

**MACC** MEETING AND ART CRAFT CENTER

CAMERA DI COMMERCIO DI PISA  
Auditorium Rino Ricci  
Piazza Vittorio Emanuele II, 5  
56125 Pisa

**Comitato organizzatore**

Prof. Simone Bertini  
Prof.ssa Petra Cagnardi  
Prof. Roberto Ciarcia  
Prof. Mauro Dacasto  
Prof.ssa Flavia Girolami  
Prof.ssa Valentina Meucci  
Prof.ssa Claudia Zizzadoro

**Responsabile scientifico**

Prof.ssa Valentina Meucci

**Segreteria scientifica**

Prof.ssa Valentina Meucci  
Dott.ssa Lucia De Marchi

CONVEGNO  
**SIFTVet**

PISA 10/11 Aprile

**2025**



## Convegno SIFTVet 2025 10-11 Aprile Pisa

### 10 Aprile

- Ore 10.00 Registrazione partecipanti
- Ore 11.00 Saluti (Prof. Miragliotta e Prof. Bertini) e Apertura lavori
- 11.30-12.15 Farmaci in ambiente. Dr.ssa Ines Lacchetti (ISS Roma)
- 12.15-13.30 Comunicazioni orali - Moderatori: Prof.ssa Sara Damiano, Prof. Nicola Pugliese
- 13.30-14.30 Pranzo e visita poster
- 14.30-16.00 Comunicazioni orali - Moderatori: Prof. Alessandro Di Cerbo, Prof.ssa Mery Giantin
- 16.00-17.30 Assemblea Società
- Ore 17.30 Visita Piazza dei Miracoli
- Ore 20.00 Cena Ristorante La Pergoletta

### 11 Aprile

- Ore 09.00 Inizio attività scientifiche
- 9.00-09.45 Solfuro di Idrogeno: Dal Veleno alla Terapia Prof.ssa Valentina Citi (Università di Pisa)
- 09.45-11.30 Attività partecipativa su didattica innovativa - Prof. Francesco Di Iacovo (Università di Pisa)
- 11.30-12.00 Coffee break
- 12.00-13.30 Attività partecipativa su didattica innovativa: restituzione
- 13.30-14.30 Pranzo e visita poster
- 14.30-15.30 Attività società su presentazione progetti ricerca
- 15.30-16.45 Comunicazioni orali - Moderatori: Dott. Carmine Merola, Prof.ssa Cristina Vercelli
- Ore 17.00 Fine congresso e Premiazioni

### 12 Aprile

- Attività per chi rimane
- Trekking sui monti pisani alla scoperta di eremi e orchidee selvatiche



---

## ABSTRACT COMUNICAZIONI ORALI

**10 Aprile 12.15-13.30**

**Moderatori: Prof.ssa Sara Damiano - Prof. Nicola Pugliese**

---

C1\_DEXMEDETOMIDINE AND ALFAXALONE INTRAVENOUS CO-ADMINISTRATION FOR THE INDUCTION OF GENERAL ANAESTHESIA IN BITCHES UNDERGOING C-SECTION: IMPACT ON NEWBORN VIABILITY AND PHARMACOKINETICS

*Federica Di Cesare*

C2\_ASSESSING OXIDATIVE STRESS IN *PROCAMBARUS CLARKII* ACROSS POLLUTED ENVIRONMENTS

*Alicia Maria Carrillo Heredero*

C3\_SALINITY-DRIVEN ALTERATIONS IN TETRACYCLINE EXPOSED *MYTILUS GALLOPROVINCIALIS*: A METABOLOMICS PERSPECTIVE

*Alessio Lenzi*

C4\_ELECTROCHEMICAL DETECTION OF BISPHENOL A IN ZEBRAFISH USING AN ALGAE PAPER SENSOR

*Carmine Merola*

C5\_BEYOND THE TREATMENT: ENVIRONMENTAL RISKS AND TOXICITY OF ECTOPARASITICIDES USED IN PETS

*Lucia De Marchi*

---

**10 Aprile 14.30-16.00**

**Moderatori: Prof. Alessandro Di Cerbo - Prof.ssa Mery Giantin**

---

C6\_QUANTIFICATION OF PFAS IN CANINE SERUM AND FELINE PLASMA: A TOOL FOR EXPOSURE ASSESSMENT AND PRELIMINARY HEALTH MONITORING

*Anisa Bardhi*

C7\_EFFECTS OF *PASSIFLORA*, *WHITHANIA SOMNIFERA* AND *TARAXACUM OFFICINALE* ON MARKERS OF INFLAMMATION AND OXIDATIVE STRESS IN OLD DOGS

*Consiglia Longobardi*

C8\_COMPARATIVE SHORT-TERM TOXICITY OF OCHRATOXIN A, ZEARALENONE, AND FUMONISIN B1 IN BROILERS

*Matteo Cuccato*

C9\_HUMAN-ANIMAL LIFE SHARING: IS THERE ANY ANTIMICROBIAL RESISTANCE RISKS FOR PEOPLE? A STUDY IN A PET CLINIC IN NORTHWEST ITALY

*Michela Amadori*

C10\_TOXIC AND ESSENTIAL METALS IN HONEY SAMPLES OF DIFFERENT ORIGIN AND THEIR ANTIOXIDANT ACTIVITY

*Clara Naccari*

C11\_ESTABLISHING THE IPEC-J2 CELL LINE AS IN VITRO MODEL FOR OKADAIC ACID TOXICITY ASSESSMENT

*Hanane Maghrebi*

---

**11 Aprile 15.30-16.45**

**Moderatori: Dott. Carmine Merola – Prof.ssa Cristina Vercelli**

---

C12\_ FROM DOMESTIC TO ENVIRONMENTAL: THE COMBINED DANGER OF PFAS AND NEONICOTINOIDS AND THEIR CARDIOTOXIC EFFECTS ON FRESHWATER ORGANISMS

*Alessio Filippo Peritore*

C13\_ A PRELIMINARY STUDY OF THE IMPACT OF NSAIDS AND SAIDS ON GENE EXPRESSION IN BOVINE GRANULOSA CELLS: IMPLICATIONS FOR REPRODUCTIVE HEALTH

*Susanna Draghi*

C14\_ ASTAXANTHIN MITIGATES 5-FLUOROURACIL-INDUCED HEPATOTOXICITY AND OXIDATIVE STRESS IN MALE RATS

*Mario Nicotra*

C15\_ INTERACTIVE EFFECTS OF ENROFLOXACIN AND OCEAN WARMING ON THE HEALTH AND ENERGETIC BALANCE OF THE MEDITERRANEAN MUSSEL (*MYTILUS GALLOPROVINCIALIS*)

*Joanna Giannessi*

C16\_ ANTIMICROBIAL RESISTANCE IN THE YELLOW-LEGGED GULLS (*LARUS MICHAELLIS*) OF TARANTO: AN ANTHROPOGENIC PHARMACOLOGICAL FOOTPRINT IN THE ENVIRONMENT?

*Nicola Pugliese*

---

## ABSTRACT POSTER SESSION

---

P1\_ISOLATION OF STAPHYLOCOCCUS XYLOSUS FROM PRE-CLINICAL AND CLINICAL MASTITIS (MILK) AND EVALUATION OF ITS SUSCEPTIBILITY TO ERYTHROMYCIN AND PLANT-DERIVED ESSENTIAL OILS

*Cristian Piras*

P2\_WHICH ANTIBIOTICS ARE STILL EFFECTIVE TO TREAT URINARY TRACT INFECTIONS IN CANINE AND FELINE PATIENTS?

*Sara Crimi*

P3\_IDENTIFICATION OF MYCOTOXINS IN *TRIGONELLA FOENUM-GRÆCUM* AND RELATED CLINICAL SYMPTOMS IN RUMINANTS FROM SICILY

*Fabio Bruno*

P4\_ANTIBIOTICS AND HEAVY METALS DETECTION IN PET FOOD SAMPLES FROM THE ITALIAN MARKET

*Mario Nicotra*

P5\_BEHAVIORAL EFFECTS OF DIETARY CANNABIDIOL SUPPLEMENTATION IN RABBITS

*Valeria Iervolino*

---

## COMUNICAZIONI ORALI

## C1\_DEXMEDETOMIDINE AND ALFAXALONE INTRAVENOUS CO-ADMINISTRATION FOR THE INDUCTION OF GENERAL ANAESTHESIA IN BITCHES UNDERGOING C-SECTION: IMPACT ON NEWBORN VIABILITY AND PHARMACOKINETICS

*Federica Di Cesare (1), Susanna Draghi (1), Federica Alessandra Brioschi (1), Vanessa Rabbogliatti (1), Martina Amari (1), Giulia Sala (2), Roberto Villa (1), Giuliano Ravasio (1), Alessandro Pecile (1), Elisa Giussani (1), Petra Cagnardi (1)*

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

*(2) Dipartimento di Scienze Veterinarie, Università degli Studi di Pisa, Pisa, Italy*

