UNIVERSITÀ DEGLI STUDI DI MILANO

VAS Days

divas
DIPARTIMENTO DI MEDICINA VETERINARIA E SCIENZE ANIMALI

SEPTEMBER 2023
Room L03

27th

Morning session:
Genetics, immunology, and biotechnology for innovative models
Afternoon session:
Embryology and Reproduction

28th

Morning session:
Nutrition, Sustainability, and One-Health
Afternoon session:
Industrial PhD Students

29th

Session:
Veterinary clinical and pathological sciences
Panel discussion:
Tackling Antibiotic Resistance in Veterinary Medicine and Animal Sciences

CONFIRMED SPEAKERS:

Prof. Christian Maltecca, *North Carolina State University, USA*
Dr. Rita Vassena, *Fecundis Lab, Spain*
Prof. Marìa José Ranilla, *Universitad de Leon, Spain*
Prof. Mabrouk Elsabagh, *Niğde Ömer Halisdemir University, Turkey*
Prof. Micaela Sgorbini, *Università di Pisa, Italy*
Prof. Francesca Bonelli, *Università di Pisa, Italy*

Participant registration at 9:15

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Structural Variation in the Aosta cattle breeds

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Copy Number Variants (CNVs) are structural variants affecting genetic diversity that influence phenotypic expression[1,2]. Scientific community has underlined relationship between CNV and adaptation to different environmental. Aosta cattle (Aosta Red Pie – ARP; Aosta Black Pied/Chestnut – ABC and Mixed Chestnut-Herèn – ACH) are autochthonous dual-purpose breeds, well-adapted to be reared in the Alps Mountain area. The aim of this study was to characterize Aosta population through the CNVs detection and investigate their relation to the adaptative selection to the mountain farming system. ANABORAVA provided raw genotyping data of 3,114 females (2,030 – ARP; 927 – ABC; 157 – ACH), obtained with the NEOGEN's GGP Bovine100K, that were used to call CNV on autosomes using the SVS 8.9 (Golden Helix®) CNAM module. Due to the SNP genotyping data the genetic similarity between ABC and ACH was discovered so all the statistics were calculated considering these two breeds as a unique one (ABCH). CNVs were aggregated into CNV regions (CNVRs) based on at least 1 bp overlap; only CNVRs identified in at least 2% of the cows were considered to infer statistics at breed level (ABCH vs ARP). After quality control performed on the obtained calling, a total of 83,824 CNVs and 2,319 CNVRs were identified. Cows of the ABCH and ARP showed similar relationship existing between the CNV count and the CNVs mean length. In the PCA performed using the called CNVs, ABCH and ARP also appear overlapping, without any defined cluster highlighting the adaptive selection to the alpine environment shared by all breeds. In fact, about 63.7% of the CNVRs identified in at least 2% of samples (n. 310 regions) resulted in common between the two breeds. Instead, only 20% (ABCH) and 13.2% (ARP) CNVRs were proper of each breed. Annotated genes (n. 662) and QTL (n. 581) overlapping the CNVRs associated with productive, functional and health traits. Funded by PSRN DUAL BREEDING_Fase_2.

Keywords: CNV, Valdostana, Genome

References:
[1] Gamazon ER, Stranger BE. The impact of human copy number variation on gene expression. Brief Funct Genomics. 2015;14(5):352–7. DOI: 10.1093/bfgp/elv017
[2] Redon R, Ishikawa S, Fitch K, Feuk L, Perry G, Andrews T, et al. Global variation in copy number in the human genome. Nature. 2006;444:444–54. DOI: 10.1038/nature05329

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Immunohistochemical characterization of cells of the mononuclear phagocyte system in the mouse

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The mononuclear phagocyte system (MPS) represents a heterogeneous system of cells composed of circulating monocytes, macrophages, and dendritic cells involved in several physiologic and pathologic processes [1]. Immunohistochemistry has been proven useful to detect MPS cells in the spatial context of tissues in different experimental settings, but no comprehensive references exist in literature for tissue MPS under steady state conditions [2,3]. The aim of the study was to immunohistochemically characterize MPS cells in a selected set of murine healthy tissues and in a subset of representative pathological conditions.

Sections from liver, spleen, kidneys, adrenal glands, lymph nodes and lungs were obtained from 25 C57BL/6 mice. Sections from representative inflammatory conditions (spontaneous necro-suppurative hepatitis and experimentally induced *Pneumocystis murina* pneumonia) and neoplastic conditions (human fibrosarcoma xenograft and murine mammary carcinoma syngraft) were included. All sections were stained for Iba1, MARCO, CD206, Ym1, iNOS, HO1, F4/80, MHC-II and Arginase 1. For each organ, the microanatomical localization, cellular morphology (round, spindle or stellate) and the number and staining intensity of the positive cells according to a semi-quantitative grading system were evaluated.

Results confirmed the morphological and immunophenotypic heterogeneity of murine MPS cells. We successfully characterized mononuclear phagocytes across different mouse tissues classifying them based on immunohistochemical positivity, microanatomical

location and morphology, providing useful morpho-functional clues on MPS cells in tissues in both physiologic and pathologic conditions.

The results provide valuable data on microanatomical localization, morphology, and staining intensity of MPS in murine healthy tissues and selected pathologic conditions, which could be useful for data analysis, results interpretation and future studies planning in translational research.

Keywords: mononuclear phagocyte, macrophage, macrophage heterogeneity

References:

[1] Gordon S and Pluddemann A. The mononuclear phagocytic system. Generation of diversity. Front Immunol. 2019, 10:1893. Doi: 10.3389/fimmu.2019.01893.

[2] Guilliams M, et al. Dendritic cells, monocytes and macrophages: a unified nomenclature based on ontogeny. Nat Rev Immunol. 2014, 14(8): 571-578. Doi: 10.1038/nri3712.

[3] Rheg J, et al. The Utility of Immunohistochemistry for the Identification of Hematopoietic and Lymphoid Cells in Normal Tissues and Interpretation of Proliferative and Inflammatory Lesions of Mice and Rats. Tox Path. 2012, 40: 345-374. Doi: 10.1177/0192623311430695

VAS Days, 27-29th September 2023

Genome Wide Association studies in the Aosta cattle breeds

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Aosta cattle has a key role in the Aosta valley economy for: i) the meat and milk production; ii) the maintenance of landscapes and mountain environment during summer pasture; iii) the cultural value of the "*Battailes des Reines*". The milk is mostly entirely used to produce the PDO cheese Fontina and milk quality has always been considered as selection criteria [1]. To implement a genomic selection breeding plan, it is important to understand the genomic bases of the traits of interest. Genome Wide Association Studies (GWAS) are the gold standard to identify genomic regions harbouring Quantitative Traits Loci (QTL) associated with complex traits. For this study 3191 female genotypes and EBVs for production traits of Aosta cattle have been used. Genotypes have been obtained with the GGP bovine 100K SNP chip by Neogen. EBVs of each animal for milk, fat and protein yield and contents were provided by the national breeders association, ANABORAVA. Genotypes QC left a total of 73,435 SNPs for the analyses. The GWAS have been performed with the Mixed Linear Model Analysis module of SVS v8.9.1, with the additive model and using MLMM method including the genomic kinship matrix [2]. To correct for multiple testing Bonferroni and FDR thresholds where set at 5% genome wide. The positions of significant SNPs have been used to identify genes and functional elements. For fat and protein contents some interesting QTL have been identified. For the fat percentage, QTL have been found on chromosomes 3, 5 and 14, and for the protein percentage we identified QTL on BTA 5, 6 and 27 with significative markers that laid in the TBC1D22A, CSN1S1, CSN2, HSTN and ZNF385D genes. Since these analyses have been realized on the actual cow population, the results represent the loci currently segregating in the population. QTL for the fat percentage have an intragenic SNP lying in the MGST1 gene, already associated with milk fat in other studies [3]. Moreover, in the QTL region on BTA14 harbouring many candidate genes, two of them, CYHR1 and VPS28, had intragenic significant SNPs and they are only 100kb away from the DGAT1 gene. Furthermore, among the QTL identified for the protein percentage, the one on BTA5, harbouring the TBC1D22A gene, has been already identified in other association studies for milk protein content.

Keywords: GWAS, autochthonous cattle, milk EBVs

Acknowledgments: Funded by PSRN-DUALBREEDING_2.

References:
[1] Strillacci MG, Vevey M, Blanchet V, Mantovani R, Sartori C, Bagnato A. The Genomic Variation in the Aosta Cattle Breeds Raised in an Extensive Alpine Farming System. Animals. 2020;10(12):2385.
DOI: 10.3390/ani10122385
[2] VanRaden PM. Efficient methods to compute genomic predictions. J Dairy Sci. 2008;91(11):4414–23.
DOI: 10.3168/jds.2007-0980
[3] Cruz VAR, Oliveira HR, Brito LF, Fleming A, Larmer S, Miglior F, et al. Genome-wide association study for milk fatty acids in holstein cattle accounting for the dgat1 gene effect. Animals. 2019;9(11):997.
DOI: 10.3390/ani9110997

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Development of an ELISA assay for the detection of the antibodies production against F4 adhesive fimbriae: follow-up of the new inactivated oral vaccine against enterotoxigenic *Escherichia coli* in post-weaning piglets.

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F4 and F18 *E. coli* adhesive fimbriae are involved in enterotoxigenic (ETEC) infections, leading to post-weaning diarrhoea (PWD) in piglets [1]. Vaccination of pregnant sows with inactivated *E. coli* stimulates the production of specific antibodies passed into colostrum and milk for the protection of suckling piglets against ETEC diarrhoea. However, passive protection is rapidly lost after weaning, and piglets can rapidly become susceptible to disease. Triggering gut mucosal immunity could represent a valid approach to prevent PWD [2]. Piglets born from two unvaccinated sows were divided into two groups: one was orally vaccinated with an inactivated *E. coli* vaccine expressing F4 and F18 fimbriae and one remained untreated. The ELISPOT results suggested that F4 fimbria is more immunogenic than F18. The present study aimed to further clarify the immunogenicity of the vaccine. Different samples (colostrum, blood, saliva, faeces) from sows and piglets were analysed with a specific ELISA set up for the detection of anti-F4 IgA and IgG. The preliminary findings showed that our ELISA provides reliable and repeatable results. In the colostrum of the unvaccinated sows, it was possible to identify specific F4 immunoglobulins regardless of the vaccine, suggesting the *E. coli* circulation in the farm environment. Statistically significant differences ($p<0,05$) were observed in the serum IgA between the vaccinated and control groups and within the unvaccinated group. Unvaccinated group exhibited IgG levels higher than vaccinated one ($p<0,05$), confirming a possible circulation of the pathogen in the farm. Faecal and saliva samples of vaccinated piglets revealed post-vaccination anti-F4 IgA (especially in earlier ones), but not IgG. The analysis of piglets' IgA and IgG active

responses, along with maternally derived colostrum antibodies, could help in evaluating ETEC vaccine immunogenicity and understand how environmental *E. coli* can interfere with the efficacy of the vaccination.

Keywords: *E. coli*, ELISA, Post-weaning diarrhoea

References:
[1] Luppi A. Swine enteric colibacillosis: diagnosis, therapy and antimicrobial resistance. Porcine Health Manag. 2017;3:16. DOI:10.1186/s40813-017-0063-4.
[2] Matías J, Berzosa M, Pastor Y, Irache JM, Gamazo C. Maternal Vaccination. Immunization of Sows during Pregnancy against ETEC Infections. Vaccines (Basel). 2017;5(4):48. DOI:10.3390/vaccines5040048.

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Copy Number Variants in commercial dairy farms

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Copy Number Variants (CNV) are modifications affecting the structure of DNA, for instance, duplications and deletions of a considerable number of base pairs (i.e., greater than 1000 bp, up to millions of bp). Although the phenomenon of the creation of CNV is not well known, the impact on the variation of the phenotypic traits has been widely demonstrated; like monogenic mutation affects phenotypes, the same occurs by CNV. Furthermore, quantitative production traits of farmed animals are influenced by the expression of genes located in CNV. In addition, CNV are a class of markers useful to visualize the genetic biodiversity among populations related to adaptation to the environment. It is well known in fact that environmental pressure causes the formation of CNV. A total of 3809 animals were collected in commercial farms and DNA of each sample was extracted and genotyped with the GGP 100K bovine SNP chip. The Log R ratio value has been used to detect CNV using the CNAM module of SVS 8.9 of Golden Helix. CNV regions (CNVRs) are genomic tracts where several population individuals exhibit CNV that overlap.
Principal Component Analysis (PCA) was performed in order to group the individuals according to their CNV similarities. A total of 123,815 CNV were called and aggregated into 1,397 CNVRs. PCA result, as expected, showed that the variability among animals is not strong, probably because the analysis refers to only one breed and the fact that farmers are selecting for the same goals and are farming animals in similar environmental conditions. The annotation of genes mapped in CNVRs, the identified QTL and the gene function highlight the productions' goals of farmers, such as milk production and quality, udder conformation and body morphology.
Even though there are no strong breakthroughs, the information emerging from the study is a new insight into CNV related to the Italian Holstein cows where no CNV map was available.

Keywords: Copy Number Variation (CNV), Holstein, Genetic diversity

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Young extracellular matrix-based bio-scaffolds boost and maintain miR-200-induced rejuvenated phenotype in senescent fibroblasts

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Aging is a degenerative pathophysiological process characterized by a gradual decline of cellular, tissue and organ functions. To date, several strategies have been proposed to slow or even reverse cellular aging, ranging from telomerase reactivation to the use of epigenetic modulators [1]. Recent evidence demonstrated that the microRNA-200 (miR-200) family is endowed with the ability to induce a high plasticity state in terminally differentiated somatic cells [2]. Here we investigate whether this family of miRNAs ameliorates aging hallmarks in senescent cells.
The results obtained demonstrate that miR-200b/c are able to erase signs of senescence in fibroblasts isolated from individuals with age comprised between 82-96 years. In particular, cells, grown in standard monolayer conditions and exposed to miR-200b/c, significantly decrease β-galactosidase and reactive oxygen species (ROS), downregulate senescence-related markers, P16, P21 and P53, while upregulating cell proliferation markers and mitochondrial activity-related genes. However, this rejuvenating action is transient and reversible, and cells do reacquire their aged original phenotype within 10 days of culture. In contrast, miR-200b/c-mediated effects are boosted and stably maintained when treated cells are plated onto 3D ECM-based scaffolds obtained from young porcine decellularized ovaries [3].
These results indicate that miR-200b/c regulate molecular mechanisms directly involved in cell rejuvenation. In parallel, biomechanical cues deriving from a young microenvironment have the ability to boost and stabilize the rejuvenating effect, thus suggesting a functional cooperation among molecular effectors and ECM-related stimuli driving the aging process.

Keywords: Extracellular matrix, rejuvenation, microRNA

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This research is part of the project "MIND FoodS HUB (Milano Innovation District Food System Hub): Innovative concept for the eco-intensification of agricultural production and for the promotion of dietary patterns for human health and longevity through the creation in MIND of a digital Food System Hub", cofunded by POR FESR 2014-2020_BANDO Call HUB Ricerca e Innovazione, Regione Lombardia.

References:
[1] Rosen, R.S., Yarmush, M.L., Current Trends in Anti-Aging Strategies. Annu Rev Biomed Eng. 2023; 25:363-385. DOI: 10.1146/annurev-bioeng-120122-123054.
[2] Balzano, F., Cruciani, S., Basoli, V., Santaniello, S., Facchin, F., Ventura, C., & Maioli, M. miR200 and miR302: Two Big Families Influencing Stem Cell Behavior. Molecules. 2018, 23(2), 282. DOI: 10.3390/molecules23020282
[3] Pennarossa, G., De Iorio, T., Gandolfi, F., Brevini, T.A.L. Impact of Aging on the Ovarian Extracellular Matrix and Derived 3D Scaffolds. Nanomaterials. 2022; 12(3):345. DOI: 10.3390/nano12030345.

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Preclinical assessment of the CNS toxicity in paediatric subjects: histological, histochemical and immunohistochemical evaluation in Sprague Dawley newborn rats

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Anaesthetics administered in the perinatal period can cause neurotoxic effects, so preclinical toxicity studies in juvenile animals are required by the Regulatory Authorities to identify possible adverse effects of new drugs [1]. The postnatal period between days 7-10 in rats is the most adequate to investigate toxic effects induced by drugs to be administered to a paediatric population [2]. The brain histopathology is the best preclinical approach to assess the potential neurotoxic effects of new compounds in newborn animals. This study aimed to develop a histopathological method to evaluate the potential neurotoxicity of new anaesthetics in the paediatric population. 7 days-old Sprague Dawley rats were intraperitoneally injected with Vehicle (4.34% lactose solution) or Ketamine (20 mg/kg) five times every 90 minutes. This treatment schedule allowed to maintain the sedation for 6 consecutive hours to mimic the paediatric clinical situation. 2 hours and 16 hours post-treatment 2 animals per group were sacrificed, the brain was harvested, formalin-fixed and processed for histology, histochemistry and immunohistochemistry to evaluate neuronal damage and glial response in the cortex and hippocampus through a digital image analysis software (QuPath). The routine H&E staining, and the immunostaining with Cleaved Caspase 3 and γH2AX revealed that Ketamine induced neuronal apoptosis, that was more prominent at 2h post-treatment than at 16h. A diminished positive area for Iba1+ microglia and an increase of GFAP+ signal were detected at 16h. No consistent results were obtained with Fluoro-Jade B. Even this study was a preliminary investigation, the set-up of this histopathological method to evaluate Ketamine-induced neuronal damage allowed to highlight the stainings' efficacy and specificity in newborn rats. This staining panel could be used to characterize the neurotoxicity of new anaesthetics with action on the CNS neonatal target in the newborn rat.

