

UNITE

RESEARCH MONTH

I Edition - September 2024

IDEAS in action



Artificial Intelligence applied to Biomedical Science

18 SEPT.
2024

THESIS ROOM
S. SPAVENTA
BUILDING

PhD Annual Meeting of
**Veterinary Medical Sciences,
Public Health and Animal Welfare**

Keynote Speaker: Prof. Angelo Porrello
University of Modena and Reggio Emilia

- **Oral presentations:** PhD students XXXVII cycle
- **Poster presentations:** PhD students XXXVIII & XXXIX cycle
- **Award ceremony:**
Best oral presentation for PhD students XXXVII cycle
Best poster presentation for PhD students XXXVIII & XXXIX cycle





18 SEPT.
2024

THESIS ROOM
S. SPAVENTA
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Artificial Intelligence applied to Biomedical Science

PROGRAMME

Chairpersons: Angela Di Cesare, Giuseppe Marruchella, Melania Giammarco

8:30 – 9:00 **Registration**

9:00 – 9:15 **Welcome address and Opening remark**

9:15 – 10:30 **Plenary Lecture:** Artificial intelligence applied to Biomedical Science

Keynote Speaker: Prof. Angelo Porrello | UNIMORE

10:30 – 11:20 **Oral Presentation Session – part 1**

Speakers: De Bonis Andrea, De Camillis Antonio,
Castelli Francesco, De Rose Cristina, Alice Musi

Abstracts 1 - 5

11:20 – 11:40 **Coffee break**

11:40 – 12:20 **Poster Presentation session – part 1**

Speakers: Bianchi Amanda, Bandera Lorenza, Boffa
Francesca, Bracco Claudia, Lanci Laura,
Coetzee Lauren Michelle, Carosi Alessia

Abstracts 6 - 12

12:20 – 13:00 **Oral Presentation Session – part 2**

Speakers: Di Matteo Ivano, Moncada Margherita,
Lo Sterzo Martina, Jalal Hassan

Abstracts 13 - 16

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PROGRAMME

13:00 – 14:00 **Lunch**

14:00 – 14:40 **Oral Presentation Session – part 3**

Speakers: Yaseen Muhammad Ahsan,
Albanese Valeria, Cito Francesca,
Paolino Virginia

Abstracts 17 - 20

14:40 – 15:20 **Poster Presentation session – part 2**

Speakers: Capretti Chiara, Pompei Luigi, Sciota
Domenico, Di Vittori Chiara, Astuti Chiara

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15:20 – 15:50 **Award Ceremony and Closing**

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**B-FLOW AND CONTRAST ENHANCED ULTRASOUND (CEUS) FEATURES OF
SUBCUTANEOUS NODULAR LESIONS IN DOGS**

Andrea De Bonis¹, Francesco Simeoni¹, Andrea Paolini¹, Martina Rosto¹, Francesca Del Signore¹, Roberto Tamburro¹, Massimo Vignoli¹

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Subcutaneous nodules in dogs are common in clinical practice. This prospective clinical study aims to compare B-flow ultrasound and CEUS techniques for the characterization of subcutaneous nodules evaluating their usefulness to distinguish benign subcutaneous nodules from potentially malignant ones. Dogs were prospectively enrolled and ultrasound cine-loops were achieved in B-mode, Color Doppler, Power Doppler, B-Flow and CEUS. Lesions vascularization highlighted through B-Flow and CEUS were classified into five patterns: P1, absence of contrast uptake; P2, enhancement only in the peripheral area of the lesion; P3, thin (<2 mm) and few vessels (<5/field); P4, thinner (>2 mm) and more numerous vessels (>5/field); P5 enhancement with a reticular aspect and both thick and thin bands inside. Patterns highlighted with B-flow and CEUS were compared to a histological diagnosis of subcutaneous nodules. A total of 16 dogs and 22 subcutaneous nodules were included and divided into three groups: 4 non-neoplastic, 11 benign tumours (8 lipomas, 3 haemangiomas) and 7 malignant tumours (4 soft tissue sarcoma, 2 Mast Cell tumours and one carcinoma). The pairwise comparison test displayed a p-value of 0.02 for B-Flow and 0.037 for CEUS to differentiate benign from malignant tumours. There were no differences with CEUS or B-flow to compare non-neoplastic nodules to malignant and benign tumours. B-flow and CEUS had an agreement of 86%. This study showed that B-flow and CEUS could differentiate benign tumours from malignant ones. B-Flow and CEUS displayed similar ability to evaluate different patterns and could be helpful in the evaluation of subcutaneous nodules.

Keywords: subcutaneous; B-Flow; CEUS

1. De Marchi A, Prever EBD, Cavallo F, Pozza S, Linari A, Lombardo P, Comandone A, Piana R, Faletti C. Perfusion pattern and time of vascularisation with CEUS increase accuracy in differentiating between benign and malignant tumours in 216 musculoskeletal soft tissue masses. *Eur J Radiol.* 2015; 84(1):142-150.
2. De Marchi A, Pozza S, Charrier L, Cannone F, Cavallo F, Linari A, Piana R, Geniò I, Balocco P, Massè A. Small Subcutaneous Soft Tissue Tumors (<5 cm) Can Be Sarcomas and Contrast-Enhanced Ultrasound (CEUS) Is Useful to Identify Potentially Malignant Masses. *Int J Environ Res Public Health.* 2020; 17(23):8868.

**DAILY VARIABILITY OF SELECTED RENAL BIOCHEMICAL PARAMETERS IN
HEALTHY ADULT MARTINA FRANCA DONKEYS***Antonio De Camillis¹**¹Department of Veterinary Medicine, University of Teramo, Teramo, Italy*

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 urinalysis. On serum samples, concentrations 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 donkey, in the morning and in the afternoon, the concentration of the following parameters was 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.

Keywords: donkey, renal biomarkers, urinalysis, breed conservation

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GESTATION LENGTH IN MARES OF SALERNITANO AND PERSANO BREEDS

Francesco Castelli¹, Brunella Anna Giangaspero¹, Salvatore Parrillo¹, Ippolito De Amicis¹, Domenico Robbe¹,
 Augusto Carluccio¹

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The Salernitano and Persano are two horse breeds submitted to the programmes for biodiversity conservation, for increasing reproductive efficacy.

The knowledge of normal gestation length and duration of parturition stages is necessary for rationale management of birth.

In the literature, the gestation length of most widespread breeds has been studied with an average of 342 days and a variable range (1). Correct prediction of foaling and management of phases can be a discriminating factor for the survival of foal (2).

This study was aimed to analyse the gestation length (GL) in Persano and Salernitano breed mares correlated to determining factors such as the foetal gender (FG), the age and reproductive conditions of the mares (RM), and the month of conception (CB). Furthermore, the evaluation of the stages of foaling included the dilating stage (DS), the expulsive phase (EP), and the foetal membrane expulsion (SP).