Based on our dexmedetomidine (DEX) and alfaxalone (AFX) mass spectrometry quantification data in maternal serum and placental tissue, the present study aims to assess the impact of these drugs on pups' viability and to determine the pharmacokinetic profile of DEX and AFX following an IV co-administration for general anaesthesia induction in bitches undergoing cesarean section. At the Veterinary Teaching Hospital of the University of Milan, fourteen client-owned bitches underwent cesarean section. General anaesthesia was induced with an IV co-administration of DEX at 2 µg/kg and AFX at 1.2 mg/kg. DEX and AFX determinations in maternal serum and placental tissue were used to: a) assess the relationship between pups' Apgar score [1] and both drugs' concentrations in placental tissue and maternal serum ( $p < 0.05$  was considered statistically significant); b) analyze serum drug concentrations over time to define the pharmacokinetic profiles of both drugs in bitches, using a dedicated software (Phoenix WinNonLin V.8.3, Pharsight Corporation, USA). Among 68 pups born via C-section, 88.2% were vigorous at birth (Apgar scores 10–14). The mortality rate within 48 hours was 1.4% and unrelated to the anaesthetic protocol. Placental DEX and AFX concentrations were positively correlated and influenced by pups' extraction time ( $p < 0.05$ ), with AFX showing higher placental retention compared to other injectable anaesthetics [1]. Higher AFX placental concentrations were associated with increased neonatal body temperature, suggesting reduced sedation in the offspring ( $p < 0.05$ ). Maternal serum pharmacokinetic analysis revealed a rapid decline of DEX and AFX concentrations following administration. The half-life  $t_{1/2}$  resulted in  $47.36 \pm 30.61$  and  $55.82 \pm 33.06$  minutes for DEX and AFX, respectively. This study demonstrated the feasibility and safety of DEX and AFX IV co-administration for general anesthesia induction in bitches undergoing C-section, ensuring an elevated neonatal viability, with better clinical outcomes for the offspring than the other protocol previously tested by the same group of authors [1]. Further research is needed to determine the canine placental protective mechanisms involving DEX and AFX.

### References

[1] Groppetti et al. 2024. Maternal and neonatal wellbeing during elective C-section induced with a combination of propofol and dexmedetomidine: how effective is the placental barrier in dogs? *Theriogenology* 129, 90-98.

## C2\_ASSESSING OXIDATIVE STRESS IN PROCAMBARUS CLARKII ACROSS POLLUTED ENVIRONMENTS

*Alicia Maria Carrillo Heredero, Tiziano Iemmi, Giuseppina Basini, Roberto Ramoni, Stefano Grolli, Simona Bussolati, Simone Bertini*

*Dipartimento di Scienze Medico-Veterinarie, Università di Parma, Parma, Italy*

The red swamp crayfish (*Procambarus clarkii*, *P. clarkii*) is an invasive freshwater species known for its adaptability, rapid reproduction, and tolerance to extreme conditions, enabling it to outcompete native species and disrupt aquatic habitats [1]. Despite its ecological impact, *P. clarkii* serves as a valuable bioindicator for heavy metal pollution monitoring [2,3]. The present study assesses oxidative stress parameters in *P. clarkii* from three sites with varying pollution levels: a highly anthropized flatland area with presumed heavy contamination, a moderately polluted site in the hills, and a remote mountain habitat with minimal human impact. Oxidative stress biomarkers were analyzed across five body districts—gills, digestive system, gonads, caudal muscle, and hepatopancreas. Indicators included reactive oxygen metabolite derivatives (d-ROMs), ferric reducing ability of plasma (FRAP), superoxide anion (WST), nitric oxide (NO), lipid peroxidation (LPO), catalase (CAT), superoxide dismutase (SOD), malondialdehyde (MDA), and glutathione peroxidase (GPX), all normalized to protein content according to the bicinchoninic acid (BCA) protein assay. Our results highlight differences in oxidative stress responses across sites, likely influenced by metabolic activity, pollutant exposure, and environmental factors such as UV radiation and temperatures. Notably, the FRAP assay revealed high peaks in the gut of individuals from the mountain site, while a similar trend was observed for NO levels in their hepatopancreas. Conversely, MDA levels were highest in the hepatopancreas and gonads of flatland specimens. GPX showed the lowest values in the muscle and gastrointestinal tract of mountain specimens. These findings emphasize the impact of environmental contamination on oxidative stress and underscore the importance of tissue-specific analyses in ecotoxicological assessments. By comparing populations from habitats with different levels of anthropogenic pressure, this study suggests that monitoring oxidative status in *P. clarkii* could be a useful biomonitoring tool clarifying oxidative stress responses in invasive freshwater crustaceans. The study was supported by LIFE18NAT/IT/000806CLAW grant. Animal sampling procedures were authorized by the Parma University's Board for Animal Welfare.

### **References**

- [1] Guareschi et al. 2024. Some Like It Cold: Long-Term Assessment of a Near-Global Invader, *Ecol. Evol.* 14, e70760.
- [2] Heras et al. 2018. Analysis of lipid peroxidation in animal and plant tissues as field-based biomarker in Mediterranean irrigated agroecosystems. *J. Environ. Sci. Health B.* 53(9), 567–579.
- [3] Gago-Tinoco et al. 2014. Metabolic signatures associated with environmental pollution by metals in Doñana National Park using *P. clarkii* as bioindicator, *Environ. Sci. Pollut. Res.* 21,13315–13323.

### C3\_SALINITY-DRIVEN ALTERATIONS IN TETRACYCLINE EXPOSED *MYTILUS GALLOPROVINCIALIS*: A METABOLOMICS PERSPECTIVE

Alessio Lenzi (1), Marta Cunha (2), Constança Figueiredo (2), Carla Leite (2), Lucia De Marchi (1), Gianfranca Monni (1), Valentina Meucci (1), Tania Russo (3), Gianluca Polese (3), Amadeu M.V.M. Soares (1), Rosa Freitas (1)

(1) Department of Veterinary Science, University of Pisa, Pisa, Italy

(2) Department of Biology & CESAM, University of Aveiro, Aveiro, Portugal

(3) Department of Biology, University of Federico II, Naples, Italy

Over the past few decades, mussels have increasingly struggled with environmental changes driven by climate change. Among the key abiotic factors influencing their survival and adaptability, salinity fluctuations stand out as of primary importance [1]. Moreover, these organisms filter large volumes of water bioaccumulating pollutants, thereby facilitating the monitoring of their exposure to a variety of chemical contaminants that may cause shifts in their metabolism [2]. Tetracyclines (TCs) are among the most widely used antibiotics, thanks to their broad spectrum of activity, high quality, and affordability. These compounds play a crucial role in both human and animal healthcare, helping treat infections and serving as feed additives in livestock, poultry, and aquaculture [3]. Nowadays, liquid chromatography-tandem mass spectrometry (LC-MS/MS) is a powerful, high-throughput tool that provides a comprehensive understanding of the metabolic state of biological systems. In this study, we aimed to evaluate the effects of TC exposure on *M. galloprovincialis* and examine how salinity fluctuations (20, 30, and 40) influence organism's metabolism. Whole homogenized mussel tissue samples were extracted using a modified two-step biphasic extraction protocol. The supernatant containing polar metabolites was then analyzed for metabolomics. Approximately 300 compounds were identified using METLIN library database. Principal Component Analysis (PCA) revealed no significant metabolic shifts in mussels exposed to TC compared to control individuals across different salinities. On the other hand, when examining the effects of salinity independently of TC exposure, individuals subjected to a salinity of 20 exhibited a distinct metabolic shift. Specifically, mussels exposed to salinity 20 showed a downregulation of phosphatidylinositols, negatively charged phospholipids known to bind cations under higher salinity conditions. These results suggest an adaptation of mussels' lipid metabolism in response to chronic exposure to environmental stressors. Our findings may pave the way for studying the impact of external factors on mussel metabolism, while emphasizing the need for further research to better characterize the metabolome of these marine organisms.

#### References

- [1] Mohsenet et al. 2024 Sci Total Environ, vol. 928.
- [2] Cocci et al. 2017. Biomarkers, vol. 22
- [3] Antos et al. 2024. Chemosphere, vol. 353

## C4 ELECTROCHEMICAL DETECTION OF BISPENOL A IN ZEBRAFISH USING AN ALGAE PAPER SENSOR

*Carmine Merola (1), Filippo Silveri (2), Annalisa Scroccarello (2), Flavio Della Pelle (2), Christian Fantilli (1), Giulia Caioni (2), Michele Amorena (1), Dario Compagnone (2)*

*(1) Dipartimento di Medicina Veterinaria, Università di Teramo, Teramo, Italy*

*(2) Dipartimento di Bioscienze e Tecnologie Agro-alimentari e Ambientali, Università di Teramo, Italy*

This study developed a stencil-printed electrochemical sensor on Algae paper, a sustainable material derived from seaweed biomass waste. This sensor was employed to measure Bisphenol A (BPA) bioconcentration in zebrafish larvae and monitor BPA levels in their culture medium. The suitability of Algae paper as a stencil-printing substrate was evaluated using various graphitic inks as conductive elements. Zebrafish larvae were exposed to varying BPA concentrations (2.19, 4.38, 8.76, 17.52, 35.04  $\mu\text{M}$ ) following OECD guideline number 236. A static and semi-static waterborne exposure was conducted for 4 days. The Algae paper sensor demonstrated strong performance for detecting BPA in both the zebrafish culture medium and the larvae. The sensor showed good sensitivity (LOD = 48 nM and 57 nM, respectively) and a linear range (0.25 – 6  $\mu\text{M}$ ), with quantitative recoveries ranging from 95% to 113%. Notably, significantly higher BPA concentrations were detected in the zebrafish larvae, with values increasing exponentially in relation to the nominal concentrations in the medium. Specifically, the measured BPA levels were almost two orders of magnitude higher than the nominal concentrations, and the results showed satisfactory reproducibility. Sublethal concentrations of BPA were also identified and confirmed [1]. This finding indicates the bioconcentration of BPA in zebrafish larvae, confirmed by the calculated bioconcentration factor. The Algae paper sensor successfully quantified BPA levels in zebrafish larvae at the end of the exposure period (96 hours post-fertilization). The sensor effectively distinguished between undetectable levels and sublethal effects, showing significantly higher bioconcentration factors in cases of visible developmental delay. This study highlights the potential of Algae paper for fabricating efficient electrochemical sensors, offering a reliable tool for environmental and in vivo BPA monitoring in zebrafish.

