confirming similar evidence reported in cows by Kor (2014).

[1] Błaszczyk, B., Stankiewicz, T., Udala, J., Gaczarzewicz, D., Lasota, B., Blaszczyk, P. (2006) Free Thyroid Hormones and Cholesterol in Follicular Fluid of Bovine Ovaries. Bulletin of the Veterinary Institute in Pulawy, 50, 189-193.
[2] Kor NM. (2014) The effect of corpus luteum on hormonal composition of follicular fluid from different sized follicles and their relationship to serum concentrations in dairy cows. Asian Pac J Trop Med. Sep;7S1:S282-8.

76° CONVEGNO SISVET
BARI, 21-22-23 GIUGNO 2023

EFFECT OF MELATONIN IMPLANT ON LOCOMOTOR ACTIVITY, BODY TEMPERATURE AND GROWTH PERFORMANCE OF POST-WEANING LAMBS

Viola Irene (1), Canto Muñoz Francisco Eduardo (2), Abecia Martinez Alfonso Jose (2)

(1) Department of Veterinary Science, University of Turin (UNITO), Largo Paolo Braccini 2, Grugliasco 10095, Turin, Italy
(2) IUCA, Departamento de Producción Animal y Ciencia de los Alimentos, Universidad de Zaragoza, Miguel Servet, 177, 50013 Zaragoza, Spain

Corresponding Author: **Irene Viola** - irene.viola@unito.it

Melatonin is an endogenous hormone that plays a pivotal role in mediating several patterns of animal physiology and behavior. Melatonin's effect on the sheep reproductive system has been deeply investigated as well as its action in reducing neonatal mortality and increasing survival rate at weaning, due to a higher colostrum quality and brown adipose tissue production. Nowadays how melatonin is involved in the lamb's growth and development is still unclear.
In light of this, the present study supports the potential role of melatonin in improving performance outcomes in fattening lambs. Therefore, this work aimed to explore the effect of melatonin implant on growth performance and physiological parameters in post-weaning lambs.
Sixty Rasa Aragonesa lambs were divided into 4 groups, depending on gender and the implantation or not of two 18 mg-melatonin implants (Melovine, CEVA, Spain), and reared for 6 weeks from weaning to slaughter under the same management conditions. During the whole experimental period, body weight and the amount of feed consumed were used to calculate feed conversion rate (FCR). An acceleration sensor was applied on a neck collar to define the locomotor activity (LA) and its circadian rhythm (CR), in terms of MESOR, amplitude, and acrophase. Finally, the surface temperature (T) was taken by a thermographic camera the last week of fattening, and the subcutaneous fat thickness (FT) over the longissimus dorsi muscle was ultrasound-scanned.
Regarding the performance parameters, FCR was not affected by the treatment, although melatonin-treated lambs consumed a lower amount of feed, especially female MEL ($p<0.001$), in which also FT was significantly higher ($p<0.05$) compared to CTR, while no effect was shown in male lambs. The thermography revealed a lower T in MEL for all the body regions analyzed ($p<0.01$). Overall LA was lower in MEL ($p<0.001$), and female lambs always exhibited more movement ($p<0.001$). Moreover, the CR was influenced by treatment since MEL displayed a progressive increase of MESOR over time ($p<0.01$), whereas CTR showed a delay in the acrophase every week ($p<0.001$).
In conclusion, the study relieves that melatonin affects physiological parameters during the post-weaning period of lambs. The results underline the interaction effect between melatonin and gender, showing a positive impact on melatonin-treated female lambs.

[1] Abecia J.A., Canto F. 2023. Circadian, feeding and locomotor activities of artificial raredlambs mesured by actigraphy. Joumal of Applied Animal Research, 51 (1), 234-241. DOI: 10.1080/09712119.2023.2186884
[2] Abecia J.A., Canudo C., Palacios C., Canto F., 2022. Measurig lamb activity during lactation by actigraphy. The Journal of Biological and Medical Rhyhm Research, 39, 1638-1380. DOI: 10.1080/07420528.2022.2108715.
[3] Flinn T., Kleemann D.O., Swinbourne A.M., Kelly J.M., Weaver A.C., Walker S.K., Gatford K.L., Kind K.L., van Wettere W.H.E.J. 2020. Neonatal lamb mortality: major risk factors and the potential ameliorative role of melatonin. Journal of Animal Science and Biotechnology 5, 11(1),107. DOI: 10.1186/s40104-020-00510-w.
[4] Ma W., Wu H., Li G., Yan L., Wang L., Zhao M., Guan S., Xu S., Guo X., Liu F.,Ji P., Wusiman A., Liu G. 2022. Melatonin promotes the growth and development of lambs by increasing growth hormone and testosterone, targeting on apoptosis signaling pathway and intestinal microflora. Frontiers in Endocrinology, 13:966120. DOI 10.3389/fendo.2022.966120.

SUPEROVULATION IN COWS: IS THERE AN EFFECT ON THE ANTI-MÜLLERIAN HORMONE?

Isabella Pividori (1), Susy Urli (1), Francesca Corte Pause (1), Alessio Cotticelli (1,2), Aloma Zoratti (1), Alberto Prandi (1), Tanja Peric (1)

(1) Università degli Studi di Udine, Dipartimento di Scienze Agroalimentari, Ambientali e Animali
(2) Università degli Studi di Napoli Federico II, Dipartimento di Medicina Veterinaria e Produzioni Animali

Corresponding Authors: **Aloma Zoratti** - zoratti.aloma@spes.uniud.it

Anti-Müllerian Hormone (AMH) is a reliable molecular marker, recognized by the scientific community, of the ovarian follicular pool and follicular responsiveness to superovulatory treatments in cow [1]. However, many aspects still need to be investigated to clarify the effect of superovulatory treatments on AMH concentrations. Therefore, this study aimed to measure the plasmatic AMH concentrations in Pezzata Rossa Italiana cows subjected to Multiple Ovulation and Embryo Transfer (MOET). Blood was collected from 3 cows at the time of the CIDR insertion, during 2 consecutive superovulation protocols, via coccigeal venipuncture using vacutainer tubes (EDTA anticoagulant). Samples were centrifuged at 1500 rpm for 15 minutes and the plasma was aliquoted into Eppendorf tubes (1 mL) before being stored at -20°C until analysis. AMH was determined by a commercially available ELISA sandwich kit (Ansh Labs®, cat. no. AL-114, Ansh Labs, USA). In our study two repeated superovulation treatments resulted in a decrease in the average plasma AMH concentrations from 607.6 ± 114.7 pg/ml to 535.1± 40.5 pg/ml (mean ± SD). In particular, after the second treatment, the AMH showed a decrement of 7.8%, 21.3% and 3.1% in the three cows. It has been already reported that the measurement of AMH concentrations can help to predict cows follicular and ovulatory responses to gonadotropin treatment [2]. We suggest that it can also be informative of the decreasing ovarian reserve subsequent to the repeated gonadotropin administrations during MOET. Given that little is known about the regulation of AMH production [3] further in larger future studies are needed to investigate the AMH decrement post-gonadotropin treatment. Therefore, the evaluation of the AMH concentrations could help to analyse the relationship between ovarian reserve and a superovulation treatment.

[1] Liang, A., Salzano, A., D'Esposito, M., Comin, A., Montillo, M., Yang, L., Campanile, G., & Gasparrini, B.; 2016. Anti-Mullerian hormone (AMH) concentration in follicular fluid and mRNA expression of AMH receptor type II and LH receptor in granulosa cells as predictive markers of good buffalo (Bubalus bubalis) donors. Theriogenology, 86(4), 963–970. https://doi.org/10.1016/j.theriogenology.2016.03.020
[2] Rico, C., Drouilhet, L., Salvetti, P., Dalbiès-Tran, R., Jarrier, P., Touzé, J. L., Pillet, E., Ponsart, C., Fabre, S., & Monniaux, D.; 2012. Determination of anti-Müllerian hormone concentrations in blood as a tool to select Holstein donor cows for embryo production: from the laboratory to the farm. Reproduction, fertility, and development, 24(7), 932–944. https://doi.org/10.1071/RD11290
[3] Rico, C., Médigue, C., Fabre, S., Jarrier, P., Bontoux, M., Clément, F., & Monniaux, D.; 2011. Regulation of anti-Müllerian hormone production in the cow: a multiscale study at endocrine, ovarian, follicular, and granulosa cell levels. Biology of reproduction, 84(3), 560–571. https://doi.org/10.1095/biolreprod.110.088187

76° CONVEGNO SISVET
BARI, 21-22-23 GIUGNO 2023

THE EXTREME PHENOTYPES OF CORTISOL LEVELS REVEAL DIFFERENT EXPRESSION PATTERNS OF MIRNAS IN SHEEP SALIVA

Isabella Manenti (1); Irene Viola (1); Ugo Ala (1); Elisabetta Macchi (1); Mario Baratta (2); Eugenio Martignani (1); Silvia Miretti (1)

(1) Dipartimento di Scienze Veterinarie, Università di Torino, Largo Paolo Braccini 2, Grugliasco (TO) 10095, Italia
(2) Dipartimento di Scienze Chimiche, Scienze della Vita e Sostenibilità Ambientale, Università di Parma, Via Università 12, Parma 43124, Italia

Corresponding Author: **Isabella Manenti** - isabella.manenti@unito.it

Farm procedures have an impact on animal health and production by activating the hypothalamic-pituitary-adrenal axis (HPA). This activation induces the physiological adaptation responses that allow the animal to cope with environmental variations. The resulting metabolic and molecular changes can be quantified (1).
The magnitude of the response is individual and reflects genetic predisposition and experiences affecting epigenetic factors (2).
MiRNAs are small noncoding RNA molecules with a key role in the regulation of homeostasis, and recently identified as potential biomarkers of stress and resilience in animals (3).
This study aims to define the salivary miRNAs profiles associated with variable stress responses in ewes during farm management.
Saliva samples were obtained from 20 animals: 3 sampling before (PRE) and 3 samplings after (POST) introducing a ram in the flock.
Cortisol and miRNAs were extracted and analysed with ELISA assay and RT-qPCR respectively.
Based on salivary cortisol levels, the ewes classified as LOW (N=4) and HIGH (N=4) showed the same phenotype in both collections (PRE and POST).
Based on literature related to adaptation responses and circulating miRNAs, 17 miRNAs were selected. Among them, miR-16b, miR-21, miR-24, miR-26a, miR-27a, miR-99a and miR-223 were analysed and their expression compared between the two phenotypes. Mann-Whitney U test highlighted that the HIGH ewes were characterized by a lower expression of miR-99a in PRE while miR-16b and miR-21 expression was lower in POST samples ($p<0.05$). Additional analysis will be performed to check association between salivary miRNAs and cortisol phenotypes and, if present, to evaluate their possible predictive values.
In conclusion, several miRNAs were found in sheep saliva. Among them, the expression patterns of miR-16b, -21 and -99a were associated to different cortisol phenotypes.
In humans, changes in miR-21 and miR-16 expressions were induced by acute psychological social stress (4) and miR-16 was described as involved in serotonin transporter gene regulation and correlated with the individual perception of stressors. MiRNAs are characterized by high homology among species, so they might participate in the stress response in sheep as well. Thus, genetic selection of ewes could benefit from salivary miRNAs to identify and breed resilient individuals.

[1] Cockrem, J.F. Individual Variation in Glucocorticoid Stress Responses in Animals. Gen. Comp. Endocrinol. 2013, 181, 45–58.
[2] Pant, S.D.; You, Q.; Schenkel, L.C.; Voort, G.V.; Schenkel, F.S.; Wilton, J.; Cain, L.; Karrow, N.A. A Genome-Wide Association Study to Identify Chromosomal Regions Influencing Ovine Cortisol Response. Livest. Sci. 2016, 187, 40–47.
[3] Shandilya, U.K; Sharma, A.; Naylor, D.; Canovas, A.; Mallard, B.; Karrow, N.A. Expression Profile of miRNA from High, Middle, and Low Stress-Responding Sheep during Bacterial Endotoxin Challenge. Animals 2023, 13, 508
[4] Wiegand, C.; Heusser, P.; Klinger, C.; Cysarz, D.; Büssing, A.; Ostermann, T.; Savelsbergh, A. Stress-associated changes in salivary microRNAs can be detected in response to the Trier Social Stress Test: An exploratory study Sci. Rep. 2018, 8, 7112

ENVIRONMENTAL NEBULIZATION OF LAVENDER ESSENTIAL OIL (L. ANGUSTIFOLIA): WELFARE ASSESSMENT IN GROWING PIGS

Alberto Elmi (1), Eleonora Nannoni (1), Domenico Ventrella (1), Giovanna Martelli (1), Nadia Govoni (1), Niccolò Ian Vannetti (1), Maurizio Scozzoli (2), Luca Sardi (1), Maria Laura Bacci (1)

(1) Department of Veterinary Medical Sciences, Alma Mater Studiorum - University of Bologna
(2) APA-CT S.r.l., Forlì, Italy

Corresponding Author: **Alberto Elmi** - alberto.elmi2@unibo.it

Intensive farming conditions expose pigs to different type of physical and psychological stressors, such as limited possibility of expressing their natural behaviour and lack of stimuli, that determine a reduction in welfare and the appearance of stress-related signs (tail biting, stereotypies, etc.). Lavender essential oil (Lavandula angustifolia; LaEO) is natural compound highly employed for medical and pharmaceutical purposes as it shows antibacterial and pesticide activity and, above all, for its carminative and sedative capabilities. Its positive effects, including relaxation and anxiety reduction, are reported in several species such as rat, horse, dog, human and pig [1-3]. Aim of the study was to analyse stress-related physiological and behavioural parameters in growing pigs exposed to environmental LaEO nebulization. The trial was performed in a porcine experimental facility. Ninety (n=90) crossbred barrows with undocked tails were allotted to three experimental groups, housed in different rooms. In the LaEO group room, a 1% solution of lavender essential oil was vaporized for 10min twice a day. Sham group (SHAM) was exposed to nebulization of the solution vehicle without LaEO, while Control (C) group did not receive any treatment. The trial lasted 63 days. Hair was shaved the day before the beginning of trial, and then sampled at the end to evaluate Cortisol (CORT) and Dehydroepiandrosterone (DHEA) by Radioimmunoassay. Individual behaviour, body weight and skin and tail lesions were recorded at three timepoints: beginning, middle and end of the trial. ANOVA and Chi-squared tests were used to analyse data. Hair CORT and DHEA did not show any statistically relevant differences, but CORT/DHEA ratio was lower in LaEO group ($p=0.0212$). No differences emerged between groups in growth parameters. Total number of body lesions was significantly reduced in LaEO group compared SHAM and CTR ($p<0.0001$) as well as the severity of tail lesions ($p=0.0254$). The overall level of activity was not different between C and LaEO. Although the positive effects of LaEO nebulisation were not strong enough to modify the overall behaviour and physiological balance, a decrease in animals' aggressiveness and tail/skin damage was recorded overtime, also supported by the decrease in CORT/DHEA ratio. It can hypothesized that performing such trial in an experimental facility, rather than under farming conditions, may have impaired the results by not inducing high stress levels.