Data were obtained through videosurveillance from 39 eutocic foalings of Persano and Salernitano. GL in these breeds is 331.2 days on average, ranging from 313 to 350 days. The mean is lower than other breeds (1,2). Among the factors influencing GL the statistically significant parameter was FG, with an average of 329 in females and 334 in males ($p < 0.05$). The various phases of foaling had an average duration of 30 minutes SD, 31 minutes EP and 75 minutes SP.

The statistical analysis of the data showed a real significance between the GF and EP ($p < 0.05$), showing an increase in this phase in females (36.7 minutes) compared to males (17.7 minutes).

In conclusion, the gestation length in the Persano and Salernitano breeds showed a shorter mean duration of gestation than in most of the previous studies (1,2). The reasons for this phenomenon probably are linked to genetic, management, nutrition, climatic conditions, and latitudes.

Keywords: gestation length, Salernitano, Persano

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**INTESTINAL MICROBIOME AND METABOLOME IN SEPTIC DOGS AFFECTED BY
PARVOVIRAL ENTERITIS**

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Canine Parvovirus (CPV-2) enteritis is a predisposing factor for sepsis, making infected puppies a suitable model as far as sepsis is concerned (1). The impact of both sepsis and CPV-2 enteritis on the canine gut microbiome is still poorly investigated.

The aim of this study is to evaluate the gut microbiome and metabolome in septic dogs with CPV-2 infection. Fourteen CPV-2 infected puppies (P group) and twelve healthy puppies (H group) admitted to the Veterinary Teaching Hospital of Teramo (2020-2022) were prospectively enrolled in this study, excluding those with any different known microbiome alteration. The median age was 5.50 months (3-9) in H dogs and 4.5 months in P dogs (2-11). The median weight was 16 kg (7-25) in H dogs and 6 kg (2-23) in P dogs. Fecal samples were collected at presentation (H and P1) in both groups, and on third (P2) and seventh (P3) days of hospitalization for group P. 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). Unconjugated fecal bile acids, ursodeoxycholic acid, fatty acids, and sterols were measured with a targeted metabolomic approach (3). Datasets were tested for normal distribution. One-way ANOVA was used for statistical purposes (GraphPad Prism 9.0). *Metaboanalyst* was used to assess heatmaps for metabolomics data. *Blautia* and *Faecalibacterium* taxa abundance changed between group H and P1. *Streptococcus* taxa progressively decreased in infected puppies. Statistically significant changes in DI (p 0.04) occurred between P1 and P3. Most fatty acids increased in the P group, while the H group presented an increase in sterols and bile acids. Despite the acute onset, these results suggest a lipid imbalance during CPV infection that aligns with those already reported in dogs with chronic enteropathy.

Keywords: sepsis; metabolomics; lipidomics

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IDENTIFYING POTENTIAL NEW THERAPIES IN MUCOSAL MELANOMA*Alice Musi¹, Alain de Bruin^{2,3}, Laura Bongiovanni^{1,2}**¹Department of Veterinary Medicine, University of Teramo, Teramo, Italy; ²Department of Biomolecular Health Sciences, Faculty of Veterinary Medicine, Utrecht University, Utrecht, the Netherlands; ³Department of Pediatrics, University Medical Centre Groningen, University of Groningen, Groningen, The Netherlands*

Mucosal melanoma (MM) is an aggressive tumor in humans as well as in dogs, where the incidence is much higher. In both species, adjuvant radiotherapy is often used after surgery to improve locoregional control and to treat locally the mucosal melanomas. Recurrently mutated cancer-driver genes are shared between the two species and, basing on their molecular profile, MMs can be classified in “hot immune” and “cold immune” melanomas, the latter potentially sensitive to targeted therapy. Effective targeted therapies are lacking, and the frequent development of resistance to immunotherapy highlight the urgent need to find new effective compounds. Aim: To identify effective compounds for the treatment of human and canine MM and to find a compound able to increase the sensitivity towards radiotherapy. Methods: A highthroughput multidrug screening was performed on two human MM, five canine MM and one control canine keratinocytes cell lines. To select the chemotherapeutic and targeted compounds with the highest sensitivity, cells were treated with 224 drugs (more than half already used in standard-of-care protocols or undergoing clinical trials). Next, a follow-up combinatorial multi-drug screening was performed, testing the whole library in combination with the MDM2- inhibitor Idasanutlin. Drug-response curves were established and effective compounds were identified using the IC50 and the areas under the curves (AUCs). Additionally, Idasanutlin was tested in combination with various radiation doses. Cell apoptosis was detected using FACS with Annexin V and propidium iodide staining. Results: MDM2-inhibitors showed high efficacy in both species, especially in a metastatic cell line overexpressing MDM2. Increased efficacy was observed when Idasanutlin was combined with compounds targeting the PI3KAkt signaling-pathway. Additionally, an higher number of apoptotic cells was observed, when cells were treated with Idasanutlin before irradiation. Conclusions: Idasanutlin administered in combination with compounds targeting PI3K-Akt signaling pathway represents a potential candidate for the treatment of canine and human MM. The combination with radiation may also represent a valuable option, enhancing radio-sensitivity of the cells. Validation experiments to investigate these potential treatments are currently ongoing.

Keywords: mucosal melanoma, therapy, dog

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Acknowledgement: the present study was conducted in the framework of the Project “Demetra” (Dipartimenti di Eccellenza 2018–2022, CUP_C46C18000530001), funded by the Italian Ministry of Education, University and Research.

**EVALUATION OF SURGICAL SITE INFECTION RATE AFTER ANTIBIOTIC-FREE
LAPAROSCOPIC OVARIECTOMY IN DOGS: PRELIMINARY RESULTS***Amanda Bianchi¹, Andrea Paolini¹, Roberto Tamburro¹**¹Department of Veterinary Medicine, University of Teramo, Teramo, Italy*

Laparoscopy is a minimally invasive surgical technique; compared to traditional laparotomy, it has several advantages such as less tissue exposure and damage, reduced post-surgical pain, infection rate, and hospitalization time. In human medicine, patients undergoing elective laparoscopic procedures do not receive antibiotic prophylaxis to reduce antimicrobial use. Antibiotic therapy was found to be an important predisposing factor to the development of antimicrobial resistance. The present study aimed to evaluate the incidence of surgical site infection (SSI) in dogs undergoing antibiotic-free laparoscopic ovariectomies. The study was approved by the Ethics Committee of the University of Teramo (Prot. N. 24213). All dogs included were presented to the Small Animals Teaching Hospital of the University of Teramo for elective two-port laparoscopic neutering. Dogs were prepared with an aseptic technique and randomly divided into two groups: A and B. Group A received a 22mg/kg dose of Cefazolin intravenously 30 minutes before skin incision, and an analog dose every 90 minutes of surgery.