### **References**

[1] Vremere et al. 2022. Oxysterols profiles in zebrafish (*Danio rerio*) embryos exposed to bisphenol A. 165, Food Chem. Toxicol.113166.

## C5\_BEYOND THE TREATMENT: ENVIRONMENTAL RISKS AND TOXICITY OF ECTOPARASITICIDES USED IN PETS

*Lucia De Marchi (1), Matteo Oliva (2), Carlo Pretti (1,2), Valentina Meucci (1), Marzia Vasarri (3), Donatella Degl'Innocenti (3), Ines Lacchetti (4), Laura Mancini (4), Mario Carere (4), Luca Maria Chiesa (5), Maria Nobile (5), Roberto Villa (5), Luigi Intorre (1)*

(1) Dipartimento di Scienze Veterinarie, Università di Pisa, Pisa, Italy

(2) CIBM, Livorno, Italy

(3) Dipartimento di Scienze Biomediche, Sperimentali e Cliniche 'Mario Serio', Università degli Studi di Firenze, Firenze, Italy

(4) Dipartimento Ambiente e Salute, Istituto Superiore di Sanità, Roma, Italy

(5) Dipartimento di Medicina Veterinaria e Scienze Animali, Università degli Studi di Milano, Milano, Italy

Ectoparasitocidal veterinary medicinal products (VMPs), such as fipronil (FIP) and imidacloprid (IMID), play a crucial role in pet health but pose potential environmental risks due to contamination from direct and indirect practices, including handwashing, bathing, and washing pet bedding [1]. Although the VICH GL6 guideline, developed by the International Cooperation Veterinary Medicinal Products (VICH), considers these risks negligible, the increasing pet population and evolving management practices challenge this assumption. To investigate these concerns, a suite of ecotoxicological tests, using environmental relevant concentrations (ERCs) of both FIP and IMID [1], was conducted on non-target species from both freshwater and marine environments. These included algal growth inhibition assays, toxicity tests on crustaceans, and biochemical analyses in *Mytilus galloprovincialis* gills and digestive glands (lipid peroxidation (LPO) levels, acetylcholinesterase (AChE) activity), and Fish Embryo Toxicity (FET) tests on *Danio rerio* larvae [2]. Additionally, *in vitro* cytotoxicity and cell viability analyses were performed on human keratinocyte cells (HaCaT) using a multi-biomarker approach [3], employing product concentrations specified in package inserts to simulate human-animal interaction. In marine water, sub-acute exposure (7 days) of the copepod *Acartia tonsa* revealed EC<sub>10</sub> and EC<sub>20</sub> values for FIP at 1.7 and 2.8 µg/L, and for IMID at 2.6 and 7.6 µg/L, respectively. Mussel gills exhibited significantly LPO induction starting at 0.5 µg/L for FIP and 1.5 µg/L for IMID. In freshwater, IMID at 2 µg/L altered *Daphnia magna* locomotor activity, while an LC<sub>50</sub> concentration of 1.57 µg/L caused spinal deformities, equilibrium loss, and depigmentation in *D. rerio* larvae. *In vitro* tests on HaCaT human keratinocyte cells revealed concentration-dependent cytotoxicity induced by both FIP and IMID, marked by increased reactive oxygen species (ROS) production, elevated lipid peroxidation (LPO) levels, and reduced cell viability (MTT assay). These results suggest that exposure to FIP and IMID could pose potential health risks to humans, especially those living in close proximity to animals. Obtained results underscore the need for further research on their long-term effects, as well as the implementation of stricter regulations and the establishment of safe exposure limits to protect both public health and ecosystems.

### References

- [1] EMA-Committee for Medicinal Products for Veterinary Use (CVMP), Reflection paper on the environmental risk assessment of ectoparasiticide veterinary medicinal products used in cats and dogs, EMA/CVMP/ERA/31905/2021. 2023.
- [2] Giannessi et al. 2023. Subcellular tissue-specific responses of *Mytilus galloprovincialis* to fluoroquinolone antibiotics. Environ. Toxicol. Pharmacol, 104, 104306.
- [3] Leri et al. 2023. The Protective Role of Oleuropein Aglycone against Pesticide-Induced Toxicity in a Human Keratinocytes Cell Model. Int. J. Mol. Sci, 24(19), 14553.

## C6\_QUANTIFICATION OF PFAS IN CANINE SERUM AND FELINE PLASMA: A TOOL FOR EXPOSURE ASSESSMENT AND PRELIMINARY HEALTH MONITORING

*Anisa Bardhi, Francesca Ferretti, Elena Serrantoni, Andrea Barbarossa*

*Department of Veterinary Medical Sciences, Alma Mater Studiorum - University of Bologna, Ozzano dell'Emilia, Bologna, Italy*

Per- and polyfluoroalkyl substances (PFAS) are persistent environmental contaminants with well-documented toxicological effects [1,2]. However, data on their bioaccumulation in companion animals are limited. Due to their close cohabitation with humans, dogs and cats serve as valuable sentinels for household contamination and associated health risks [3,4]. This study aimed to develop a single liquid chromatography-tandem mass spectrometry approach for quantifying eleven PFAS compounds (PFBA, PFPeA, PFHxA, PFHpA, PFOA, PFNA, PFDA, PFBS, PFHxS, PFOS, GenX) in canine serum and feline plasma, combining a simplicity, speed, and optimal analytical performance. The subsequent application of the method allowed to conduct a preliminary evaluation of PFAS concentrations in canine serum and their potential association with endocrine disorders. Protein precipitation was performed on 200 µL of serum/plasma, followed by evaporation of the supernatant under nitrogen stream, reconstitution in mobile phase and UHPLC-MS/MS analysis in negative electrospray ionization mode. The method was validated according to European Medicines Agency guidelines [5], demonstrating excellent selectivity, linearity ( $R^2 \geq 0.99$ ), precision (CV% <15%), accuracy (within  $\pm 15\%$ ), and high recovery (80.0-114.9%). Process efficiency (80.7-114.0%) was consistent across matrices, with minimal matrix effect and carry-over. The technique was successfully applied to samples from 23 healthy dogs and 4 healthy cats, as well as from 24 dogs with adrenal tumors, 10 dogs with hypothyroidism, and 21 dogs with pituitary-dependent hypercortisolism (PDH). PFAS concentrations in dog serum samples was in the 0.1-0.4 µg/L for range for PFBS, 0.1-0.7 µg/L for PFHxA, 0.1-3.8 µg/L for PFHxS, 0.1-8.1 µg/L for PFOA, 0.2-23.5 µg/L for PFOS, 0.1-3.1 µg/L for PFNA, and 0.1-4.6 µg/L for PFDA. In this preliminary investigation, a comparison of PFAS concentrations between healthy dogs and dogs with endocrine disorders revealed no significant correlation between PFAS levels and the presence of endocrine diseases. In feline plasma samples, measured concentrations were in the 0.2-0.5 µg/L range for PFBA, 0.3-1.1 µg/L for PFBS, 0.4-2.8 µg/L for PFHxS, 0.3-1.1 µg/L for PFOA, 1.1-1.6 µg/L for PFOS, 0.2-0.7 µg/L for PFNA, and 0.1-0.4 µg/L for PFDA. In conclusion, this method provides a reliable tool for large-scale PFAS monitoring in companion animals, offering valuable insights into their environmental exposure and the identification of potential correlations with endocrine disorders.

### **References**

[1] Fenton et al. 2021. Per- and Polyfluoroalkyl Substance Toxicity and Human Health Review: Current State of Knowledge and Strategies for Informing Future Research. Environ. Toxicol. Chem. 40, 606–630.

- [2] Dickman & Aga 2022. A Review of Recent Studies on Toxicity, Sequestration, and Degradation of per- and Polyfluoroalkyl Substances (PFAS). *J. Hazard. Mater.* 436, 129120.
- [3] Ruiz-Suárez et al. 2016. Are Pet Dogs Good Sentinels of Human Exposure to Environmental Polycyclic Aromatic Hydrocarbons, Organochlorine Pesticides and Polychlorinated Biphenyls? *J. Appl. Anim. Res.* 44(1), 135-145.
- [4] Andrews et al. 2023. Discussion. Has the Human Population Become a Sentinel for the Adverse Effects of PFAS Contamination on Wildlife Health and Endangered Species? *Sci. Total Environ.* 901, 165939.