Keywords: Ketamine, newborn rats, neurotoxicity

References:
[1] ICH guideline S11 on nonclinical safety testing in support of development of paediatric pharmaceuticals – September 26th 2020.
[2] Rao D.B., Hoberman A.M., Brown P.C., Varela A., and Bolon B. Regulatory Perspectives on Juvenile Animal Toxicologic Pathology. Toxicologic Pathology. 2021, 49, 1393-1404.
DOI: 10.1177/01926233211046869.

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Identification and Characterisation of *Gamma-herpesviruses* in *Artiodactyla* species using a Long-range PCR approach

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Mammalian gamma-herpesviruses are commonly identified and characterised using a Nested Pan-herpes PCR, which employs degenerate and inosine-substituted primers capable of binding to conserved regions of the DNA polymerase gene (*DPOL*) of all mammalian herpesviruses [1]. The Pan-herpes PCR however amplifies a very short PCR region (~200 bp), which does not allow accurate identification and results in a less-than definitive viral identification, with low percentage of identity [2]. Consequently, it is necessary to extend the *DPOL* partial sequence to improve diagnosis and this could be done using a Long-range PCR. The first step to develop this technique is to look for a conserved gene adjacent to the *DPOL* gene, and within the same orientation [3].
I carried several studies to achieve the first goal. 33 gamma-herpesvirus genomes available in GenBank were analysed by SnapGene to find an adjacent gene with the same orientation as the *DPOL* gene. Subsequently, I employed a gamma-herpesvirus gB-specific Nested-PCR [3] to test 25 zoological samples previously resulted positive by the classical Pan-herpes PCR. The positive PCR products were purified, sequenced, identified by BLAST analysis and translated into polypeptides, then a phylogenetic analysis was performed by maximum-likelihood (ML) method to study the degree of the *gB* gene conservation.
My results show that the *gB* gene is a good candidate to implement the Long-range PCR, it is located immediately up-stream and in the same orientation as the *DPOL* gene and encodes amino acid sequence sufficiently conserved to create good quality alignments appropriate for phylogenetic analysis.

Keywords: Nested Pan-herpes PCR; Long-range PCR; glycoprotein B gene.

References:
[1] Ehlers, B., Borchers, K., Grund, C., Frölich, K., Ludwig, H., Buhk, H.J. Detection of new DNA polymerase genes of known and potentially novel herpesviruses by PCR with degenerate and deoxyinosine-substituted primers. *Virus Genes*, **1999**, *18*, 211-220.
doi: 10.1023/a:1008064118057.
[2] Prepens, S., Kreuzer, K.A., Leendertz, F., Nitsche, A., Ehlers, B. Discovery of herpesviruses in multi-infected primates using 811 locked nucleic acids (LNA) and a bigenic PCR approach. *Jornal Virology*, **2007**, *308*: 317-329.
doi: 10.1186/1743-422X-4-84.
[3] Ehlers, B., Dural, G., Yasmum, N., Lembo, T., de Thoisy, B., Ryser-Degiorgis, M.-P., Ulrich, R.G., McGeoch, D.J. Novel mammalian herpesviruses and lineages within the *Gammaherpesvirinae*: Cospeciation and interspecies transfer. *Journal Virolology*, **2008**, *82*, 3509–3516.
doi: 10.1128/JVI.02646-07.

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Short and long-term effects on gut microbiota of oregano essential oil in dairy calves.

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This study aimed to determine how an early supplementation with EO in milk replacement in dairy calves during pre-weaning and at adult age would influence the uncultured microbiota. For this aim, 16 dairy calves (3 days of age) were randomized to two groups (8 each): the control group (CON) and the treated (EO) group (providing 0.23 ml of oregano EO for 45 days). After weaning, calves were kept in a feedlot and fed *ad libitum* with a total mixed ration formulated to cover their nutritional requirements. The animals were weighed, and fecal samples were collected on days 45 (T1) and 370 (T2) to determine microbiota composition. The sequencing of the V3-V4 regions of the bacterial 16S rRNA gene was analysed through Qiime v. 1.9 and allowed the identification of 2 531 distinct OTUs. Further analysis revealed that the difference between the experimental groups (α-diversity) was significant when considering the time effect and treatment effect alone and combined. Within groups (β-diversity), significant clusters were found considering time but not treatment; therefore, diversity significance indices underlined that treatment was crucial for differences between groups, while time was for diversity within groups. Taxonomy evaluation showed instead 38 significantly differentially expressed genera: e.g., at T1, the EO group had a lower abundance of *Ruminococcaceae UCG-014*, *Faecalibacterium*, *Blautia*, and

Alloprevotella, and an increase of *Allistipes* and *Akkermansia* when compared to T2. The data suggest that an early EO supplementation in milk modulated the microbiota during pre-weaning and at adult age.

Keywords: Essential oils, dairy calves, microbiota

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Breed and temperature: can they be considered drivers of muscle development?

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Skeletal muscles develop under the control of early and late Myogenic Regulatory Factors (MRFs). Their expression can be influenced by genetic and environmental factors [1], such as the breed, already observed *in vivo* [2], and the temperature, studied *in vitro* but to be clarified *in vivo* [3].
This study aimed to evaluate the *in vivo* influence of different genetic backgrounds and seasons on the level of muscle development at birth, using newborn piglets as models. *Examined breeds:* rustic Italian Nero di Lomellina (NL), highly selected Commercial Hybrid (CH). *Considered seasons:* winter, summer.
Animals were not sacrificed for research purposes (OPBA_89/2021): newborn samples of *Longissimus dorsi* were collected from piglets of the two breeds crushed at birth under the sow and specimens were involved in morphological and molecular analyses. The effect of the temperature was assessed through the evaluation of specific myogenesis-related Heat Shock Proteins (HSPs).
Morphology: in summer NL showed bigger and more mature muscle fibres than NL in winter and CH in summer ($p<.05$). *Molecular-MRFs:* in winter NL showed a higher expression of early MRFs than NL and CH in summer ($p<.05$); on the other hand, in summer NL overexpressed late MRFs, compared to NL in winter ($p<.0001$) and CH in summer ($p<.001$)
Molecular-HSPs: Myogenesis-related HSPs resulted significantly overexpressed in summer in both breeds but they were always more expressed in NL than in CH ($p<.05$; $p<.01$)
These findings showed that breed and especially temperature can influence the level of myogenesis at birth in pigs, with an acceleration in muscle development in summer, mainly

evident in the rustic breed. This may represent a starting point to better investigate the epigenetic mechanisms involved in muscle development. Further studies are required to better clarify the joint role of breed and temperature in muscle development.
Further studies are required to better clarify the joint role of breed and temperature in muscle development.

Keywords: Myogenesis, breed, temperature

References:
[1] J. Chal and O. Pourquié, "Making muscle: skeletal myogenesis in vivo and in vitro," Development, vol. 144, no. 12, pp. 2104–2122, Jun. 2017, doi: 10.1242/dev.151035.
[2] S. S. Sodhi, "Differential Expression Profiling of Myogenic Regulatory Factor Genes in Postnatal Longissimus dorsi Muscle of Indigenous and Large White Yorkshire Breeds of Pigs," J. Anim. Res., vol. 11, no. 1, 2021, doi: 10.30954/2277-940x.01.2021.2.
[3] S. Pomella, M. Cassandri, F. Antoniani, S. Crotti, L. Mediani, B. Silvestri, M. Medici, R. Rota, A. Rosa, S. Carra, "Heat Shock Proteins: Important Helpers for the Development, Maintenance and Regeneration of Skeletal Muscles." Muscles 2023, 2, 187-203. https://doi.org/10.3390/muscles202001

VAS Days, 27-29th September 2023

So you think you got something? Translational research and entrepreneurship in reproduction

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This talk will give a bird eye view of the process of bringing a research discovery to the market through dissection and discussion of the following aspects: identification and protection of intellectual property, building the company, the founders team, funding and financing options, advisors and directors, product development, regulatory path and market choice, client research and market fit. Each aspect will be discussed through examples from companies in the reproductive field with a special emphasis of innovative start-ups in the field of reproductive health and reproductive medicine.

Keywords: Reproduction, entrepreneurship, business

VAS Days, 27-29th September 2023

Development of a procedure for the isolation, identification, and quality assessment of bovine round spermatids

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Round spermatid injection (ROSI) into the oocyte has been applied in cattle [1]. However, the success rate is still low, and no offspring have been born so far. One of the reasons for the limited efficiency is the ambiguous definition of a round spermatid (RS) that hampers its selection. Aims of this work are: to set up a protocol for RS isolation from bull testis; to define the morphological features for proper RS identification; to evaluate whether Spermatid Flower-Like Structure Protein (SPERT) [2]; can be used as a marker of RS in cattle, to assess RS quality immediately after their isolation (0 h) and after 24 h of culture (24 h).
RS were isolated with enzymatic tissue dissociation, discontinuous Percoll gradient, and using 10 μm mesh cell strainers. Morphological analysis was carried out using haematoxylin/eosin and DAPI staining. SPERT positivity was assessed both on testis and isolated cells. RS were cultured at 37°C in 5% CO_2 incubator. Their quality was studied by cell viability, DNA integrity and mitochondrial activity at 0 and 24 h. Statistical analysis was run using one-way ANOVA.
Morphological analysis revealed that 60% of the isolated cells were RS. They ranged between 7.0 - 12.0 μm in size, with centrally located nuclei and 1-3 nucleoli. SPERT signal was detected in the spermatid stages, both in testicular tissues and isolated cells. At 0 and 24 h, all cells were viable and had an intact DNA. Mitochondrial activity significantly increased when RS were cultured for 24 h, suggesting the activation of metabolic process and/or oxidative stress responses. Overall, our results indicate the possibility to combine different isolation steps to obtain an RS enriched population in the bovine. At the end of our protocol, cells were viable and preserved their morphology after 24 h, suggesting the possibility to culture RS before ROSI. The present work provides preliminary data for the development of an effective ROSI protocol in cattle.

Keywords: bovine, round spermatids, ROSI

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References:

[1] S. A. Ock et al., 'In vitro development of bovine oocytes reconstructed with round spermatids', Theriogenology, vol. 65, no. 7, pp. 1242–1253, Apr. 2006, doi: 10.1016/j.theriogenology.2005.08.005.

[2] D'Amours O, Calvo É, Bourassa S, Vincent P, Blondin P, Sullivan R. Proteomic markers of low and high fertility bovine spermatozoa separated by Percoll gradient. Mol Reprod Dev. 2019;86:999–1012. https://doi.org/10.1002/mrd.23174

VAS Days, 27-29th September 2023

Ovarian tissue banking in animal models

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Ovarian tissue (OT) banking is an emerging technique for fertility insurance which suffers from lack of optimization. The general aim was to establish successful OT banking protocols in feline and bovine models. Specific objectives were to: 1. establish a suitable fixative and evaluation technique for OT. This was achieved through testing of three different fixation protocols (Bouin, neutral buffered formalin and form-acetic). It was concluded that form-acetic (a new compound fixative) maintained a reliable OT architecture with excellent follicular morphology and good immunohistochemical signals [1]. 2. establish OT vitrification and culture protocols for bovine model. This was realized with vitrification of bovine OT and comparing two culture systems (agarose and culture inserts) for vitrified-warmed OT fragments. It was concluded that vitrification of culture-suitable fragments is achievable despite damaging effects of cryoprotectants, and in vitro culture is significantly affected by culture system employed. Here, agarose inserts maintained good follicle morphology, low follicle activation, and low apoptosis in OT parenchyma [2]. 3. establish and optimize a suitable protocol for vitrification and culture of feline OT. This objective was attempted by comparing three vitrification protocols (A, B and C) with different equilibration temperature and time for feline OT followed by melatonin supplementation in both vitrification and culture media. In all cases, morphology and expression of proliferative (Ki-67, MCM-7) and apoptotic markers (activated caspase-3) were evaluated. Here, it was partially concluded that, protocol C, with lower equilibration time at room temperature, has maintained a significant proportion of intact follicle population up to the final day of culture and is the most suitable for vitrification of feline OT [3] while evaluation of melatonin supplementation is ongoing.

Keywords: Fixation, vitrification, culture

Acknowledgments: part of this work was carried out at NOLIMITS, an advanced imaging facility established by the Università degli Studi di Milano.

References:

[1] Alkali, I. M., Colombo, M., Rodak, O., Nizanski, W., and Luvoni, G. C. Effect of fixatives and fixation period on morphology and immunohistochemistry of feline ovarian tissue. Manuscript in preparation for submission to Theriogenology.

[2] Alkali, I. M., Colombo, M., Luciano, A. M., Nizanski, W., Ali Hassan, H., Dziegiel, P., & Luvoni, G. C. Culture of vitrified bovine ovarian tissue on agarose gel inserts maintains follicle integrity. Reproduction, online ahead of print 2023, DOI: https://doi.org/10.1530/REP-23-0089.

[3] Alkali, I. M., Colombo, M., De Iorio, T., Piotrowska, A., Rodak, O., Prochowska, S., Luvoni, G. C. Feline ovarian tissue vitrification: fixation may affect follicle grading. Proceeding of ISCFR-EVSSAR 2020+2 – June 30th- July 2nd, 2022, p64, Milan, Italy.

VAS Days, 27-29th September 2023

Amniotic-derived extracellular vesicles improve fertility of mares affected by chronic degenerative endometritis

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Early embryo loss is a common cause of pregnancy failure in equines [1]. During the first stages of pregnancy, maternal and embryo cells establish a strict interaction by the secretion of specific factors into the surrounding environment, including extracellular vesicles (EVs), acknowledged to be essential players in this communication. EVs are lipidic bi-layered particles actively secreted by cells to transfer lipids, proteins, DNA and RNAs, able to influence the biological activity of recipient cells [2]. Pathological alterations of endometrial tissue might hinder this exchange of signals, compromising embryo adhesion and development. Chronic degenerative endometritis (CDE) is a major responsible of reduced fertility in mares and is characterized by progressive and severe alteration of glandular structures accompanied by fibrosis of the surrounding stroma [3]. The decline in fertility observed in CDE-affected mares might be due also to a deficiency in fetal-maternal crosstalk. For this reason, the aim of this work is to restore a proper interaction between embryo and maternal counterparts and possibly to improve the condition of the endometrium altered by CDE through the intrauterine administration of EVs. Amniotic-derived EVs (AMC-EVs) were isolated and characterized before their use for treatment, which consisted in the intrauterine administration of $20x10^9$ EV diluted in 50 ml of sterile saline solution. On a total of n=12 mares enrolled in this study, with a history of failed reproductive attempts, n=11 had conception. The histological classification of their endometrial condition generally did not improve, meaning AMC-EVs were not able to induce a regeneration of the damaged tissue. However, it is possible to hypothesize that the integration of AMC-EVs probably drove a significant restoration of the intercellular

communication in an altered uterine environment where EVs production or delivery were defective.

Keywords: extracellular vesicles, chronic degenerative endometritis, fetal-maternal communication

References:

[1] Merkt, H.; Günzel, A. -R. A Survey of Early Pregnancy Losses in West German Thoroughbred Mares. *Equine Vet. J.*, **1979**, *11*. DOI: 10.1111/j.2042-3306.1979.tb01359.x

[2] Camussi, G.; Deregibus, M.C.; Bruno, S.; Cantaluppi, V.; Biancone, L. Exosomes/Microvesicles as a Mechanism of Cell-to-Cell Communication. *Kidney Int.*, **2010**, *78*, 838–848. DOI: 10.1038/ki.2010.278

[3] Rebordão, M.R.; Galvão, A.; Szóstek, A.; Amaral, A.; Mateus, L.; Skarzynski, D.J.; Ferreira-Dias, G. Physiopathologic Mechanisms Involved in Mare Endometrosis. *Reprod. Domest. Anim.*, **2014**, *49*, 82–87. DOI: 10.1111/rda.12397

VAS Days, 27-29th September 2023

Does energetic imbalance affect the preimplantation development of bovine embryos in vitro?

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Both under- and over-nutrition negatively impact the offspring's health, in agreement with the Developmental Origins of Health and Disease [1]. However, the definition of the critical window(s) of exposure and the underlying causative mechanisms are still a matter of debate. Major events of nuclear and epigenetic remodeling occur during the preimplantation embryo development, when waves of DNA de-methylation/re-methylation take place. Starting from this consideration, the present project aims at addressing the emerging hypothesis that nutrient excess or restriction during the pre-implantation embryo development is sufficient to induce changes in the epigenetic profile of the embryos that can be propagated up to the post-natal life and adulthood. To address this hypothesis, we developed an in vitro model to study the exposure to nutrient imbalance in preimplantation bovine embryos. Then, we conducted parallel isolation of DNA and RNA from the same embryos. After quality control, gene expression was investigated by RNAseq. Two bioinformatic pipelines were developed generating lists of differentially expressed genes. We found that only a handful of transcripts were differentially expressed. Validation experiments are in progress to disentangle which pipeline better capture the biological differences. Furthermore, specification of cell lineages (inner cell mass, trophectoderm, and primitive endoderm) was tracked using immunofluorescence of lineage markers and image analysis. This experiment, aiming to uncover if changes in the energetic substrates favor the development of a specific cell lineage compared to the others, did not show differences. Thus far, our experiments seem to indicate that energetic imbalance does not affect the cell specification and gene expression of in vitro cultured bovine blastocysts. We are currently testing if changes in the epigenetic profiles may be already in place at this stage, without triggering evident effects on gene expression yet.