Group B did not receive prophylactic antibiotics. Medical records were reviewed; data collected included timing and dosage of antimicrobial administration, duration of surgery and anesthesia, and signs of SSI. Dogs were discharged 24 hours post-surgery after clinical evaluation of surgical wounds. Clinical follow-up was performed 14 days post-surgery and dehiscence, fistula formation, and/or purulent discharge were recorded. Telephone interviews with owners were conducted 30 days post-surgery to evaluate clinical outcomes.

Data from two groups were presented as percentages and compared with Fisher's exact test, significance was set at $p \leq 0.05$. The overall SSI rate was 0/20 (0%) in the group A and 1/20 (5%) in the group B ($p=1$).

The use of antibiotic prophylaxis during elective laparoscopic surgery appears avoidable as there is no statistically significant difference in the rate of SSI among dogs who received or not received prophylaxis.

Keywords: laparoscopy, antibiotic prophylaxis, antibiotic free surgery

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3. Menz BD, Charani E, Gordon DL, Leather AJM, Moonesinghe SR, Phillips CJ. Surgical Antibiotic Prophylaxis in an Era of Antibiotic Resistance: Common Resistant Bacteria and Wider Considerations for Practice. *Infect Drug Resist* 2021; 14:5235-5252.

**EFFECTS OF TECAR THERAPY ON SUBCLINICAL POST-ANAESTHETIC MYOPATHY
IN HORSES UNDERGOING ELECTIVE AND EMERGENCY PROCEDURES:
PRELIMINARY DATA**

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¹Department of Veterinary Medicine, University of Teramo, Teramo, Italy

Post-anaesthetic myopathy is one of the most common peri-anaesthetic complication in horses and may affect muscular groups. Tecar is an endogenous thermotherapy used in rehabilitation due to its diathermic effect secondary to blood circulation increase that could help muscle oxygenation. Aim of the study was to determine the effects of tecar on muscles after general anaesthesia in horses undergoing elective and emergency procedures. Horses included in the study receiving tecar therapy were assigned to Group A-T after elective surgery, to Group B-T after emergency surgery. A non-treated control group was provided for A-T and B-T (Group A-C and Group B-C). Treatment consisted of 3 tecar applications on the muscles involved in the recumbency at regular intervals in the two days following the anaesthesia. A blood sample was collected before the induction of the anaesthesia and 6h, 24h and 48h after induction in all horses, and the concentration of serum muscle enzymes were assessed for each sample. Descriptive statistic and T-test were performed to compare the trend of muscular enzymes in between treated and non-treated horses and to compare the same variables between Group A-T and Group A-C and between Group B-T and Group B-C. Fifteen horses were included in the study. Some notable differences for AST and LDH concentrations were found when comparing all treated horses with all control horses. T-test comparing Group A-T and Group A-C highlighted a difference for AST, while the comparison between Group B-T and Group B-C found a significance for LDH. The results of our study demonstrate that tecar is safe and well-tolerated, and can help to limit the muscular damage in horses undergoing general anaesthesia for elective or emergency surgeries. Further studies involving a larger number of horses are required to establish the optimal treatment protocol and its effects on subclinical PAM in horses.

Keywords: horse; myopathy; tecar

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2. Ribeiro S, Henriques B, Cardoso R. The effectiveness of tecar therapy in musculoskeletal disorders. *International Journal of Public Health and Health Systems* 2018; 3(5):77-83.
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IN VITRO DIFFERENTIATION OF DEFINITIVE ENDODERM FROM SHEEP EPIBLAST*Francesca Boffa¹, Marta Czernik¹, Pasqualino Loi¹, Domenico Iuso¹, Ramiro Alberio²**¹Department of Veterinary Medicine, University of Teramo, Teramo, Italy; ²School of Biosciences, University of Nottingham, Sutton Bonington Campus, UK*

The gastrulation process is a pivotal event in mammalian embryo development, marking the emergence of three primary somatic germ layers: ectoderm, mesoderm, and definitive endoderm. Investigations into embryos with a typical flat embryonic disc, such as those of humans, rabbits, and ungulates, remain scarce (1). Expanding on previous findings by Simpson et al. in 2023 (2), which highlighted the critical balance between WNT and Activin/NODAL signaling for endoderm fate acquisition in pigs, our study aims to elucidate the role of these pathways in driving the in vitro differentiation of definitive endoderm (DE) from sheep epiblast cells (sEpiSCs).

Blastocysts obtained from in vitro matured and fertilized oocytes underwent immunosurgery to remove the trophoblast layers, resulting in isolated inner cell masses (ICMs). These ICMs were cultured on Matrix Laminin 511-coated wells in N2B27 medium supplemented with Activin, FGF, and a WNT inhibitor to induce epiblast formation. While 80% of ICMs adhered to the plate and proliferated in vitro, only 25% successfully established the epiblast in culture.

Upon reaching the epiblast stage, cells were cultured with a higher concentration of Activin for varying durations, and DE cells were identified through immunofluorescence, specifically targeting Sox17 and FoxA2 markers while excluding other mesoderm markers. This confirmed the direct differentiation of sEpiSCs into DE cells without a transition through a mesendodermal progenitor.

The DE layer represents a crucial initial germ cell layer in the development of vital visceral organs such as the gut tube, liver, lungs, and pancreas (3). Our study contributes to bridging gaps in gastrulation research, particularly in species with mammary typical flat embryonic discs, and provides insights into the early stages of visceral organ development, with potential future applications in regenerative medicine.

Keywords: Definitive Endoderm, sheep, Embryonic Stem Cells

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ULTRASOUND-BASED TECHNOLOGIES FOR MANAGING AND PROMOTING DOG REPRODUCTIVE HEALTH

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Reproductive health and fertility are essential for effective breeding management in dogs. A thorough understanding and evaluation of both male and female reproductive systems are critical for ensuring optimal breeding outcomes. The breeding soundness examination (BSE) is essential for evaluating the reproductive capability of dogs (1), involving clinical and ultrasonographic examinations, along with cytologic and hematologic assessments. The aim of this doctoral project is to enhance reproductive assessments in breeding dogs by developing a comprehensive BSE and integrating conventional and advanced ultrasonographic techniques. Methods: The project focuses on two primary lines: male and female reproduction. Male dogs of various breeds, ages and sizes are enrolled with no restrictions on fertility status; homogeneity and reliability in sample collection were guaranteed thanks to a partnership with ENCI breeder, and experimental groups were formed based on age and semen quality. Female intact dogs were recruited in four-month period, in each stage of the estrous cycle, with a specific focus on the follicular phase and the periovulatory timing. Patient with health issues were excluded from the study. Before each procedure, informed consent was obtained and anamnesis recording, general clinical examination and specific BSE were performed. Various techniques were used to conduct ultrasonography in male dogs, and semen quality was evaluated. In female dogs, a gynecological examination was performed to identify the time of ovulation. After data collection, the relationship between ultrasonographic features and normal and pathological conditions was investigated, and correlations between laboratory and echo/elastographic parameters was verified. Results: The initial part of the work focused on male's testicles performing elastography, laboratory evaluations and histology demonstrated that elastography can detect lesions that conventional ultrasound is unable to identify. Moreover, the study found a correlation between changes in tissue stiffness and semen quality/histopathological changes (2). The female-focused part of the work allowed for an in-depth and detailed investigation of the canine cervix using advanced ultrasound, representing the groundwork for defining an operational protocol and establishing reference values specifically concerning the normal elastographic appearance of the cervix during the different phases of the estrous cycle.