## C7\_EFFECTS OF PASSIFLORA, WHITHANIA SOMNIFERA AND TARAXACUM OFFICINALE ON MARKERS OF INFLAMMATION AND OXIDATIVE STRESS IN OLD DOGS

*Consiglia Longobardi, Valeria Iervolino, Arianna Delle Ville, Salvatore Florio, Roberto Ciarcia, Sara Damiano*

*Dipartimento di Medicina Veterinaria e Produzioni Animali, Università di Napoli "Federico II", Napoli, Italy*

Aging in dogs is a complex process characterized by metabolic, immunological and neurological changes that can lead to chronic inflammation and oxidative stress. These factors contribute to cellular degeneration and the onset of age-related pathologies, such as osteoarthritis, cognitive decline and metabolic dysfunctions [1]. The use of phytotherapeutics with antioxidant and anti-inflammatory properties represents a promising approach to improve the quality of life in elderly dogs. The aim of this study was to investigate the beneficial effects of a mix of phytoextracts on geriatric dogs (n=7; weighing 50-80 Kg; 8-10 years old). In particular, the mix was constituted by *Passiflora* (10 mg/Kg b.w), *Whitania somnifera* (10 mg/Kg b.w) and *Taraxacum officinale* (15 mg/Kg b.w.) administered per os for 40 days in addition to their diet. The whole blood and serum have been collected at the beginning of the experimental phase (T0), after 20 days (T1) and at the end point of the experiment, i.e., 40 days (T2), and used for biochemical analysis, detection of interleukins by ELISA, and for malondialdehyde (MDA) and Total antioxidant capacity (TAC) assay. Results revealed that C-reactive protein (CRP) before treatment (T0) was significantly higher than T1 and T2 ( $p < 0.01$  and  $p < 0.001$ ). Moreover, a decrease in IL-6 and IL-10 cytokines at both T1 ( $p < 0.05$ ) and T2 ( $p < 0.01$ ) was observed, probably due to mitigation of chronic inflammation in the treated geriatric dogs. The addition into the diet of the abovementioned extracts promoted TAC and reduces lipid peroxidation in the sera of aged dogs, especially at T2 ( $p < 0.001$ ). Interestingly, liver parameters (ALT, ASP, Lipase), that resulted altered at T0, returned within physiological range at the endpoint of the treatment. The integration of these herbal medicines into dietary or therapeutic regimens could improve the quality of life, promoting the general well-being of old dogs.

### **References**

[1] Cohen et al. 2018. Aging across the tree of life: The importance of a comparative perspective for the use of animal models in aging. *Biochim. Biophys. Acta Mol. Basis Dis.* 1864(9 Pt A), 2680-2689.

## C8\_COMPARATIVE SHORT-TERM TOXICITY OF OCHRATOXIN A, ZEARALENONE, AND FUMONISIN B1 IN BROILERS

*Matteo Cuccato (1), Neenu Amminikutty (1), Achille Schiavone (1), Giuseppina Avantageggiato (2), Carlo Nebbia (1), Flavia Girolami (1)*

(1) Department of Veterinary Sciences, University of Turin, Torino, Italy

(2) Institute of Sciences of Food Production, National Research Council of Italy, Bari, Italy

The EU Commission Recommendation (EU) 2016/1319 sets guidance values for mycotoxin (MYCO) contamination in feed, including ochratoxin A (OTA), fumonisin B1 (FB1), and zearalenone (ZEA). OTA, produced by *Penicillium* and *Aspergillus* fungi, can induce hepatotoxic, nephrotoxic, and immunotoxic effects [1]. Differently, ZEA and FB1, produced by various *Fusarium spp.*, can result in reproductive toxicity binding to estrogen receptors, or lipid peroxidation by the disruption of ceramide and sphingolipid metabolism, respectively [2]. This study compared the adverse effects of a short-term dietary exposure to OTA, ZEA, and FB1 in broilers at concentrations slightly exceeding EU recommended limits to find common biomarkers of exposure. Thirty-two 18-day-old male broilers (ROSS 308), receiving water and standard basal diet (BD) *ad libitum*, were divided into 4 experimental groups (n=8 each) and dietary exposed for 10 days to: BD, OTA (0.3 mg/kg feed), FB1 (50 mg/kg feed) or ZEA (6 mg/kg feed) (approval number = 319508/2017-PR). At the end of the trial blood, liver and intestine were collected. The serum antioxidant capacity (SAC), and the following hepatic parameters were measured: malondialdehyde (MDA) levels, reduced glutathione content (GSH), and the activities of glutathione peroxidase (GPx), DT-diaphorase (DT-D) and total and mu-class glutathione S-Transferases (GSTs). The gene expression of antioxidant and biotransformation enzymes (Cytochrome P450 – CYP – and GSTs) and drug transporters (ATP-binding cassette – ABC) in liver and intestine samples was evaluated via qRT-PCR. All the investigated MYCO reduced the hepatic GSH content ( $p < 0.001$ ), and the activities of selenium-dependent GPx, DT-D, and total-GSTs ( $p < 0.001$ ), while increasing the MDA levels ( $p < 0.001$ ). Only OTA significantly reduced the SAC ( $p < 0.001$ ). The hepatic gene expression of nuclear factor erythroid 2-related factor 2 (Nrf2) ( $p < 0.001$ ), GPx1 ( $p = 0.003$ ), GSTA2 ( $p < 0.05$ ) and CYP2A6 ( $p = 0.001$ ) was significantly downregulated by OTA and FB1, while the mRNA levels of CYP3A4 were increased by ZEA and FB1 ( $p = 0.007$ ). In the liver, OTA downregulated ABCB1 ( $p = 0.031$ ), whereas ZEA and FB1 upregulated ABCC2 ( $p = 0.0059$ ). In the intestine, all the three MYCO upregulated ABCC2 ( $p = 0.01$ ) and downregulated Nrf2 ( $p = 0.01$ ). Only ZEA significantly downregulated the intestinal expression of superoxide dismutase (SOD1) ( $p = 0.007$ ), catalase (CAT) ( $p = 0.003$ ), CYP1A4 ( $p = 0.02$ ) and CYP1A5 ( $p = 0.001$ ). OTA, ZEA and FB1 induced oxidative stress in broilers to different extents even following a short exposure period. Noteworthy, ZEA showed stronger intestinal gene modulation.

### References

[1] Abdelrahman et al. 2022. Antioxidant and antiapoptotic effects of quercetin against ochratoxin A-induced nephrotoxicity in broiler chickens. *Environ Toxicol Pharmacol.* 96, 103982.

[2] Tardieu et al. 2021. Fumonisin and zearalenone fed at low levels can persist several days in the liver of turkeys and broiler chickens after exposure to the contaminated diet was stopped. *Food Chem Toxicol.* 148, 111968.

## C9\_HUMAN-ANIMAL LIFE SHARING: IS THERE ANY ANTIMICROBIAL RESISTANCE RISKS FOR PEOPLE? A STUDY IN A PET CLINIC IN NORTHWEST ITALY

*Michela Amadori (1,2), Marco Peradotto (3), Graziana Gambino (2), Antonio Curtoni (1,3), Sara Crimi (2), Chiara Caruso (4), Federica Pregnolato (4), Davide Danieli (1), Giovanni Re (2), Cristina Costa (1,3), Cristina Vercelli (2)*

(1) Dept. of Public Health and Pediatric Sciences, University of Turin, Turin, Italy

(2) Dept. of Veterinary Sciences, University of Turin, Turin, Italy

(3) University Hospital Città della Salute e della Scienza, Turin, IT (4) CVT -Centro Veterinario Torinese di Rivoli, Rivoli, Turin, Italy

The *One Health* approach is currently applied to face antimicrobial resistance (AMR): the close relationship between people and pets could be an underestimated risk for AMR transmission [1]. To descriptively analyze antimicrobial susceptibility testings of canine and feline patients in a pet hospital, from 2013 to 2023. 697 samples were collected: 430 positives in dogs and 129 in cats. The isolates derived from skin lesions - pyoderma and otitis (67.7%; 37.2%), cavitory liquids (5.1%; 16.3%), respiratory (2.8%; 14.7%) and urogenital tracts (4.4%; 0.8%), in dogs and cats respectively. In dogs the most isolated pathogens were: 117 *Staphylococcus (S.) intermedius/pseudintermedius*, 74 *Escherichia (E.) coli*, 43 *Pseudomonas (P.) aeruginosa*, 28 *Proteus mirabilis*, 22 *Enterococcus (E.) faecalis*, 17 *Klebsiella (K.) pneumoniae*, 14 *S. aureus*, 13 *Enterobacter cloacae*, 8 *Malassezia spp.* In feline patients, the most isolated bacteria were: 22 *E. coli*, 18 *S. intermedius/pseudintermedius*, 14 *Pasteurella multocida*, 13 *S. aureus*, 9 *P. aeruginosa*, 8 *Streptococcus canis*, 7 *K. pneumoniae*. Enterobacterales showed susceptibilities to ampicillin (31.2%; 22.6%) amoxicillin-clavulanic acid (53%; 45.9%), cefalexin (69.7%; 72.2%) and enrofloxacin (55.9%; 36.1%), in dogs and cats respectively. Imipenem remains highly susceptible: 92.9% in dogs and 97% in cats. Extended spectrum beta-lactamase (ESBL) were 22.3%. 38.8% of Stafilococci resulted resistant to oxacillin screening. The ceftiofur screening resulted positive for 46.4% of dogs and cats. *Pseudomonas spp.* showed lower susceptibility for enrofloxacin (21.4%; 50%, in dogs and cats, respectively) than for marbofloxacin (61.9%; 90%). The antibiotic administration in dogs and cats is mainly due to skin disease, urogenital and respiratory pathologies and treatments are repeated considering their long-life expectancy [1,2]. Penicillins, 1st generation cephalosporins and macrolides are commonly used both in human and veterinary medicine, increasing the risk of cross-resistance. The AIFA 2022 report highlighted 59% and 10% of antibiotics are used in dogs and cats, confirming the resistance prevalence of this study [3]. Monitoring the pathogens and their resistance pattern in pets appears to be a strategic measure to face AMR.