[1] Cavanagh, H. M. A., Wilkinson J.M. «Biological Activities of Lavender Essential Oil». Phytotherapy Research 16, fasc. 4 (2002): 301–8.
[2] Shaw, D., K. Norwood, J. C. Leslie. «Chlordiazepoxide and Lavender Oil Alter Unconditioned Anxiety-Induced c-Fos Expression in the Rat Brain». Behavioural Brain Research 224-1(2011): 1–7.
[3] Direksin, K., S. Nopwinyoowong, S. Seesupa. «Influence of Lavender Essential Oil Inhalation on Aggressive Behavior of Weaned Pigs.» Journal of Applied Animal Science 10, fasc. 1 (2017): 47–56.

76° CONVEGNO SISVET
BARI, 21-22-23 GIUGNO 2023

PRIMARY CULTURES OF GÖTTINGEN MINIPIG MAMMARY EPITHELIAL CELLS FOR THE ESTABLISHMENT OF AN IN VITRO MODEL FOR STUDYING THE EPITHELIAL BARRIER. A CONTRIBUTION FROM THE IMI CONCEPTION CONSORTIUM

Debora La Mantia (1), Augusta Zannoni (1), Roberta Salaroli (1), Monica Forni (2), Chiara Bernardini (1)

(1) Università di Bologna, Dipartimento di Scienze Mediche Veterinarie
(2) Università di Bologna, Dipartimento di Scienze Mediche e Chirurgiche

Corresponding Author: **Debora La Mantia** - debora.lamantia2@unibo.it

The use of drugs is not uncommon during pregnancy and breastfeeding but often data concerning the drugs transport across blood/milk barrier (and so their safety) are not robust or even completely missing [1]. The development of a non-clinical platform based on in vivo, in vitro and in silico models, is a fundamental step in generating reliable and translatable data. The European project ConcePTION selected the swine as the most appropriate animal model and, specifically, the Göttingen Minipig breed, that provides genetic and microbial control improving reproducibility [2]. To limit the use of animals for experimentation, ConcePTION Project explored the parallelisms of results between in vivo and in vitro studies, in full compliance with both, international legislations and the 3Rs principles [3]. The aim of the present research is to establish an in vitro model to predict the transfer of drugs from blood to milk across the mammary barrier, using primary cultures of mammary epithelial cells derived from Göttingen Minipigs (mpMECs). For this purpose, three primary cell lines of mpMECs, isolated and immunophenotypically characterized as previously described [4], were expanded until the passage 10. To study the epithelial barrier, the mpMECs were cultured on 24-well PET membrane transwells at different seeding densities (0.1, 0.15, 0.2 and 0.3 × 10^6 cells/insert). The epithelial monolayer integrity was tested by measuring the trans-epithelial electrical resistance and the sodium fluorescein transport in function of time. The immunofluorescence analysis of the cells, cultured on transwells, was performed to check the presence of the tight junctions' proteins zonula occludens-1 (ZO-1) and occludin (OCL). The cells showed typical epithelial morphology during the expansion phase. The mean doubling time of all the three primary cell lines was 30±3 h; however, a significative difference resulted between MG9 and MG12, this last with a faster growth rate. All the three primary cell lines resulted able to form a compact monolayer, even if with different kinetics at the seeding density tested. Overall, the cells created an appropriate epithelial barrier between day 3 and 4 of culture, also confirmed by the abundance and correct localization of ZO-1 and OCL. In conclusion, the present research has defined the appropriate culture conditions to obtain a stable epithelial barrier to study the transfer of drugs across the blood/milk barrier in the minipig model.

[1] Nawaelaerts N. et al. A comprehensive review on non-clinical methods to study transfer of medication into breast milk – A contribution from the ConcePTION project, Biomedicine & Pharmacotherapy, 136: 111038, 2021.
[2] Forster R. et al. The minipig as a platform for new technologies in toxicology, Journal of Pharmacological and Toxicological Methods, 62:3 227-235, 2010.
[3] EU regulations on animal research: https://www.eara.eu/animal-research-law?lang=it (accessed on 13 March 2023).
[4] Bernardini, C. et al. Development of a Pig Mammary Epithelial Cell Culture Model as a Non-Clinical Tool for Studying Epithelial Barrier—A Contribution from the IMI-ConcePTION Project, Animals (Basel), 5;11(7):2012, 2021.

76° CONVEGNO SISVET
BARI, 21-22-23 GIUGNO 2023

PRELIMINARY ANALYSIS FOR THE OPTIMIZATION OF 3D AIRWAY SMOOTH MUSCLE VISUALIZATION IN MICRO- AND SUBMICRON-CT IMAGES

Beatrice Sica (1), Annapaola Parrilli (2), Giulia Memoli (1), Alessandra Romolo (1), Michela Bullone (1)

(1) Department of Veterinary Sciences, University of Turin, Italy
(2) Center for X-ray Analytics, Empa - Swiss Federal Laboratories for Materials Science and Technology, Dübendorf, Switzerland

Corresponding Author: **Michela Bullone** - michela.bullone@unito.it

The study of airway smooth muscle (ASM) structure and function relationship in vivo is complicated by its unreachability and a paucity of tool for its indirect assessment. The aberrant activation of ASM is a feature of asthma, and its cause remains to date elusive [1]. Most ASM structure studies are limited to histological examination of 2D slides, with important negative implications in terms of in vivo relevance. In vivo, indeed, ASM is a 3D tissue disposed in spiral bundles along the bronchial tree and its contraction causes both a reduction in the airway lumen and a shortening of the airway itself, with significant ripercussions on lung function.
We have recently observed a new, aberrant disposition of ASM cells along the ASM bundles in endobronchial biopsies of asthmatic horses [2]. Whether this contributes to asthma pathology or contrasts the ASM ability to shorten has still to be established. To do so, a tool for 3D assessment of ASM structure is needed so that real data can be inserted into in silico models.
We present here the results of preliminary experiments performed to obtain 3D micro to nano-CT images of equine bronchial specimens in which ASM cell orientation and nucleus can be identified. To this aim, formalin fixed and paraffin embedded samples of equine ASM have been scanned at different resolution at the Center for X-ray Analytics, Empa - Swiss Federal Laboratories for Materials Science and Technology, Dübendorf, Switzerland. Prior to embedding, tissues underwent one of four staining procedures overnight based on optimized in house protocols (PMA, PTA, Lugol, and negative control, as described in [3]). Preliminary results on a limited number of 16 specimens (4 per group) revealed a small to moderate penetration of all stainings in deep bronchial tissues, however the ASM layer was always reached. PTA only allowed for an adequately contrasted CT image that permitted a 3D reconstruction of the tissue with cell orientation inside. Cell nuclei were not visible at this stage, and future steps in this direction are warranted.

[1] Hassoun D, Bronchial smooth muscle cell in asthma: where does it fit? BMJ Open Respir Res, 2022. 9:e001351.
[2] Bullone M, The equine asthma model of airway remodeling: from a veterinary to a human perspective. Cell Tissue Res, 2020. 380:223-236.
[3] Nierenberger et al, Assesing the three-dimensional collagen network in soft tissues using contrast agents and high resolution microCT: application to porcine iliac veins. CR Biologies, 2015. 338:425-433.

76° CONVEGNO SISVET
BARI, 21-22-23 GIUGNO 2023

THE PHARMACOLOGICAL INHIBITION OF THE REFLEX ACTIVATION OF SYMPATHETIC NERVES DURING A SYSTEMIC INFECTION ENHANCES THE BACTERIAL CLEARANCE IN VIVO IN PIGS

Domenico Ventrella (1), Alberto Elmi (1), Alessandra Occhinegro (2), Ludovico Taddei (2), Emiliana Piscitiello (2), Alessandra Cappellini (2), Silvia Felici (3), Tiziana Lazzarotto (3), Maria Laura Bacci (1), Davide Martelli (2)

(1) Department of Veterinary Medical Sciences, Alma Mater Studiorum - Università di Bologna;
(2) Department of Biomedical and NeuroMotor Sciences, Alma Mater Studiorum - Università di Bologna.
(3) Department of Medical and Surgical Sciences, Alma Mater Studiorum - Univeristà di Bologna

Corresponding Author: **Domenico Ventrella** - domenico.ventrella2@unibo.it

During an infection the nervous system has the ability of inhibiting, in a negative feedback manner, the inflammatory response via two main mechanisms: i) humorally, upon release of glucocorticoids; ii) neurally, upon activation of a specific neural reflex, known as the "inflammatory reflex" [1]. Recent studies have proved, in rodents and sheep, that the efferent arm of the inflammatory reflex is purely sympathetic, running in the greater splanchnic nerves [2]. Surgical resection of the splanchnic sympathetic nerves induced in sheep an exaggerated inflammatory response, leading to resolution of a systemic bacterial infection in less than 90 minutes instead of days, as observed in sham operated animals. Better understanding of the physiological mechanisms underlying the sympathetic-mediated inhibitory action on immune function, may represent a pivotal point in the field of sepsis research. As a matter of fact, the immunosuppression characterizing the late phases of sepsis may be mediated by the nervous system itself [3]. Aim of this study was to investigate the sympathetic influence on immunity in a porcine model of bacterial infection obtained by intravenous E.coli administration. To increase the translational value of the study, the resection of the nerves was substituted by the administration of a non-selective β-blocker, propranolol, capable of disrupting communication between sympathetic nerves and leukocytes. Pigs were divided into 2 groups: propranolol (3mg/kg TID per OS) and control, and were equipped with a long-term catheter for blood samplings and a subcutaneous thermo-logger. Starting from the bacterial challenge, for 14 days, animals were strictly monitored and blood was collected daily for blood culture, complete blood count (CBC), clinical chemistry and lactatemia, and cytokines quantification. At the end of the trials, animals were sacrificed for necroscopy. The preliminary results show that treatment with propranolol allowed for a quicker bacterial clearance compared to control pigs, probably due to increased phagocytic activity of circulating phagocytes. Serum biomarker often used in bacterial infection and sepsis, like lactatemia, did not show differences between groups. Overall, the study provided new insight into the sympathetic influence on immunity in a large animal non-ruminant model, and the results seems to strengthen the hypothesis that pharmacological suppression of the sympathetic activity may enhance innate immune function.

[1] McAllen RM, McKinley MJ, Martelli D. Reflex regulation of systemic inflammation by the autonomic nervous system. Auton Neurosci 237:102926, 2022.
[2] Martelli D, Farmer DG, and Yao ST. The splanchnic anti-inflammatory pathway: could it be the efferent arm of the inflammatory reflex? Experimental physiology 101: 1245-1252, 2016.
[3] Lankadeva YR, May CN, McKinley MJ, Neeland MR, Ma S, Hocking DM, Robins-Browne R, Bedoui S, Farmer DGS, Bailey SR, Martelli D, and McAllen RM. Sympathetic nerves control bacterial clearance. Sci Rep 10: 15009, 2020.

SOIPA

76° CONVEGNO SISVET
BARI, 21-22-23 GIUGNO 2023

LONG-TERM SURVEY ON *TRICHINELLA* SPP. INFECTION IN RED FOXES (*VULPES VULPES*) OF CAMPANIA REGION

Scarcelli S. (1), D'Alessio N. (2,3), Buono F. (1), Castaldo E. (1), Ottaviano M. (1), Sgroi G. (1), Argenio F. (1), Fioretti A. (1,3), Veneziano V. (1,3).