Conclusions: The findings of this study demonstrate the potential of advanced ultrasound-based techniques to enhance reproductive assessment in breeding dogs. This comprehensive approach represents a significant step forward in veterinary reproductive medicine, enhancing breeding management and outcomes. Future research should refine imaging technologies and explore new markers to improve understanding and application in clinical practice.

Keywords: dogs; ultrasound elastography; breeding management

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**ANTIBIOTIC-RESISTANCE-GENES (ARGS) SPREADING IN MARINE ECOSYSTEM:
 EXPLORING THE RELATIONSHIP BETWEEN ENVIRONMENTAL FACTORS AND
 RESISTOME, GROWTH AND
 WELL-BEING OF FARMED MUSSEL**

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Currently, diverse bivalve-based monitoring programs exist in many countries (1), but only few studies are focused on mussel microbiome/resistome and the resulting knowledge is still fragmentary. This research project aims to advance scientific knowledge on the relationships between environmental variables and microbiota/resistome profiles (2,3), growth and well-being of farmed mussels. In this sense, comprehensive environmental monitoring techniques as satellite data, in-situ sensors and hydrographic measurements are correlated to mussel growth. Additionally, microbiome and resistome of farmed mussels are compared with those of environmental samples collected from river estuaries. Methods: Monthly mussel samples are collected from a mussel farm along the Abruzzo coast and analyzed to calculate condition index and evaluate microbiota/resistome profiles, histopathological data and valve gaping behaviour. Environmental parameters (temperature, salinity, chlorophyll, dissolved oxygen, nitrates, phosphates, sea currents, wind) are monitored both by satellite and in-situ probe and correlated with river hydrometric and rainfall data. Furthermore, environmental samples from river estuaries are analyzed to identify ARGs and comparing these findings with mussel microbiota/resistome profiles. Results: The condition indices from the last two years showed divergent trends: in 2023, they increased until the end of the production cycle, while in 2024, they decreased until May and then increased in June. A comparison between satellite data and in-situ sensor measurements highlighted a strong correlation in temperature readings but revealed the satellite system's inadequacy in providing precise biogeochemical measurements at specific depths, emphasizing the need for site-specific observations. Nevertheless, environmental data have been graphically correlated with mussel growth, aligning with observations regarding growth patterns. Moreover, behavioural gaping data, histological observations and microbiota/resistome analyses are ongoing. Molecular biology and isolation results for ARG evaluation from river samples are currently under analysis. Conclusions: These findings highlight the necessity of continuous and comprehensive monitoring to understand and, consequently, manage, the factors influencing ARGs spread in marine ecosystems.

Keywords: Mussel Growth, Antibiotic-Resistance Genes, Environmental Monitoring

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HIGH DETECTION FREQUENCY AND GENETIC DIVERSITY OF PORCINE CIRCOVIRUS 3 (PCV-3) IN NAMIBIAN BACKYARD FARMS AND WARTHOGS

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Since first identification in 2015, porcine circovirus 3 (PCV-3) has been reported worldwide with a high frequency, accompanied by of several clinical conditions, although its impact on pig health and productivity is still debated. Data on the presence of PCV-3 in Africa are, however, limited. A previous study performed on commercial pigs in Namibia failed to identify the pathogen. In the present study, the viral circulation in backyard farms, characterised by lower biosecurity measures and frequent animal exchange between farms, was assessed. The susceptibility of warthogs to PCV-3 infection and their potential epidemiological role were also evaluated. Tonsils from 77 pigs from backyard piggeries and 55 warthogs were collected in different regions of Namibia and tested by PCR. Positive samples were sequenced and compared to PCV-3 strains circulating globally. Forty-two out of 77 pigs (54.54 %) and 12 out of 55 warthogs (21.82 %) tested positive, demonstrating the presence of PCV-3 in the country. The partial ORF2 gene was successfully sequenced in samples from 27 pigs and 6 warthogs. Genetically, the identified strains were part of 3 distinct groups which included both backyard pigs and warthogs from different regions of Namibia. There is also evidence for the occurrence of multiple introduction events most likely from Asian countries, either directly into Namibia or through other African countries. Considering the strict Namibian regulations on live animal importation, understanding the source of viral introduction is challenging, although semen importation or the habit of feeding backyard pigs with human food waste might have played a role. Pig exchanges between farms for breeding purposes or wildlife movements could also have been involved in PCV-3 dispersal within Namibia. Despite the significant advances in the field, further studies should be undertaken to properly understand PCV-3 epidemiology in Namibia and its impact on pig productivity and wildlife health.

Keywords: PCV-3; Pigs; Molecular epidemiology

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THE PHENOTYPE OF THE MARTINA FRANCA DONKEY: AN ENDANGERED ITALIAN DONKEY BREED

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The Martina Franca (MF) donkey breed (*Equus asinus*) primarily inhabits the rural areas surrounding the homonymous municipality (1).

The objective of this study was to assess the current phenotype through the evaluation of 27 morphologic measurements (2, 3).

The objective of this study was to assess the current phenotype through the evaluation of 27 morphologic measurements. The study was conducted on 73 female and 18 male breeding animals from eight different herds located in central and southern Italy. Statistical analysis was performed to demonstrate statistical differences between males and females, as well as the sexual dimorphism and uniformity of all measurements in both genders.

The results demonstrated that the mean of three parameters used for the evaluation of an MF donkey (height at withers, circumference of thorax, and shin circumference) are highly representative of the population, with minimal dispersion ($C_v^2 = 0.05-0.06$ up to a maximum of 10%, as evidenced by relatively low standard deviations across observed measurements in both genders. Regarding sexual dimorphism, a statistically significant difference was found between males and females.