Keywords: developmental origins of health and disease, bovine embryo, energetic substrates

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References:

[1] Barker DJ. The origins of the developmental origins theory. J Intern Med. 2007;261(5):412-7. doi: 10.1111/j.1365-2796.2007.01809.x. PubMed PMID: 17444880.

VAS Days, 27-29th September 2023

Bioinformatics approaches to investigate the regulation of maternal mRNA translation in bovine oocytes

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In oocytes resumption and completion of meiosis I are largely driven by post-transcriptional mechanisms on a background of repressed mRNA transcription [1]. However how translation is regulated in maturing oocytes, especially in higher-order mammals, has not been addressed in depth, mainly for methodological constraints in isolating polysome-associated mRNAs from oocytes. With this is mind, we exploited deposited datasets to gain information on mRNAs polysome association (GSE56603 and GSE196484) and polyadenylation (GSE61717) in immature (GV) and mature (MII) bovine oocytes. GEO-retrieved datasets were re-analysed using R-Studio. Differential expression was determined using edgeR (Bioconductor – Software packages). AdjP<0.05 and LogFC>2 were considered. GO analyses were conducted https://david.ncifcrf.gov/. Correlation between datasets was analysed by Pearson's $\chi 2$ test [2].
While the overlap of differentially polyadenylated/polysome-associated genes between datasets was limited when comparing MII oocytes, all comparisons conducted in GV oocytes were substantial. These results indicate that all the experimental approaches yield comparable results for immature oocytes, but that this homogeneity is somehow lost with in vitro maturation (IVM), thus suggesting that the different in vitro culture conditions are responsible for a deviation in the pattern of maternal mRNA translation. This hypothesis has been addressed *in silico* by comparing the previously mentioned datasets with the pattern of polysome-association in mature mouse oocytes in response to the activation of the EGF network (GSE46640). Indeed, only one dataset showed a possible association with the follicular EGF activation.
Our meta-analyses seem to point out that the inability of recreating a cultural microenvironment that supports the program of maternal mRNA translation may be a

factor that contributes to the lower embryo development observed with in vitro matured oocytes compared to in vivo matured ones.

Keywords: In vitro maturation, mRNA translation, oocyte quality.

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References:

[1] Thelie A, Papillier P, Pennetier S, Perreau C, Traverso JM, Uzbekova S, et al. Differential regulation of abundance and deadenylation of maternal transcripts during bovine oocyte maturation in vitro and in vivo. BMC Dev Biol. 2007;7:125. Epub 20071107. doi: 10.1186/1471-213X-7-125. PubMed PMID: 17988387; PubMed Central PMCID: PMC2211488.

[2] Rabaglino MB, Conrad KP. Evidence for shared molecular pathways of dysregulated decidualization in preeclampsia and endometrial disorders revealed by microarray data integration. FASEB J. 2019;33(11):11682-95. Epub 20190807. doi: 10.1096/fj.201900662R. PubMed PMID: 31356122; PubMed Central PMCID: PMC6902722.

VAS Days, 27-29th September 2023

Gene expression profiling of isolated bovine primordial follicles for the improvement of in vitro culture systems for fertility preservation

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In several mammalian species, the pool of resting primordial follicles is the source of developing and fertilizable ova for the entire female reproductive life span. Only a limited number of primordial follicles are recruited into the growing follicle pool, and the remaining primordial follicles are either maintained in a quiescent state or die directly from this dormant state. Current Assisted Reproductive Technologies (ARTs) have successfully been employed from the early antral stage (>500µm) onwards in large mammals, like bovine [1]. However, to exploit the preantral follicular reserve, the *in vitro* follicle growth from the primordial follicle (PMF) stage (<40µm) in bovine is still experimental and has only been achieved in mice [2]. This is due to high follicle mortality after a culture period following PMF isolation from the surrounding tissue [3]. Our study aims to identify programmed cell death mechanisms triggered in the isolated bovine primordial follicles after a short period in culture by contrasting the transcriptome profiles of freshly isolated against cultured PMF. Our findings showed a significant reduction in follicle viability after 16 hours of culture, while no significant differences were observed between 16 and 24 hours ($p<0.0001$ and $p=0.9753$, respectively, two-way ANOVA followed by Tukey's test). Transcriptome analysis of freshly isolated and 16-hour cultured PMF revealed 1430 differentially expressed genes (FDR<0.05).
Here, we report for the first time the gene expression profiling of isolated bovine PMF at collection and after a short period of culture following the triggering of cell death. We hypothesize that activated cell death signalling networks could be delineated through the transcriptome analysis and subsequently inhibited to improve current culture systems. This would contribute to advancing culture strategies targeting preantral follicle development to pursue female fertility preservation interventions.

Keywords: primordial follicle, in-vitro folliculogenesis, programmed cell death

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References:
[1] Garcia Barros, R., et al., A refined culture system of oocytes from early antral follicles promotes oocyte maturation and embryo development in cattle. Reproduction, 2023. 165(2): p. 221-233. DOI: 10.1530/REP-22-0277
[2] Eppig, J.J., Development in vitro of mouse oocytes from primordial follicles. Biology of Reproduction, 1996. 54(1): p. 197-207. DOI: 10.1095/biolreprod54.1.197
[3] Laronda, M.M., et al., Alginate encapsulation supports the growth and differentiation of human primordial follicles within ovarian cortical tissue. J Assist Reprod Genet, 2014. 31(8): p. 1013-28. DOI: 10.1007/s10815-014-0252-x

VAS Days, 27-29th September 2023

Investigation on the putative physiological function of ROS during oogenesis

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Reactive Oxygen Species (ROS) are highly reactive molecules that result from the intermediate steps of oxygen reduction by electrons during cellular metabolism. Embryos are known for three free radicals during their normal aerobic metabolism: hydrogen peroxide (H2O2), superoxide anion (O2 −) and hydroxyl radical (OH−). Oxidative stress refers to the imbalance of the redox system. In this state the free radicals notably escalate to a level surpassing the capacity of the endogenous antioxidant system. This stress is known to be the cause of many cellular alterations, such as protein, lipid, and DNA damage. However, their importance in physiological processes in oogenesis and embryogenesis is frequently overlooked. ROS also act as second messengers in physiological cell signaling and control pathways [1] , therefore they have an important role in embryonic development, cell proliferation and differentiation. Our study aims to investigate the physiological role of ROS during the final phase of oocyte growth and maturation and the effect on oocyte quality and competence of different ROS concentrations. To achieve this, it is essential to utilize a ROS detection and localization system that reports ROS levels in real time using the technology that is available to us. Literature has offered different possibilities, but a common limitation of the probes used in these studies is the absence of specificity and ability to track ROS fluctuations accurately. Comparison and optimization of such systems is a priority and the first step for this project, being followed by the investigation of factors that can induce and inhibit or scavenge ROS in the oocyte. Preliminary steps to address this issue will be presented. Work supported by MSCA-ITN-ETN 2019 EUROVA n. 860960.

Keywords: Reactive oxygen species, oocyte, oogenesis

References:

[1] Jamil, M., Debbarh, H., Aboulmaouahib, S., Filali, O. A., Mounaji, K., Zarqaoui, M., Saadani, B., Louanjli, N., & Cadi, R. (2020). Reactive oxygen species in reproduction: harmful, essential or both? Zygote, 28(4), 255-269. https://doi.org/10.1017/s0967199420000179

VAS Days, 27-29th September 2023

Establishing an experimental protocol to detect reactive oxygen species in bovine early embryonic development: preliminary data

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Reactive oxygen species (ROS), and especially H_2O_2, are often considered wrongdoers; however, recent studies have uncovered the importance of ROS as secondary messengers in many physiological processes. In particular, the interplay between Calcium and ROS signalling is emerging as a key regulator in many biological functions. However, little is known about this interplay in mammalian early development. One of the limitations is the lack of standardized methods to detect ROS fluctuations in gametes and early embryos. To address this issue, we standardized an experimental model to specifically detect H_2O_2 during early embryonic development using the ratiometric and genetically encoded redox indicators (GERI's) [1]. In our experimental setup, we used the mitochondrially targeted Hyper7 plasmids commercially available from Addgene (USA) [2] to produce mRNA through in vitro transcription. The mRNA was then microinjected it into the GV/MII bovine oocytes. To validate the ability of the probe in detecting changes in H_2O_2 content in the oocytes, the microinjected oocytes were cultured in the presence of exogenous pro-oxidants (H_20_2 and t-BOOH) and the ratiometric signal was detected using time lapse - spinning disk microscopy using appropriate excitation/emission settings. Our preliminary experimental data suggest that we have successfully established a protocol for the real-time monitoring of mitochondrial H_2O_2 in bovine oocytes/zygotes. We also established a method to detect calcium oscillations in bovine zygotes using Fluo4 AM [3]. In the future, we plan to integrate these two methods to decipher the role of calcium and ROS interplay in early bovine embryonic development.

Keywords: ROS, Hyper7-MLS, GV/MII bovine oocytes.

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References:
[1] Murphy MP, Bayir H, Belousov V, Chang CJ, Davies KJA, Davies MJ, et al. Guidelines for measuring reactive oxygen species and oxidative damage in cells and in vivo. Nat Metab. 2022;4(6):651-62. Epub 20220627. doi: 10.1038/s42255-022-00591-z. PubMed PMID: 35760871.
[2] Pak VV, Ezerina D, Lyublinskaya OG, Pedre B, Tyurin-Kuzmin PA, Mishina NM, et al. Ultrasensitive Genetically Encoded Indicator for Hydrogen Peroxide Identifies Roles for the Oxidant in Cell Migration and Mitochondrial Function. Cell Metab. 2020;31(3):642-53 e6. Epub 2020/03/05. doi: 10.1016/j.cmet.2020.02.003. PubMed PMID: 32130885; PubMed Central PMCID: PMC7088435.
[3] Wang F, Meng TG, Li J, Hou Y, Luo SM, Schatten H, et al. Mitochondrial Ca(2 +) Is Related to Mitochondrial Activity and Dynamic Events in Mouse Oocytes. Front Cell Dev Biol. 2020;8:585932. Epub 20201027. doi: 10.3389/fcell.2020.585932. PubMed PMID: 33195238; PubMed Central PMCID: PMC7652752.

VAS Days, 27-29th September 2023

In vitro methods for simulating ruminal fermentation

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Ruminants can transform vegetable food, which cannot be used directly by humans, into high quality animal products (milk and meat). This process is possible thanks to the complex microbial population in the rumen. The rumen and everything that happens in it has been an important scientific challenge for researchers. It is a fluctuating system, in which fermentation, secretion and absorption processes of products and flows occur simultaneously in different directions and at different speeds, and as an ecosystem, it hosts a constant action-reaction relationship between biotic factors (rumen microorganisms) and abiotic factors (such as temperature, pH, etc.) and in turn with the host animal. Although the rumen is accessible by surgical means, the study of these processes *in vivo* is complex due to the difficulty of controlling all the factors involved.
As a result of such complexity of the rumen, the difficulty of working with fistulated animals and the increasing public awareness of animal rights, several techniques to simulate the *in vitro* ruminal fermentation have been developed over the years. These *in vitro* systems are used for assessing the nutritive value of animal feeds, to optimize the rumen fermentative processes, to improve the quality of the products, to increase the animal welfare and to reduce the contaminant emissions, basically methane and nitrogen. A better knowledge of the process that occurs in the rumen and the improvement of such *in vitro* systems will allow a better simulation of the ruminal fermentative processes. This presentation focus on two *in vitro* systems widely used (batch cultures of ruminal microorganisms and RUSITEC fermenters) and on different factors affecting *in vitro* rumen fermentation.

Keywords: *in vitro* rumen fermentation, batch cultures, RUSITEC

VAS Days, 27-29th September 2023

Evaluation of the difference in contamination by per- and poly-fluorinated substances (PFASs) in wild boar and swine tissues sampled in the same area.

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Wild boars are considered bioindicators of environmental pollution since they can be easily exposed to persistent organic contaminants through the oral route. Domestic pigs belong to the same Sus scrofa species and may present the same exposure route and accumulation patterns for many xenobiotics, including PFASs. Due to their physico-chemical properties PFASs resist biodegradation. PFASs exposure lead to disruption of endocrine functions, cause mutagenic and carcinogenic effects [1]. Monitoring the presence of PFASs in the environment only may be not sufficient to determine the real exposure of the biota and biomonitoring could be more useful. This study aimed to quantify PFASs concentrations in muscle and liver from wild boars and swine bred in a semi-extensive way in a specific area of Northern Italy. Muscle and liver samples from 20 wild boars, killed during the hunting activity, and from 20 slaughtered swine were collected. The detected substances were PFOA and PFOS, PFBS and NEtFOSAA. The compounds were quantified with LCMSMS. All compounds were detected in all samples, except NEtFOSAA which resulted in traces in swine liver. PFOA content resulted statistically higher in wild boar liver than swine with a concentration of 18.85 ± 5.41 $\mu g \cdot kg^{-1}$ and 12.7 ± 6.34 $\mu g \cdot kg^{-1}$ respectively. NEtFOSAA resulted significantly higher in wild boar liver with mean concentrations 14.61 ± 23.34 $\mu g \cdot kg^{-1}$ and detected in traces in swine liver. No differences in muscle concentration for all PFASs were identified. The overall higher content of PFASs in liver and muscle from wild boar could be attributable to different patterns of accumulation, related to the different attitudes of these animals, stronger bound to the natural environment and the agricultural activity and with a longer lifespan [2]. Wild boar resulted more contaminated by these emerging environmental pollutants and could be used as bioindicator tools to assess their presence in an area, avoiding its use as pasture.

Keywords: bioindicators; ecotoxicology, endocrine disruptors

References:

[1] Parsons, J.R., Sáez, M., Dolfing, J., de Voogt, P., 2008. Biodegradation of perfluorinated compounds. Rev Environ Contam Toxicol 196, 53–71. https://doi.org/10.1007/978-0-387-78444-1_2.

[2] Pizzurro, D.M., Seeley, M., Kerper, L.E., Beck, B.D., 2019. Interspecies differences in perfluoroalkyl substances (PFAS)toxicokinetics and application to health-based criteria. Regulatory Toxicology and Pharmacology 106, 239–250. https://doi.org/10.1016/J.YRTPH.2019.05.008

VAS Days, 27-29th September 2023

Nutritional interventions to modulate the gut microbial core and ameliorate young monogastrics and ruminants health.

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Weaning is a complex process in which changes in the gut environment could markedly affect animals health during the production cycle. Both in young monogastrics and ruminants, the gut microbiota plays a key role in conditioning the health of the host. Given that, optimizing feed additives administration to modulate gut microbiota, and increasing gut health represent an ideal strategy to contain enteric diseases and ameliorate production and health [1;2].
In a first trial aimed to evaluate the effects on gut health of different dietary ratios of Zn and Cu, 120 piglets were divided into 4 treatments after weaning: a positive control (PC, 2500 ppm of Zn through ZnO) and 3 groups in which Cu and Zn were supplemented through potentiated Zn and monovalent Cu at European levels of inclusion (EU, 120 ppm of Zn; 140 ppm of Cu) and non-European (non-EU^{+}, 300 ppm of Zn; 200 ppm of Cu and non-EU^{-} 300 ppm of Zn; 140 ppm of Cu). Fecal samples were collected at 14 and 28 d and sequenced in V3-V4 region of the 16S rRNA gene. Alpha-diversity was higher in EU samples if compared to non-EU^{-} 14 days after weaning. Increased abundances of *Prevotella*, *Prevotellaceae* and *Lachnospiraceae* and decreased *Sutterella* were detected in EU in comparison with non-EU^{-} at 14 d.
In a second study focused on the safety and efficacy of *Bacillus Coagulans* DSM 32016, 20 female Holstein calves were divided into 2 groups after weaning: a control group (CTR), fed with a standard milk replacer (MRP) plus concentrate and a treated group (T) fed with a supplementation of *Bacillus coagulans* DSM 32016 at 10^{9} CFU/kg of MRP. Fecal samples were collected on day 0, 28 and 56 on trial and subjected to 16S rRNA sequencing and then analysed. *Bifidobacterium* and *Lactobacillus* genera were enhanced by the treatment, while potentially harmful *Clostridium sensu stricto* 1 decreased in T. In conclusion, these nutritional interventions revealed positive effects on gut health of young monogastrics and ruminants.

Keywords: Nutrition, Weaning, Microbiota

References:

[1] Pluske JR, Turpin DL, Kim JC. Gastrointestinal tract (gut) health in the young pig. Anim Nutr. 2018 Jun;4(2):187-196. doi: 10.1016/j.aninu.2017.12.004. Epub 2018 Jan 10. PMID: 30140758; PMCID: PMC6104527.