### References

[1] Vercelli et al. 2022. Implications of Veterinary Medicine in the comprehension and stewardship of antimicrobial resistance phenomenon. From the origin till nowadays. Vet. Anim. Sci. 16, 100249.

- [2] Pomba et al. 2017. Public health risk of antimicrobial resistance transfer from companion animals. *J. Antimicrob. Chemother.* 72(4), 957–968.
- [3] L'uso degli antibiotici in Italia. – Rapporto Nazionale anno 2022. Available at: <https://www.aifa.gov.it/-/l-uso-degli-antibiotici-in-italia-rapporto-nazionale-anno-2022> (Last access: 05.03.2025).

## C10\_TOXIC AND ESSENTIAL METALS IN HONEY SAMPLES OF DIFFERENT ORIGIN AND THEIR ANTIOXIDANT ACTIVITY

*Clara Naccari (1), Vincenzo Ferrantelli (2), Gaetano Cammilleri (2), Federica Lucia (2), Ernesto Palma (1,3)*

*(1) Dipartimento di Scienze della Salute, Università "Magna Græcia" di Catanzaro, Catanzaro, Italy*

*(2) Istituto Zooprofilattico Sperimentale della Sicilia "A. Mirri", Palermo, Palermo, Italy*

*(3) CIS - Centro Servizio Interdipartimentale - IRC-FSH "Centro di Ricerche Farmacologiche, Sicurezza degli alimenti e Salute ad alto contenuto tecnologico", Università "Magna Græcia" di Catanzaro, Catanzaro, Italy*

Honey is an apicultural product used in human and animal for its various beneficial properties (probiotic, anti-inflammatory, antioxidants, etc.) but, at the same time, is considered a bio-indicator of environmental pollution, due to the presence of contaminants, particularly toxic metals [1-3]. This study has been carried on fresh honey samples (n.=56) of different origin (Wildflower, Citrus, Acacia, Chestnut, Honeydew, Erika and Eucalyptus) collected in apiaries of Calabria (Italy). Samples, previously digested in acid mixture, were analysed in Inductively Coupled Plasma - Mass Spectrometry (ICP-MS) for the qualitative-quantitative determination of toxic metals and metalloids (Cd, Pb, As) and essential microelements (Cu, Zn, Se, Fe, Mn, Cr, Co, Mo). On the same samples, the antioxidant/free radical scavenging capacity was tested by redox-based assays: total polyphenols content (TPC), 2,2-diphenyl-1-picrylhydrazyl (DPPH) and ferric reducing/antioxidant power (FRAP). All analyses were repeated in triplicate for each sample; data were expressed as mean values  $\pm$  S.D. and considered significant with  $P < 0.05$  and  $P < 0.01$ . A comparative analysis has been conducted to correlate the toxic and essential metals content in honey samples and their antioxidant activity. The results obtained showed the presence of toxic metals and metalloids in all honey samples, with levels of Pb (range 0.081-0.127 mg/kg) in several samples higher than the Maximum Residual Level (MRL 0.001 mg/Kg) for honey and beekeeping products [4], and the abundance of essential metals (Mn>Zn>Fe>Cu>Se>Cr>Co>Mo). All samples showed a high TPC content (range: 131.92-53.27 mg Gallic Acid/100g honey) according to their origin and a good radical scavenger activity on DPPH radical, particularly those of Eucalyptus, Acacia and Wildflowers. The best antioxidant activity on FRAP was observed in honey of Honeydew and Wildflowers ( $9.39 \pm 1.29$  and  $5.22 \pm 0.81$  mmol Fe<sup>2+</sup>/kg, respectively), probably correlated to their high content of essential metals. The presence of toxic metals in honey samples is due to the environmental pollution of areas where apiaries are sited. The content of essential microelements, correlated to origin of honey, could influence its antioxidant/free radical scavenger activity.

### References

- [1] Ajibola et al. 2012. Nutr Metab (Lond). 9,61.
- [2] Vogt et al. 2021. Front Vet Sci. 7, 618301.
- [3] Naccari et al. 2014. J. Food Research 3(2).
- [4] EU Regulation 2023/915 Off. J. European Union L 119/103

## C11\_ESTABLISHING THE IPEC-J2 CELL LINE AS IN VITRO MODEL FOR OKADAIC ACID TOXICITY ASSESSMENT

*Hanane Maghrebi (1), Silvia Iori (1), Alessia Zoroaster (2), Sara Rinaldi (1), Elisabetta Notariello (1), Marianna Pauletto (1), Mery Giantin (1), Mauro Dacasto (1)*

*(1) Dipartimento di Biomedicina Comparata e Alimentazione, AURORA lab, Università degli Studi di Padova, Padova, Italy*

*(2) Dipartimento di Medicina animale, Produzioni e Salute, Università degli Studi di Padova, Padova, Italy*

Okadaic acid (OA), a prevalent marine biotoxin known to accumulate in shellfish, is the leading cause of Diarrhetic Shellfish Poisoning (DSP), resulting in severe gastrointestinal distress [1]. The present study assessed the suitability of the porcine intestinal epithelial cell line (IPEC-J2) as an in vitro model for studying OA-induced toxicity, with a focus on constitutive expression of target genes, barrier integrity and cytotoxic effects. IPEC-J2 cells were cultured for 46 days post-seeding on 12-well inserts at two different cells densities ( $1.25 \times 10^5$  and  $2.00 \times 10^5$  cells), and the monolayer integrity was evaluated via trans-epithelial electrical resistance (TEER) measurements. The constitutive expression of genes coding for tight junction proteins (ZO-1, OCLN and CLDN1), mucins (MUC1 and MUC2), efflux transporters (ABCG2 and ABCB1), and drug metabolizing enzymes (CYP3A) was analysed 21 days post-seeding. OA cytotoxicity (IC<sub>50</sub>) at 24h and 48h was assessed using Alamar Blue (AB) and Neutral Red Uptake (NRU) assays. TEER measurements confirmed optimal monolayer integrity at 21 days post-seeding, with resistance stabilizing at  $5,500 \Omega \cdot \text{cm}^2$ . Gene expression analysis showed a progressive functional cell differentiation over 21 days, with a significant time-dependent upregulation of ZO-1, CLDN1, MUC1, MUC2, ABCG2, and ABCB1. Moreover, CYP3A expression increased, reflecting an improved metabolic capacity. Cytotoxicity assays showed a time-dependent increase in OA toxicity, with IC<sub>50</sub> values decreasing from 115.9 nM (24h) to 47.11 nM (48h) in AB assay, and from 77.87 nM (24h) to 36.74 nM (48h) in NRU assay. The lower IC<sub>50</sub> values in the NRU assay indicate that OA preferentially affects the cell cycle rather than overall metabolic activity. Our experiments demonstrated a significant OA-induced toxicity in the IPEC-J2 cells, validating IPEC-J2 cells as a robust in vitro model for studying the marine toxin toxicity, with optimal morphological and functional differentiation reached at 21 days of culture. Future work will compare OA toxicity mechanisms in IPEC-J2 and human Caco-2 cells to assess its potential impact on human health. Study funded by PRIN 2022 (Grant number 2022MYJX94) and University of Padua (PhD grant) to M.D. and H.M., respectively.

### References

[1] EFSA, 2008. Scientific opinion of the panel on contaminants in the food chain on a request from the European commission on marine biotoxins in shellfish - okadaic acid and analogue, EFSA J. 589,1–62.

## C12\_FROM DOMESTIC TO ENVIRONMENTAL: THE COMBINED DANGER OF PFAS AND NEONICOTINOIDS AND THEIR CARDIOTOXIC EFFECTS ON FRESHWATER ORGANISMS

*A.F. Peritore (1), S. Natale (2), F. Capparucci (2), R. Crupi (1), D. Britti (3)*

*(1) Department of Veterinary Science, University of Messina, Italy*

*(2) Department of Chiobiofaram, University of Messina, Italy*

*(3) Department of Health Sciences, University of Catanzaro, Italy*

Perfluorooctanesulfonate (PFOS) is a widely used chemicals that accumulate in living things and the environment, especially aquatic [1]. Different substances known as forever chemicals are hardly found in the environment. such as imidacloprid (IMI), an insecticide belonging to the neonicotinoid family [2]. The objective of the study was to investigate the effect of co-exposure of PFOS and IMI. We first analyzed different nominal concentrations of PFOS (from 0,1 to 10  $\mu$ M) and IMI (from 75 to 1000  $\mu$ M) on zebrafish (*Danio rerio*) embryo/larvae at 96 hpf. We assessed the morphological effects of exposure alone and mixture, and after the oxidative stress and apoptosis with qPCR and Acridine orange. Co-exposure at two individually nontoxic concentrations resulted in increased toxicity in terms of morphological alterations, with increased cell death in the pericardium. Molecular investigations confirmed the cardiotoxicity accompanied by cell death, showing over expression of apoptosis (caspase 3, bax and bcl-2) and a dysregulation of oxidative stress (cat, sod1 and gstp2). The results suggest that IMI could potentiate PFOS cardiotoxicity by alteration of antioxidative and apoptosis balance.

### **References**

- [1] Cheng et al. 2013. Perfluorooctane sulfonate (PFOS) induced embryotoxicity and disruption of cardiogenesis. *Toxicol In Vitro* 27(5), 1503-1512.
- [2] Prestes et al. 2021. Biochemical and behavioral responses in zebrafish exposed to imidacloprid oxidative damage and antioxidant responses. *Arch Environ Contam Toxicol*. 81(2), 255-264.