(1) Department of Veterinary Medicine and Animal Productions, Università Degli Studi Di Napoli "Federico II", Naples, Italy
(2) Istituto Zooprofilattico Sperimentale del Mezzogiorno, Portici, Italy
(3) Osservatorio Faunistico Venatorio - Regione Campania, Naples, Italy

Corresponding Author: **Stefano Scarcelli** - stefano.scarcelli@unina.it

Trichinella spp. are cosmopolitan parasites infecting many animal species such as domestic and wild mammals (including humans) by the consumption of raw or undercooked infected meat through predation, cannibalism and scavenger attitude. Wild animals represent the main reservoir hosts of this zoonotic nematode, especially carnivores such as foxes (*Vulpes vulpes*) and wolves (*Canis lupus*) [1]. *Trichinella britovi* is the most prevalent species among wild canids of Europe, including Italy [2]. This survey aims to investigate the role of foxes as one of the main reservoirs of this parasitosis, contributing to the maintenance of the sylvatic cycle and the interconnection with the domestic one in Campania region. From 2017 to 2022, as part of the health monitoring plan in the Campania region (southern Italy), 325 carcasses of foxes were collected. Samples of 10 g of diaphragm or tibialis muscles from each animal were analysed individually using the magnetic stirrer method with artificial digestion, in accordance with Regulation (EU) 2015/1375. Larvae from positive samples, were kept in 90% ethyl alcohol and send to European Union Reference Laboratory for Parasites (EURLP) for species identification.
Out of 325 carcasses examined, 4 foxes were positive, and all isolated larvae have been identified as *T. britovi*. The overall prevalence during the six years was 1.23%. The foxes' positivity was related to the average altitude of the municipality where the carcasses were found: 3/97 were found above 400 m (3,09%) (95% CI 0-6,54) and 1/228 under 400 m (0,44%) (95% CI 0-1,30). Foxes' prevalence show a significant difference between the altitudes of the position where the carcasses were found (P-value = 0,05). This survey confirms the circulation of *T. britovi* and the epidemiological role of foxes in maintaining sylvatic cycle and the contribution in generating the interface with the domestic cycle of *Trichinella* spp. in the investigated area, according to results obtained in central Italy [3]. The increasing trend of positivity in the last years are comparable to results in Abruzzi region [4]. The low prevalence in foxes compared to wolves [3] is due probably to greater foxes' presence in anthropized areas and different feeding habits of these two species of wild canids.

[1] Gottstein B., Pozio E., Nöckler K. (2009). Epidemiology, Diagnosis, Treatment, and Control of Trichinellosis. Clinical Microbiology Reviews, 22(1): 127-145.
[2] Badagliacca, P.; Di Sabatino, D.; Salucci, S.; Romeo, G.; Cipriani, M.; Sulli, N.; Dall'Acqua, F.; Ruggieri, M.; Calistri, P.; Morelli, D. (2016). The role of the wolf in endemic sylvatic *Trichinella britovi* infection in the Abruzzi region of Central Italy. Vet. Parasitol.2016,231,124–127.
[3] Ricchiuti L., Petrini A., Interisano M., Ruberto A., Salucci S., Marino L., Del Riccio A., Cocco A., Badagliacca P., Pozio E. (2021). First report of *Trichinella pseudospiralis* in a wolf (*Canis lupus italicus*). Int J Parasitol Parasites Wildl. (2021) May 24; 15:195-198. doi: 10.1016/j.ijppaw.2021.05.002. PMID: 34136345; PMCID: PMC8182262.
[4] Badagliacca P., Di Sabatino, D., Salucci, S., De Massis, F., Cocco, A., Tieri E.E., Romeo, G., Dominicis N.D., Ricci, L., Petrini A. (2021). *Trichinella britovi* Larval Biomass in Wild Canids in the Abruzzi Region, Italy. Biol. Life Sci. Forum (2021), 5, 1. https://doi.org/10.3390/ blsf2021005001

DEVELOPMENT OF AN INTEGRATED APPROACH FOR MONITORING TOXOPLASMOSIS IN SOUTHERN ITALY

Pepe P. (1), Bosco A. (1), Mangieri G. (1), Bambacaro E. (1), Quaranta P. (1), Ciccone E. (1), Lattero N. (1), Santaniello M. (1), D'Orilia F. (2), Sarnelli P. (3), Pinto R. (3), Maurelli M.P. (1), Cringoli G. (1), Rinaldi L. (1)

(1) Department of Veterinary Medicine and Animal Production, CREMOPAR, University of Naples Federico II, Naples, Italy
(2) Centro di Riferimento Regionale Sanità Animale (CReSan), Regione Campania, Salerno, Italy
(3) UOD Prevenzione e Sanità Pubblica Veterinaria Regione Campania, Naples, Italy

Corresponding Author: **Paola Pepe** - paola.pepe@unina.it

Toxoplasmosis is a widespread worldwide zoonotic infection caused by the intracellular protozoan *Toxoplasma gondii* (Dubey et al., 2020). This protozoan infection is considered one of the most important food-borne parasitic zoonoses globally (Gabriel et al., 2022). Beyond its impact on public health, toxoplasmosis has also important veterinary implications, because it causes in livestock miscarriage or congenital malformations with negative economic impacts (Stelzer et al., 2019). In order to reduce the spread of this infection, an integrated monitoring programme aimed to deepen the epidemiological data on toxoplasmosis and to identify the risk factors which may favour T. gondii infections in animals and humans was conducted in an endemic area of southern Italy. Since 2019, an integrated approach to control toxoplasmosis has been implemented in a highly endemic area of continental southern Italy (Campania region). Innovative procedures and tools have been developed and exploited during the control programme based on the following strategies: (i) parasitological analysis and risk factors for *T. gondii* in livestock (sheep, goats, cattle and water buffalo) farms; (ii) serological and molecular monitoring in meat activities; (iii) biosecurity and pest management practices in positive farms; (iv) hospital discharge records (HDRs); (v) outreach activities (information, dissemination and health education) to farmers, vet practitioners, school-age children and pregnant women.
The present study shed light on the current epidemiological situation of *T. gondii* in the Campania region of southern Italy, confirming a very high seroprevalence of this infection in livestock (up to 93.1 % in sheep farms) in southern Italy (Pepe et al., 2021) and highlighting the potentially significant public health risk in this area and the need of valid control strategies based on comprehensive and transdisciplinary actions according to the One Health approach.

[1] Dubey JP, Cerqueira-Cézar CK, Murata FH, Kwok OC, Hill D, Yang YR, Su C. All about *Toxoplasma gondii* infections in pigs: 2009–2020. Vet. Parasitol. 2020, 288, 109185.
[2] Gabriël S, Dorny P, Saelens G, Dermauw V. Foodborne Parasites and Their Complex Life Cycles Challenging Food Safety in Different Food Chains. Foods. 2022, 12(1),142.
[3] Stelzer S, Basso W, Benavides Silván J, Ortega-Mora LM, Maksimov P, Gethmann J, Conraths FJ, Schares G. *Toxoplasma gondii* infection and toxoplasmosis in farm animals: Risk factors and economic impact. Food Waterborne Parasitol. 2019, 15, e00037.
[4] Pepe P, Bosco A, Capuano F, Baldi L, Giordano A, Mancusi A, Buonanno M, Morena L, Pinto R, Sarnelli P, Cringoli G, Rinaldi L. Towards an Integrated Approach for Monitoring Toxoplasmosis in Southern Italy. Animals (Basel). 2021, 11(7), 1949

76° CONVEGNO SISVET
BARI, 21-22-23 GIUGNO 2023

EPIDEMIOLOGICAL DEVELOPMENTS OF TOXOPLASMOSIS IN BUFFALO FARMS

Cappelli G. (1), Bosco A. (2), Martucciello A. (1), Di Vuolo G. (1), Vecchio D. (1), Smeraldo S. (1), Guerra M. (1), Napoletano M. (1), Rinaldi L. (2), De Carlo E. (1)

(1) CReNBuf Istituto Zooprofilattico Sperimentale del Mezzogiorno, Portici, Italy
(2) Department of Veterinary Medicine and Animal Production, University of Naples Federico II

Corresponding Author: **Giovanna Cappelli** - giovanna.cappelli@izsmportici.it

Toxoplasmosis is one of the world's most common parasitic zoonoses, caused by the protozoan *Toxoplasma gondii*. In addition to its public health impact, toxoplasmosis also has important veterinary implications, as it causes abortions, foetal mummification, stillbirths and weak offspring in cattle, with negative economic impacts Water buffaloes are considered resistant to the clinical disease due to *T. gondii*, so there are studies reporting only serological evidence of natural infection in these animals. Due to the increase in the use of buffalo meat and milk for human consumption, there has been growing concern over the last 10 years about the sanitary conditions on buffalo farms. In order to reduce the risk of human infection with *T. gondii*, knowledge of epidemiological data and identification of potential risk factors associated with infection in farm animals are of paramount importance. Therefore,the objectives of this study were (i) to determine the seroprevalence of *T. gondii* infection in water buffaloes and (ii) to assess the risk factors associated with infection in water buffalo farms. The study was conducted from March 2022 to December 2022 and 104 farms in the province of Salerno and 9,712 lactating and non-lactating animals were analysed, of which 1,492 were positive for *Toxoplasma gondii* (15%; 95% Confidence Interval [CI] = 14.6-16). For the province of Caserta 80 farms were analysed for a total of 3,937 animals analysed, of which 1,201 were positive (30%; 95% Confidence Interval [CI] = 29-31.9). Farms were selected on the basis of production orientation, type of housing and number of animals milked. Serological screening was performed using blood samples collected during status prophylaxis and analysed with an indirect ELISA kit (ID Screen®, Indirect Toxoplasmosis Multi-Species, IDVET, France), according to the manufacturer's instructions. A questionnaire designed to assess risk factors was administered to each farm. It included questions on several management variables (type of production, number of animals, presence of cats and any rodent control measures) and on the presence of abortions on water buffalo farms. Overall, out of 13649 water buffalo animals tested, 184 (19.7%; 95% Confidence Interval [CI] = 19.7-24.6) were seropositive for *T. gondii*. The results of this study highlight the current epidemiological situation of *T. gondii* on buffalo farms in the Campania region, confirming the presence of a very high seroprevalence.

This work was supported by RC IZSME10/21 Finaced by Italian Ministry of Health

[1] Hill et al., 2015. Anim Health Res Rev, 6: 41-61.
[2] Dubey 2010. General Biology, 1-72.
[3] Pepe P. et al. 2021.Animals (Basel), Jun 30;11(7):1949.

FIRST APPLICATION OF A DROPLET DIGITAL PCR FOR DETECTION OF TOXOPLASMA GONDII IN MUSSELS

Mancusi A. (1), Proroga Y.T.R. (1), Giordano A. (1), Girardi S. (1), D'Orilia F. (2), Pinto R. (3), Sarnelli P. (3), Rinaldi L. (2,4), Capuano F. (1), Maurelli M.P. (4)

(1) Istituto Zooprofilattico Sperimentale del Mezzogiorno, Department of Food Security Coordination
(2) Centro di Riferimento regionale per le malattie degli animali domestici (C.Re.San.)
(3) Regione Campania, UOD Prevenzione e Sanità Pubblica Veterinaria
(4) University of Naples Federico II, Department of Veterinary Medicine and Animal Production.

Corresponding Author: **Andrea Mancusi** - andrea.mancusi@izsmportici.it

Toxoplasmosis, caused by the protozoan *Toxoplasma gondii*, is considered one of the most important food-, water- and soil-borne zoonotic disease worldwide [1]. In the last twenty years many papers were published on the transmission of *T.gondii* by marine animals, including mollusks [2], that can concentrate the oocysts and release them with faeces after several days of ingestion from the water. Sporulated oocysts can remain viable and infective for 18 months in sea water. Therefore, raw or undercooked bivalve mollusks represent a risk for humans [3]. The aim of this study was to develop and validate a digital droplet polymerase chain reaction (ddPCR) protocol to obtain a sensitive diagnostic tool for detection and quantification of *T. gondii* in mussels. To evaluate the limit of detection at 95% (LOD95) of ddPCR four concentration levels: 8000 genomic copy (gc)/µl, 800 gc/µl, 80 gc/µl, 8 gc/µl of a *T. gondii* reference DNA were used. DNA was extracted from 80 pools of mussels (*Mytilus galloprovincialis*), using a QIAamp DNA Mini kit, according to the manufacturers' instructions. Forty pools were contaminated with reference DNA of *T. gondii* and used as positive controls, while other 40 pools were used as negative controls. The ddPCR reaction was performed using the protocol, primers and probe to amplify the region Toxo-529bp repeat element of the parasite described by Mancusi et al. (2022) [4]. The ddPCR was performed in a QX200 system. The LOD95 obtained for ddPCR was 8 gc/µl. The sample was considered positive if showed ≥ two droplets. All infected replicates resulted positive, while no positive droplets were detected in the samples used as negative controls. The number of droplets generated for reaction ranged from 8,828 to 14,075, with an average of 12,627 droplets. The sensitivity and specificity of ddPCR were 100% (95%CI= 94.3-99.9). To validate the protocol 100 mussel pools collected in Gulf of Naples were used. Of these 16% resulted positive (95% Confidence Interval (CI)= 9.7-25.0) for *T. gondii*. Samples were tested also by real-time PCR [4] and no positive samples were found. The ddPCR data revealed good separation between negative and positive droplets with few interface droplets supporting a higher primer specificity and reaction efficiency than real-time PCR. This approach could be very useful for a rapid detection of small quantity of *T. gondii* in mussels aimed to reduce the risks of toxoplasmosis infection in humans.