[2] Slanzon GS, Ridenhour BJ, Moore DA, Sischo WM, Parrish LM, Trombetta SC, McConnel CS. Fecal microbiome profiles of neonatal dairy calves with varying severities of gastrointestinal disease. PLoS One. 2022 Jan 4;17(1):e0262317. doi: 10.1371/journal.pone.0262317. PMID: 34982792; PMCID: PMC8726473.

VAS Days, 27-29th September 2023

Dietary energy source on Italian Holstein heifers feed efficiency

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Improving feed efficiency of animals in livestock production is one of the objectives for the near future of the agri-food sector, to improve the sustainability of farms and the producer profitability [1]. The study of the relationship between feed efficiency, dry matter intake and methane production is the basis of possible selective choices for more efficient animals. The objective of the trial was to study the impact of diets characterized by different sources of energy on feed efficiency and methane emissions in Italian Holstein heifers. Sixteen heifers were divided in two homogenous groups and included in a crossover design. The trial lasted in total 10 weeks: after one week of adaptation, a 4-weeks treatment period was applied followed by one week of adaptation and a second 4-weeks treatment period. Two TMR isoenergetic and isonitrogenous diets were administrated during the experimental period, a maize flour-based diet or a hydrogenated fat-integrated diet. Feed intake was measured daily and live body weight and BCS weekly. At the beginning and at the end of each experimental period, feces, urine, rumen content, and saliva pH were recorded. Contextually methane emissions were measured using a laser detector methane, 4 times a day for 5 minutes/animal. From collected data residual feed intake (RFI) was calculated for each diet and for each animal. According to RFI cows were grouped in high RFI (RFI>0.5), medium RFI (-0.5<RFI<0.5) and low RFI (RFI<-0.5). Data were analyzed by a MIXED procedure of SAS and significance was declared at $P<0.05$. Feed intake was higher ($P<0.05$) when heifers were fed hydrogenated fat compared with corn-based diet. No differences were detected for LBW and BCS between treatments. Fecal and urine pH values were lower when animals were fed corn-based diet compared with fat-included diet. ADG and FCR were not affected by the treatment ($P>0.05$). Methane emissions were lower when animals were fed corn-based diet compared with fat-included diet ($P<0.05$). Comparing the RFI values of the two diets we observed that 20% of the animals experienced a strong change in RFI from high to low RFI or vice versa, 60% of the animals experienced less RFI change while the remaining animals did not undergo changes in RFI. In conclusion RFI and methane emissions may vary based on the source of energy included in the diet.

Keywords: Feed efficiency, RFI, methane

References:
[1] VandeHaar, M.J., et al., Harnessing the genetics of the modern dairy cow to continue improvements in feed efficiency. J Dairy Sci, 2016. 99(6): p. 4941-4954.

VAS Days, 27-29th September 2023

Air Quality Assessment in Pig Farming: The Italian Classyfarm

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The ClassyFarm protocol provides guidelines for assessing animal welfare on Italian farms. It is a risk assessment tool that allows the evaluation of animal welfare and management from different points of view.
The risk categorisation introduced by ClassyFarm in pig farms is based on the EU's requirement to avoid tail docking in piglets and minimize stressful aspects that can lead to aggressive behaviour in pigs. Good management and proper infrastructure in intensive systems are key factors in improving animal welfare [1].
The protocol covers various aspects of farm management. Still, we focus on the environmental quality assessment because animal confinements can present high levels of harmful gasses that could threaten animal safety, causing discomfort and distress, thus reducing their welfare and health [2]. Specific indications are given on the microclimatic requirements of livestock indoor environment (temperature, relative humidity, dustiness), expression of air quality, especially regarding harmful gases such as ammonia (NH_3) and carbon dioxide (CO_2), and the correct procedure of measuring dust and gasses.
This review aims to frame ClassyFarm's guidance for conducting a farm risk assessment based on pig welfare, examining the assessment of environmental quality on pig farms and its impact on animal health and welfare, and describing how to evaluate the environmental quality in farms.

Keywords: air quality assessment; animal welfare; pigs

References:
[1] Gade, P.B. Welfare of animal production in intensive and organic systems with special reference to Danish organic pig production. Meat Sci. 2002, 62, 353–358. DOI: 10.1016/s0309-1740(02)00123-7.
[2] Ni, J.-Q.; Erasmus, M.A.; Croney, C.C.; Li, C.; Li, Y. A critical review of advancement in scientific research on food animal welfare-related air pollution. J. Hazard. Mater. 2021, 408, 124468. DOI: 10.1016/j.jhazmat.2020.124468

VAS Days, 27-29th September 2023

Evaluation of *Phaseolus vulgaris* LPA and *Lupinus albus* as alternative protein sources to soybean in weaned piglets

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Due to rapid population growth and the limited self-sufficiency of protein sources for livestock, becomes essential to look for alternatives to conventional protein used in feed formulation [1]. In this scenario, the re-introduction of grain legumes into crop production system may represent a valid option. [2]. The overall objective of this study was to evaluate the replacement of soybean meal in piglet diet by two varieties of legumes, *Phaseolus vulgaris LPA* and *Lupinus albus,* genetically selected for reduced content of anti-nutritional factors (phytic acid (LPA) and alpha-amylase (αAI)) [3].
Firstly, the two varieties were characterized *in vitro* for i) nutritional profile, ii) digestibility, and iii) Effect on cell viability on IPEC-J2 cells. *In vitro* analyses confirm the known nutritional value of meals tested. Also, highlighting that they did not adversely affect the viability of the intestinal epithelium. Then, the effect of the dietary inclusion of *Phaseolus vulgaris* or *Lupinus albus* meals in weaned piglets' diet was evaluated *in vivo*. With this purpose, two experimental facilities and a total of 34 animals were involved in the trial for 28 days. Animals were divided into four experimental groups: LUP (n: 10), receiving 10% of *L. albus*; FAG (n: 7), receiving 10% of *P. vulgaris* LPA; CTRL 1 (n:10) and CTRL 2 (n: 7), receiving basal diet with 7.3% soybean. Following parameters were analyzed: i) zootechnical performances, ii) protein digestibility, iii) mineral content in feces, and iv) fecal microbiota. *In vivo* trials revealed that treated groups grew less than the control (LUP: 17.63±5.86 Kg; FAG: 16.91±2.71 Kg; CTRL1: 24.80±4.42 Kg; CTRL2: 22.74±2.35 Kg). Nevertheless, the analyses performed on the fecal samples showed similar contents in terms of protein in both experimental groups confirming that the introduction of the considered legumes does not affect the protein intake of animals.
In conclusion, the introduction of *Phaseolus vulgaris* LPA and *Lupinus albus* in animals' diets could be considered in order to reduce protein dependence from soybean for a local production particularly suitable for organic farming.

Keywords: Legumes, Protein source, Piglets

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References:

[1] Rauw, W.M., Gómez Izquierdo, E., Torres, O. et al. Future farming: protein production for livestock feed in the EU. Sustain Earth Reviews, 2023, 6, 3. DOI: 10.1186/s42055-023-00052-9

[2] Ferreira H., Pinto E., and Vasconcelos M.W. Legumes as a Cornerstone of the Transition Toward More Sustainable Agri-Food Systems and Diets in Europe. Front. Sustain. Food Syst. 2021, 5, 694121. DOI: 10.3389/fsufs.2021.694121

[3] Campion, B., Sparvoli, F., Doria, E. et al. Isolation and characterisation of an lpa (low phytic acid) mutant in common bean (Phaseolus vulgaris L.). Theor Appl Genet, 2009, 118, 1211–1221. https://doi.org/10.1007/s00122-009-0975-8

VAS Days, 27-29th September 2023

Traditional diet vs Circular diet: effect of the partial replacement of corn and soybean meals with bakery former food products and wet distillers grains on production performance, health status, digestibility and environmental sustainability in fattening beef heifers

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The beef cattle sector is facing many sustainability challenges, such as greenhouse gases (GHG) emissions, land and water use and pollution, human-edible resources consumption and increase in the demand of meat. Losses along the food chain represent another dilemma. Using alternative resources such as former food products instead of traditional feeds can valorise those losses, reduce food–feed competition and mitigate the environmental impact of beef cattle.
The effects of the partial substitution of corn and soybean meals with bakery former food products (BFF) and liquid wheat distillers grains (WDGs), in beef cattle on environmental sustainability and production efficiency were evaluated. Limousine heifers (n=408) were divided in two balanced groups: (i) Traditional diet; (ii) Circular diet with average as-fed 1.5 kg BFF and 1.5 kg WDGs as substitute for 1.6 kg corn and 0.3 kg soybean meals. The environmental impact of the feed substitution was analysed considering GHG emissions (kg CO_2 eq), water (H_2O, L), and land use (LU, m^2) as well as inclusion of human-edible feeds (HE, kg). Animals were weighted individually at day 0, day 92 and day 145. The average daily gains (ADG) were calculated. The pen feed intake (FI) was evaluated weekly. Pen feed conversion rate (FCR) was calculated. Apparent total tract digestibility (aTTD) was evaluated monthly. Carcass weights and muscle and fattening scores were recorded individually. Colorimetric characteristics and pH were evaluated in 20 animals per group at 24 h post-mortem. The Circular diet led to a reduction per kg of cold carcass weight (CCW) of 1.00 kg CO_2 eq of GHG, 72.38 L of H_2O, 1.20 m^2 of LU, and 0.95 kg of HE ($p<0.0001$).

Growth performances and carcass characteristics. The aTTDs of sugar and pectin were higher ($p<0.0001$) in the Circular group. Replacing traditional feeds with BFF and WDGs reduced the environmental impact and the food competition, in accordance with circular economy principles.

Keywords: alternative feeds; circular economy; beef cattle.

VAS Days, 27-29th September 2023

Evaluation of rainbow trout (Oncorhynchus mykiss) organotypic intestinal platforms: cellular responses after long-term exposure to *in vitro* digested feed

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Reliable and predictive *in vitro* models would support the search for new raw materials that can improve current fish diets. We recently developed some rainbow trout (RT) cell-based organotypic intestinal models demonstrating that the platform type modulates the degree of cell differentiation achieved *in vitro* [1]. Here, we studied whether such differentiation correlates with their response to a prolonged exposure to a diet rich in fish meal. We seeded the RTpi-MI and RTdi-MI cell lines derived respectively from the proximal or the distal intestine on (1) the polyethylene terephthalate (PET) culture inserts ThinCert™ (TC); (2) the TC coated with RT fibroblasts embedded within Matrigel® (MMfb); and (3) the highly porous polystyrene scaffold Alvetex™ also populated with fibroblasts (AV). Platforms have been exposed for 21 days to increasing concentrations of feed pellets digested *in vitro* by gastric and intestinal RT enzymes (IVD). Cells exposed to culture medium without IVD were used as controls. Trans-epithelial electrical resistance significantly increased when IVD was supplemented. At the end of culture, epithelial cells formed multilayers irrespective of cell line or platforms if exposed to IVD, but not in the controls. This proliferative activity followed a dose-dependent pattern in the AV, did not vary in MMfb, and was highly variable in the TC. Moreover, IVD induced the formation of a few goblet-like cells characterized by rounded vacuoles. These changes suggest a de-differentiation of

the enterocytes and their partial differentiation towards the secretory lineages. In summary, the three platforms reacted differently to a pronged exposure to IVD: TC quenched most of the cell responses, MMfb generated overly sensitive reactions, while the AV reacted mostly in a dose-dependent manner. Overall, AV generated more physiological results, being the most promising to perform nutritional studies.

Keywords: aquaculture, *in vitro*, organo-typic culture

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References:

[1] Verdile, N.; Camin, F.; Pavlovic, R.; Pasquariello, R.; Stuknyt˙ e, M.; De Noni, I.; Brevini, T.A.L.; Gandolfi, F. Distinct Organotypic Platforms Modulate Rainbow Trout (Oncorhynchus mykiss) Intestinal Cell Differentiation In Vitro. Cells 2023, 12, 1843. https://doi.org/10.3390/cells12141843

VAS Days, 27-29th September 2023

Evaluation of Antioxidant and Bacterial Growth Inhibitory activities of in vitro digested nutraceutical products

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In 2050, one of the major causes of mortality will be untreatable bacterial infections due to the spread of antibiotic resistance [1]. The aim of the present research is the study of bioactive compounds and their combination for the development of innovative nutraceutical products and the reduction of antibiotic administration [2], [3]. The study evaluates possible differences in antioxidant activity and growth inhibition capacity against porcine Escherichia coli O138 of Uriach S.r.l. nutraceuticals products (TFA and KP) and verify the activities even following the in vitro digestion. ABTS Radical Cation Decolorization Assay was used to evaluate the antioxidant activity at different concentrations. A microplate assay was performed to assess the pathogen inhibitory growth capacity, and Escherichia coli O138 was considered pathogen model for its verocytotoxic properties. The results confirmed an antioxidant activity among nutraceuticals. The non-digested products reached up to 90% free radical inhibition, the digested products around 50% with an increased antioxidant activity during the intestinal phase of the in vitro digestion of KP due to the production of new and unknown bioactive compounds. The outcomes underlined a bacterial growth inhibitory effect of the non-digested products up to 1:16 for TFA and KP (respectively $p<0.0027$; $p<0.0132$). Results on digested TFA suggest an inhibitory effect until the third hour of incubation at 1:4 concentration ($p< 0.0198$). Digested KP maintains a statistically significant difference in the first hour of incubation at 1:8 ($p<0.0001$), after that, the E. coli O138 growth of treatment and control was comparable. In conclusion, the products maintained antioxidant capacity in a dose-dependent manner both in digested and non-digested conditions, suggesting the maintenance of bioactive properties even during the simulated intestinal transit. Non-digested TFA and KP maintained growth inhibition activity of E. coli O138 over time and at different concentrations, even up to 6 hours; the digested products maintained the inhibitory activity only at the very first hours, suggesting a lower time-lasting activity.

Keywords: Nutraceutics; Antioxidant, Antimicrobial

Acknowledgments

References:

[1] Frazzini, S.; Scaglia, E.; Dell'Anno, M.; Reggi, S.; Panseri, S.; Giromini, C.; Lanzoni, D.; Sgoifo Rossi, C.A.; Rossi, L. Antioxidant and Antimicrobial Activity of Algal and Cyanobacterial Extracts: An In Vitro Study. *Antioxidants* **2022**, *11*, 992. https://doi.org/10.3390/antiox11050992

[2] Pandey, Manisha, Rohit K. Verma, and Shubhini A. Saraf. "Nutraceuticals: new era of medicine and health." *Asian J Pharm Clin Res* 3.1 (2010): 11-15.

[3] Aslam B, Khurshid M, Arshad MI, Muzammil S, Rasool M, Yasmeen N, Shah T, Chaudhry TH, Rasool MH, Shahid A, Xueshan X and Baloch Z (2021) Antibiotic Resistance: One Health One World Outlook. *Front. Cell. Infect. Microbiol.* 11:771510. doi: 10.3389/fcimb.2021.771510

VAS Days, 27-29th September 2023

Food inspection for consumer's safety: incidence of perfluoroalkyl substances in commercial eggs from Northern Italian markets.

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According to the dietary exposure pathway, eggs are known to play an important role in a balanced diet because they are a rich source of protein and several essential nutrients; however, eggs and egg products are also recognized by the European Food Safety Authority as one of the main sources of poly and per-fluoroalkyl substances [1]. For food safety and consumer health purposes, in this study, the presence of PFASs was analysed in eggs produced by hens raised in Northern Italian regions (Piedmont, Lombardy, Veneto, Emilia Romagna, and Friuli Venezia Giulia), which are known to be PFASs contaminated areas [2]. The analysis was performed on 65 samples by high-performance liquid chromatography coupled to high-resolution mass spectrometry.
6 out of 17 PFASs were detected in the samples: PFBA, PFOS, PFNA, PFOA, PFUndA, and PFDoA. The greatest presence of PFASs was found in eggs from farms of Veneto and Emilia Romagna and the most detected PFASs were PFBA and PFOS, with mean concentrations of 0.30 ± 0.15 and 0.05± 0.00 ng g^{-1}, respectively. Considering the most recent updates (European Regulation (EU) 2022/2388) [3], the values detected in the samples analysed in the present study were well below the maximum limits value set by the European Union (EU). Furthermore, for the sum of the main four PFASs (PFOA, PFOS, PFNA, and PFHxS), the highest concentration found in the analysed samples was 0.05 ng g^{-1}, well below the maximum limit set by EU. Risk characterization confirmed that the consumption of eggs from hens raised in PFASs contaminated areas should not represent a risk for Italian consumers.

Keywords: Food safety; PFASs; HPLC-HRMS.

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References:

[1] European Food Safety Authority. (2020). Risk to human health related to the presence of perfluoroalkyl substances in food. EFSA Journal, 18(9), e06223.
DOI: 10.2903/j.efsa.2020.6223.
[2] World Health Organization (WHO), 2007. Keeping Our Water Clean: The Case of Water Contamination in the Veneto Region, Italy. https://www.euro.who.int/__data/assets/pdf_file/0018/340704/FINAL_pfas-report-20170530-h1200.pdf.
[3] Commission Regulation (EU) 2022/2388 of 8 December 2022 amending Regulation (EC) No 1881/2006.