## C13\_A PRELIMINARY STUDY OF THE IMPACT OF NSAIDS AND SAIDS ON GENE EXPRESSION IN BOVINE GRANULOSA CELLS: IMPLICATIONS FOR REPRODUCTIVE HEALTH

*Susanna Draghi, Federica Di Cesare, Giulio Curone, Daniele Vigo, Anna Lange  
Consiglio, Petra Cagnardi*

*Dipartimento di Medicina Veterinaria e Scienze Animali, Università degli Studi di Milano,  
Milano, Italy*

This preliminary study evaluates the cellular effects of steroidal (SAIDs) and non-steroidal (NSAIDs) anti-inflammatory drugs on bovine granulosa cells. Focusing on ketoprofen, flunixin (NSAIDs), and dexamethasone (SAID), it investigates gene expression modulation related to prostaglandin pathways and glucocorticoid receptor activation, emphasizing potential reproductive implications. Granulosa cells were isolated from bovine ovaries and treated with ketoprofen, flunixin, and dexamethasone at three concentrations, representing the potential range of tissue concentrations after systemic treatment from low (below potential tissue levels), medium (comparable to *in vivo* treatment levels), and high (potentially cytotoxic). Cellular samples were collected at 0, 4, 8, 12, 24, and 48 hours after treatment, plus a non-treated control sampled at corresponding times. Gene expression analysis targeted ABCG2 (membrane transport protein) and EP receptors (EP1–EP4) for prostaglandin E2 (PGE2). The expression of NR3C1, encoding the glucocorticoid receptor, was specifically analyzed to assess the genomic response to dexamethasone. Real-time PCR quantified gene expression and evaluated pharmacodynamic profiles. Ketoprofen and flunixin, as non-selective COX inhibitors, showed effects on prostaglandin synthesis. Flunixin notably modulated ABCG2 and PGE2 receptor gene expression [1]. Dexamethasone influenced gene expression via NR3C1 activation, affecting genomic pathways linked to inflammation and stress [2]. Although observable effects were detected, no statistically significant differences emerged among treatment groups under the current conditions, highlighting the preliminary nature of the study. Preliminary findings indicate that NSAIDs and SAIDs exhibit distinct cellular effects on bovine granulosa cells, potentially influencing reproductive outcomes. NSAIDs primarily affected prostaglandin pathways, while SAIDs demonstrated broader genomic impacts. Future research should increase sample sizes, test additional time points, and explore broader concentration ranges to detect significant differences and better understand the pharmacodynamics of these drugs. Such insights are crucial for developing strategies that optimize reproductive health and minimize adverse effects in bovine species.

### **References**

- [1] Wongrakpanich et al. 2018. A Comprehensive Review of Non-Steroidal Anti-Inflammatory Drug Use in The Elderly. *Aging and disease* 9(1), 143–150.
- [2] Wu et al. 2023. NR3C1/Glucocorticoid receptor activation promotes pancreatic  $\beta$ -cell autophagy overload in response to glucolipototoxicity. *Autophagy* 19(9), 2538–2557.

## C14\_ASTAXANTHIN MITIGATES 5-FLUOROURACIL-INDUCED HEPATOTOXICITY AND OXIDATIVE STRESS IN MALE RATS

*Mario Nicotra (1), Yasin Öztürk, (2) Merve Öztürk (2), Carlotta Marini (1), Gian Enrico Magi (1), Alessandro Di Cerbo (1)*

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

*(2) Dipartimento di Farmacologia e Tossicologia Veterinaria, Università di Necmettin Erbakan, Turkey*

Cancer is one of the most diffused diseases globally. Among the chemotherapeutic drugs that are used in the management of this potentially fatal illness is 5-fluorouracil (5-FU). However, its employment has been associated with increased Reactive Oxygen Species, leading to oxidative stress and inflammation, especially in the liver. Astaxanthin is a natural xanthophyll carotenoid pigment proven to possess antioxidant, anti-inflammatory, anti-cancer, and anti-apoptotic properties and approved by the US Food and Drug Administration as a dietary supplement. Because of these characteristics, this study aimed to assess the potential therapeutic effect of astaxanthin against 5-FU-induced hepatotoxicity. Thirty Male Wistar albino rats were divided into 5 groups receiving 1 ml saline daily by gavage (group I), 5-FU intraperitoneally (group II), Astaxanthin orally (group III), 5-FU intraperitoneally + 16 or 32 mg/kg/BW/day Astaxanthin orally (groups IV and V, respectively). Saline and Astaxanthin were administered for 14 days, while groups II, IV, and V received a single dose of 5-FU on the first day. Then, the concentrations of liver function biomarkers and pro- and antioxidant biomarkers were estimated in serum and hepatic tissue, respectively. Hepatic damage was further characterized through histopathological and immunohistochemical analyses. While 5-FU alone was responsible for an increase in the serum concentration of the liver function biomarkers, astaxanthin determined a reduction in their concentration in a dose-dependent manner and improved the redox balance. Concerning the histopathological changes, the administration of 5-FU caused degenerative changes (vacuolar degeneration, coagulative necrosis, and vascular changes) and an increase in the expression of TNF- $\alpha$  and 8-OHdG. Conversely, astaxanthin reduced the severity of these lesions and TNF- $\alpha$  and 8-OHdG expression. The present investigation proves that astaxanthin can obstruct 5-FU-induced hepatic damage, thus it can be considered a new potential therapeutic agent to be used during cancer treatment to counteract 5-FU-induced hepatotoxicity.

### **References**

- [1] Guidetti et al. 2016. In Vitro Effects of Some Botanicals with Anti-Inflammatory and Antitoxic Activity. *J. Immunol. Res.* 2016, 5457010.
- [2] Di Cerbo et al. 2017. Functional foods in pet nutrition: Focus on dogs and cats. *Res. Vet. Sci.* 112,161-166.
- [3] Abou-Zeid et al. 2021. Astaxanthin mitigates thiacloprid-induced liver injury and immunotoxicity in male rats, *Mar. Drugs* 19, 525.

## C15\_INTERACTIVE EFFECTS OF ENROFLOXACIN AND OCEAN WARMING ON THE HEALTH AND ENERGETIC BALANCE OF THE MEDITERRANEAN MUSSEL (*MYTILUS GALLOPROVINCIALIS*)

*Joanna Giannessi* (1), *Valentina Meucci* (1), *Luigi Intorre* (1), *Alessia Cuccaro* (2), *Rosa Freitas* (2), *Lucia De Marchi* (1), *Gianfranca Monni* (1), *Mariella Baratti* (3), *Carlo Pretti* (1,4)

(1) Department of Veterinary Sciences, University of Pisa, Pisa, Italy

(2) Centre for Environmental and Marine Studies (CESAM) & Department of Biology, University of Aveiro, Aveiro, Portugal

(3) Institute of Biosciences and Bioresources, IBBR-CNR, Firenze, Italy

(4) CIBM, Livorno, Italy

Marine organisms face growing exposure to anthropogenic stressors such as rising seawater temperatures and antibiotic pollution. Fluoroquinolones like enrofloxacin (ENR), widely used in veterinary medicine, are prevalent in aquatic environments [1]. While temperature increases impact marine species' physiological processes [2], the combined effects of these stressors are still poorly understood. This study aims to evaluate the combined impact of ENR contamination and elevated temperatures on the physiological and biochemical responses of the ecologically and economically important marine bivalve *Mytilus galloprovincialis*. Mussels were exposed to relevant environmental concentrations of ENR (5 and 500 ng/L) for 14 days at 20 °C (current) and 25 °C (projected warming). After exposure, they underwent a 14-day recovery period in the absence of ENR. Physiological responses were assessed via respiration and filtration rates, while biochemical analyses included metabolic activity, total energy reserves, and DNA integrity. The Independent Action (IA) model was applied to predict interactive effects. Chemical analyses quantified ENR concentrations in mussel tissues and seawater, revealing considerable variation in ENR bioaccumulation across tissues. Respiratory and filtration rates decreased post-exposure, with temperature influencing recovery. Biochemical evaluations indicated that higher temperatures increased metabolic capacity and energy storage but also raised energy expenditure ( $p < 0.001$ ). ENR exposure caused DNA single-strand breaks in both gill and digestive gland tissues ( $p < 0.001$ ), with temperature influencing DNA repair efficiency. The IA model predicted mostly additive effects, though antagonistic interactions were observed in filtration rates ( $p < 0.01$ ), suggesting compensatory respiration. Energy reserves declined significantly ( $p < 0.01$ ) despite increased metabolic activity, indicating an energy imbalance. Our findings reveal that antibiotic contamination and ocean warming act as compounding stressors to marine bivalves. The observed metabolic trade-offs and energy depletion may have long-term consequences for mussel health and survival, underscoring the need for further research and mitigation efforts.

### References

[1] Freitas et al. 2019. The influence of temperature and salinity on the impacts of lead in *Mytilus galloprovincialis*. Chemosphere, 235, 403-412.

[1] Shen et al. 2023. Occurrence, bioaccumulation, metabolism and ecotoxicity of fluoroquinolones in the aquatic environment: a review. *Toxics*, 11(12), 966.

## C16\_ANTIMICROBIAL RESISTANCE IN THE YELLOW-LEGGED GULLS (*LARUS MICHAELLIS*) OF TARANTO: AN ANTHROPOGENIC PHARMACOLOGICAL FOOTPRINT IN THE ENVIRONMENT?