[1] Almeria S, Dubey JP (2021). Foodborne transmission of *Toxoplasma gondii* infection in the last decade. An overview. Res Vet Sci 135: 371-385.
[2] López Ureña NM et al. (2022). Contamination of Soil, Water, Fresh Produce, and Bivalve Mollusks with *Toxoplasma gondii* Oocysts: A Systematic Review. Microorganisms 10(3): 517.
[3] Dubey JP (2022). Toxoplasmosis of animals and humans. Third Edition, CRC press Taylor & Francis Group (Boca Raton, Florida, USA.
[4] Mancusi et al. (2022). Development of a droplet digital polymerase chain reaction tool for the detection of *Toxoplasma gondii* in meat samples. Parasitol Res. 121(5): 1467-1473.

76° CONVEGNO SISVET
BARI, 21-22-23 GIUGNO 2023

MOLECULAR SURVEY OF VECTOR-BORNE DISEASES IN SHELTERED DOGS FROM ROMANIA

Napoli E. (1), Palazzolo V. (1), Di Bella S. (2), Blanda V. (2), Guercio A. (2), D'Amico G. (3), Gabrielli S. (4), Brianti E. (1)

(1) Dipartimento di Scienze Veterinarie, Università degli Studi di Messina, Italia
(2) Istituto Zooprofilattico Sperimentale della Sicilia "A. Mirri", Italia
(3) Departement of Parasitology and Parasitic Disease, University of Agricultural and Veterinary Medicine, Romania

Corresponding Author: **Ettore Napoli** - enapoli@unime.it

Most of the Vector Borne Diseases (VBDs) of zoonotic concern are regarded as emerging pathologies, and a main role is played by canine leishmaniosis (CanL). The spreading of this disease is influenced mainly by climatic features that drive the spread of its sand fly vectors and in the uncontrolled movements of infected/diseased dogs. CanL was endemic in Countries of the Mediterranean Basin, while in Northern and Central Europe it was considered sporadic. However, an expansion of the disease toward North-East Europe was observed in the last decades; but data on this zoonotic parasitosis are, so far, scant and scattered. Aim of the present study was to investigate the epidemiology of CanL and other VBDs in sheltered dogs in Romania.
The study has been conducted in a cohort of 156 dogs (67 male and 89 female) whit a mean age of 40.3 ± 42.5 months (range: 3-192 months), hosted in 4 shelters. The first shelter (S1) was located site in Mureș district, North Romania, where 59 Ukrainian dogs recently rehoused in Romania due to the war were sampled. The second shelter (S2) was in the Argeş district, Central Romania while the last two shelters (Cernavodă S3 and Nisipari S4) were in the district of Constanţa, in southern part of the country. For each included dog a blood sample was collected and stored in K3EDTA, genomic DNA was then extracted using a commercial kit and analyzed through end-point PCRs for Anaplasma spp., Rickettsia spp. and Babesia spp. and through qPCR for the detection of Leishmania spp.
Out of the 156 dogs, 42 (26.9%) tested positive for *Leishmania spp*. The presence of dogs positive to *Leishmania spp*., was observed in all the sites with the highest prevalence (i.e., 40%; 12/30) in S3, followed by S1 (28.8% 17/59), S2 (20.7% 6/29) and S4 (18.4% 7/38), any statistical differences was observed in the study sites. Furthermore, 2.6% of the tested dogs was positive for *Rickettsia spp.*
Results of this survey confirm the presence of *Leishmania spp.* in sheltered dogs in Romania with an average prevalence of 27%, that was higher compared to what already reported in the same area (1). suggesting an increase in the circulation of the parasite in Country. Moreover, considering that the Ukrainian dogs arrived in Romania only two months before the sampling, the detection of the parasite in the 28.8% of the dogs suggests a spread also in Ukraine and underlines as the uncontrolled movement of animals is one of the major drivers of spreading the disease.

[1] Cazan CD, Ionică AM, Matei IA, D'Amico G, Muñoz C, Berriatua E, Dumitrache MO. Detection of Leishmania infantum DNA and antibodies against Anaplasma spp., Borrelia burgdorferi s.l. and Ehrlichia canis in a dog kennel in South-Central Romania.

A RECENT OUTBREAK OF ZOONOTIC LEISHMANIASIS IN BOLIVIAN CHACO

Napoli E.(1), Macchioni F. (2), Cosmi F. (3), De Benedetto G. (1), Ustarez Medina W. (4), Mendosa Moreno M. (4), Brianti E. (1), Gabrielli S. (5)

(1) Dipartimento di Scienze Veterinarie, Università degli Studi di Messina, Italia
(2) Dipartimento di Scienze veterinarie, Università di Pisa, Italia
(3) Convenio de Salud, Camiri, Bolivia
(4) Facultad Integral del Chaco de la Universidad Autónoma Gabriel René Moreno (U.A.G.R.M.)
(5) Dipartimento di Sanità Pubblica e Malattie Infettive, Sapienza Università di Roma, Italy

Corresponding Author: **Ettore Napoli** - enapoli@unime.it

Leishmaniases are a group of protozoan diseases transmitted to mammals, including humans, by the bite of sandflies. Globally, about 350 million people are at risk of infection and nearly 2 million of new cases occur annually. The disease is present in many regions of South America including the Pluractional State of Bolivia. This Country has the highest incidence of Cutaneous Leishmaniasis (CL) and Mucocutaneous Leishmaniasis (ML) in Latin America caused by *Leishmania braziliensis*, whereas Visceral leishmaniasis (VL) is rather rare and, so far, restricted to one single focus (1). Also, although CL was originally regarded as a 'sylvatic' disease in Bolivia, there are few evidence suggesting the presence of a peri-domestic transmission cycle because of urbanization and deforestation.

The present study describes a recent outbreak of zoonotic leishmaniasis observed in owned dogs of Bolivian Chaco, investigated by serological and molecular methods. The study was conducted in 2 rural communities, i.e., Camiri and Villamontes where a total of 46 and 143 dogs, respectively were examined. Data on sex, breed, living conditions, age, and clinical signs compatible with leishmaniasis of each dog were recorded. Dogs' sera were tested for the detection of antibodies against *Leishmania spp*. by a rapid immunochromatographic assay (Cypress Diagnostics, BE) and by a commercial indirect ELISA kit (VET-Innovate ID Diagnostics, France). Genomic DNA was extracted from the whole blood and tested by qPCR (Genesig®, UK).

Sixty-three out of 189 sera (33.33%) scored positive to *Leishmania spp*. by serology while Leishmania DNA was detected in the blood of 9 dogs (4.76%).

In the Bolivian Chaco, few studies have been conducted on animals, especially dogs, as reservoirs of zoonotic parasites (2). In the context of the One Health concept, surveys to determine the epidemiological scenario of zoonotic pathogens are of great importance. The results of this survey confirmed the role of dogs as an important reservoir of leishmaniasis in the domestic environment, as a high seroprevalence (about 33%) was demonstrated in owned dogs. Considering the poor sanitation conditions in rural communities and the lack of systematic vector control and prevention measures, further studies are needed to assess the risk to humans in such a close and low-income environment where many interactions between humans and infected/diseased dogs occur.

[1] García AL, Parrado R, Rojas E, Delgado R, Dujardin JC, Reithinger R. Leishmaniases in Bolivia: comprehensive review and current status. Am J Trop Med Hyg. 2009 May;80(5):704-11.
[2] Gabrielli S, Spinicci M, Macchioni F, et al. Canine Trypanosoma cruzi infection in the Bolivian Chaco. Parasit Vectors. 2018;11(1):632.

76° CONVEGNO SISVET
BARI, 21-22-23 GIUGNO 2023

HUMAN MYIASIS CAUSED BY *OESTRUS OVIS*: A QUESTIONNAIRE-BASED SURVEY ON MEDICAL AWARENESS AND DISEASE'S MANAGEMENT

Scala A. (1), Tamponi C. (1), Mignacca S. (2), Carta C. (1), Cavallo L. (1), Porcu F. (1), Nonnis F. (1), Ahmed F. (1), Pentcheva P. (1), Sini M.F. (1), Varcasia A. (1)

(1) Dipartimento di Medicina Veterinaria, Università degli Studi di Sassari, Sassari, Italy
(2) Pathology Division, Department of Agriculture, Food and the Marine, Celbridge (Dublin), Ireland

Corresponding Author: **Antonio Scala** – scala@uniss.it

Oestrus ovis is the most common cause of ophthalmo-myiasis in humans, and its infection is often misdiagnosed by medical doctor (MD) [1]. Although it typically affects farmers, it has also been reported in urban areas [2]. The aim of this study is to assess the knowledge of the disease among MDs.

Between January 2021 and September 2022, an online survey to MDs working in Italy was carried out.

A total of 100 completed questionnaires were returned, mainly from South Italy, where this parasite is ubiquitous, and small ruminant husbandry is prominent. The average number of patients for each MD was 1,686.25 (range 1,000-12,000), in a mean period of 11.7 years (range 1-45 years) of activity. 35% of the MDs had already knowledge of the myiasis, 18% had heard about it only, and 31%had no knowledge of it whatsoever. The cases observed from each MD per year were 1 (14%), 2 (9%), 3 (2%), 4 (4%), or more than 10 (3%).

Infections were registered from spring to autumn. 18% of the patients worked outdoors in contact with animal, 6% worked outdoors without being in contact with animal, 3% worked indoors with some outdoor activities (i.e. trekking), and 1% worked indoors without any outdoor activities. Presumably, the infection was contracted in rural areas in 23% of the cases (only in 6% of the cases the patient reported contact with ovine), whilst in 7% infestation was presumed to have been contracted at the beach. The diagnosis was confirmed based on either the symptoms and/or the anamnesis only (16%), or after parasite identification (14%). 25% of MDs did not request any further diagnosis, whilst 7% and 2% asked for morphological identification, and molecular biology/serological tests. The myasis were ocular (26%), otherwise oral-pharyngeal (1%), nasal (1%), ocular and oral/nasal-pharyngeal (3%); surprisingly, also auricular (2%). 12% of MDs prescribed ophthalmic topic antibiotic and corticosteroid cream, alone or associated with povidone-iodine (7%), 6% reported the efficacy of a mercuric oxide ointment, 9% performed larval removal only. The duration of symptoms ranged 1 (3%), 2-7 (18%), 7-10 (3%), 10-20 days (3%), or ended after larval removal (2%). 9% of the physicians reported repeated infestations in the same patients.

Recent global warming together with increasing rural tourism predispose an increase of *O. ovis* infection in humans [3]. This should raise the DVMs and DMs' awareness level and the need for an active surveillance of the disease.

This research was funded by Fondazione di Sardegna 2017 – "Epidemiologia e approcci sperimentali per il controllo della parassitosi da Oestrus ovis negli animali e nell'uomo" by Prof. Antonio Scala.

[1] Ahaduzzaman M. (2019) The global and regional prevalence of oestrosis in sheep and goats: a systematic review of articles and meta-analysis. Parasite and Vectors, 12, 346.
[2] Tamponi, C., Pasini, C., Ahmed, F., Dessì, G., Contu, E., Porcu, F., Gaglio, G., Brianti, E., Scala, A., Volkhard, K.A.J., Jó zsa, K., Varcasia, A. (2022) External ophthalmomyiasis by Oestrus ovis in tourists visiting Italy. Report of three cases and a literature review. Travel Medicine and Infectious Disease, 46, 102279.
[3] Francesconi F., Lupi O. (2012) Myiasis. Clinical Microbiology Reviews, 25, 79-105.

76° CONVEGNO SISVET
BARI, 21-22-23 GIUGNO 2023

FURTHER EVIDENCE OF PARASITIC RISKS ASSOCIATED WITH TRANSLOCATION OF FISH: FIRST REPORT OF PHILOMETROIDES SANGUINEUS (NEMATODA: PHILOMETRIDAE) IN GOLDFISH (CARASSIUS AURATUS) FARMED IN NORTHERN ITALY

Pirollo T. (1), Tedesco P. (1), Cantori A. (1), Caffara M. (1), Tentoni E. (2), Tarocchi L. (2), Fioravanti M.L. (1), Gustinelli A. (1)

(1) Dipartimento di Scienze Mediche Veterinarie (DIMEVET), Alma Mater Studiorum - Università di Bologna
(2) APA-CT s.r.l., Forlì

Corresponding Author: **Marialetizia Fioravanti** - marialeti.fioravanti@unibo.it

The trade and production of ornamental fish species represent a field of global economic interest, leading them to be among the most translocated animals in the world. A direct consequence of these movements is the increased risk of spreading transmissible pathogens in geographic areas where they are not usually present. We report here for the first time in Italy the presence of Philometrid nematodes (Philometridae) in goldfish (*Carassius auratus*) farmed in northern Italy. After some reports from consultants and operators in the sector, a sample of 10 goldfish was sent to the Fish Pathology unit of DIMEVET for parasitological analysis. All the fish showed the presence of 1-3 reddish round worms that moved slowly in the thickness of the skin between the bony rays of the caudal fin. Some of them were straight and others were arranged in a "U" shape with the ends directed toward the caudal margin of the fin. After sedating the fish by anaesthetic (MS-222), the worms were extracted by gentle pressure, and fixed in alcohol 70% or in 10% buffered formalin to be subjected to analyses useful for species identification. The morphological study allowed to identify them as gravid females (3,6-6 cm × 0,9-1,2 mm) of Philometroides sanguineus, a non-zoonotic nematode species already described in the literature [1] as specific to the genus *Carassius* and initially reported in Eastern Europe [2], then in other European and extra-European countries [3, 4] following fish translocations. The life cycle of P. sanguineus is indirect and includes copepod crustaceans as intermediate hosts in which the first-stage larvae expelled by the gravid females reach the infective third-stage. After ingestion of the infected copepods by a fish belonging to the genus Carassius, larvae migrate through the intestinal wall to the abdominal cavity and the swimbladder, where they will develop in adults and mate; after mating, the gravid females migrate through the body to the fins, with the caudal fin as preferred site. The life cycle takes approximately a year, with a different seasonality depending on the conditions of the ecosystems involved. Parasitological surveys should be conducted urgently to establish the current distribution in Italy of this newly introduced parasite and assess the risks for national fish populations.