Upon comparing the measurement averages with those from Montanaro's 1930 study (2), the statistical approach revealed significant variations. Notably, for females, there was a decrease in the width of auditory meatuses, sternum-to-ground distance, knee-to-ground distance, and body weight, while there was an increase in the width between the temporal angles of the eyes, ear length, and thorax width. Conversely, males exhibited increases in trunk length, head length, thorax circumference, and various other measurements, alongside a decrease in sternum-to-ground distance and live weight.

In conclusion, our findings suggest that the phenotype of the Martina Franca donkey has been largely preserved over time. While no substantial differences were observed in female donkeys, male breeding stock displayed greater robustness, albeit with a decrease in weight across both sexes.

Keywords: Martina Franca donkey morphology; phenotype; body measurements

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STUDY OF THE EFFECTS OF AN INACTIVATED DIVA VACCINE FOR LUMPY SKIN DISEASE IN DAIRY COWS

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Lumpy skin disease (LSD) is a viral disease that naturally affects bovine, especially the young and dairy cows in the peak of lactation (Tuppurainen et al., 2017; Namazi and Tafti, 2021). The aetiological agent of LSD, called lumpy skin disease virus, belongs to the Poxviridae family and Capripoxvirus genus, and it is primarily transmitted through hematophagous biting midges (WOAH, 2023), which act as mechanical vectors rather than biological ones (Clemmons et al., 2021; Sanz-Bernardo et al., 2021). Since 2012, many LSD outbreaks have been reported in Middle East regions, until the first LSD incursion occurred, in 2015/2016, in South-Eastern Europe (Greece, Bulgaria, Serbia, Albania and Montenegro) (EFSA, 2018; 2020). To contain LSD in Europe, different measures have been carried out by authorities: culling of all infected animals, prohibiting animal movement and vaccination is one of the actions to prevent and limit the spread of the disease. Commercial vaccines available are live attenuated but their use is considered unsafe in non-endemic countries for the possibility of having adverse effects, such as reduced milk production. The aim of this study was the evaluation of the inactivated DIVA vaccine immunogenicity and its effects in dairy cows, concerning the quantity and quality of produced milk. Moreover, qualitative and quantitative properties of the milk produced by vaccinated cows showed no differences when compared to the experimental control group, demonstrating the properties of vaccine not only in cattle, but also in dairy cows.

Keywords: lactation, DIVA, LSD

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THE CRUCIAL DYNAMICS OF LIPID DROPLETS IN EARLY OVINE EMBRYONIC DEVELOPMENT

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Our research has revealed a critical aspect of lipid metabolism in pre-implantation sheep embryonic development. While lipid droplets (LDs) are known to play vital roles in peri-implantation events in mice (1), their specific function during pre-implantation is less understood despite their abundance in early embryos, particularly in farm animals. In our study, sheep zygotes subjected to mechanical delipidation (DEL) developed into blastocysts at a significantly lower rate than the control group (CTR) (24.44% for DEL vs. 40.82% for CTR, $P=0.0097$). However, these delipidated zygotes were able to restore LDs, showing no significant difference from the control when stained at the blastocyst stage with BODIPY (Fluorescent signal: 0.92 in DEL fold change over CTR; $P>0.05$). We explored lipid anabolism pathways in DEL and CTR embryos, focusing on citrate and acetate derived Acetyl-CoA by inhibiting ATP-citrate-lyase (ACLY) and Acetyl-CoA-synthetase (ACSS2), critical enzymes in de novo lipogenesis (DNL). ACLY and ACSS2 inhibition did not significantly affect blastocyst development (17.2% of CTR ACLYi-ACSS2i vs. 12.9% of CTR; 13.33% for DEL ACLYi-ACSS2i vs. 24.44% for DEL, $P>0.05$) or LD restoration (BODIPY signal: 0.47 in DEL ACLYi-ACSS2i fold change over DEL, $P>0.05$). These findings underscore the LD organelle's essential role in proper pre-implantation development and highlight an unconventional DNL metabolic pathway in early embryos. Inhibiting β -oxidation, blocking ATP production from fatty acids, reduced LDs per blastocyst, likely affecting Acetyl-CoA-carboxylase (ACC1) through AMPK. We speculate that Acetyl-CoA used for LD production via ACC1 in embryos comes from sources other than citrate and acetate. Understanding lipid metabolism in sheep pre-implantation development has significant implications for human assisted reproduction. *In vitro* deregulation of cellular metabolism can prevent embryos from developing into newborns, with developmental arrest influenced by metabolic and epigenetic dysfunctions (2). Our findings prompt further investigation into the relationship between lipid metabolism and epigenetic regulation, crucial during the morula to blastocyst transition.

Keywords: IVF; lipid metabolism; development

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EXOGENOUS EXPRESSION OF LATE EMBRYOGENESIS ABUNDANT (LEA) PROTEIN ENHANCES COLD TOLERANCE IN MAMMALIAN CELLS BY COUNTERING OXIDATIVE STRESS

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Understanding the molecular mechanisms that confer cold resistance in mammalian cells is essential for advancing medical applications such as organ and cell transport. This study aimed to mimic the function of Late Embryogenesis Abundant (LEA) proteins, which are known to provide resistance to low temperatures in extremophiles and plants, by exogenous expression of two of them in mammalian cells: cold acclimation protein WCOR410 from *Triticum aestivum*, that targets the plasma membrane, and the dehydrin-1dhn RAB17 from *Zea mays*, found in the nucleo-cytoplasm.

LEA-transfected fibroblasts revealed significantly higher cell viability and proliferation rates compared to un-transfected control when exposed to cold stress. Both WCOR410 and RAB17 proteins mitigated cold-induced mitochondrial stress and the overproduction of reactive oxygen species (ROS), leading to enhanced cytoskeleton stability and decreased in DNA damage.

Given the observation of the robust antioxidant effect of LEA's proteins, we next set out to verify whether the employment of α -Tocopherol (also known as Vitamin E) a potent antioxidant, might biomimicry LEA proteins in conferring cold stress resistance to fibroblasts. Accordingly, α -Tocopherol supplementation improved cold stress tolerance in fibroblasts by decreasing ROS production and DNA damage, and enhancing cold stability of mitochondria and actin microfilaments in sheep fibroblasts. In conclusion, the transient expression of WCOR410 and RAB17 proteins in sheep fibroblasts provides an efficient model for transferring cold-resistance mechanisms from plants to mammals. This finding underscores the potential of LEA proteins in inducing cellular cold resistance by counteracting oxidative stress and highlights α -Tocopherol as a promising antioxidant for future application, such as cell transport at low temperature.