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Goat Hair as a Bioindicator of Environmental Presence of Heavy Metals and Trace Elements and Hypothalamic-Pituitary-Adrenal Axis Activation During Vertical Transhumance

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Livestock autochthonous breeds are considered a pivotal genetic resource for agriculture, rural development, and food and nutrition security [1]. In the Italian Alps, they are maintained using the traditional alpine farming system based on vertical transhumance, with the use of alpine pastures from late spring to autumn, and indoor housing with a hay-based diet for the remaining part of the year. Because of their tight link with the territory of origin, local breeds could be used to biomonitor environmental contaminations. Animal welfare should also be monitored in animals which are exposed to a sudden farming system change and different kinds of stressors. This study aimed to assess the response of an Italian local goat breed to the change from indoor housing to alpine pasture in summer in terms of hair concentrations of (i) trace elements and heavy metals, and of (ii) cortisol. Regrown hair of Frisa goats was monthly collected for 2 consecutive years once before vertical transhumance and twice after that event. Hair was then analysed for trace elements, heavy metals, and cortisol by inductively coupled plasma-optical emission spectrophotometer (ICP-OES) and enzyme immunoassay (EIA), respectively. Results showed an increase of As during alpine pasture, probably reflecting the soil and water As content of the grazing area [2], while Mg, Zn, and Al followed the opposite trend, decreasing in the second month after vertical transhumance. Hair cortisol concentrations increased during the 2 months of alpine pasture, indicating an increase in the activation of the HPA axis, in agreement with previous studies [3]. Future investigations should consider (i) a longer study period, (ii) the validation

of goat hair as a bio-indicator of environmental contamination by trace elements and heavy metals by measuring their concentration also in soil, water, and plants, and (iii) the development of *ad hoc* animal welfare indicators.

Keywords: autochthonous breed, small ruminant, hair cortisol.

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References:
[1] Scherf, B.; Pilling, D. The Second Report on the State of the World's Animal Genetic Resources for Food and Agriculture; Scherf, B.D., Pilling, D., Eds.; FAO: Rome, Italy, 2015; ISBN 978-92-5-108820-3.
[2] Zuzolo, D.; Cicchella, D.; Demetriades, A.; Birke, M.; Albanese, S.; Dinelli, E.; Lima, A.; Valera, P.; De Vivo, B. Arsenic: Geochemical distribution and age-related health risk in Italy. Environ. Res. 2020, 182, 109076, doi:10.1016/j.envres.2019.109076.
[3] Comin, A.; Prandi, A.; Peric, T.; Corazzin, M.; Dovier, S.; Bovolenta, S. Hair cortisol levels in dairy cows from winter housing to summer highland grazing. Livest. Sci. 2011, 138, 69–73, doi:10.1016/j.livsci.2010.12.009.

VAS Days, 27-29th September 2023

A Randomized Controlled Trial on Use of Antibiotic Dry-Goat Treatments and Its Effect on Intramammary Infection and Milk Microbiome

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Intramammary infection (IMI) is one of the most common diseases on dairy goat farms, with a significant economic impact on the dairy industry globally. The aims of current study are to (i) evaluate the status of somatic cells, (ii) identify the bacterial isolates and (iii) evaluate the impact of Dry Goat Therapy (DGT) on the milk microbiome at dry-off and post-partum periods in heathy and infected Camosciata delle Alpi goats located in Lombardy. A total of 60 goats (30 healthy: negative on bacteriological analysis and 30 infected: positive for one or two pathogens on bacteriological analysis) were included in this study based on the results obtained from bacteriological analysis of collected milk prior to dried-off from 106 goats. During the dry-off period, these 60 goats were randomized homogeneously into following 4 groups (15 goats in each group): healthy untreated (HNT), infected untreated (INT), healthy treated (HT), infected treated (IT). Each half-udder of each goat from HT and IT group were treated with intramammary suspension of Cefazolin 250mg (Cefovet A®, Dopharma, Italy, s.r.l) at the dry-off period. Pool milk samples were collected at three different time points from the goat farm and time points were dry-off (T1), kidding day (T2) and 5-10 days in milk (T3) for the somatic cell count, microbiological analysis and microbiome studies by 16S rRNA-gene sequencing. Of the total 30 infected goats before dry off, non-aureus staphylococci (NAS) were found to be the most isolated group of pathogens (80%); among them, *Staphylococcus (S.) equorum* was found to be the predominant species (50%), followed by *S. caprae* (45.8%). *Serratia (Se.) marcescens* was the only Gram-negative

bacterium identified, with a frequency of 16.7%, while 10% of the goats were infected by S. aureus. On the other hands, 5-10 DIM milk samples were dominated by *S. caprae* (35%) followed by *S. equorum* (31%), *Se. marcescens* (17%) and *S. aureus* (11%). Moreover, colostrum samples were dominated by *S. equorum* (66.7%) and *Se. marcescens* (33.3%). We also found significance decreases of Somatic Cell Count (SCC) at different time point of sampling. However, microbiome studies of the samples were done, and data will be analyzed. In the conclusion, this study evaluation will allow us to determine the bacterial composition and diversity of milk taken from healthy and infected goats, and to verify any correlation with antibiotic dry-goat therapy.

Keywords: Dry Goat Therapy, Milk, Microbiome

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Determination of persistent organic pollutants in fish tissues by accelerated solvent extraction technique (EXTREVA ASE™) and GC-MS/MS to support food safety

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Halogenated hydrocarbons as PCBs, OCPs, and (PBDEs) are, together with PAHs, part of the persistent organic pollutants (POPs) family [1]. The primary source of POPs exposure is food, especially fish [2]. Lake fish represents a niche consumption, but for local populations, it can reach high consumption levels. The aim of this study was to develop and validate an analytical method for the determination of POPs in fish tissues using the new EXTREVA ASE™ to assess its capability to be used for monitoring plans supporting food safety official controls. Overall, 30 Mediterranean shad were provided by the fish market of Milan. 6 PCBs, 16 OCPs, 7 PBDEs and 4 PAHs were determined in fish tissues. Two key improvements of the method were introduced: freeze-drying and in-line cleanup with Z-Sep. The method showed good linearity (R2>0.99), repeatability (4-19%) and recovery (84-109%) for all the compounds, fulfilling the validation parameters defined by SANTE 2021 [3]. The one-step accelerated solvent extraction and purification is both rapid and cost-effective and minimizes waste generation. PCBs were detected in all the samples (1.09-11.8 ng/g) as well as PBDE 47. PBDE 99, 28 and 100 were detected in over 70% of the samples; PBDE 153, 33 and 154 in less than 40% of the samples (1.05-5.72 ng/g). Despite its ban, DDT was detected in 70% of the samples, while its reductive dechlorination products DDD and DDE were detected in 100% of the samples. With 87% and 70% of occurrence hexachlorobenzene and Endosulfan I were the two other more OCPs found. The concentrations for OCPs ranged from 1.03 to 14.81 ng/g. PAHs were detected between 83 and 7%, while the concentrations were all below the LOD. This is the first reported example of an in-line cleanup on the EXTREVA ASE system. All results obtained confirm the efficacy of the method for the determination of multiresidue pollutants in fish tissues that could be pivotal to support food safety controls involved in monitoring plans.

Keywords: Accelerated solvent extraction, Persistent organic pollutants, GC-MS/MS

References:

[1] Lallas, P.L. The Stockholm Convention on persistent organic pollutants. Am. J. Int. Law 2001, 95, 692– 708.
[2] Schlummer, M.; Moser, G.A.; McLachlan, M.S. Digestive tract absorption of PCDD/Fs, PCBs, and HCB in humans: Mass balances and mechanistic considerations. Toxicol. Appl. Pharmacol. 1998, 152, 128–137. DOI: 10.1006/taap.1998.8487
[3] European Commission. Analytical Quality Control and Method Validation Procedures for Pesticide Residues Analysis in Food and Feed (SANTE 11312/2021). 2022. Available online: https://www.eurl-pesticides.eu/docs/public/tmplt_article.asp?CntID=727

VAS Days, 27-29th September 2023

Role of precision animal nutrition in combating antimicrobial resistance

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Antimicrobials have been used to promote growth, prevent disease, and control infections in food animals. However, concerns regarding their overuse and abuse in veterinary practices have arisen due to the emergence of antimicrobial resistance (AMR). This issue poses a significant threat to public health, sustainable animal production and the global economy. It is estimated that AMR could lead to 10 million deaths annually and a 3.8% decline in the gross domestic product by 2050 if effective actions are not taken to address it [1]. Combating AMR in animals must receive more attention because most emerging infectious diseases in humans are of zoonotic origins, and global antimicrobial consumption in animals is three times higher than in humans [2]. Additionally, antimicrobial-resistant microbes can spread from animals to humans through various pathways.

When combined with optimal housing and hygiene practices, precision animal nutrition emerges as a promising strategy to combat AMR. It goes beyond meeting specific nutrient requirements to optimize productivity and profitability while enhancing critical functions required for host defense and resilience. A healthy, well-balanced diet is essential for maintaining proper gut function and preserving microbiome homeostasis. This is necessary to keep animals healthy and resilient against infection, thereby reducing the need for antimicrobial use [3].

Nevertheless, simply replacing antimicrobials with a blend of functional feed additives is insufficient to reduce antimicrobial use. Instead, a holistic nutritional approach, taking into account overall animal health and welfare while optimizing for their genetic potential, should be implemented through the collaboration among animal nutritionists, veterinarians and farm managers [3]. Unfortunately, animal nutrition expertise is often overlooked in efforts aimed at combating AMR. Therefore, it should be integrated into national AMR mitigation action plans and supported through legal frameworks [4]. Furthermore, there is an urgent need for interdisciplinary research to identify precision nutrition biomarkers. This would enable the development of tailored feeding strategies aimed at enhancing animal

health and welfare while advancing our understanding of nutrition's role in addressing AMR.

Keywords: Precision nutrition, antimicrobial resistance, biomarkers, food animals

References:

[1] O'Neill J. Tackling drug-resistant infections globally: final report and recommendations. Government of the United Kingdom; 2016.
[2] Van Boeckel TP, Pires J, Silvester R, Zhao C, Song J, Criscuolo NG, et al. Global trends in antimicrobial resistance in animals in low- and middle-income countries. Science 2019;365:eaaw1944. https://doi.org/10.1126/science.aaw1944.
[3] Smits CH, Li D, Patience JF, Hartog LA den. Animal nutrition strategies and options to reduce the use of antimicrobials in animal production. vol. 2021. Rome: Food and Agriculture Organization of the United Nations; 2021. https://doi.org/10.4060/cb5524en.
[4] Contribution of advanced feeding strategies to enhanced and circular sustainable livestock and aquaculture production | FEFAC n.d. https://fefac.eu/newsroom/news/contribution-of-advanced-feeding-strategies-to-enhanced-and-circular-sustainable-livestock-and-aquaculture-production/ (accessed September 23, 2023).

VAS Days, 27-29th September 2023

Evaluation of antibiotic use and intramammary infections in farms belonging to the Consortium of Parmigiano Reggiano Cheese

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Objectives
In front of a global concern about Antimicrobial Resistance (AMR), a growing interest in preventing Antimicrobial Use (AMU) has reached as far Parmigiano Reggiano farms. This study investigated how bovine udder health and dairy herd management are related to AMU, with the aim to support dairy farmers in milk quality and Parmigiano Reggiano cheese production.

Materials and methods
A total of 14 farms in Parmigiano Reggiano area was randomly selected for this study, based on the availability of DHIA (Dairy Herd Improvement Association) data provided by ARAER (Associazione Regionale Allevatori Emilia Romagna), and of 2021 mastitis and treatment records. Each farm visit included an initial farmer interview to fill out checklist, followed by visiting cows and environment for cleanliness assessment. Information on biosecurity, housing and bedding management, udder health performance, AMU, and milking routines were assessed using a questionnaire.

Results
The questionnaire results revealed that the most used screening test for mastitis diagnosis in lactating dairy cows was CMT (California Mastitis Test; 59%), followed by SCC (Somatic Cell Count; 36%), and on-farm culture (5%). Strep. uberis was the most prevalent CM (Clinical Mastitis) causative agent, with a frequency of 50%, followed by Staph. aureus

(20%), E. coli (20%), and Strep. agalactiae (10%). The most frequent antimicrobial classes for CM cure were beta-lactams (penicillin; 100%), sulfonamides (50%) and macrolides (43%). In 93% of farms, treatment protocols included systemic, local, and combination treatments. At dry-off, SDCT (Selective Dry Cow Therapy) was performed in 71% of farms, while it was not applied in the remaining ones due to the presence of contagious microorganisms. In all farms, internal teat sealant was used alone or combined with antimicrobials, mostly first-generation cephalosporins (53%) and penicillin (41%).

Conclusions

The data showed that the use of antibiotic doses varied widely among dairy farms studied. It ranged from 0.2 to 3.6 treatments per cow/year under risk. Here, the use of antibiotic doses was not related to udder health indicators, which could be calculated from DHI cell counts. The treatment concepts applied on individual farms seemed to be more important for antibiotic use than the udder health situation of the herds and the wide range could offer considerable room for reducing antibiotic doses in mastitis control.

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Effect of a lime-based conditioner on pH, dry matter, and microbiological counts of recycled manure solids as dairy cow bedding substrates

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Manure-derived materials are a good alternative to conventional bedding substrates in a circular economy perspective. However, the control of microbial proliferation is crucial to avoid health issues [1]. Lime-based bedding conditioners can be used for this purpose. To our knowledge, no studies assessed the impact of different lime amounts on the microbial population of manure-derived bedding substrates. We evaluated different concentrations (0-10-15-20%) of VF10, a lime-based product, on separated raw manure solids (SRMS) and anaerobically digested manure solids (ADMS). Samples were analyzed immediately and after storage at 28°C for 1, 3, and 7 days. Dry matter (DM), pH, and microbial populations including total bacterial counts (TBC), Gram-negative bacteria, and streptococci were assessed. pH and DM increased according to VF10 concentrations. Additionally, a decrease in pH was observed over time, while no differences were observed in DM. In general, a decrease in microbial proliferation was observed for both materials with increasing VF10 concentrations. TBC increased observed over time in untreated samples, while it dropped in treated samples after 1 day, followed by a gradual increase at 3 and 7 days. Gram-negative bacteria increased in untreated ADMS after 3 days and decreased at day 7. In untreated SRMS and 10%-SRMS, Gram-negatives showed an initial increase at day 1 followed by a decrease at day 3 and another increase at day 7. In 15-20% treated ADMS and SRMS, Gram-negative bacteria were seldom isolated. In untreated ADMS and 15%-ADMS, streptococci increased until day 3 and decreased at day 7. In 10-20%-ADMS and 20%-SRMS the counts increased over time, but in 20%-ADMS the growths were observed starting from day 3. In untreated SRMS and 10-15%-SRMS, streptococci showed an initial increase at day

1 followed by a decrease at days 3 and 7. In conclusion, VF10 controlled microbial proliferation in both substrates, with better control over Gram-negative bacteria compared to streptococci. The product also demonstrated efficacy over time.

Keywords: bedding materials, bedding conditioner, microbiological counts

References:

[1] Leach, K.A., Archer, S.C., Breen, J.E., Green, M.J., Ohnstad, I.C., Tuer, S., and Bradley, A.J. Recycling manure as cow bedding: Potential benefits and risks for UK dairy farms. The Veterinary Journal, 2015, 206:123–130. doi:10.1016/j.tvjl.2015.08.013

VAS Days, 27-29th September 2023

Occurrence of perfluoroalkyl substances in canned tuna and their impact on food safety

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Fish is commonly recognized as a healthy food, indicated for its nutritional properties [1]. At the same time, the continued use of chemical compounds for industrial purpose and their subsequent spillage into water has made this matrix particularly susceptible to phenomena such as bioaccumulation of various contaminants [2]. Perfluoroalkyl substances (PFASs) are molecules of particular concern for their toxicity as endocrine disruptors, immunodepressants, possible carcinogens and liver and kidney toxicity. Thanks to their different physical and chemical properties, such as heat stability and repellency, their use is widespread in various commercial applications resulting in a great distribution and spread in different environments [3]. Water and food, in particular fish, represent the most important source of PFAS assumption. Considering their impact on human health, the EFSA CONTAM Panel in 2020 published a scientific opinion aimed the focusing on four different PFASs: perfluorooctane sulfonic acid (PFOS), perfluorooctanoic acid (PFOA), perfluorononanoic acid (PFNA) and perfluorohexane sulfonic acid (PFHxS). The conclusion of the CONTAM Panel was a Tolerable Weekly Intake (TWI) of 4.4 ng kg^{-1} b.w. per week for their sum [4]. In this study, the presence of 18 different PFASs in 75 canned tuna samples and their corrispective pre-cooked loins, was evaluated through ultra-high performance liquid chromatography-tandem high-resolution mass spectrometry (UHPLC-HRMS). Among the investigated PFASs only 7 were detected in the analyzed samples and just two of the four PFASs of which EFSA established a TWI, i.e., PFOS and PFNA. The risk characterization related to canned tuna resulted in an intake value much lower than the TWI for the average Italian consumer. Considering 34% of intake for high consumers, is important to consider that canned tuna is not the only source of PFASs intake.