[1] Molnar K., Buchmann K., Székely C. (2006). Chapter 12 - Phylum Nematoda. In: Fish Diseases and Disorders Vol. 1 (ed. P.T.K. Woo): 414-440.
[2] Wierzbicki K. (1960). Philometrosis of crucian carp. Acta Parasitologica Polonica 8: 181–196.
[3] Pegg J., Williams C.F., Cucherousset J., Britton J.R. (2011). What are the consequences of infection by the introduced parasite Philometroides sanguineus for threatened crucian carp Carassius carassius populations in England? Ecology of Freshwater Fish, 20: 598–604.
[4] Moravec F. (1994). Parasitic Nematodes of freshwater fishes in Europe. Kluwer Academic Publishers Dordrecht (NL): 291-296.

76° CONVEGNO SISVET
BARI, 21-22-23 GIUGNO 2023

EPIDEMIOLOGICAL STUDY OF ENDOPARASITES IN PIGS AT THE BEGINNING OF THE FATTENING PERIOD IN NORTHERN ITALY

Allievi C. (1), Villa L. (1), Valleri M. (1), Zanzani S.A. (1), Manfredi M.T. (1)

(1) Dipartimento di Medicina Veterinaria e Scienze Animali, Università degli Studi di Milano, Via dell'Università, 6 26900 Lodi, Italy

Corresponding Author: **Maria Teresa Manfredi** - mariateresa.manfredi@unimi.it

In Italy pig farming is mainly related to the heavy pig industry; in this breeding context, parasitic diseases may often present a subclinical pattern resulting in a decrease in animal welfare and a huge economic damage (1). Further, some porcine parasites may pose a risk of infection for professionals involved in the food chain for their zoonotic potential (2). Within this framework, this study attempts to evaluate prevalence of the main parasites in this animal species at the beginning of the fattening period. For this purpose, 320 individual fecal samples from fattening pigs, weighing between 35 kg and 85 kg, were analyzed from 16 farms located in Lombardy (11 farms), Piedmont (3 farms) and Emilia-Romagna (2 farms). The samples, at the rate of 20 per farm, were taken directly from the rectal ampulla of the animals upon their arrival at the fattening farms and before the anthelmintic treatment. For the detection of helminth eggs and coccidian oocysts, the FLOTAC double technique®, was used (3). Two different flotation solutions, FS2 (Sodium chloride, NaCl; s.g. = 1200) and FS7 (Zinc sulfate, ZnSO4; s.g. = 1350) were employed. Subsequently, for the direct detection of *Balantioides* coli was performed the sedimentation technique (4).
Balantioides coli was the most frequently found parasite, with an overall prevalence of 86.6% (277/320 positive samples). In addition, *Ascaris suum* (P= 1.56%) and *Cystoisospora* suis (P=2.19%) were reported, although with low prevalences. Moreover, 3 farms and 1.87% of the samples (6/320) were positive for eggs of cestodes morphologically similar to those of the genus *Hymenolepis*. Subsequently, Dna from isolated eggs of this genus were extracted and an end-point PCR was performed with sequencing on NADH gene. Sequencing revealed the presence of *Hymenolepis diminuta*.
This study provided current information on the prevalence and loadings of gastrointestinal parasites of fattening pigs in the early stages of the production cycle. Regarding *B. coli*, data obtained in this survey suggest the need to evaluate the impact of *B. coli* on animals and humans. Further investigations will be directed both to assess the circulation of the gastro-intestinal parasites at the end of the fattening period and the presence of zoonotic genotypes of *B. coli* aimed to characterize the risk to humans.

[1] Roepstorff, A.; Mejer, H.; Nejsum, P.; Thamsborg, S.M. (2011). Helminth parasites in pigs: new challenges in pig production and current research highlights. Veterinary Parasitology 180(1-2):72-81.
[2] Giarratana, F.; Nalbone, L.; Napoli, E.; Lanzo, V.; Panebianco, A. (2021). Prevalence of Balantidium coli (Malmsten, 1857) infection in swine reared in South Italy: A widespread neglected zoonosis. Veterinary World 14(4):1044-1049.
[3] Cringoli, G.; Rinaldi, L.; Maurelli, M.P.; Utzinger, J. (2010). FLOTAC: new multivalent techniques for qualitative and quantitative copromicroscopic diagnosis of parasites in animals and humans. Nature Protocols 5(3):503-515.
[4] Ministry of Agriculture, Fisheries and Food (1986). Manual of Veterinary Parasitological Laboratory Techniques, Third Edition. HMSO, UK.

76° CONVEGNO SISVET
BARI, 21-22-23 GIUGNO 2023

TOXOPLASMA GONDII AND *NEOSPORA CANINUM* IN BIRDS OF PREY FROM CENTRAL ITALY

Veronesi F. (1), Zanet S. (2), Giglia G. (1), Pinto Baptista C.R. (3), Morganti G. (1), Mandara M.T. (1), Vada R. (2), Madeira De Carvalho L.M. (3), Ferroglio E. (2)

(1) Department of Veterinary Medicine, University of Perugia, Italy
(2) Department of Veterinary Science, University of Turin, Italy
(3) Centre for Interdisciplinary Research in Animal Health, Faculdade de Medicina Veterinária, Universidade de Lisboa, Avenida da Universidade Técnica, Lisboa, Portugal

Corresponding Author: **Fabrizia Veronesi** - fabrizia.veronesi@unipg.it

Toxoplasma gondii and *Neospora caninum* are apicomplexan protozoa of major concern in livestock and *T. gondii* is also considered one of the major threats and public health concern [1,2]. These protozoa have a wide range of intermediate hosts, including birds. Birds of prey, due to their specific feeding habits, are particularly exposed to horizontal transmission by feeding on infected small mammals and other birds or through the consumption of sporulated oocysts through contaminated water or food sources [3]. Information on circulating of *T. gondii* and *N. caninum* across wild bird populations in Italy are scarce and only one study [4] was carried out in Northern regions to genotype the strains of *T. gondii* from raptors. Therefore, the present study aimed to assess the prevalence of those 2 parasites in birds of prey in Central Italy, and to expand the available information on the population structure and molecular epidemiology of *T. gondii*. The skeletal muscle and myocardium of 159 birds of prey from Central Italy, belonging to 19 species and recovered across 6 Wildlife Recovery Centers along the Italian migratory route, were collected specifically for molecular (PCR) to detect *T. gondii* and *N. caninum* and for histopathological analysis. Results from the histology showed few protozoal tissue cysts in skeletal muscle (n. 4) and hearts (n. 2). For the molecular analysis, genomic DNA was extracted. The DNA was tested by sequence typing, targeting GRA6, 529bp repeated element, B1, PK1, BTUB, SAG2, alt.SAG2 and APICO genes for *T. gondii* and to end-point PCR targeting NC5 gene for *N. caninum.* Thirty-seven out of the 159 analyzed samples tested positive for *T. gondii* with a prevalence of 23.27% and 9 for *N. caninum*, with a prevalence of 5.66%. Thirty-two sequences were obtained from the 37 isolates of *T. gondii*. Among these, 26 belonged to allele I in one or more loci, 4 to allele II and 2 consisted in atypical strains. The results of the study confirmed the circulation of both the parasites in birds of prey from Central Italy. However, the molecular analysis did not allow to draw sound conclusions about the population structure and molecular epidemiology of *T. gondii*, since it was not possible to complete the genotyping. Considering the prevalence of infection found and the non-univocal genetic patterns detected, further research is necessary to understand the role of predatory birds and their preys in the maintenance and spread of both the parasites.

[1] Reichel, M.; Ayanegui-Alcérreca, M.A.; Gondim, L.; Ellis, J. What is the global economic impact of Neospora caninum in cattle The billion-dollar question. Int. J. Parasitol. 2013, 43, 133–142.
[2] Aguirre, A.A.; Longcore, T.; Barbieri, M.; Dabritz, H.; Hill, D.; Klein, P.N.; Lepczyk, C.; Lilly, E.L.; McLeod, R.; Milcarsky, J.; Murphy, C.E.; Su, C.; VanWormer, E.; Yolken, R.; Sizemore, G.C. The One Health Approach to Toxoplasmosis: Epidemiology, Control, and Prevention Strategies. Ecohealth 2019, 16(2),378-390.
[3] Sato, A.; Goulart, M.; Konell, A.; Koch, M.O.; Fonseca, F.; Morel, A.; Locatelli-Dittrich, R. Serosurvey of Toxoplasma gondii, Neospora caninum and Sarcocystis neurona in raptors and risk factor analysis. Parasitol. Int. 2021, 82, 102312.
[4] Gazzonis, A.L.; Villa, L.; Lubian, E.; Ressegotti, S.; Grilli, G.; Raimondi, S.; Zanzani, S.A.; Manfredi, M.T. Molecular Survey on Toxoplasma gondii and Neospora caninum infection in wild birds of prey admitted to recovery centers in Northern Italy. Microorganisms 2021, 9, 736.

76° CONVEGNO SISVET
BARI, 21-22-23 GIUGNO 2023

NEOSPORA CANINUM ANTIBODIES IN TANK BULK MILK FROM DAIRY CATTLE FARMS IN ITALY: SPATIAL ANALYSIS AND EFFECTS ON REPRODUCTIVE AND PRODUCTIVE PERFORMANCES

Villa L. (1), Allievi C. (1), Di Cerbo A.R. (1), Sommariva F. (2), Zanini L. (2), Gazzonis A. (1), Zanzani S. (1), Mortarino M. (1), Manfredi M.T. (1)

(1) Dipartimento di Medicina Veterinaria e Scienze Animali, Università degli Studi di Milano, Via dell'Università, 6 26900 Lodi, Italy
(2) Associazione Regionale Allevatori della Lombardia, Via Kennedy, 30 26013 Crema, Italy

Corresponding Author: **Maria Teresa Manfredi** - mariateresa.manfredi@unimi.it

Among the available diagnostic techniques, antibody detection in bulk tank milk (BTM) represents a useful tool to estimate and monitor *Neospora caninum* herd prevalence.
To evaluate the prevalence of *N. caninum* and the effect of parasite infection on herd performances, BTM samples collected from 586 dairy herds located in one of the largest dairy production areas in Italy (Lombardy) were analyzed by an indirect ELISA (Innovative Diagnostics, France). Univariate generalized linear models (GLMs) were developed. A purely spatial analysis scanning for clusters with high or low rates for *N. caninum* using the Bernoulli model (SaTScan vers.10.1) was performed. *A maximum* entropy approach (MaxEnt vers.3.4.4) was used to estimate the probability of distribution of the pathogen based on occurrence records together with 19 environmental variables (BIO01-BIO19) obtained from WorldClim.
Overall, 180 herds resulted positive to *N. caninum* antibodies on tank bulk milk (P=30.7%). A higher risk of infection was evidenced in the provinces of Milano, Cremona, Brescia and Bergamo (P=32-40%); a lower risk was evidenced in Lodi, Pavia and Mantova (P=13-24%). A higher risk of parasite infection was revealed in small-medium farms (101-300 animals) and in older animals with more than 4 years.
The effect of *N. caninum* infection on herd performances was evaluated. Indeed, the number of inseminations for conception were higher and the period from calving to conception were longer in positive farms. Besides, *N. caninum* positive herds presented lower head daily milk production and somatic cell counts higher than 300000 cells/ml.
Spatial analysis showed that the geographical distribution of *N. caninum* positive farms with the highest level of infection probability were located in central and the western sectors of the Po valley. Two significant clusters were evidenced: one high-risk cluster with a prevalence of 50% and relative risk of 2.1 and one low risk cluster with no infection cases and expected value of 20.6%. For positive farms the climate variables with the higher risk when used in isolation were related to both temperature and precipitation.
Neosporosis is widely distributed in Italian dairy herds and an impact of the parasite on herd performances could be hypothesized. Even if the role of *N. caninum* in alterations of reproductive and productive parameters should be further explored, veterinarians and farmers should be aware of neosporosis, and control plans should be adopted.