Keywords: Reactive Oxygen Species (ROS), cold-stress, α -Tocopherol

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COMPREHENSIVE EVALUATION OF MANGO AND AVOCADO FRUIT BYPRODUCTS: NUTRITIONAL POTENTIAL, IN VITRO DIGESTIBILITY, RUMEN FERMENTATION, INCLUDING ENCAPSULATED PHENOLIC EXTRACTS AS FEED SUPPLEMENTS FOR RUMINANTS

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Intensified food-feed competition and environmental concerns drive research on unconventional ruminant feed sources. In this study, we explored the potential use of mango peel (MP), mango seed kernel (MSK), mango seed coat (MSC), avocado peel (AP), and avocado seed (AS) in two experiments. Experiment 1 evaluated the feed potential of these fruits byproducts by assessing their chemical composition, in vitro true digestibility, gas production, and volatile fatty acid production. In vitro true digestibility was determined using the Ankom DaisyII incubator. Among the various byproducts, MP and AP exhibited higher total phenolic content ranging from 121.50 to 243.69 (mg GAE/g) and antioxidant capacity from 342.92 to 366.63 (mg TE/g), indicating their potential to positively influence the rumen ecosystem. MP, MSK, and AS showed higher digestibility (87.51– 89.5%), increased gas production, and elevated metabolizable energy (8.20-10.50 MJ/kg DM), while MSC and AP exhibited lower values. Acetate-to-propionate ratio, a key methane indicator, was higher in AS (3.96), MSC (3.70), and MSK (3.53), and lower in AP and MP (3.07mmol/l). Experiment 2 was conducted to assess the effectiveness of incorporating mango and avocado by-products encapsulated extracts as feed additives, in comparison to alfalfa hay used as a control diet (200 mg DM) across all groups. The findings demonstrated that the MS and AS extract group exhibited the highest 24-hour gas production (42.44-42.22 ml/0.2 g DM) when compared to both the control and other extract groups (42.11 to 40.06 ml/0.2 g DM). MP and AP extracts significantly reduced acetate to propionate ratio and methane production compared to other groups ($P < 0.001$). Furthermore, the extract groups exhibited a lower ammonia nitrogen concentration compared to the control. In conclusion, MP, MS, and AS show promising results as feed ingredients. Peel extracts of mango and avocado emerge as feed additives, modulating rumen fermentation parameters effectively.

Keywords: phenolic compounds, encapsulated extracts, ruminal fermentation

IS TRIM25 REQUIRED FOR BTN3A3-MEDIATED RESTRICTION OF AVIAN INFLUENZA VIRUSES?*Muhammad Ahsan Yaseen^{1,2}, Massimo Palmarini², Siddharth Bakshi²**¹Department of Veterinary Medicine, University of Teramo, Teramo, Italy; ²MRC Centre for Virus Research, University of Glasgow, UK*

Avian influenza viruses pose a significant global health threat, and understanding the molecular mechanisms that restrict these viruses from crossing species barriers is crucial. Previous studies have identified BTN3A3, a member of the butyrophilin family, as a key inhibitor of avian influenza virus replication. This study aimed to determine whether an antiviral protein TRIM25, an E3 ubiquitin ligase involved in the innate immune response, is required for BTN3A3-mediated restriction of avian influenza viruses. Mass spectrometry analysis revealed that TRIM25 was a prominent binding partner of BTN3A3. Knockout experiments of TRIM25 in BTN3A3-overexpressing human A549 cells showed that while TRIM25 knockdown did not immediately rescue the replication of a BTN3A3-sensitive avian Mallard wildtype virus over 6 hours of infection at high MOI, extended multi-cycle growth assays over 48 hours demonstrated a significant increase in viral growth by approximately two logs when knockout TRIM25. This suggests that TRIM25 plays a supportive role in BTN3A3-mediated restriction, particularly in the later stages of infection. However, we started noticing that constitutive high levels of expression of BTN3A3 resulted in a gradual disappearance of the phenotype with the Mallard wild-type virus. So, TRIM25 knockout clones were first followed by the addition of BTN3A3 and/or TRIM25 generated using a doxycycline-inducible system. However, further experiments using inducible expression systems for TRIM25 and BTN3A3 did not yield significant results, likely due to lower protein expression levels compared to constitutive overexpression. Additional research is needed to confirm the critical interaction between TRIM25 and BTN3A3 in sustaining the restriction of avian influenza virus replication. These insights could inform strategies for enhancing antiviral defences against avian influenza viruses.

Keywords: BTN3A3, TRIM25, Avian Influenza viruses

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COMPARISON OF SEVERAL TECHNIQUES FOR PELVIC FLEXURE ENTEROTOMIES IN HORSES

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The goal of pelvic flexure enterotomy in equine gastrointestinal surgery is to allow for evacuation of the large colon of its contents. This procedure is performed most often to remove impacted ingesta in the large colon that may be too large or firm to pass with medical management. An enterotomy is also commonly performed in cases of small colon obstructions, to reduce bulk of ingesta moving from the large colon through the inflamed or injured bowel aboral and avoid recurrence. In cases of caecal impactions, a pelvic flexure enterotomy may facilitate outflow of ingesta from the intestinal tract oral to it and help minimise the chances of recurrence. In cases of enterolithiasis or fecaliths, if the enterolith(s) or fecalith(s) can be moved to the pelvic flexure, they are removed through a pelvic flexure enterotomy as well. Another indication for performing a pelvic flexure enterotomy is to facilitate manipulation of the large colon for correcting a displacement or a volvulus. Additionally, pelvic flexure enterotomy has been linked to a decreased incidence of post-operative ileus in some studies. Although generally considered benign, the procedure is not without risk. Enterotomy increases surgical time and manipulation of the bowel. It implies opening the bowel thus changing a clean procedure into a clean-contaminated procedure. It can also leave exposed suture material on the serosal surface of the bowel, which can cause local inflammation and adhesions. A two-layer closure with absorbable suture material is recommended, but a consensus has not been established. Many different techniques have been tested to evaluate surgical time, luminal diameter reduction, bursting pressure and post-operative complications including adhesion formation. The results of these studies vary within and between enterotomy techniques. The objective of our research was to evaluate, summarise and compare relevant published literature on pelvic flexure enterotomy techniques in the horse.