Keywords: PFASs, Tuna, Food safety, Risk characterization

References:
[1] Daviglus, M., Sheeshka, J., & Murkin, E. (2002). Health benefits from eating fish. *Comments on Toxicology*, *8*(4-6), 345-374. https://doi.org/10.1080/08865140215064.
[2] Domingo, J. L. (2016). Nutrients and chemical pollutants in fish and shellfish. Balancing health benefits and risks of regular fish consumption. *Critical reviews in food science and nutrition*, *56*(6), 979-988. https://doi.org/10.1080/10408398.2012.742985.
[3] Evich, M. G., Davis, M. J., McCord, J. P., Acrey, B., Awkerman, J. A., Knappe, D. R., Lindstrom, A. B., Speth, T. F., Tebes-Stevens, C., Strynar, M. J., Wang, Z., Weber, E. J., Henderson, W. M., & Washington, J. W. (2022). Per-and polyfluoroalkyl substances in the environment. *Science, 375*, 6580. doi: 10.1126/science.abg9065
[4] EFSA Panel on Contaminants in the Food Chain (EFSA CONTAM Panel), Schrenk, D., Bignami, M., Bodin, L., Chipman, J. K., del Mazo, J., ... & Schwerdtle, T. (2020). Risk to human health related to the presence of perfluoroalkyl substances in food. *EFSA Journal*, *18*(9), e06223. https://doi.org/10.2903/j.efsa.2020.6223

VAS Days, 27-29th September 2023

Antibiotic susceptibility and biofilm formation in ESBL-/AmpC-/carbapenemase-producing *Escherichia coli* isolated from fecal samples in dogs

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A serious public health threat is represented by extended-spectrum β-lactamase (ESBL)-, AmpC β-lactamases- and carbapenemases-producing *Escherichia coli*. Moreover, biofilm-producing *E. coli* are highly resistant to disinfection and sanitation procedures and contribute to the transfer of antimicrobial resistance genes between bacteria. The aims of this study were to assess the presence of biofilm-forming ability and antibiotic resistance rates in 14 ESBL-/AmpC-/carbapenemase-producing *E. coli* isolated from dog fecal samples harboring *bla*$_{CTX-M}$ (64.3%), *bla*$_{TEM}$ (64.3%), *bla*$_{SHV}$ (0%), *bla*$_{CMY-2}$ (28.6%), cAmpC (7.1%) and *bla*$_{OXA-48}$ (7.1%) genes [1, 2]. The phenotypic detection of biofilm production was performed with the microtiter plate method and biofilm-associated genes (*csgA*, *sdiA*, *rpoS*) were investigated by PCR [3]. The minimum inhibitory concentration (MIC) was determined by the broth microdilution method.
One (7.1%) strain was able to form biofilm and was classified as weak biofilm producer. The biofilm-producing *E. coli* was an ESBL-producing *E. coli*, confirmed by the presence of *bla*$_{CTX-M}$ gene. All strains possessed the *csgA*, *sdiA* and *rpoS* biofilm-associated genes. Multidrug resistance was observed in 13 (92.9%) isolates.
The high presence of multidrug resistance in ESBL-/AmpC-/carbapenemase-producing *E. coli* from dogs has been previously reported. Biofilm formation in ESBL-producing *E. coli* in fecal samples of dogs, suggesting the association with biofilm production previously reported in *E. coli* harboring *bla*$_{CTX-M}$ gene, highlights the need to consider biofilm production in future studies aimed to improve knowledge on factors to reduce the global spread of resistant bacteria.

References:
[1] Monstein, H. J., Ostholm-Balkhed, A., Nilsson, M. V., Nilsson, M., Dornbusch, K., & Nilsson, L. E. Multiplex PCR amplification assay for the detection of blaSHV, blaTEM and blaCTX-M genes in Enterobacteriaceae. APMIS: acta pathologica, microbiologica, et immunologica Scandinavica, 2007, 115(12), 1400–1408.
DOI: 10.1111/j.1600-0463.2007.00722.x
[2] Rehman, M. A., Hasted, T. L., Persaud-Lachhman, M. G., Yin, X., Carrillo, C., & Diarra, M. S. Genome Analysis and Multiplex PCR Method for the Molecular Detection of Coresistance to Cephalosporins and Fosfomycin in Salmonella enterica Serovar Heidelberg. Journal of food protection, 2019, 82(11), 1938–1949.
DOI: 10.4315/0362-028X.JFP-19-205
[3] Adamus-Białek, W., Kubiak, A., & Czerwonka, G.. Analysis of uropathogenic Escherichia coli biofilm formation under different growth conditions. Acta biochimica Polonica, 2015, 62(4), 765–771.
DOI: 10.18388/abp.2015_1127

VAS Days, 27-29th September 2023

Developing tailor-made strategies in age- and follicular stage-dependent approaches for preantral follicles rescue in female fertility preservation programs

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Fertility preservation programs are severely limited to the number of rescuable gametes. This limitation relies on the individual's age effect and the limited number of oocytes that can be currently employed in assisted reproductive technologies (ARTs). In particular, the ovarian reserve is finite and is established during fetal life when it decreases until menopause [1]. Only follicles from the early antral stage can be developed in vitro [2]. However, for fertility preservation programs, this is just a tiny portion compared to the reproductive potential enclosed in the ovary that remains unexploited due to the lack of technologies capable of growing preantral follicles.
Using the bovine model, we aim to devise innovative strategies to increase the efficiency of ARTs by studying the dynamics of the follicular reserve during reproductive life and the characterization of the physiological mechanisms regulating the differentiation of the heterogenous population of preantral follicles.
For this purpose, we proceeded toward two specific objectives. The first objective was quantitatively and qualitatively defining the follicular reserve during reproductive life. The second objective was to isolate specific stages within the population of preantral follicles (primordial, primary, and secondary) to define distinct transcriptions profiles for each follicular stage.
Our preliminary results show a drastic decrease in follicle reserve in cows (40-96 months) compared to heifers (12-24 months) ($p<0.0001$, two-way ANOVA followed by Sidak's test). The transcriptomic analysis indicates 68 genes differentially expressed on comparing primary versus primordial follicles and 1301 genes between secondary and primary follicles with FDR<0.05.

Collectively, our data can contribute to developing tailored stage-specific culture systems concerning the reproductive age, providing valuable tools in ARTs for animal breeding schemes, biodiversity preservation programs, and treating human infertility.

Keywords: Folliculogenesis, Ovarian Reserve, Transcriptome

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References:
[1] Ge, W., et al., Establishment and depletion of the ovarian reserve: physiology and impact of environmental chemicals. Cell Mol Life Sci, 2019. 76(9): p. 1729-1746. DOI: 10.1007/s00018-019-03028-1
[2] Garcia Barros, R., et al., A refined culture system of oocytes from early antral follicles promotes oocyte maturation and embryo development in cattle. Reproduction, 2023. 165(2): p. 221-233. DOI: 10.1530/REP-22-0277

VAS Days, 27-29th September 2023

The toxicity of organic compounds on PBMCs

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The demand for animal products and greenhouse gas emissions from livestock are on the rise. Cattle are known to account for 65% of all animals emitting greenhouse gases (GHG), significantly impacting the global environment. Due to their highly adapted digestive systems, ruminants can consume types of fibers that monogastric animals cannot, making them non-competitive with human resources and feed in the coming years. The breakdown of complex carbohydrates occurs in the rumen, which is home to a complex ecosystem composed of anaerobic bacteria, protozoa, fungi, methanogenic archaea, and phages. A substantial amount of GHGs, primarily methane, is produced during digestion, contributing significantly to global warming. Various strategies are being explored to reduce livestock emissions without affecting animal performance, including using feed additives and higher-quality forages, such as essential oils. Organic acids can modulate the rumen microbiome by affecting methanogenic archaea development and increasing animal hydrogen consumption. This, in turn, reduces methane production and increases the production of volatile fatty acids (VFAs), ultimately promoting animal growth. One hypothesis gaining traction in recent years is using essential oils and organic acids as additives in cattle feed to mitigate greenhouse gas emissions. Our study aims to evaluate the immunomodulatory activity of Essential Oils alone and in combination with Organic Acids on bovine mononuclear cells. We conducted viability and apoptosis assays to assess these compounds' toxicity levels. In terms of viability, the results indicated that essential oil number 1 exhibited toxicity at a concentration of 1000 μM ($P < 0.001$), while EO 2 and EO 3 showed significant differences at concentrations of 400 ($P < 0.01$) and 1000 μM ($P < 0.001$). EO 4 and EO 5 were toxic at concentrations of 200 ($P < 0.05$), 400 ($P < 0.01$), and 1000 μM ($P < 0.001$). Since 1000 μM appeared highly toxic for all oils, this concentration was excluded from further experiments. However, the compounds did not significantly affect cell apoptosis (NS). After determining the optimal concentration of EOs (25 μM), we combined them with the best concentration of OAs (100, 150, and 200 μM, as determined previously). The viability results demonstrated that none of the mixtures significantly affected the

viability of PBMCs (NS). However, the mixtures did negatively impact cell apoptosis – Mixture 1 and 2 ($P < 0.001$), Mixture 3 and 5 ($P < 0.01$), and Mixture 4 ($P < 0.05$). In conclusion, our report shows that the selected essential oils are not toxic to cells at low concentrations, whether used alone or in combination with organic acids. However, these mixtures significantly affected the apoptotic capacity of cells, as evidenced by an increase in Caspase 3-7 activity. This difference in viability and apoptosis assay results may be explained by the ongoing cellular processes during apoptosis, including converting WST-1 salt into formazan.

Keywords: Organic compounds, nutrition, methane

References:

Nazzaro, F., Fratianni, F., Coppola, R., & De Feo, V. (2017). Essential oils and antifungal activity. In Pharmaceuticals (Vol. 10, Issue 4). MDPI AG. https://doi.org/10.3390/ph10040086

Nehme, R., Andrés, S., Pereira, R. B., Jemaa, M. Ben, Bouhallab, S., Ceciliani, F., López, S., Rahali, F. Z., Ksouri, R., Pereira, D. M., & Abdennebi-najar, L. (2021). Essential oils in livestock: From health to food quality. In Antioxidants (Vol. 10, Issue 2, pp. 1–42). MDPI. https://doi.org/10.3390/antiox10020330

Patra, A. K., & Yu, Z. (2012). Effects of essential oils on methane production and fermentation by, and abundance and diversity of, rumen microbial populations. Applied and Environmental Microbiology, 78(12), 4271–4280. https://doi.org/10.1128/AEM.00309-12

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The effect of hydrolysed yeast on production performance and gastrointestinal health in broilers

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The goal of the present study was to ascertain the effects of dietary inclusion of hydrolysed yeast (HY) on the growth performance, meat quality, and gastrointestinal health of broilers. A total of 320 male 1-day-old chicks (ROSS 308) were used in this experiment. The animals were homogeneously separated into two groups and given either the basal diet (CTR) or the basal diet supplemented with yeast (TRT, 500 mg/kg HY). Each experimental group was composed of 160 birds, which were distributed among eight replicates (20 birds per replicate). Growth performance and body lesions (hock burn and foot pad dermatitis) were evaluated at the beginning, at each feeding phase change, and at the end of the trial (i.e., 0, 10, 21, 42). On day 42, all the animals were taken to a slaughterhouse for slaughter and sample collection to further determine meat quality (water holding capacity, pH, or color) and gastrointestinal health (gene expression). Statistical Analysis System software (SAS version 9.4; SAS Institute Inc., Cary, NC, USA) applying a MIXED procedure, the GLM procedure, and PROC FREQ was used for the analysis of the data. Differences between groups were considered statistically significant at $p < 0.05$, whereas a trend for a treatment effect was noted for $0.05 \leq p < 0.10$. The results revealed no difference between the CTR and TRT groups both in terms of production performance and meat quality ($P > 0.05$). Furthermore, the mRNA expression studies on the Adiponectin system genes (AdipoQ, Adipo Receptor 1, and Adipo Receptor 2) and tight junctions (Zonula occludens ZO-1, Occludin, Claudin-3) showed no significant difference ($P > 0.05$) between the CTR and TRT groups.

Keywords: (Hydrolysed yeast, gut health, adiponectin system)

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Which animal-based measures (ABMs) of consciousness can be influenced by a poor stunning in lambs in an industrial abattoir?

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According on EU legislation on the protection of the animal at the time of killing [1], the efficiency of stunning method must be evaluated through a list of ABMs. However, different slaughtering conditions can affect their feasibility and there is the need to identify the combination of ABMs that can be used in different abattoir context [2]. The aim of this study was to investigate which ABMs of consciousness in lambs were the most observed in an industrial abattoir and whether their presence was influenced by poor quality of the stun (tongs position and duration). Data were collected during normal slaughter routine, on 100 lambs (LW 6.0-8.0 kg) in one Italian slaughterhouse in October 2022. Lambs were manually restrained and stunned with head-only and hoisted on the rail. Two fixed action video cameras were used: one recorded the stunning and hoisting phases, while the second recorded the bleeding during post-cutting period up to 60s. Videos were analysed with BORIS by a trained observer to evaluate the positioning of the electrodes, the duration of the stun, the stun-to-stick interval, and the ABMs related to a poor stun (absence of tonic seizure, righting reflex, movements of the ears, the head, and the nostrils) [3,4]. In 60 lambs the position of the electrodes was incorrect. When the stun was poor the lambs exhibited: ear movements (48%), movements of the head (37%), movements of nostrils (53%), righting reflex (43%) and absence of tonic seizures (18%). The high presence of poor stunned animals encourages further analysis focused on the correlation between each ABM and other parameters that can affect the quality of the stunning, such as the stun-to-stick interval. Furthermore, it would be useful to identify possible automation solutions, which may inform the operator of the incorrect placement of the electrodes.

References:
[1] European Commission (EC). Council Regulation (EC) 1099/2009 of 24 September 2009 on the protection of animals at the time of killing. O_. J. Eur. Union. 1–30, 2009.
[2] Comin, M., Barbieri, S., Minero, M., & Dalla Costa, E. (2023). The Feasibility of Animal-Based Indicators of Consciousness and Unconsciousness for Stunning in Sheep: A Systematic Review. Animals, 13(8), 1395.

[3] Nielsen, S.S.; Alvarez, J.; Bicout, D.J.; Calistri, P.; Canali, E.; Drewe, J.A.; Garin-Bastuji, B.; Gonzales Rojas, J.L.; Gortázar Schmidt,C.; Herskin, M.; et al. Welfare of Sheep and Goats at Slaughter. EFSA J. 2021, 19, e06882.
[4] von Holleben, K.; (Bsi-Schwarzenbek—Training and Consultancy Institute for Animal Welfare at Transport and Slaughter,Schwarzenbek, Germany). Personal communication, 2022.

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In vitro studies on the antimicrobial, antioxidant, and prebiotic activities of biochar derived from chestnut and vine residues.

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Biochar has been used in agriculture to enhance soil fertility and improve plant growth. As a feed ingredient biochar can potentially improve animal health, and livestock housing climate [1]. The variability in physical-chemical-biological properties was influenced by the waste used for their production. In the present work, two different biochar from chestnut waste (CB) and vine residues (VB) were characterized for chemical composition and functional properties. The CB and VB biochar were maintained in hot water (90°C for 3h head-over-heels) and the extracts were analyzed for inorganic/organic components by QTOF-MS. The antioxidant potential was determined by the ABTS assay [2]. The extracts were tested for their effects on *in vitro* growth inhibitory activities against enterotoxigenic *E. coli* sp. *E. coli* was inoculated into 20 mL of Luria-Bertani medium supplemented with 0, 25, 50, and 100 µl/mL of biochar water extracted. Tubes were cultured at 37°C and the bacterial growth rate was determined by optical density at 600 nm for 6 hours [3]. The same concentrations of biochar extract were tested on the growth of *L. plantarum* and *L. reuteri* cultured in MRS broth. The optical density was checked for a total of 8 hours. The biochar extracts showed a polyphenol profile characterized by low molecular weight compounds. The antioxidant activity of VB biochar was significantly higher than CB biochar (110 TE/gr vs 54 TE/gr; $p \leq .01$). Antimicrobial screening showed that both biochar extracts exerted a significative inhibitory activity against *E. coli* strains ($p \leq .01$); the maximum percentage of inhibition (-29% of bacterial cells) was observed after 2 hours of incubation with 100 µl/mL of biochar extracted. The growth of *L. plantarum* and *L. reuteri* was not negatively influenced. These preliminary results suggest that biochar from chestnut and vine residue may be interesting in inhibiting the growth of pathogenic *E. coli* and counteract oxidative stress. Biochar can be considered a valid alternative for the reduction of the use of antibiotics.

Keywords: biochar, animal nutrition, alternative to antibiotics

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References:

[1] Osman, A.I., Fawzy, S., Farghali, M. *et al.* Biochar for agronomy, animal farming, anaerobic digestion, composting, water treatment, soil remediation, construction, energy storage, and carbon sequestration: a review. *Environ Chem Lett*, 2022, 20, 2385–2485. DOI:10.1007/s10311-022-01424-x

[2] Benkhaira, Nesrine & Saad, Ibnsouda & Fikri Benbrahim, Kawtar. In vitro Methods to Study Antioxidant and Some Biological Activities of Essential Oils: a Review. Biointerface Research in Applied Chemistry, 2021, 12, 3332 – 3347. DOI: 10.33263/BRIAC123.33323347.