76° CONVEGNO SISVET
BARI, 21-22-23 GIUGNO 2023

INSIGHTS INTO HOOKWORM INFECTIONS IN DOGS IN SOUTHERN ITALY: RETROSPECTIVE ANALYSIS (2011-2021 YEARS) ON PREVALENCE AND IDENTIFICATION OF HOOKWORM SPECIES

Illiano S. (1), Ciuca L. (1), Maurelli M.P. (1), Pepe P. (1), Caruso V. (1), Bosco A. (1), Pennacchio S. (2), Pompameo M. (2), Rinaldi L. (1)

(1) Department of Veterinary Medicine and Animal Production, University of Naples Federico II, via Delpino, 1, 80137, Italy
(2) ASL Napoli 1 Centro, Veterinary Hospital, via Marco Rocco di Torrepadula, 13, 80145 Naples, Italy

Corresponding Author: **Sergio Illiano** - sergio.illiano@unina.it

Hookworms are among the most important soil-transmitted parasites in the world. Considering their veterinary and sanitary importance in dogs and their role in public health due to their zoonotic risk and the recent changes in the molecular epidemiology of some hookworm species as well, it is mandatory to constantly monitor the molecular and epidemiological scenario of these parasitosis (Traub et al., 2021; Traversa et al., 2012). The present study aimed to update the epidemiological scenario of hookworm infections in both owned and stray dogs in southern Italy and discriminate between different hookworm species (*Ancylostoma caninum* and *Uncinaria stenocephala*) through molecular analyses. For this, a total of 7008 owned dogs and 5642 stray dogs referred to our lab from June 2011 to June 2021 for copromicroscopical examination with FLOTAC technique (Cringoli et al., 2010) were included in the study for a retrospective analysis. Moreover, 72 faecal samples, from dogs naturally infected to hookworms, were used to discriminate between *A. caninum* and *U. stenocephala* using two PCR protocols (A-described by Traub et al., 2004; B-the same protocol with modifications of the thermal profile). Prior molecular analyses, a subsample of 40/72 positive faecal samples were used for morphometric investigations on hookworm eggs. The results of the ten years retrospective analysis (2011-2021) showed an overall prevalence of hookworm infections equal to 9.2%, specifically, 5.1% in owned dogs and 14.2% in stray dogs. The logistic regression identified a significant association between positivity to hookworms and the variable "puppies" both in stray (13.84%; OR=2.4) and owned (7.07%; OR=2.2) dogs. Results of molecular analyses showed that only 25/72 were confirmed with both protocols (A, B). Specifically, 6 samples were confirmed by using protocol A and 19 with protocol B. The sequencing revealed 17 samples of *U. stenocephala* and 8 of *A. caninum*. The findings of this study showed a high prevalence of hookworm infections in dogs in southern Italy, updating its epidemiological scenario for the last ten years. This study revealed the first identification of hookworm species in dogs in Italy through molecular studies, despite further studies are needed.

[1] Traub RJ, Zendejas-Heredia PA, Massetti L, Colella V. Zoonotic hookworms of dogs and cats - lessons from the past to inform current knowledge and future directions of research. Int J Parasitol. 2021; 51(13-14):1233-1241.
[2] Traversa D. Pet roundworms and hookworms: a continuing need for global worming. Parasit Vectors. 2012; 5:91.
[3] Cringoli et al. FLOTAC: new multivalent techniques for qualitative and quantitative copromicroscopic diagnosis of parasites in animals and humans. Nat Protoc. 2010; 5(3): 503-515
[4] Traub RJ, Robertson ID, Irwin P, Mencke N, Thompson RC. Application of a species-specific PCR-RFLP to identify Ancylostoma eggs directly from canine faeces. Vet Parasitol. 2004; 123(3-4):245-55.

76° CONVEGNO SISVET
BARI, 21-22-23 GIUGNO 2023

PRELIMINARY TRIALS ON EFFECTIVENESS OF COLD PLASMA TREATMENTS ON CANDIDA ALBICANS BIOFILMS

Bordoni T. (1), Neretti G. (2), Tedesco P. (1), Luci V. (1), Galuppi R. (1)

(1) Dipartimento di Scienze Mediche Veterinarie (DIMEVET), Alma Mater Studiorum - Università di Bologna
(2) Dipartimento di Ingegneria dell'Energia Elettrica e dell'Informazione "Guglielmo Marconi" (DEI) Alma Mater Studiorum - Università di Bologna

Corresponding Author: **Roberta Galuppi** - roberta.galuppi@unibo.it

Candida albicans, due also to its morphological plasticity and the ability to form biofilms [1,2], is able to cause serious infections in both humans and animals. In this study, a protocol was developed for the in vitro formation of *C. albicans* biofilms on petri dishes, on which an indirect non-thermal plasma treatment was carried out by using a PSJA device (Plasma Synthetic Jet Actuator). A field strain of C. albicans isolated from human vaginal swab was used for the tests. The strain was maintained in culture at 37°C through periodic transplants on Sabouraud Dextrose Agar (BD) supplemented with 0.05g/l of Chloramphenicol (Sigma-Aldrich) (SAB-CAF). For biofilm production, the strain was transplanted on SAB-CAF and incubated for 24 hours at 37°C. Some loopfuls of the grown colonies were transferred into Sabouraud Dextrose broth and then incubated at 37°C for 48 hours. The broth cultures were centrifuged at 2000 rpm for 10 minutes and the sediment was washed twice with PBS, and then resuspended in PBS until an opacity of an intermediate value between 1 and 2 of the McFarland scale was obtained. Petri dishes of 6mm diameter were used as substrate, in each of which 3 ml of suspension of C. albicans were placed. The Petri dishes were incubated under continuous agitation at 37°C for 90 minutes [3, 4] to favour the adhesion phase and then washed twice with PBS to remove non-adherent cells. Six milliliters of RPMI 1640 [4] were then added to each plate and incubated at 37°C. After 12 days, the Petri dishes with the biofilm were emptied of the culture medium after gentle stirring and brought to the DEI Plasma Technology Laboratory (PTL), and subjected to treatment with cold plasma for 5 and 15 minutes or kept untreated as a control. Each treatment was performed in quadruplicate in order to evaluate in duplicate their morphology under inverted microscope and SEM, the number of live cells (CFU/ml), the Reduction Factor and the Inactivation Rate. The test was repeated three times.
After 15 min. of treatment a morphological alteration of the biofilm structure was observed. A significant reduction of the CFU/ml was observed with increasing treatment time. In particular, after 15 minutes the Inactivation Rate reached 99.9% -100%.
The present study was carried out within a research field that is still evolving, also with reference to the various plasma sources, nevertheless these preliminary results are promising and suggest a need for further investigation.

[1] Gow Neil A. R,. Van de Veerdonk Frank L., Brown Alistair J. P., Netea Mihai G. (2011): Candida albicans morphogenesis and host defence: discriminating invasion from colonization. Nature Reviews Microbiology, 10(2): 112–122
[2] Pereira R, Dos Santos Fontenelle RO, de Brito EHS, de Morais SM. Biofilm of Candida albicans: formation, regulation and resistance. J Appl Microbiol. 2021 Jul;131(1):11-22. doi: 10.1111/jam.14949. Epub 2020 Dec 9. PMID: 33249681.
[3] Kuhn D.M., Chandra J., Mukherjee P.K., Ghannoum M.A. (2002): Comparison of biofilms formed by Candida albicans and Candida parapsilosis on bioprosthetic surfaces. Infection and Immunity, 70(2):878-88
[4] Handorf O., Welhe T., Bekeshus S., Graf A.C., Schnabel U., Riedel K., Ehlbeck J. (2018): Nonthermal plasma jet treatment negatively affects the viability and structure of Candida albicans SC5314 biofilms. Applied Environmental Microbiology, 84: e 01163-18.

76° CONVEGNO SISVET
BARI, 21-22-23 GIUGNO 2023

FIRST DESCRIPTION BY SCANNING ELECTRON-MICROSCOPY OF SIMONDSIA *PARADOXA* INFESTING NEBRODI BLACK PIGS IN SICILY

Di Marco Lo Presti V. (1), Ippolito D. (1), Lembo F. (1), Calogero R. (1), Biondo M. (1), Romeo G. (1), Pruiti Ciarello F. (2), Fiasconaro M. (1)

(1) Istituto Zooprofilattico Sperimentale della Sicilia, Area Territoriale Barcellona Pozzo di Gotto, Viale S. Andrea 96, 98051, Barcellona Pozzo di Gotto (ME)
(2) Azienda Sanitaria Provinciale di Ragusa, Sanità animale

Corresponding Author: **Francesca Lembo** - francesca.lembo@tiscali.it

In "non-industrial" swine breeding system where non-specific parasitic prophylaxis is often used it is possible to find ancient and neglected species of parasites. Here we report the first scanning electron-microscopy (SEM) of *Simondsia paradoxa* (*Nematoda, Spirocercidae*). It is a gastric nematode of suids discovered in the 1800s and today there are rare reports with different morphological descriptions depending on the observation method employed. During an abattoir survey, the stomachs of a typical native breed of Sicilian pig "The Nebrodi Black pig" reared in free-range system in the Nebrodi park, were collected. Gross gastric lesions were recorded, samples were taken and fixed in 10% formalin for histological investigations. The collected parasites were fixed in 70% ethanol for morphological identification by light microscopy (LM) and scanning electron-microscopy (SEM). Microscopic observation by LM was performed after clarification with lactophenol. Concurrently, fixation in 2.5% glutaraldehyde and dehydration in ethanol. The parasites were then dried in liquid C02 in a critical point apparatus and coated with gold in an ion-coating sputter coater. The observation was performed with an EM30-COXEM Scanning Electron microscope and photographs were taken with a digital acquisition system. The parasitized stomachs showed moderate to severe catarrhal inflammation and chronic granulomas in the mucosa and submucosa. Due to the marked sexual dysmorphism of *S.paradoxa* it is possible to clearly distinguish the male from the female. The front part is similar in both sexes, with a cylindrical shape, a transversely striated surface and two simple lateral wings. The caudal end of the male is coiled and has papillae, the number of which presents discrepancies in the few studies carried out. In the central part, the female has an enormous spherical growth of a globular shape, with a cuticula covered by tiny spots. This part with a characteristic alveolar shape houses the extremely developed uterus, from which the uterine branches branch off, containing the ellipsoidal eggs, which continue with the uterine horns. Few reports are present in the literature about *S.paradoxa* in in feral pigs or pigs bred in a free-range system. However, to the best of our knowledge this is the first descriptive SEM report of this ancient and perhaps neglected parasite. Further studies are needed to clarify the morphology, the role and the life cycle (arthropod-borne) in free-range suids.

[1] Oshmarin PG, Oshmarin AP, Ryzhikov KM and Khrustalev AV (1983) Structure and aspects of the nutrition of female Simondsia paradoxa (Nematoda, Spirurata), a gastric parasite of pigs. Paraziologiya 17:448.
[2] Spratt, D. M., & Pavlov, P. M. (1996). Simondsia paradoxa: newly recorded nematode parasite from feral pigs in Australia. Australian veterinary journal, 74(5), 394–395. https://doi.org/10.1111/j.1751-0813.1996.tb15454.x

POSTER

AIPVET

1

Comparison between hot spot fields and whole slide digital image analysis methods for the evaluation of immune cell infiltrates in a murine model of caerulein-induced acute pancreatitis

Bertola L., Canesi S., Pepe G., Dolce A., Scanziani E., Vegeto E., Ressel L., Recordati C.

2

Monoclonal antibody Cetuximab: a new promising therapy against invasive feline oral squamous cell carcinoma

Altamura G., Matrone A., Borzacchiello G.

3

Effects of ketoconazole and diethylstilbestrol on the uterus of 90-day-old rats after perinatal oral exposure: histological and immunohistochemical evaluation

Canesi S., Cappelleri A., Kossa M., Johansson H.K.L., Boberg J., Scanziani E., Recordati C.

4

Gross and histopathological findings in European hedgehogs in Piedmont region: preliminary data

Prandi I., Colombino E., Perotti M., Cardello M., Ippolito D., Vacchetta M., Bertolotti L., Tomassone L., Zanet S., Mauthe Von Degerfeld M., Quaranta G., **Capucchio M.T.**

5

Bilateral ovarian hamartoma in an aborted foal

Cuccato M., Bertuglia A., Bollo E., Scaglione F.E.

6

Massive infestation by Taenia crassiceps in a captive Lemur catta: first report in Italy

Cuccato M., Rubiola S., Rossi L.,Piga S., Scaglione F.E.

7

Relation between post-mortem interval and RNA degradation in tissues of decomposed cats

Divari S., Pregel P., Ricci E., Ingriccini S., Ottonello E., Nicoletti A., Ressel L., Scaglione F.E.

8

Histiocytic sarcoma in a captive hybrid orangutan (Pongo sp.): morphological and immunohistochemical features

Galietta V., Fonti N., Cocumelli C., Raso C., Di Cerbo P., Parisi F., Bovi E., Parmigiani R., Friedrich K. G., Eleni C.

9

T-Cell Lymphoma in a roe deer (Capreolus capreolus): a case report

Ranucci A., Lepri E., Gobbi M., Manuali E., de Ferrari P., Ottone E., Brustenga L., Mandara M.T., D'Avino N.

10

Scoring ear lesions in slaughtered pigs: preliminary investigation

Sciota D., Rosamilia A., **Marruchella G**.

11

Cecal infarction in a Holstein Friesian calf

Spagnolo M., Negroni N., Marruchella G.

12

Blood exo-miRNAs in Bubaline alphaherpesvirus 1 affected Water buffaloes

Zamboni C., Dadi Y., Lecchi C., Ceciliani F., Calogero R., Arigoni M., Cappelli G., Balestrieri A., Grassi C.,

Vecchio R., Galiero G., De Carlo E., Petrini S., **Martucciello A.**

13

Optimization of a canine osteosarcoma cell model for FGFRs translational pharmacology

Massimini M., Romanucci M., Alvarez C.E., De Maria R., Della Salda L.

14

Gross lesions and histological changes in an accidentally electrocuted cat

Cocco A., **Merola C**., Cantelmi M.C., Tieri E.E., Badagliacca P., D'Innocenzo V., Martino M., Rulli M., Tinelli A., Averaimo D., Defourny S.V.P.,Petrini A.,Di Teodoro G.