Keywords: horse; colic; enterotomy

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***STREPTOCOCCUS EQUI* SUBSP. *ZOOEPIDEMICUS* EPIDEMIOLOGICAL AND GENOMIC FINDINGS OF AN EMERGING PATHOGEN IN CENTRAL ITALY**

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Streptococcus equi subsp. *zooepidemicus* (SEZ) is a significant concern in equine veterinary medicine. Typically a commensal in horses, SEZ can cause severe diseases including respiratory infections, septicemia, and reproductive tract infections under certain conditions. Recent evidence suggests that SEZ may also cause severe diseases in humans through direct contact with animals or consumption of unpasteurized milk and dairy products (1,2). This study investigates SEZ strains isolated from equidae nasal swabs in central Italy in 2023 to describe the epidemiology and genomic characteristics of circulating strains. A sampling plan was implemented to randomly collect nasal swabs from equid farms in the Abruzzo and Molise regions. Additionally, a sampling form was designed to gather information on risk factors related to the presence of the bacterium and the potential development of respiratory disease. Relative risk was employed to measure the association between the presence of SEZ in the samples and various variables. The swabs were analysed using PCR, and the positive samples were further tested for isolation. To confirm the identification and characterize the isolated strains, the isolates were fully sequenced by next generation sequencing (NGS) through Illumina platform (Illumina, CA). A total of 457 animals from 95 farms were sampled with 134 animals tested positive for SEZ (74% horses, 25% donkeys, 1% mules). Forty-five percent of the farms examined were found to be positive for SEZ. Monitoring the clonal spread of SEZ is crucial for understanding the ecology of this emerging zoonotic pathogen, assessing the risk, and implementing effective control measures. Furthermore, genomic assessments are recommended to examine the pathogenicity of circulating strains. This study provides a comprehensive understanding of the epidemiology and genomic diversity of SEZ strains isolated in central Italy.

Keywords: *Streptococcus equi* subsp. *zooepidemicus*, Whole Genome Sequencing, epidemiology

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THE ANALGESIC VALIDITY OF AN OPIOID-FREE ANESTHESIA PROTOCOL IN FEMALE CATS UNDERGOING OVARIECTOMY

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Opioid-free protocols are increasingly used in veterinary medicine although there are few studies concerning the feline species. In 2022, Rufiange et al. (1) compared the analgesic power of an opioid-free protocol with that of a standard protocol (using opioids) in ovariohysterectomized female cats, evaluating the degree of analgesia and sedation using the Feline Grimace Scale (FGS), obtaining as a result an equivalence between the opioid-free and standard protocols in the management of intraoperative pain and the degree of anesthesia achieved. Among the most commonly used drugs in opioid-free protocols is dexmedetomidine. Gupert A. et al. (2), have shown that this causes no side effects to the respiratory system, as well as having an analgesic, sedative and anxiolytic effect. The use in continuous intraoperative infusion of dexmedetomidine in cats was studied in 2018 by authors who demonstrated its usefulness also in reducing the volatile anaesthetic agent used to maintain general anaesthesia (isoflurane), thus decreasing its undesirable effects systemically (3).

The objective of the study is to evaluate the analgesic and anesthesiologic capacity of an opioid-free protocol using dexmedetomidine, alfaxalone, and lidocaine intra-operatively in female cats undergoing ovariectomy, comparing it with a standard protocol. The study is based on 30 subjects randomly assigned to experimental group 1 and standard group 2. Group 1 received administration of dexmedetomidine at 15 µg/kg IM and alfaxalone at 2 mg/kg IM for induction of anesthesia, followed by maintenance of anaesthesia in isoflurane and administration by continuous infusion (CRI) of dexmedetomidine at 1 µg/kg/h IV. Physiological parameters were measured for each patient during nine well-defined stages of surgery. Patients in standard group 2 received an additional dose of methadone at 0.1 mg/kg IM during anaesthesia induction and did not receive intraoperative dexmedetomidine CRI. Both groups received a dose of intra-ligamentous lidocaine during surgery before ovariectomy. The parameters recorded for both groups and the FGS resulted in the good outcome from both anesthesiological and analgesic point of view of the experimental group compared with the control group, demonstrating in fact the analgesic power of intraoperative dexmedetomidine and of the experimental protocol.

Keywords: opioid-free, dexmedetomidine, ovariectomy

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**IDENTIFICATION OF TOOLS FOR IMPROVING AND SUPPORTING SUSTAINABILITY
AND INNOVATION PROCESSES IN THE DAIRY SUPPLY CHAIN***Chiara Capretti¹, Jorgelina di Pasquale¹, Giorgio Vignola¹**¹Department of Veterinary Medicine, University of Teramo, Teramo, Italy*

The sustainable development of livestock sector is one of the greatest challenges for next years. Within the development of a sustainable food system, animal production plays a debated role, thus its sustainable transition is particularly difficult and controversial. This transition is recognised as a key element for the future development of the planet by the European Green Deal and the Italian Recovery Plan. Strong efforts are currently being made to convert livestock farms to more sustainable and technological management, but there is still a long way to go.

In the context of sustainability evaluation, several methods based on Life Cycle Thinking are being developed with the aim of framing the three dimensions of sustainability (Environmental, Economic and Social); interest in the application of this methods in agriculture has been increasing in recent years due to the implications of this sector on climate change.

In the procedures tools nowadays used to assess the sustainability of farms, animal health and welfare are usually neglected or not organically integrated.

In this context, the primary aim of the PhD project presented here was to assess the environmental impact of dairy farms, incorporating an approach that considers animal health and welfare. This was achieved using a Life Cycle Assessment (LCA) method with a “cradle to factory gate” analysis. Following an extensive literature review, an innovative and comprehensive LCA inventory was developed to collect primary data from four dairy farms within the Parmigiano Reggiano PDO supply chain involved in the research. This inventory included aspects such as animal health and welfare, feeding, production, and management. The farms were monitored, and farmers were engaged through questionnaires.

Upon completion of a more extensive farm sampling and data analysis, customized management strategies for climate change mitigation will be identified and implemented for each farm involved in the research.

Keywords: dairy sector, LCA, sustainability

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**INCLUSION OF WINERY AND OLIVE WASTE BY-PRODUCTS IN SHEEP DIETS:
NUTRITIONAL PROPERTIES AND METHANE MITIGATION POTENTIAL**

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The valorization of winery and olive wastes and by-products in a circular economy perspective and their use as animal dietary supplement or alternative feed resource meets the multiple efforts in reducing the carbon footprint of the livestock industry. Moreover, their application enables the sustainable use of high-added value bioactive ingredients inside the food chain with positive effect on human health in a farm to fork approach. The aim of the present study was to evaluate the effect Grape pomace (GP), Grape skin (GS) and Olive leaves (OL) at the inclusion level of 10% on DM basis in a standard sheep diet, on the nutritional properties, rumen digestibility, in vitro gas/methane production. Polyphenols content was determined as described by Oliva et al. (2021). The NDF digestibility was measured using the filter bag technique with a fiber analyzer (ANKOM Technology, NY, USA), as described by Van Soest et al. (1991). GP was characterized by a higher percentage of tannins compared to GS and OL. In vitro gas production was monitored by the Gas Endeavour® system which allows to measure in real time the total gas and methane produced during ruminal fermentation. All the experimental diets showed a good digestibility (C: 85.5%; GP: 84.3%; GS: 84.3%; OL: 86.3%). The grape enriched diets showed a significant reduction ($P < 0.01$) of methane production: 38.5 ml/d DM (GP), 39.5 ml/d DM (GS), compared to OL (49.1 ml/g DM) and C (48.0 ml/g DM). Preliminary results showed that condensed tannins in the GP may have altered rumen degradation by decreasing methane production without adversely affecting digestibility. These by-products deserve to be investigated in more details as potential agents able to increase sustainability of ruminant nutrition also by mitigating methane emissions. This has to be confirmed in in vivo studies.