Giuseppe Crescenzo, Olimpia Lai, Chiara Intermite, Claudia Zizzadoro, Antonio Camarda, Nicola Pugliese

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

Antimicrobial resistance in wildlife is an emerging problem that reflects the impact of human activities on environmental microbial communities [1,2]. This study focused on bacterial strains isolated from cloacal swabs of yellow-legged gulls (*Larus michahellis*) that have colonized the rooftops in the historical center of Taranto. The study aimed to identify any bacteria resistant to enrofloxacin (ENR) and cefepime (FEP), antibiotics that should be avoided in veterinary practice because they are considered critically important for human medicine. The strains were isolated from cloacal swabs cultured on TSA supplemented with ENR or FEP and were identified using MALDI-TOF. The minimum inhibitory concentrations (MICs) were determined for ENR and FEP, while disk diffusion tests were performed to evaluate susceptibility to other antimicrobial agents. Conjugation experiments were conducted to assess the potential horizontal transfer of resistance to ENR or FEP. The thirty isolated strains included members of the *Bacillus cereus* group and, commensal Gram-negative species of the *Enterobacter cloacae* complex. A significant number of isolates exhibited multidrug resistance (MDR) profiles, including resistance to tetracycline (TET), chloramphenicol (CHL), sulfamethoxazole-trimethoprim (SXT), and streptomycin (STR). In particular, the conjugation experiments did not yield transconjugants, suggesting that the resistance mechanisms may be chromosomal or associated with non-transferable elements. The detection of MDR profiles in both commensal Gram-negative and Gram-positive bacteria from the Taranto gulls is particularly surprising. The resistance to ENR and FEP, together with widespread resistance to chloramphenicol, SXT, and streptomycin, highlights a significant anthropogenic influence. These results suggest that urban pharmaceutical pollution and the terrestrial use of antibiotics may indirectly impact adjacent marine wildlife [3], positioning urban gulls as potential reservoirs and vectors of multidrug-resistant (MDR) bacteria [4]. Genomic investigations will be essential to clarify the resistance mechanisms and to understand the epidemiological and eco-pharmacological implications.

### References

- [1] Wellington et al. 2013. The role of the natural environment in the emergence of antibiotic resistance in gram-negative bacteria. *Lancet Infect Dis.* 13, 155-165.
- [2] Martinez et al. 2009. A global view of antibiotic resistance. *FEMS Microbiol Rev.* 33, 44-65.
- [3] Lai et al. 2025. Observed prevalence and characterization of fluoroquinolone-resistant and multidrug-resistant bacteria in loggerhead sea turtles (*Caretta caretta*) from the Adriatic Sea. *Animals* 14, 252.

[4] Bonnedahl et al. 2009. Dissemination of *Escherichia coli* with CTX-M type ESBL between humans and yellow-legged gulls in the south of France. PLoS One 18, 4:e5958.

## POSTER SESSION

## **P1\_ ISOLATION OF STAPHYLOCOCCUS XYLOSUS FROM PRE-CLINICAL AND CLINICAL MASTITIS (MILK) AND EVALUATION OF ITS SUSCEPTIBILITY TO ERYTHROMYCIN AND PLANT-DERIVED ESSENTIAL OILS**

Rosario De Fazio (1), Giacomo Di Giacinto (2), Paola Roncada (1), Domenico Britti (1), Rosangela Odore (2), Paola Badino (2), Cristian Piras (1)

Bovine mastitis is a major infectious disease in dairy farming, often caused by opportunistic pathogens such as *Staphylococcus xylosus* (*S.xylosus*). The increasing prevalence of antimicrobial resistance (AMR) among mastitis-causing bacteria poses a significant challenge to effective treatment, necessitating alternative antimicrobial strategies. Essential oils (EOs) with antimicrobial properties have emerged as potential candidates for mitigating AMR-related infections. This study aimed to isolate *S. xylosus* from raw bovine milk and evaluate its susceptibility to erythromycin and an EO blend composed of *Myrtus communis*, *Salvia officinalis*, and *Cistus ladanifer* to assess its potential as an alternative therapeutic agent for bovine mastitis. *S. xylosus* was isolated from raw bovine milk and identified through mass profile analysis using MALDI-TOF technology (MALDI Biotyper) to ensure strain confirmation. The EO blend was tested at concentrations of 100 mg/mL, 50 mg/mL, and 25 mg/mL, while erythromycin was tested at concentrations of 1.5, 3, and 6 µg/mL using the disk diffusion method. Zones of inhibition were measured to determine antibacterial efficacy. The results demonstrated dose-dependent inhibitory effects for both the EO blend and erythromycin, with erythromycin exhibiting superior activity. The EO blend showed moderate inhibition, with zones ranging from 9 mm to 13.3 mm, suggesting potential antimicrobial activity. These findings suggest that the tested EO blend possesses antimicrobial properties that could be further explored for its potential role in managing bovine mastitis, particularly in the context of AMR. While erythromycin displayed stronger inhibitory effects, the EO blend exhibited moderate antimicrobial activity, indicating possible complementary or alternative therapeutic applications. Future studies should investigate the mechanisms of action, synergistic potential with conventional antibiotics, and in vivo efficacy of this EO blend in mastitis treatment strategies.

## P2 WHICH ANTIBIOTICS ARE STILL EFFECTIVE TO TREAT URINARY TRACT INFECTIONS IN CANINE AND FELINE PATIENTS?

*Sara Crimi (1), Marco Peradotto (2), Graziana Gambino (1), Federica Pregnolato (3), Chiara Caruso (3), Michela Amadori (1,4), Antonio Curtoni (2,4), Davide Danieli (1), Giovanni Re (1), Cristina Costa (2,4), Cristina Vercelli (1)*

(1) Dept. Veterinary Sciences of Turin - Division of Pharmacology and Toxicology, Turin, Italy

(2) University Hospital Città della Salute e della Scienza, Turin, IT

(3) CVT-Centro Veterinario Torinese di Rivoli, Rivoli, Turin, IT

(4) Dept. of Public Health and Pediatric Sciences University of Turin, Turin, Italy

The close contact between people and pets can cause interspecies transmission of bacteria and/or of resistance genes. Urinary tract infections (UTIs) commonly required antibiotic treatment. A *One Health* approach is crucial, and antibiotics should be administered according to the Antimicrobial Advice Ad Hoc Expert Group (AMEG) and the World Health Organization (WHO) guidelines, which encourage a limited use of antibiotics and banned some drugs in veterinary medicine to safeguard humans [1]. Urine susceptibility testings (USTs) of owned canine and feline patients in a pet clinic in the Northwest Italy, were descriptively analyzed from 2013 to 2023. Results: 643 USTs were considered. 237 were positive in dogs with the following isolates: 108 *Escherichia (E.) coli*, 37 *Proteus mirabilis*, 19 *Klebsiella (K.) pneumoniae*, 17 *Enterococcus faecalis*, 13 *Staphylococcus (S.) pseudintermedius*, 11 *Pseudomonas aeruginosa*. In 118 positive bacterial isolated in cats, the pathogens were: 57 *E. coli*, 10 *Enterococcus faecalis*, 8 *K. pneumoniae*, 8 *S. pseudintermedius*. The sensitivity to antibiotics was: amoxicillin-clavulanic acid 62.75%, gentamicin 85.16% (both AMEG C), nitrofurantoin 66.96%, trimethoprim/sulfamethoxazole 62.30% (both AMEG D). Antibiotic resistance was: ampicillin 54.58% (AMEG D), pradofloxacin 33.22%, cefovecin 27.04% (both AMEG B), imipenem 7.98% (AMEG A), oxacillin 25%. A moderate percentage of Extended-Spectrum Beta-Lactamases were isolated (24.60%). It is clear the presence of bacteria responsible of UTIs that are common in humans and pets. Most of them are Gram-negative bacteria insensitive to several antibiotics (defined as multidrug resistant bacteria), making treatment more challenging. Data presented in this study are encouraging, because they show that rigorous management of clinical cases of UTIs according to the proposed antimicrobial stewardship guidelines has resulted in maintaining good sensitivity to the most commonly used antibiotics (i.e., amoxicillin-clavulanic acid suggested as an empirical treatment by the UTIs treatment guidelines) [2]. Low resistance to antibiotics reserved to humans included in AMEG A (i.e., 7.98% imipenem) persists.

### References

[1] European Medicines Agency. Categorisation of antibiotics for use in animals for prudent and responsible use. Available at: [https://www.ema.europa.eu/en/documents/report/infographic-categorisation-antibiotics-use-animals-prudent-and-responsible-use\\_en.pdf](https://www.ema.europa.eu/en/documents/report/infographic-categorisation-antibiotics-use-animals-prudent-and-responsible-use_en.pdf) (last accessed: 05.03.2025).

[2] Weese et al. 2019. International Society for Companion Animal Infectious Diseases (ISCAID) guidelines for the diagnosis and management of bacterial urinary tract infections in dogs and cats. *Vet. J.* 247, 8-25.