15

Granulomatous hepatitis in rainbow trout (Oncorhynchus mykiss) associated with the discovery of an unrecognized nidovirus

Orioles M., Eldar A., Karniely S., Berkowitz A., Salinas I., Volpatti D., Beraldo P., Galeotti M.

16

InfoFaunaFVG: a novel progressive web application for wildlife surveillance

Tomè P., Pesaro S., **Orioles M**., Pascotto E., Cadamuro A., Galeotti M.

17

Erythrocyte sedimentation rate (ESR) in dogs: frequency in routine clinical practice and possible pathogenic mechanisms

Paltrinieri S., Ferrari R., Tagliasacchi F., Diamanti D., Scavone D.

18

Hyaline glomerulopathy in mice: a histochemical and immunohistochemical study

Recchia M., Sabbioni L., **Canesi S.**, Busnelli M., Colombo A., Franchi E., Recordati C., Scanziani E.

19

Calprotectin expression in canine hepatoid gland tumors and correlation with beta-catenin expression and macrophage cell infiltrate

Rifici C., Napoli E., Di Giorgio S., Giambrone G., Marino G., Mazzullo G., Sfacteria A.

20

Multi bacterial infections and pre-weaning piglet mortality in a Calabrian farm

Rifici C., Attili A.R., Alessi V.E.,Puleo V., Scaramozzino C., Mannarino C., Foti F., Sfacteria A., Mazzullo G.

21

Extratesticular anaplastic carcinoma in a Mexican kingsnake (*Lampropeltis Mexicana*)

Rifici C., Spadola F., Villa A., Minniti S., Giambrone G., Rossi G., Mazzullo G.

22

Pathological findings in condemned pig carcasses: a large scale survey in a high-throughput Italian slaughterhouse

Rosamilia A., Capezzuto S., Base G., Cirasola M.V., Benedetti S., Pipistrelli M.V., Perri M., **Marruchella G.**

23

Bacterial investigation and somatic cell count cut off value in ewe subclinical mastitis

Montagnani M., Gasparoni E., Vichi F., Santini A., Pietrini R., Pacini M., Ciofi L., Galli T**., Saralli G**., Fichi G.

24

Polysaccharide storage myopathy1 occurence in specific equine populations: preliminary results

Rosone F., **Ricci I.**, Linardi B., Minniti C.A., Carvelli A., Manna G., Tilesi E., Conti R., Marcario G., Zini M., Tofani S., Scicluna M.T.

25

Anatomopathological investigations of non-experimentally induced seminomas in adult zebrafish

Tinelli A., Pugliese N., Leone R., Crescenzo G., Zizzadoro C., Perugini M., Amorena M., Caioni G., Merola C.

26

Effects of local anaesthetics on equine tenocytes and protective effects of Platelet-Rich Plasma (PRP)

Tognoloni A., Pellegrini M., Cagiola M., Di Salvo A., Sforna M., Nannarone S., Beccati F., Chiaradia E.

27

MiRNA profiles in dogs affected with Splenic Angiosarcoma

Zamboni C., Avallone G., Roccabianca P., De Matos L.G., Dadi Y., Valenti P., Ceciliani F., Lecchi C.

28

Cytological recognition of keratinization patterns assists the preliminary diagnosis of canine hair follicle tumors and tumor like-lesions: assessment of diagnostic accuracy

Zamboni C., Ghisleni G., Caniatti M., Pirvu A.M., Dell'Aere S., Roccabianca P.

AISMEVEM

29

A mysterious 1826 manuscript on equine and bovine anatomy and medicine

Farinelli A., Bo P., Zaghini A., **Grandis A.**

30

Historia magistra vitae. The meaning of a History of Veterinary Medicine course in a university study plan.

Associazione Italiana di Storia della Medicina Veterinaria e della Mascalcia (A.I.S.Me.Ve.M.) – **Grandis A.**

AIVI

31

Antimicrobial susceptibility and prevalence of Salmonella spp. and commensal Escherichia spp. isolated from hunted wild boars and farmed pigs in Umbria

Di Giacinto G., Musa L., Ranucci D., Cai D., Toppi V., Spata N., Casagrande Proietti P.

32

The odor of Bolinus brandaris ed Hexaplex trunculus after harvesting: is it a marker of freshness?

Nalbone L., Cincotta F., Condurso C., Giarratana F., Panebianco A.

33

NIR Spectroscopy and Multivariate Data Analysis to Identify Illicit Conservation Treatments in Fresh Fish

Pennisi F., Esposito G., Benedetto A., Pezzolato M., Bozzetta E., Brizio P.

34

Buffalo milk cheeses produced with different coagulant: results of a preliminary study.

Campagna M.C., Proietti A., Briganti P., D'Amici L., Manocchio A., Matterazzo A., Zottola T., **Saralli G**.

35

Validation of a Real-Time PCR for the detection of fish allergen in food samples

Cau S., Daga C., **Spanu C**., Giagnoni L., Pinna F., Soro B., Tedde T., Salza S., Melillo R., Piras G., Virgilio S., Vodret B., Mudadu A.G.

36

The use of DCC to identify intra-mammary infections in Sarda dairy sheep

Spanu C., Acciaro M., Decandia M., Cabiddu A., Molle G., Comunian R., Dupré I., Caredda M., Giovanetti V.

37

Comparison of legal provisions for veterinary drug residues in dairy products in Italy and Armenia

Visciano P., Haykanush A.

38

Assessment of dietary exposure to ochratoxin A related to its concentrations in different foods

Annunziata L., Campana G., De Massis M.R., Aloia R., Scortichini G., **Visciano P.**

AMV

39

Tertiary Lymphoid Tissue in the lung: a single-cell characterization in young and aged mice

Ciccimarra R., Ragionieri L., Zoboli M., Gazza F., Cacchioli A., Stellari F., Ravanetti F.

40

Effect of oleuropein on induced senescence in canine skeletal muscle cells

Polacchini G., **Colitti M**.

41

Investigation on the presence and amounts of microtubule-associated proteins in rat glioblastoma C6 cells.

Lepore G., Succu S., Zedda M., Farina V., **Gadau S.D.**

42

Brain aging in cultured sheep cortical neurons

Gadau S., Farina V., Zedda M., Succu S., **Lepore G**.

43

Glucoerucin diet supplementation determines adipose tissue histological changes in mice

Lazzarini G., Flori L., Pirone A., Lenzi C., Testai L., Calderone V., Miragliotta V.

44

Anterior Digestive Tract of Adult Nile Tilapia Oreochromis niloticus: morphological characterization

Scocco P., Palladino A., De Felice E., Attanasio C., Barone C.M.A., Crasto A., D'Angelo L., Giaquinto D., Lambiase C., Serrapica F., Maruccio L.

45

Osteogenesis dynamics during early development in the ovine species

Succu S., Contu E., Bebbere D., Gadau S.D., Falchi L., Nieddu S.M., Ledda S.

ANIV

46

Evaluation of the presence of a urinary bladder bacterial community in cats with feline idiopathic cystitis (FIC) using culture-dependent and sequencing methods

Urbani L., Franzo G., Bano L., Segatore S., Rizzardi A., Cordioli B., Cornaggia M., Terrusi A., Dondi F., Balboni A., Battilani M.

47

PRRS viral detection versus resistance genetic profiles in pig livestock of Central Italy

Torricelli M., Fratto A., Ciullo M., Sebastiani C., Arcangeli C., Felici A., Giovannini S., Sarti F.M., Sensi M., **Biagetti M.**

48

Mastitis outbreak in a dairy sheep farm caused by Streptococcus canis.

Brundu D., Ghisu S., Antonietta L., Bassu M., Soddu M., Dore S., Bandino E.

49

Cat vaccination & antibody titration: a standard of care

Dall'Ara Paola

50

Project TECHARE: Italian pilot on dairy sheep to test innovative technologies for welfare monitoring

Decandia M., Giovanetti V., Acciaro M., Manca C., Molle G., **Spanu C**., Martini F., Solinas A., Morgan-Davies C.

51

Recombinase polymerase amplification for the detection of equine herpesvirus type 1

Ferrara G., Iovane V., Rossi A., Pagnini U., Montagnaro S.

52

Preliminary data on the discrimination of bacillus anthracis vaccine strains from virulent ones by ir biotyper

Galante D., Serrecchia L., Rondinone V., Pace L., Fasanella A., Farina D., Aceti A., Iatarola M., Tolve F., Poppa E., Manzulli V.

53

First detection of swinepox virus in wild boar in Italy: gross and histopathologic findings

Lisa G., Varello K., **Listorti V**., Paletto S., Wolfsgruber L., Zoccola R., Montemurro V., Messana E., Bozzetta E., Acutis P., Masoero L., Razzuoli E.

54

Identification and antimicrobial resistance of bacteria from the wounds of stray dogs and cats before and after sterile physiological solution washing.

Nocera F.P., Iovane V., Masullo A., Pizzano F., Di Palma C., Cappiello S., Pompameo M., Lamagna B., De Martino L.

55

Carriage of multidrug-resistant Gram-positive and Gram-negative bacteria in healthy stray dogs and cats.

Masullo A., Pizzano F., Di Palma C., Cappiello S., Pompameo M., Lamagna B., De Martino L., **Nocera F.P.**

56

Susceptibility of monkey-derived, hamster-derived, porcine-derived continuous cell lines to ASFV strains of diverse virulence

Franzoni G., Mura L., Zinellu S., Bazzardi R., Dei Giudici S., **Oggiano A.**

57

Genotyping of Paslahepevirus balayani (Hepatitis E virus) spread in wild boars in the Emilia Romagna region, Italy

Ferretti F., Corradini C., **Preziuso S.**

58

The use of DCC to identify intra-mammary infections in Sarda dairy sheep

Spanu C., Acciaro M., Decandia M., Cabiddu A., Molle G., Comunian R., Duprè I., Caredda M., Giovanetti V.

59

IR-Biotyper and biomolecular methods: a new protocol for rapid characterization of Streptococcus suis serotypes

Zoccola R., Marra C., Goria M., Zoppi S.

ARNA

60

In vitro effect of two nutraceutical products on the modulation of canine intestinal microbiota and production of SCFAs

Belà B., **Pignataro G.,** Fusaro I., Crisi P.E. Gramenzi A.

61

A case study on the utilization of Instagram as a powerful learning and disseminating tool

Cavallini D., Muca E., Buonaiuto G., Raspa F., Valle E., Ghiaccio F., Federiconi A., Silvestrelli S., Vettori J., Colleluori R., Formigoni A.

62

Food safety of products of animal origin: Toxic and Potentially Toxic Mineral Elements of Edible Gastropods Land Snails (Mediterranean Escargot)

De Maria L., Nava V., Vadalà R., Cicero N.

63

Effect of the dietary inclusion of former food products on subcutaneous adipose tissue and on serum metabolites in post-weaning piglets

Manoni M., Luciano A., Mazzoleni S., Ottoboni M., Tretola M., Pinotti L.

RNIV

64

Cytokines and immunoglobulin profile after melatonin treatment to pregnant ewes for coccidiosis control in their offspring

Ciliberti M.G., Bouroutzika E., Valasi I., Saratsis T., SaratsisAlbenzio M., della Malva A., di Corcia M., Santillo A., Marino R., Sevi A., Caroprese M.

66

Genetic variation of the ORFV VIR gene in the islands of Sardinia and Cuba

Coradduzza E., Scarpa F., Cacciotto C., Fiori M.S., Rodriguez V.Y., De Pascale A.M., Azzena I., Casu M., Pintus D., Ligios C., Scagliarini A., Sanna D., Puggioni G.

67

Evaluation of cell-mediate immune response in water buffaloes immunized with different marker vaccines and subsequently experimentally infected with Bubaline alphaherpesvirus 1

Grandoni F., De Matteis G., Napolitano F., Signorelli F., Cappelli G., Balestrieri A., Grassi C., De Donato I., Donniacuo A., Galiero G., De Carlo E., Petrini S., **Martucciello A.**

68

Targeting Toll-like Receptor 2: polarization of porcine, equine, ovine monocyte-derived macrophages by three diverse synthetic diacylated lipopeptide

Franzoni G., Dei Giudici S., Dell'Anno F., Anfossi A.G., Mura L.,De Ciucis C.G., Fruscione F., Zinellu S., Columbano N., Oggiano A., Chessa B. **Razzuoli E.**

69-b

Biochemical and inflammatory profiles in lambs subject to two different tail docking techniques

Roccaro M., Pilo G.A., Peli A., Fresi S., **Tedde G**., Loi F.G., Franzoni G., Carta G., Secchi L., Nicolussi P.

SICLIMVET

67- b

Quadricuspid aortic valve in a 16-year-old Quarter horse: clinical and echocardiographic findings

Caivano D., Cicogna M., Orvieto S., Spitale D., Porciello F.

68 -b

Clinical findings and follow-up of suspected dysmyelinogenesis in a cane corso pup

Canal S., Rosto M., Del Signore F., Paolini A., Tamburro R., De Bonis A., Vignoli M.

69

The gastrointestinal stasis in dwarf rabbit

Capurso O.V.

70

Point Shear-Wave Elastography of normal canine spleen by linear and convex transducers: preliminary results

Caspanello T., Masucci M., De Majo M., Sinagra L., Zappone V., Santoro S., Iannelli N.M.