Keywords: By-products; methane; rumen

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DETECTING EAR LESIONS IN SLAUGHTERED PIGS USING ARTIFICIAL INTELLIGENCE*Domenico Sciota¹**¹Department of Veterinary Medicine, University of Teramo, Teramo, Italy*

Background: Recording skin lesions in slaughtered pigs is widely recognized a useful tool to assess animal welfare along the entire pork chain. Particular attention is currently given to ear and tail lesions resulting from biting, mostly after the ban on tail docking (1, 2).

Objectives: Training a convolutional neural network (CNN) to detect ear lesions at slaughter.

Methods: A total of 1415 pictures (dataset) were randomly collected along the slaughter chain. Carcasses were photographed after passing through the scalding tank, flaming and brushing. Each image included the external surface of both ears and was evaluated by two veterinarians, who agreed to classify pinnae as healthy or diseased. Moreover, ears ripped after brushing were classified as unsuitable. Thereafter, 1000 images were used to train an open-source CNN (Tensor Flow, available at <https://keras.io>) to detect any change of the auricle silhouette. The remaining 415 images were used to test CNN's accuracy, when comparing its predictions with veterinarians' assessment.

Results: Veterinarians classified 78.2% ear pinnae as healthy, 17.8% as diseased and 4.0% as unsuitable. Overall, CNN's accuracy was 95.06%. However, CNN was much less accurate to correctly identify unsuitable ears (59.45%). More in detail, CNN correctly recognized ear pinnae affected by severe artifacts (22 out of 37), while it mistakenly identified as healthy (n = 7) or diseased (n = 8) the remaining 15 unsuitable ears.

Conclusions: This study highlights that open-source, artificial intelligence-based tools could effectively fulfill well-targeted tasks. The presence of artifacts represented the greatest concern, as they were the main responsible of mistakes. We consider such an issue could be solved by increasing the dataset and/or taking pictures before brushing, whether allowed by the slaughter chain facility.

Keywords: animal welfare, slaughtered pigs, artificial intelligence

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ENHANCING FOOD SAFETY AND QUALITY THROUGH OZONE TREATMENT OF SURFACES IN COD PRODUCTION CHAIN*Chiara Di Vittori¹, Massimo Monti², Gianluigi Ferri¹, and Alberto Vergara¹**¹Department of Veterinary Medicine, University of Teramo, Teramo, Italy; ²Foods Import F.lli Monti SPA, Corropoli (TE), Italy*

In the food industry, traditional disinfection methods involve heat or liquid chemicals, which can lead to food contamination by chemical by-products that are harmful to human health and promote bacterial resistance (1). The demand for safer, higher-quality, and minimally processed food has led the food industry to explore innovative decontamination methods (2). Ozone emerges as a promising alternative, being economical, safe, and chemical-free. It effectively inactivates bacteria, moulds, yeasts, parasites, and viruses with short contact times and low concentrations (3).

This study evaluates the efficacy of ozonated water as a surface disinfectant on boxes used for soaking cod, comparing it to the conventional cleaning method (hydrogen peroxide and dishwashing machine).

A total of 25 samples were collected with sponges on dirty boxes that were used for soaking cod, after direct treatment with ozonated water (2 ppm) or traditional washing, at 7-14-21 days of storage of traditionally cleaned boxes, and following the application of ozonated water at day 21 of storage.

Total psychrophilic count (TPC) and total mesophilic count (TMC) metrics are used to assess the levels of microbial contamination.

On dirty surfaces, preliminary results show a mean reduction of 2.87 and 1.14 log CFU/cm² for TMC and 3.36 and 1.28 log CFU/cm² for TPC using the traditional wash and ozonated water, respectively. On day 21, the mean TMC growth is 1.69 log CFU/cm², which is then reduced by 1.40 log CFU/cm² after ozonated water treatment; TPC growth reaches 1.80 log CFU/cm², with complete microbial load reduction achieved by ozonated water application.

The aim of this study is to provide original data regarding the suitability of ozonated water as a safe and effective surface disinfection method.

These early results suggest that ozonated water reduces microbial loads on dirty surfaces and effectively clears them from cleaned surfaces that are recontaminated due to storage-related conditions.

Keywords: food industry; environmental sanitation; ozone

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**DIROFILARIA IMMITIS AND ANGIOSTRONGYLUS VASORUM IN DOGS:
EPIDEMIOLOGICAL STUDY IN CENTRAL ITALY**

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Dirofilaria immitis and *Angiostrongylus vasorum* are primary nematodes of dogs. In Europe, the geographical distribution of these parasites is changing due to several factors and a continuous monitoring is essential to increase the knowledge on their epidemiology. The present survey has been carried out to generate updated data on the distribution of dirofilariosis and angiostrongylosis in dogs living in central regions of Italy. Overall, 2000 dogs were included in the study: 400 in Umbria (Site A), 400 in Marche (Site B), 400 in Abruzzo (Site C), 366 in Molise/ Northern Puglia (Site D) and 434 in Lazio (Site E). Dogs were enrolled based on specific inclusion criteria: at least one year of age, not treated for at least six months with molecules effective in the prevention and/or treatment of dirofilariosis and angiostrongylosis and with outdoor access. For each animal fecal and blood samples were collected. All fecal samples were subjected to flotation and Baermanbin's test, while blood samples were used to perform the Knott's test and, if positive for microfilariae, tested with the SNAP test 4DX IDEXX. Overall, 35 dogs (1.7%) were positive for *D. immitis*, i.e. 16 (4%) in site A, 8 (2%) in site B, 2 (0.6%) in site C, 3 (0.8%) in site D and 6 (1.7%) in site E, while a total of 62 dogs (3.1%) were infected with *A. vasorum* i.e. 39 (10.2%), 12 (3%), 6 (1.5%), 2 (0.5%), 3 (0.7%) in site A, B, C, D and E, respectively. These results confirm the occurrence and the spread of *D. immitis* and *A. vasorum* in central Italy. Therefore, the present data underlines the importance of a constant vigilance and the need to adopt appropriate preventive strategies both in enzootic and non-enzootic areas, to reduce the spreading of these nematodes and safeguard the health of dogs.

Keywords: Epidemiology, Dirofilariosis, Angiostrongylosis

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