[3] Reggi, S., Giromini, C., Dell'Anno, M., Baldi, A., Rebucci, R., Rossi, L. In Vitro Digestion of Chestnut and Quebracho Tannin Extracts: Antimicrobial Effect, Antioxidant Capacity and Cytomodulatory Activity in Swine Intestinal IPEC-J2 Cells. Animals 2020, 10, 195. DOI:10.3390/ani10020195

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Evaluation of *Tenebrio molitor* larvae as alternative protein source in growing pigs

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The human population is projected to reach 10 billion by 2050 [1], and consequently, to meet the increased demand for food and feed, the needing of new sustainable sources has been arisen. To address this sustainability challenge, the use of insects in animal diets is considered a promising due to their low environmental impact and high nutritional value [2]. The aim of this study was to evaluate the replacement of soy protein concentrate with *Tenebrio molitor* meal on performance, diet digestibility, and intestinal microbiota of growing pigs. A total of 14 growing pigs (80 ± 2 days old) were randomly allotted to two groups (2 pens with 7 pigs/pen): the control group (CON) was fed a commercial diet containing 4% fermented soy protein concentrate (48% crude protein, CP), and the treatment group (TM) was fed a basal diet containing 5% of *T. molitor* larvae meal in replacement of soy protein concentrate. The study lasted for 28 days. Animals were weekly weighed; faecal and blood samples were collected for biochemical analyses and DNA was extracted from rectal swabs to evaluate the effects on the microbiota. No differences were observed in terms of growth (CON: 48.44±3.41 kg; TM: 48.41±4.52 kg at 28 days), feed efficiency, and diet digestibility for the protein and lipid components during the trial. The nitrogen concentrations in faeces showed similar levels between the two groups at day 28 (CON: 0.63±0.06 and TM: 0.65±0.23), confirming high digestibility of both diets. TM showed an increased beta diversity index suggesting a modulation of the faecal microbiota induced by the inclusion of *T. molitor* ($p < 0.05$). No differences were observed for the concentration of albumin, globulin, urea and interleukin-6 in serum of both groups (CON: 744,51±94,67 ng/L; TM: 727,77±94,03 ng/L), suggesting a good health status of pigs. These results suggest that 5% *T. molitor* meal can be successfully used as a replacement for soy protein concentrate growing pigs' diets.

Keywords: insect, *Tenebrio molitor* larvae, growing pig

References:
[1] Salter, A.M., and Lopez-Viso, C. Role of novel protein sources in sustainably meeting future global requirements. Proceedings of the Nutrition Society, 2021, 80.2 (2021): 186-194. DOI: 10.1017/S0029665121000513
[2] Moruzzo, R., Mancini, S., and Guidi, A. Edible Insects and Sustainable Development Goals. Insects, 2021, 12(6), 557. DOI: 10.3390/insects12060557

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Novel biomarkers for the diagnosis of sepsis and systemic inflammatory response syndrome (SIRS) in equine and bovine

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The expression 'systemic inflammatory response syndrome' (SIRS) describes a clinical condition representing the activation of a complex network of endogenous mediators which lead to uncontrolled and widespread inflammation and the production of reactive oxygen species (ROS). Sepsis defines the SIRS due to bacterial origin but a consensus on its definition has not been postulated for large animals (LA). Considering global trends of increasing antimicrobial resistance in both Gram + and Gram - organisms, much effort has been directed toward identification of biomarkers (BIOs) that are useful in the differential diagnosis of sepsis and other infectious conditions.
Some promising BIOs has been evaluated in LA. Procalcitonin (PCT), a glycoprotein able to distinguish septic *vs* not-septic inflammation in humans, can be considered a suitable BIOs in equine [1-5], and bovine [6-9] for different infectious conditions leading to sepsis. Levels of PCT in septic animals were significantly higher compared to the healthy ones.
Protein carbonyl content (PCC) provides an indication of the amount of oxidative stress under inflammatory conditions in LA [5,7,9-10]. PCC was higher in plasma of SIRS + horses *vs* healthy ones. PCT and PCC were tested in septic arthritis found that both BIOs were statistically higher in synovial fluid from septic joints compared to healthy [5]. Paraoxonase (POase) and butyrylcholinesterase (BChE) seemed good BIOs in distinguishing SIRS vs healthy horses [11] Finally, symmetric (SDMA) and Asymmetric (ADMA) dimethylarginines were also found to be higher in colic horses compared to healthy ones over the time, with better performance of SDMA [12].
Novel BIOs for the evaluation of SIRS and/or septic status in large animals showed promising results. Further studies will be focused on the application of these BIOs in large populations for setting reference values and for the evaluation of their potential implication in driving the antimicrobial therapy.

Keywords: equine, bovine, biomarkers

References:

[1] Bonelli, F., Meucci, V., Divers, T.J., Radcliffe, R., Jose-Cunilleras, J.,E., Corazza, M., Guidi, G., Tognetti, R., Castagnetti, C., Intorre, L., Sgorbini, M. Evaluation of Plasma Procalcitonin Concentrations in Healthy Foals and Foals Affected by Septic Systemic Inflammatory Response Syndrome. Journal of Equine Veterinary Science, 2015, 35, 645-649. https://doi.org/10.1016/j.jevs.2015.06.007

[2] Bonelli, F., Meucci, V., Divers, T.J., Wagner, B., Intorre, L., Sgorbini, M. Kinetics of plasma procalcitonin, soluble CD14, CCL2 and IL-10 after a sublethal infusion of lipopolysaccharide in horses. Veterinary Immunology and Immunopathology, 2017, 184, 29-35. DOI: 10.1016/j.vetimm.2016.12.010

[3] Bonelli, F., Meucci, V., Divers, T.J., Jose-Cunilleras, E., Corazza, M., Tognetti, R., Guidi, G., Intorre, L., Sgorbini, M. Plasma procalcitonin concentration in healthy horses and horses affected by systemic ınflammatory response syndrome. Journal of Veterinary Internal Medicine 2015, 29(6), 1689-1691. DOI: 10.1111/jvim.13640

[4] Nocera, I., Bonelli, F., Vitale, V., Meucci, V., Conte, G., Cunilleras, E.J., Gracia-Calvo, L.A., Sgorbini, M. Evaluation of plasmatic procalcitonin in healthy, and in Systemic Inflammatory Response Syndrome (SIRS) negative and positive colic horses. Animals, 2021, 11, 2015. DOI: 10.3390/ani11072015.

[5] Nocera, I., Aliboni, B., Sgorbini, M., Camisi, M., Meucci, V., Battaglia, F., Gracia-Calvo, L.A., Tapio, H., Citi, S. Evaluation of procalcitonin and carbonylated proteins concentration in equine synovial fluid: preliminary results. 31st Annual Scientific Meeting of European College of Veterinary Surgeons (ECVS),2022, 7-9 July, Porto, Portugal, p. 190.

[6] Bonelli, F., Meucci, V., Divers, T.J., Boccardo, A., Pravettoni, D., Meylan, M., Belloli, A.G., Sgorbini, M. Plasma procalcitonin concentration in healthy calves and those with septic systemic inflammatory response syndrome. The Veterinary Journal, 2018, 234, 61-65. DOI: 10.1016/j.tvjl.2018.02.003

[7] Sala, G., Orsetti, C., Meucci, V., Sgorbini, M., Bonelli, F. Endogenous Procalcitonin and carbonylated protein level in healthy cows and cows affected by subclinical mastitis. 76° SISVET Congress, 2023, Bari, 21-23 June.

[8] Bonelli, F., Madrigali, A., Sgorbini, M., Meucci, V., Battaglia, F., Guélat-Brechbuehl, M., Sala, G., Meylan, M. Case – Control study: Evaluation of plasma procalcitonin concentration as an indicator of inflammation in healthy and sick cows. Research in Veterinary Science, 2023, 155, 56-61. DOI 10.1016/j.rvsc.2023.01.004

[9] Sala, G., Pravettoni, D., Meucci, V., Sgorbini, M., Ferrulli, V., Boccardo, A., Castelli, M., Bonelli, F. Can endogenous Procalcitonin distinguish healthy calves from calves affected by broncho-pneumonia? 76° SISVET Congress, 2023, Bari, 21-23 June.

[10] Nocera, I., Bonelli, F., Meucci, V., Rinnovati, R., Spadari, A., Intorre, L., Pretti, C., Sgorbini, M. Evaluation of protein carbonyl content in healthy and sick hospitalized horses. Frontiers Veterinary Science, 2020, 7: 582886. DOI: 10.3389/fvets.2020.582886

[11] Bindi, F., De Marchi, L., Vernaccini, M., Spadari, A., Rinnovati, A., Meucci, V., Bonelli, F., Sgorbini, M. Evaluation of a panel of oxidative stress biomarkers in healthy and colic horses: preliminary study. Proceedings of the 76° SISVET Congress, 2023, Bari, Italy.

[12] Bindi, F., Nocera, I., Vallesi G., Meucci, V., Bonelli, F., Spadari, A., Rinnovati, R., Sgorbini, M. Evaluation of symmetric (SDMA) and asymmetric (ADMA) dimethylarginines in healthy and in systemic inflammatory response syndrome (SIRS) negative or positive colic horse. Proceedings of the Annual ECEIM Congress, 2023, Lyon, France.

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Detection of Ferret Coronavirus and *Hedgehog Coronavirus 1* in Domestic Ferrets and Hedgehogs in Italy

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Exotic animal, such as ferrets and hedgehogs, have become popular in recent years as pets in Italy. The susceptibility of ferrets to SARS-CoV and SARS-CoV-2 and the recent discovery of MERS-related coronaviruses (CoVs) in wild hedgehogs suggest the need of CoVs surveillance in these animals. Therefore, this study aimed at investigating the presence of CoVs in pet ferrets and hedgehogs in Italy. Fecal samples were collected from domestic ferrets and hedgehogs during 2020-2022. Partial 12S rRNA sequencing was used for hedgehog species identification [1]. Samples were analyzed by nested reverse transcriptase (RT)-PCR and phylogenetic analysis of CoVs partial sequence of the RNA-dependent RNA polymerase (RdRp) gene [2]. Positive samples were further characterized using CoVs RT-PCR targeting partial sequence of the spike (S) gene [3]. Data analysis was carried out to identify risk factors. CoVs positivity was observed in 11/59 (18.6%) pet ferrets and 2/25 (8%) pet hedgehogs. Among pet hedgehogs, CoVs were detected in 2/3 (66.7%) *Hemiechinus auritus*, while all the 22 tested *Atelerix albiventris* were negative. RdRp gene phylogeny showed that CoVs from ferrets grouped together with ferret coronavirus (FRCoV) within the genus *Alphacoronavirus*, whereas CoVs from hedgehogs clustered with other representative species of *Hedgehog coronavirus 1* (HedCoV1) identified in Europe, within subgenus *Merbecovirus* of the genus *Betacoronavirus*. Available partial S gene phylogeny showed that the CoV from ferret grouped together within FRCoV European lineage, while CoV from hedgehog clustered with other HedCoV1 identified in China. No significant association was observed between CoVs detection and age, gender or health status. Results support the circulation of CoVs among pet ferrets and hedgehogs. Further investigations are needed to genetically characterize CoVs detected in this study, considering the high mutation rate and recombination events among CoVs.

Keywords: ferrets, hedgehogs, coronaviruses

References:
[1] Kitano, T., Umetsu, K., Tian, W., and Osawa, M. Two universal primer sets for species identification among vertebrates. International journal of legal medicine, 2007, 121, 423–427. DOI: 10.1007/s00414-006-0113-y.
[2] Drzewnioková, P., Festa, F., Panzarin, V., Lelli, D., Moreno, A., Zecchin, B., De Benedictis, P., and Leopardi, S. Best Molecular Tools to Investigate Coronavirus Diversity in Mammals: A Comparison. Viruses, 2021, 13, 1975. DOI: 10.3390/v13101975.
[3] Wise, A. G., Kiupel, M., and Maes, R. K. Molecular characterization of a novel coronavirus associated with epizootic catarrhal enteritis (ECE) in ferrets. Virology, 2006, 349, 164–174. DOI: 10.1016/j.virol.2006.01.031

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On-farm application of Precision Livestock Farming technologies in pair-housed dairy calves

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The objective of this study is to identify of innovative behavioural and physiological indicators of disease and welfare, through the application of Precision Livestock Farming (PLF) technology in pre-weaned dairy calves in pair-housing conditions. Pair housing has been described as an advantageous alternative to group housing, maintaining the positive effects of social housing, yet with a major control on health status and sanitary risk [1]. Promoting social-housing systems is likely to provide positive effects (i.e., better productive and cognitive performances) but might also facilitate disease spread [2], therefore the use of PLF might contribute to the monitoring of health and welfare of young animals.
16 Italian Friesian female calves (from 2 to 70 days of life) have been included in the study and assigned to individual housing indoor (IND; N=8 subjects) or pair housing indoor (PAIR; N=8 subjects). The activity of each calf is continuously monitored using tri-axial accelerometers and the general well-being and growth of animals is assessed daily from farm staff. At 1, 3, 5 and 8 weeks of age, calves' behaviour is video recorded and startle test is performed to assess the reactivity of animals. Infrared thermography images are taken for the evaluation of body temperature in different anatomical areas and each calf is subjected to complete veterinary clinical examination. Environmental parameters are also monitored continuously.
Through the evaluation of data sampled, we aim at identifying early indicators of disease and positive welfare indicators. This research might provide a useful and non-invasive on-farm approach for pair-housed calves management, allowing a prompt intervention for improving health and welfare.

Keywords: dairy calves, pair housing, PLF

References:
[1] Mikuš, T., Marzel R., and Mikuš O. Early weaning: New in-sights on an ever-persistent problem in the dairy industry. Journal of Dairy Research, 2020, 87, 88–92. https://doi.org/10.1017/S0022029920000503

[2] Costa J.H.C., von Keyserlingk M.A.G., Weary D.M. Invited review: Effects of group housing of dairy calves on behavior, cognition, performance, and health. Journal of Dairy Science, 2016, 99, 2453–2467. https://doi.org/10.3168/jds.2015-10144

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Expression of periostin in cancer-associated fibroblasts in spontaneous canine urothelial carcinoma

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The tumor microenvironment is involved in cancer development and progression and may influence the cancer cells' behavior [1]. Periostin (POSTN) is an extracellular matrix protein, and its main functions are induction of fibrillogenesis, fibroblastic cell proliferation and migration, enhancing regeneration in normal tissue and promoting metastasis in case of neoplasia. POSTN has already been studied in various human normal tissues, inflammatory processes and tumours revealing an important role for tumor progression in several types of cancer, such as those derived from colon, lung, head and neck, breast, ovarian, and prostate [2]. In these latter, high levels of POSTN are usually associated with a more aggressive tumor behavior, tumor advanced stages and poor prognosis while, in human bladder urothelial carcinoma (BUC), unlike in most tumors, POSTN expression seems to be down-regulated [3]. In veterinary medicine research on POSTN is poor thus the aim of the present study was to immunohistochemically investigate the presence and the intensity of POSTN expression in canine BUCs, to determine a possible relationship between POSTN expression and histopathological features such as mitotic count, muscular and vascular invasion. For the present retrospective study, archived samples from 45 canine BUCs and 6 non-neoplastic canine bladders were histologically examined and immunohistochemical tested for the expression of POSTN. POSTN expression was semi-quantitatively assessed considering the percentage of the neoplastic stroma positive for POSTN and the intensity of the immunohistochemical labelling. Histologically, 38/45 tumours were papillary and 7/45 non-papillary. All tumours were infiltrating,

21 extending also to the muscle layer and a significant correlation between this feature and the presence of neoplastic emboli emerged. In normal bladder tissue, as reported in human patients, a thick strongly positive belt of POSTN was visible and, in canine BUCs, as in human ones, a general decrease in POSTN expression was observed except for a strongly labelled ring of POSTN observed around some neoplastic nodules infiltrating the muscle layer. Moreover, POSTN expression and mitotic count were significatively inversely correlated.

Keywords: Urothelial carcinoma, Periostin, Cancer-associated fibroblasts, Mitotic count

References:

[1] González-González et al. A Matricellular Protein With Multiple Functions in Cancer Development and Progression. Front Oncol. (2018) 12.8:225.
[2] Lu P, Weaver VM, Werb Z. The extracellular matrix: a dynamic niche in cancer progression. J Cell Biol. (2012) 196(4):395–406. doi:10.1083/jcb.201102147
[3] Kudo A, Kii I. POSTN function in communication with extracellular matrices. J Cell Commun Signal. (2018) Mar;12(1):301-308. doi: 10.1007/s12079-017-0422-6.