### **P3\_IDENTIFICATION OF MYCOTOXINS IN TRIGONELLA FOENUM-GRAECUM AND RELATED CLINICAL SYMPTOMS IN RUMINANTS FROM SICILY**

*Fabio Bruno, Michela Pugliese, Pietro Niutta, Patrizia Licata*

*Department of Veterinary Science, University of Messina, Messina, Italy*

*Trigonella foenum-graecum* (fenugreek) is a commonly grown legume in Mediterranean regions, used for human and animal consumption, as well as for medicinal purposes [1]. It is also a valuable component in livestock feed due to its bioactive compounds, including flavonoids, polyphenols, and proanthocyanidins, which improve the biological and nutritional value of the fodder by providing strong antioxidant properties [2]. Fenugreek has therapeutic uses in treating various health conditions such as diabetes, breast cancer (*in vitro*) and colon cancer (*in vivo*), hyperlipidaemia, inflammation, hepatotoxicity, ulcers, wounds, bacterial and fungal infections, as well as leg weakness and edema [3]. The warm temperatures and abundant rainfall in Sicily during the spring create ideal conditions for fungal growth, which may lead to mycotoxin production [4]. This study aimed to determine the concentrations of ochratoxin A, aflatoxin, deoxynivalenol, zearalenone, fumonisins and T-2 toxin in samples of *Trigonella foenum-graecum* administered as feed in cattle and sheep showing neurological signs suggestive of intoxication. The concentrations of these mycotoxins were measured in *Trigonella foenum-graecum* fed to ruminants. OTA levels were also assessed in blood samples from cattle and sheep to evaluate the potential toxicity related to the presence of these mycotoxins, based on the clinical signs observed in the animals. Mycotoxin analyses in fenugreek and blood samples were conducted using an Enzyme Immunoassay Kit (ELISA) (RIDASCREEN, R-Biopharm AG, Darmstadt, Germany). The study involved five farms in the Northwest of Sicily, with a total of 90 affected animals (50 beef cows, 21 dairy cows and 19 sheep), which reported an unusual outbreak of neurological disorders. The observed clinical signs, including reduced spinal reflexes, postural abnormalities, weakness, recumbency, and limb hyperesthesia, suggested a disorder of the peripheral nervous system. The mortality rate was alarmingly high, reaching 100% among the affected animals. The OTA concentrations found in fenugreek ranged from a minimum of 21.25 ppb to a maximum of 23.16 ppb. In the sheep serum samples the values of OTA were between 10.5 and 18.38 ppb. The OTA values found in bovine serum ranged from 1.61 to 4.57 ppb. These values are significantly higher than the Commission Regulation (EU) 2023/915 limit set at 10 µg/kg for OTA in dried herbs [5]. OTA contamination in Sicilian ruminants highlights the significant risk farms face from mycotoxins and underscores the need for preventive measures and ongoing monitoring in animal health management.

#### **References**

- [1] Visuvanathan et al. 2022. Revisiting *Trigonella foenum-graecum* L.: Pharmacology and Therapeutic Potentialities. *Plants (Basel)* 11(11).
- [2] Dixit et al. 2005. Antioxidant properties of germinated fenugreek seeds. *Phytotherapy research*. PTR 19, 977-83.

- [3] Kandhare et al. 2019. A systematic literature review of fenugreek seed toxicity by using ToxRTool: evidence from preclinical and clinical studies. *Heliyon* 5(4), e01536.
- [4] El-Sayed et al. 2022. An overview on the major mycotoxins in food products: characteristics, toxicity, and analysis. *J. Future Foods* 2(2), 91-102.
- [5] Commission E. Commission Regulation (EU) 2023/915 of 25 April 2023 on maximum levels for certain contaminants in food and repealing Regulation (EC) No 1881/2006 (Text with EEA relevance). 2023.

## P4\_ ANTIBIOTICS AND HEAVY METALS DETECTION IN PET FOOD SAMPLES FROM THE ITALIAN MARKET

*Mario Nicotra (1), Carlotta Marini (1), Yasin Öztürk (2), Merve Öztürk (2), Alessandro Di Cerbo (1)*

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

*(2) Dipartimento di Farmacologia e Tossicologia Veterinaria, Università di Necmettin Erbakan, Turkey*

Dogs and cats are increasingly showing the typical cutaneous and gastrointestinal symptoms related to Adverse Food Reactions (AFRs) [1,2]. Such phenomena deal with clinical responses that follow the ingestion of food contaminated by residues of different origins. These responses can be classified as food allergies or intolerances, and antibiotic residues, particularly the tetracycline class, have been hypothesized to be the main ones responsible for the onset of AFRs [2]. Nevertheless, the presence of heavy metals in pet food in EU and extra-EU countries and their possible involvement in these responses have only been questioned recently [4]. Thus, the present study aims to assess the presence of antibiotic residues and heavy metals in chicken-based pet food from the Italian market. Twenty-nine chicken-based kibble samples and one chicken-based treat sample were purchased from the Italian market. Antibiotic residues were assessed in all samples using quantitative LC-MS/MS (LOD/LOQ 50 µg/kg) [3], while heavy metals [Lead (Pb), Cadmium (Cd), and Arsenic (As)] were assessed using quantitative ICP-MS (LOD/LOQ 20 µg/kg) on kibble specimens. Antibiotic (Doxycycline, 90 µg/kg) and antiparasitic [Lasalocid (16 µg/kg), Monensin A (26 µg/kg), Nicarbazine (10 µg/kg), and 4,4'-Dinitrocarbanilide (7.2 µg/kg)] residues were detected only in the treat sample. Concerning heavy metals, 28 out of 29 samples contained at least one among Pb, Cd, and/or As, with a detection frequency of 83%, 52%, and 93%, respectively. Nevertheless, all detected substances resulted below the MRLs [5,6]. Considering the need for further studies to strengthen the achieved results, this research shows the absence of antibiotics, the presence of heavy metals in the analyzed commercial chicken-based kibble, and the presence of antibiotic and antiparasitic drugs in chicken-based treats. This evidence poses a serious concern about the possible chronic intake of such compounds in chicken-based pet food and their correlation with the increasing number of AFRs in pets.

### References

- [1] Mazzeranghi et al. 2017. Clinical efficacy of nutraceutical diet for cats with clinical signs of cutaneous adverse food reaction (CAFR). *Pol. J. Vet. Sci.* 20, 169-276.
- [2] Di Cerbo et al. 2018. Adverse food reactions in dogs due to antibiotic residues in pet food: a preliminary study. *Veterinaria Italiana* 54, 137-146.
- [3] Dasenaki et al. 2015. Multi-residue determination of 115 veterinary drugs and pharmaceutical residues in milk powder, butter, fish tissue and eggs using liquid chromatography-tandem mass spectrometry, *Anal. Chim. Acta.* 880, 103-121.

- [4] Kim et al. 2018. Evaluation of Arsenic, Cadmium, Lead and Mercury Contamination in Over-the-Counter Available Dry Dog Foods With Different Animal Ingredients (Red Meat, Poultry, and Fish), *Front. Vet. Sci.* 5, 264.
- [5] Directive 2002/32/EC on undesirable substances in animal feed.
- [6] Commission Regulation (EU) No 37/2010 on pharmacologically active substances and their classification regarding maximum residue limits in foodstuffs of animal origin.

## P5\_ BEHAVIORAL EFFECTS OF DIETARY CANNABIDIOL SUPPLEMENTATION IN RABBITS

*Valeria Iervolino, Consiglia Longobardi, Ruggero Amato, Maria Elena Pero, Vincenzo Mastellone, Sara Damiano*

*Dipartimento di Medicina Veterinaria e Produzioni Animali, Università degli studi di Napoli, Napoli, Italy*

The present study aimed to evaluate the effects of dietary cannabidiol (CBD) on behavioral patterns in rabbits. A total of 42 New Zealand White California rabbits (60 days, sex ratio 1:1, average weight  $1621.3 \pm 46.2$  g), were systematically allocated into 2 groups (21 animals/group), and individually housed in cages (25 x 45 x 30 cm). Both groups were fed the same commercial diet. A cannabis extract in coconut-based oil, was daily administered at a dose of 6,25 mg/kg CBD corresponding to 10 mg of CBD/animal. CBD extract was characterized according to the European Pharmacopoeia. Video recordings lasting 90 minutes each were conducted once a week for 4 weeks. The recordings were coded by 2 expert observers, with an interobserver reliability of 90-96%. In terms of resting behavior, control group spent more time lying down than CBD group, although this difference did not achieve statistical significance ( $p = 0.0850$ ). During the initial phase of the trial, treated rabbits demonstrated a significant increase in locomotor activities ( $p = 0.0192$ ). By the end of the trial, treated rabbits exhibited a significant increase in grooming behavior compared to the control group ( $p = 0.0498$ ). Overall, behavioral analyses revealed significant changes in CBD-treated rabbits in terms of generalized increases in locomotor activity and grooming behaviors. Although less evident, CBD group tended to show more pronounced "movement" behavior. Confirmation that CBD-treated rabbits spent significantly more time grooming than the control group was given at the end of the experiment. "Stretching" also increased significantly in CBD-treated rabbits, which could be associated with changes in muscle tone or comfort. Such behavioral changes could be interpreted as an improvement in psychological well-being, probably associated with the anxiolytic action of CBD (1). Overall, the data suggest that CBD significantly affects behavioral changes, proving a possible welfare improvement. Further studies are necessary to fully understand the mechanisms behind these effects in order to improve welfare and psychological state of animals, particularly important in intensive farming where animals are subjected to continuous stress.

### References

[1] Guangzhao et al. 2024. Cannabidiol exhibits anxiolytic-like effects and antipsychotic-like effects in mice models, *Neurosci Lett.* 15 (826), 137723.

Con il Patrocinio del Dipartimento di Scienze Veterinarie



Con il contributo non condizionante di