71

Unusual presentation of tricuspid valve endocarditis in a lamb: clinical, echocardiographic and postmortem findings

Cicogna M., Crociati M., Lepri E., Passamonti F., Sylla L., Barbetta A., Porciello F., Caivano D.

72

Correlation between taurine serum concentrations and echocardiographic features of systolic function in Golden retrievers

Ghilardi S., Bagardi M., Zanchi F., Polli M., Minozzi G., Faverzani S., Locatelli C., Fusi E., Brambilla P.G.

73

First isolation of asinine γ???-herpesvirus-5 (AsHV-5) in a donkey with pulmonary fibrosis in northern Italy

Imposimato I., Muscatello L., **Ellero N**., Lelli D., Mira F., Sarli G., Freccero F.

74

A case of hilar bronchial carcinoma in a dog

Maggi G., Tessadori M., Porciello F., Perrucca A.L., Caivano D., Marchesi M.C.

75

Medical management and positive outcome after prolonged recumbency in a case of Equid alphaherpesvirus-1 myeloencephalopathy

Mannini A., **Ellero N**., Imposimato I., Balboni A., Battilani M., Gialletti R., Freccero F.

76

Effect of first long term training on platelet aggregation, platelet function and nitric oxid in Thoroughbreds

Miglio A., Falcinelli E., Cappelli K., Mecocci S., Mezzasoma A.M., Gresele P., Antognoni M.T.

77

Urinary incontinence in spayed female dogs: a review

Rosace F., Caspanello T., Marino F., Iannelli D., Iannelli N.M., Masucci M.

78

Terapia multimodale in un mastocitoma metastatico

Sebastiani V.

79

Echocardiographic assessment of left ventricular function in the late pregnant mare: a pilot study

Timbò D'el Rey Dantas F., **Perina F**., Menchetti L., Castagnetti C., Mariella J., Cristofori C., Cipone M., Freccero F.

80

Serum protein electrophoresis in dogs with dirofilariasis: A pilot study

Valente C., Poser H., Guglielmini C., Romito G., Dondi F., Fasoli S., Leoncini R., Contiero B., Berlanda M.

SICV

81

Life-threatening cranial tibial artery hemorrhage developing five days after tibial plateau leveling osteotomy in a dog

Bianchi A., Paolini A., Collivignarelli F., Vignoli M., De Bonis A., Canal S., Rosto M., del Signore F., Tamburro R.

82/130

Effects of low postoperative Total Plasma Protein on outcome and short-term complications in horses undergoing surgery for small intestinal lesions

Cerullo A., Giusto G., Dal Cero B., **Gandini M.**

83

Angular deviations of the carpus in foals: evaluation of two radiographic measurement protocols of the angles of deviation

De Iasio C., Montano C., de Chiara M., Greco A., Coluccia P., Costanza D., Lamagna B., Pasolini M.P.

84

Hernioplasty with autologous flap; surgical treatment of umbilical hernias in livestock animals

Neve V.C., Costa G., Interlandi C., Spadaro A., Macrì F., Iannelli N.M., Spadola F.

85

A non-surgical approach to prolonged constipation in an Indian python (Python molurus)

Ottino C., Scandone L., Serpieri M., Bonaffini G., Prandi I., Quaranta G., von Degerfeld M.M.

86

Efficacy of the use of Asudyn ® in the therapy of canine osteoarthritis. Prospective randomized blind pilot study. Preliminary results

Riccio V., Sassaroli S., Pennasilico L.,Di Bella C., Piccionello A.P.

87

Transverse abdominal plane (TAP) block in rabbit cadavers: anatomical description and measurements of injectate spread using one- and two-point approaches

Serino F., Pennasilico L., Palumbo Piccionello A., Galosi M., Angorini A., Di Bella C.

88

A case of oral health problem in a geriatric camel (Camelus batracianus) showing behavioral changes

De Benedictis G.M., Bono L., Fiore E., Tulini R., Zanusso F.

89

Severe abdominal distension associated with a paraovarian cyst in a guinea pig (Cavia porcellus)

Zanusso F., Rametta B., Ranieri V., Torrigiani F., Bellini L., Franzoso A., De Benedictis G.M.

131

Radiofrequency ablation in horses with chronic lameness pain: description of technique and case series

Amari M., Brioschi F.A., Ravasio G., Ferrari F. ,Rabbogliatti V.

SIFTVET

90

Transcriptional biomarkers related to illicit Testosterone esters administration in pigs

Benedetto A., Stastny K., Giaccio N., Biasibetti E., Arigoni M., Calogero R., **Pezzolato M.**, Martin F., Bozzetta E.

91

Statistical evaluation of suspected poisoning cases reported in Apulia and Basilicata between 2020 and 2022

Chirizzi D., Mercadante F., Catanzariti R., Carbotti G.

92

Exploring the potential of snail secretion filtrate as a green pharmacological approach for mastitis treatment in veterinary medicine

Franco G.A., Minutoli M., Crupi R., Britti D., Gugliandolo E., Cuzzocrea S.

93

Subchronic oral exposure to polystyrene microplastic causes inflammation and alteration of antioxidant defense system in the liver of gilthead seabream (Sparus aurata)

Del Piano F., Lama A., Addeo N.F., Almorth B.C., Ferrante M.C.

94

Subcellular tissue-specific response of Mytilus galloprovincialis to fluoroquinolone antibiotics: a multi-biomarker approach to evaluate potential ecotoxicity in marine ecosystems

Giannessi J., De Marchi L., Meucci V., Intorre L., Monni G., Pretti C.

95

Analysis of cannabinoid receptors expressions and their role in inflammation involved in canine breast cancer

Marino Y., Molinari F., Crupi R., Cuzzocrea S., Gugliandolo E., **Franco G.A.**

96

Bioactivity of phaeodactylum tricornutum extract against chronic mieloid leukemia: an in vitro pilot study

Longobardi C., Damiano S., Montagnaro S., Lauritano C., Ciarcia R.

97

Mineral elements in Xiphias gladius from Strait of Messina and risks related to human health

Messina L., Bruno F., Lo Turco V., Litrenta F., Peritore A., Gugliandolo E., Crupi R., Licata P.

SIRA

98

Expression of key enzymes remodeling m6A RNA methylation is altered during in vitro maturation of low competence sheep oocytes

Coseddu C., Nieddu S.M., Succu S., Ledda S., **Bebbero D.**

99

Evaluation of the APGAR Score after anesthesia for C-section with methadone and alfaxalone: preliminary results

Ciribè F., **Bucci R.**, Straccia L., Cudini A., Parrillo S., Robbe D., Carluccio A.

99-b

Innovative Surgical and Anaesthesiological Techniques for the orchiectomy in Mouflons (Ovis aries) in the Field

Cicirelli V., Carbonari A., Burgio M., Aiudi G.G., Giannini F., Rizzo A.

100

Surgical management of penile prolapse in a domestic pig: A Case Report

Ferrulli V., Boccardo A., Giussani E., Pravettoni D., Fusi J.

101

Study about treatment of septic puerperal metritis in mares

Fischetti L., Lanci A., **Perina F**., Castagnetti C., Mariella J.

102

Isolation of mesenchymal stromal cells from cryopreserved wharton's jelly in the horse: preliminary results

Iacono E., Pezzotta A., Gugole P.M., Lanci A., Merlo B.

103

Ascorbic acid and trans-resveratrol: effects on casa parameters when added to the semen freezing extender for of italian holstein bulls used in a commercial program

Bertuzzo F., Gerratana S., Merlo B., **Iacono E.**

132

Monitoring of behavioral, hormonal and vaginal cytology aspects following contraceptive treatment in a group of Lemur catta in a controlled environment

Ponzio P. Imberti E., Bertero A., Piga S., Isaia V., Macchi E.

SOFIVET

104

First investigation on colostrum quality in autochthonous goat breeds of northern Italy

Agradi S., González-Cabrera M., Menchetti L., Argüello A., Hernández-Castellano L.E., Castro N., Salomone-Caballero M., Poquet-Blat P.M., Curone G., Faustini M., Vigo D., Brecchia G.

105

Circadian variation in leucocytes and lymphocytes subpopulation blood horse: a preliminary study

Aragona F., Giannetto C., Arfuso F., Piccione G., Fazio F.

106

Gene expression of Pregnancy-associated glycoproteins-1 (PAG-1) and Interferon stimulated genes (ISGs) during early pregnancy in buffalo cows

Casano A.B., Menchetti L., Trabalza-Marinucci M., Riva F., De Matteis G., Brecchia G., Inglesi A., Rossi E., Signorelli F., Barile V.L., Barbato O.

107

A comparative study of single and group culture of cumulus-oocyte complexes from sheep early antral follicles

Ebrahimi M., Dattena M., Mara L., Pasciu V., Chessa F., Luciano A.M., Berlinguer F.

108

Thyroid and Cortisol Response to Olive Cake Inclusion in Growing Fattening Limousines' Diet

La Fauci D., Bionda A., Fazio E., Lopreiato V., Chiofalo V., Liotta L.

109

Gillnets bycatch affect population sex ratio in a North Sardinia Mediterranean Shag (Gulosus aristotelis desmarestii) population

Satta V., Pira A., Cherchi S., Nissardi S., Rotta A., Pirastu M., Mereu P., Zedda M., Bogliolo L., **Naitana S.**, Leoni G.G.

110

CAN FEATHERS BE USEFUL TO ANALYZE REPRODUCTIVE SUCCESS IN GRIFFON COLONIES?

Rebula G., Frongia G.N., Naitana S., **Pividori I.,** Peric T., Prandi A.

111

Cyclic AMP efflux through MRP4 stimulates capacitation and fertilizing ability of pig spermatozoa

Spinaci M., Blanco-Prieto O., Ortiz-Rodriguez J.M., Bucci D.

112

Autophagy effect on trophoblast cell functionality during early placenta development in sheep.

Viola I., Manenti I., Accornero P., Baratta M., Toschi P.

SOIPA

113

Essential oils from Calabrian botanical species for the control of the honeybee parasite Varroa destructor

Bava R., Castagna F., Palma E., Marrelli M., Conforti F., Musolino V., Carresi C., Lupia C., Ceniti C., Tilocca B., Roncada P., Britti D., Musella V.

114

The role of hunters in wildlife health surveillance: systematic review and meta-analysis of the scientific literature

Benatti F., Zanet S., Poncina M., Pasetto C., Chiari M., Sorrenti M., Ferroglio E.

115

High prevalence of Hymenolepis (Rodentolepis) nana in amateur breeding facilities of chinchillas (Chinchilla lanigera) and sugar gliders (Petaurus breviceps) from Italy

Brustenga L., Morganti G., Baldoni E., Deli G., Rigamonti G., Lucentini L., Diaferia M.

118

Outbreak of cryptosporidiosis in water buffalo calves (Bubalis bubalis)

Dini F.M., Serraino A., **Galuppi R.**, Poglayen G.

119

SEM characterization of red fox intestinal helminths

Tedesco P., Barbaro A., Luci V., Cantori A., Dini F.M., **Galuppi R.**

120

Development of a biosensor for the early diagnosis of Nosema spp.: a preliminary study.

Mogliotti P., Garrone A., Guasco C., Goria M., Zoccola R., Brusa F., Ghisellini P., Rando C., Garbati P., Eggenhoffner R.

121

Prevalence of Neospora caninum in bulk milk samples from dairy cattle herd in Ragusa area (Sicily).

Neve V.C., Salonia R., Antoci F., Cascone G., Salina F.

122

Urban Dermanyssus gallinae infestation in humans and the need of interactions between urban planners and public health operators

Puccini A., Barlaam A., Cafiero M.A., Giangaspero A.

123

Insights into diagnosis of liver and rumen flukes in cattle within the PREPARE4VBD EU project.

Maurelli M.P., Bosco A., Ciuca L., Vitiello P., Cringoli G., Rinaldi L.

124

BOVINE BESNOITIOSIS (Besnoitia besnoiti) IN A CATTLE FARM IN CENTRAL ITALY

Bruni G., Bosco A., D'Onofrio V., Eleni C., Iurescia M., Fichi G., Ragionieri G., Sorbara L., Maurelli M.P., Cringoli G., Rinaldi L., **Saralli G.**

125

Cryptosporidiosis in cattle dairy farms in Sardinia: trend of prevalence in suckling calves, weaned calves, lactating cows, dry cows and heifers

Scala A., Tamponi C., Stevanato L., Coghetto A., Carta C., Cavallo L., Porcu F., Ninnis F., Fahad A., Pentcheva P., Sini M.F., Varcasia A.

126

Human myiasis caused by Oestrus ovis: a questionnaire based survey on medical awareness and disease's management

Scala A., Tamponi C., Mignacca S., Carta C., Cavallo L., Porcu F., Nonnis F., Fahad A., Pentcheva P., Sini M.F., Coghetto A., Varcasia A.

127

Effects of gold compounds on Toxoplasma gondii: preliminary results of an in vitro study

Semeraro M., Boubaker G., Scaccaglia, M., Genchi M., Kramer L., Pelosi G., Bisceglie F., Vismarra A., Hemphill A.

128

Prevalence of anti-Dirofilaria immitis antibodies in cats from Italy

Semeraro M., Morchon R., Escolar I.R., Genchi M., Colombo L., Kramer L., Vismarra A.

129

Toxoplasma gondii 20.23: serological survey in Central Italy pig farms and novel insights for genetic resistance studies

Torricelli M., Fratt A., Sebastiani C., Ciullo M., Panzieri C., Costarelli S., Torina A., Biagetti M.