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Evaluation of programmed death-ligand 1 expression in canine lymphoma using flow cytometry

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Programmed Death-Ligand 1 (PD-L1) is a well-known immune checkpoint molecule that recently has shown to be expressed in various canine neoplasms [1-3]. So far PD-L1 expression has been assessed by immunohistochemistry or polymerase chain reaction in canine lymphoma. Flow cytometry (FC) could serve as an alternative technique, as it can be conducted concurrently with the initial diagnosis without requiring additional sampling beyond that for diagnostic purposes. The aim of this study was to investigate PD-L1 expression in different canine lymphoma subtypes through FC. Surface expression of PD-L1 on neoplastic cells was assessed using excess material collected for diagnostic purpose from 70 dogs with lymphoma. The ratio between median fluorescence intensity (MFI) of neoplastic cells stained with an anti-PD-L1 antibody and an isotypic control was calculated and categorized as negative (=1), low-positive (>1 and <1.2), positive (≥1.2 and <1.7) and high-positive (≥1,7). Forty-five dogs had B-cell lymphoma (BCL) and 25 had T-cell lymphoma (TCL). Results for PD-L1 are as follows: 20(28.6%) dogs were categorized as negative (12 BCL, 8 TCL), 19 (27.1%) as low-positive (7 BCL, 12 TCL), 17(24.3%) as positive (13 BCL, 4 TCL) and 14(20%) as high-positive (13 BCL, 1 TCL). Median MFI-ratio of positive samples was 1.35 (range, 1.01-6.02), 1.39 (range, 1.01-6.02) for TCL and 1.31 (range, 1.01-3.47) for BCL. A difference in the occurrence of PD-L1 positivity categories was identified among FC phenotypes (p=.002), and positive BCL had significantly higher levels of PD-L1 compared to TCL (p=.001). This observation aligns with literature, confirming FC as a reliable technique for evaluating PD-L1 expression in canine lymphomas. Lastly, TCL can express PD-L1, although with lower prevalence and intensity than BCL. Remarkably, our study reported a single case of T zone lymphoma with an MFI-ratio=6,02. Further studies are needed to evaluate PD-L1 expression across various lymphoma subtypes.

Keywords: Flow Cytometry, Canine Lymphoma, Programmed Death 1 - Ligand 1

References:
[1] Shosu, K., Sakurai, M., Inoue, K., Nakagawa, T., Sakai, H., Morimoto, M., Okuda, M., Noguchi, S. and Mizuno, T. Programmed Cell Death Ligand 1 Expression in Canine Cancer. In Vivo, 2016, 30, 195-204. PMID: 27107075.
[2] Hartley, G., Elmslie, R., Dow, S. and Guth, A. Checkpoint molecule expression by B and T cell lymphomas in dogs. Veterinary Comparative Oncology, 2018, 16, 352-360. DOI: 10.1111/vco.12386.
[3] Aresu, L., Marconato, L., Martini, V., Fanelli, A., Licenziato, L., Foiani, G., Melchiotti, E., Nicoletti, A. and Vascellari, M. Prognostic Value of PD-L1, PD-1 and CD8A in Canine Diffuse Large B-Cell Lymphoma Detected by RNAscope. Veterinary Sciences, 2021, 8, 120-131. DOI: 10.3390/vetsci8070120.

VAS Days, 27-29th September 2023

KIT as a Potential Therapeutic Target in Canine Soft Tissue Sarcomas: Immunohistochemical Analysis and c-kit Gene Mutations

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Background: KIT (CD117) is a tyrosine kinase receptor involved in intracellular signalling and its mutated form associates to initiation and progression of several tumours [1]. Tyrosine kinase inhibitors are commonly used to treat mast cell tumours and GISTs with promising results.[2] CD117 has not been investigated in canine soft tissue sarcomas (STS). Treatment of STS is currently mostly limited to surgery and adjunctive therapies are needed.
Aim: to assess expression and detect mutations of CD117 in canine STS to assess its utility as a druggable target for adjuvant therapies.
Methods: 112 cases of canine STS we fixed, routinely processed for histology and immunohistochemistry (anti-CD117). Cases with high CD117 expression were selected for PCR analysis of exon 8, 9 and 11 mutations. Statistical correlation between CD117 expression and tumour grade was assessed with X^2 test.
Results: CD117 was expressed in 40/112 STS (35%), with predominantly diffuse cytoplasmic positivity separated by STS type as follows: rhabdomyosarcomas (5/5, 100%), fibrosarcomas (12/13. 92%), nerve sheath tumours (2/3, 66%), perivascular wall tumours (15/26, 57%) and liposarcomas (6/45, 13%); leiomyosarcomas were negative. Mutations in the c-kit gene were detected in 4 out of 14 cases.
Conclusions: canine STS histotype variably express CD117 and may harbour mutations in the c-kit gene. These data suggest that the use of tyrosine kinase inhibitors may represent a promising target therapy in selected subtypes of canine sarcoma.

Keywords: Canine, Soft Tissue Sarcoma, c-kit

References:
[1] Abbaspour Babaei, M., Kamalidehghan, B., Saleem, M., Huri, H. Z., & Ahmadipour, F. Receptor tyrosine kinase (c-Kit) inhibitors: a potential therapeutic target in cancer cells. Drug design, development and therapy, 2016, 10, 2443–2459.
DOI: 10.2147/DDDT.S89114
[2] Bonkobara M. Dysregulation of tyrosine kinases and use of imatinib in small animal practice. Veterinary journal, 2015, 205(2), 180–188.
DOI: 10.1016/j.tvjl.2014.12.015

VAS Days, 27-29th September 2023

Potential factors influencing complete functional recovery in traumatized stray cats with orthopedic lesions – a cohort study.

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Management of stray cats is being recognized as an emerging problem [1]. Little is known regarding the outcome of traumatic orthopedic injuries in this feline population [2]. Furthermore, the arrangement of these patients after recovery should not be underestimated. Indeed, complete functional recovery (CFR) should be the goal of treatment for reintroduction on the territory or adoption [3]. Aim was to evaluate potential clinical influencing factors on CFR in traumatized stray cats with orthopedic lesions. All bluntly traumatized stray cats with orthopedic lesions referred over 2 years were enrolled. Various clinical variables were prospectively collected. Nominal logistic analysis was applied to evaluate the influence of clinical variables on the CFR. A sub-sample of parvovirus-infected cats was also evaluated. Forty-eight stray cats were enrolled in the study, with median age of 24 (1-180) months and bodyweight of 3 (0.7-5) kg. Higher bodyweight and longer time from trauma to therapeutic intervention resulted significantly associated to CFR (P=0.04, both). Parvovirus infected cats resulted younger and lighter than the remaining cats enrolled. Probably, lighter cats experience more severe consequences following blunt trauma. Emergency procedures were associated to uncomplete functional recovery, possibly due to more severe lesions. Younger and lighter cats bear a higher risk of parvovirus-related death.

Keywords: stray cat, high energy trauma, causes of death.

References:
[1] Jessup DA. The welfare of feral cats and wildlife. J Am Vet Med Assoc. 2004 Nov 1;225(9):1377-83. doi: 10.2460/javma.2004.225.1377
[2] Grieco V, Crepaldi P, Giudice C, Roccabianca P, Sironi G, Brambilla E, Magistrelli S, Ravasio G, Granatiero F, Invernizzi A, Caniatti M. Causes of Death in Stray Cat Colonies of Milan: A Five-Year Report. Animals (Basel). 2021 Nov 19;11(11):3308. doi: 10.3390/ani11113308.

[3] Tanaka A, Wagner DC, Kass PH, Hurley KF. Associations among weight loss, stress, and upper respiratory tract infection in shelter cats. *J Am Vet Med Assoc*. 2012;240(5):570-576. doi:10.2460/javma.240.5.570.

VAS Days, 27-29th September 2023

MiR-30b-5p and miR-128-3p salivary expression in Cavalier King Charles Spaniels affected by myxomatous mitral valve disease

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Myxomatous mitral valve disease (MMVD) is the most common acquired canine heart disease and is reported to be hereditable in the Cavalier King Charles Spaniel (CKCS). Much is known about the structural valvular changes, but little is known about the molecular mechanisms that characterize MMVD progression. It was hypothesized that microRNAs (miRNAs) are involved in the development of MMVD[1]. MiRNAs are single-stranded non-coding RNAs that can silence or downregulate the expression of mRNA-targets. Circulating miRNAs have been detected in body fluids such as blood, saliva, urine, and breast milk. Several studies reported miRNAs to be up- or downregulated in MMVD-affected dogs in relation to the American College of Veterinary Internal Medicine (ACVIM) stages of the disease. Recently, a relationship between MMVD and plasmatic miR-30b-5p in CKCSs was established: the up-regulation of miR-30-5p is related to forms of disease that appear echocardiographically more stable over time[2]. Moreover, miR-128-3p is reported to be down-regulated in dogs affected by congestive heart failure[3]. At present, there is no study in veterinary cardiology that assesses whether miRNAs can be found in the canine saliva. The aim of this study was to detect miR-30b-5p and miR-128-3p in CKCSs' saliva and to observe how their salivary expressions modify in relation to MMVD progression. 24 CKCSs were included in the study (ACVIM stages: A n. 3, B1 n. 13, B2 n.5, C n. 3). MiR-30b-5p and miR-128-3p salivary expressions were, respectively, 2.39 (IQR_{25-75} 1.67-3.25) and 1.32 (IQR_{25-75} 0.68-2.89). Results showed a statistically significant different distribution of miR128-3p among the ACVIM stages ($p = 0.039$), particularly between stages A and C ($p = 0.028$). MiR-30b-5p does not show any statistically significant difference among the ACVIM stages ($p = 0.087$). Our results support the hypothesis that salivary miR-128-3p is upregulated in healthy CKCSs compared to MMVD-severely-affected CKCSs.

Keywords: myxomatous mitral valve disease, microRNA, saliva

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References:

[1] Reis-Ferreira, A., Neto-Mendes, J., Bras-Silva, C., Lobo, L., Fontes-Sousa, A.P. Emerging Roles of Micrornas in Veterinary Cardiology. Veterinary Sciences, 2022, 9, 533.
DOI: 10.3390/vetsci9100533.
[2] Ghilardi, S., Lecchi, C., Bagardi, M., Romito, G., Colombo, F.M., Polli, M., Franco, C., Brambilla, P.G. Prospective pilot study on the predictive significance of plasma miR-30b-5p through the study of echocardiographic modifications in Cavalier King Charles Spaniels affected by different stages of myxomatous mitral valve disease: The PRIME study. PLoS One, 2022, 17, e0274724.
DOI: 10.1371/journal.pone.0274724.
[3] Jung, S.W., Bohan, A. Genome-wide sequencing and quantification of circulating microRNAs for dogs with congestive heart failure secondary to myxomatous mitral valve disease. American Journal of Veterinary Research, 2018, 79, 163-169.
DOI: 10.2460/ajvr.79.2.163.

VAS Days, 27-29th September 2023

Urinary neutrophil gelatinase-associated lipocalin (uNGAL) in dogs: Clinical validation of a new rapid lateral flow test

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Urinary Neutrophil Gelatinase-Associated Lipocalin (uNGAL) has been identified as an early marker of acute kidney injury (AKI) in dogs [1], although its assessment, in clinical practice, is hardly feasible given the need to use ELISA assays. Furthermore, although higher levels of uNGAL are reported in AKI, similar findings are detected in other diseases [2]. The aim of this study was to evaluate the usefulness of a canine specific point-of-care (POC) lateral flow immunoassay for semiquantitative uNGAL measurement in clinical practice. Measurement of uNGAL was performed on 147 canine urinary supernatants using "Dog NGAL ELISA Kit" (Bioporto) and "PRIMA Veterinary - AKI Rapid Test canine NGAL detection" (PRIMA Lab). The analyses were performed on left-over samples previously collected for diagnostic purposes. Based on history, clinical and laboratory data, dogs were grouped as follows: controls; urinary tract infections (UTI); urolithiasis; chronic kidney disease (CKD); acute kidney injury (AKI); AKI on CKD; extrarenal inflammatory diseases. Data were analyzed by MedCalc 20.218. Kruskal Wallis and post hoc tests showed significantly higher uNGAL levels in AKI, AKI on CKD, and extrarenal inflammatory groups when compared to CKD and UTI ($p<0.05$). In the control group, the level of uNGAL was significantly lower than all others. A further grouping, based on the presence (n=30) or absence (n=117) of AKI, was applied on the canine population to perform a ROC curve using ELISA results, which are generated on a continuous scale. The cut-off of 29.7 ng/mL on the ROC curve determined a sensitivity of 96.7% and a specificity of 74.3%. Considering the POC device, the results were classified as negative (0 ng/mL), low (4 ng/mL), moderate (20 ng/mL) and high risk of AKI (90 ng/mL), based on the color chart provided by the manufacturer. A sensitivity of 97.6% and specificity of 55.6% were obtained using the cut-off of 20 ng/mL to discriminate between AKI or non-AKI dogs. Finally, Cohen's Kappa coefficient (K) of 0.72 showed good agreement between POC and ELISA results. In conclusion, good agreement was found between the POC test and the reference method in classifying patients with AKI. The relatively low specificity of both methods is due to the inherent characteristics of uNGAL, whose increase can be found in different disorders. However, based on its good sensitivity, the POC device may be a clinically relevant diagnostic tool in monitoring all those patients and/or clinical settings in which AKI may occur.

Keywords: NGAL, uNGAL, canine

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References:
[1] De Loor J, Daminet S, Smets P, Maddens B, Meyer E. Urinary biomarkers for acute kidney injury in dogs. J Vet Intern Med. 2013 Sep-Oct;27(5):998-1010. doi: 10.1111/jvim.12155. Epub 2013 Aug 19. PMID: 23952327.
[2] Nabity M, Hokamp J. Urinary Biomarkers of Kidney Disease in Dogs and Cats. Veterinary Clinics of North America - Small Animal Practice. 2023;53(1):53-71. doi:10.1016/j.cvsm.2022.07.006

VAS Days, 27-29th September 2023

Feline head and hind-limb lymphography using near infrared fluorescence with indocyanine green: an ex vivo anatomical study in 11 cats

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Animal lymphatic system is a translational model for humans, nonetheless, previous researches have been conducted only on few canine cadavers and no studies included cats[1-3]. Moreover, previous studies did not describe anatomical lymphosomes' landmarks[1]. In this context the aim of this explorative study is to map the feline lymphatic system, focusing on head and hind-limb regions whose drainage is considered complex.
Feline cadavers without neoplastic, surgical or traumatic lesion located at the anatomical region of interest were included. Topographical regions of head and hind-limb were defined based on bone landmarks. Indocyanine-green (ICG) was intradermally injected in each area. Massage and flexion-extension movements were performed to optimize the ICG migration. Near infrared fluorescence (NIRF) was used for lymphography and to guide nodal extirpation. Correspondence between detected lymphocentrum (LC) and those predicted by previous canine studies were recorded[1].
Eleven adult cats were included. All cats had body condition score <3/5 and skin thick <2 mm. One cat had pigmentated skin. A total of 16 hind-limb and 7 head regions were analysed. In 5/23 regions no LC was identified: 2 ears, 1 crural, 1 cranial-thigh, 1 finger-plantar. Six hind-limb cases drained to double lymphosome as reported in previous anatomical canine studies. In 5 cases the LC did not correspond to the expected lymphosome of those canine studies. Nineteen LCs were detected and 27 nodes extirpated. Median migration time, drainage length and nodal size were respectively 5 (1-30) min, 5 (0-11) cm and 10 (5-22) mm
The use of NIRF-ICG is feasible in feline cadavers with a low percentage of failure, probably due to cadavers' storage and conservation, and thawing process. Anatomical landmark

gives more accurate identification of the areas drained by an LC. A higher number of cases is needed to evaluate repeatability and predictability of lymphosomes.

Keywords: Feline Lymphatic System, Ex- vivo Anatomical study, Indocyanine Green

References:

[1] Suami, H., Yamashita, S., Soto-Miranda, M.A., Chang, D.W. Lymphatic Territories (Lymphosomes) in a Canine: An Animal Model for Investigation of Postoperative Lymphatic Alterations. PLoS ONE. 2013 Jul 24;8(7). doi: 10.1371/journal.pone.0069222

[2] Sánchez-Margallo, FM, Brun, M.V., Sánchez-Margallo, J.A. Identification of intra-abdominal lymphatics in canine carcasses by laparoscopic fluorescence lymphography with intradermal and intrapopliteal ICG administration. PLoS One. 2020 Nov 12;15(11):e0241992. doi: 10.1371/journal.pone.0241992

[3] Baum, H. The Lymphatic System of the Dog; Mayer, M.; Bettin, L.; Bellamy, K.; Stamm, I., Translators; University of Saskatchewan: Saskatoon, SK, Canada, 2021; pp. 26–34

VAS Days, 27-29th September 2023

Epidemiological study of endoparasites in pigs at the beginning of fattening period in northern Italy

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In Italy pig production is mainly related to the heavy pig industry, which is almost always confined indoors. Although oligosymptomatic trends predominate, diseases of parasitic origin can cause a decrease in animal welfare and a huge economic damage [1]. Moreover, some parasites may pose a risk of infection for professionals involved in the food chain (e.g., farmers and veterinarians) for their zoonotic potential [2].
Within this framework, this study attempts to evaluate the epidemiology and prevalence of the main porcine parasites at the beginning of fattening period. For this purpose, 440 fecal samples of fattening pigs, weighing between 35 kg and 85 kg, were analyzed from 22 farms located in three different regions of northern Italy.
For the detection of helminth eggs and coccid oocysts, the FLOTAC double technique®, was used [3], while for the direct detection of *Balantioides coli* was performed the sedimentation technique.
B. coli was the most frequently found parasite, with an overall prevalence of 89.1%. In addition, *Ascaris suum* (2.3%), *Cystoisospora suis* (1.6%) and *Trichuris suis* (1.4%) were reported, although with low prevalences. Moreover, 8 samples were positive for eggs of cestodes morphologically similar to those of the genus *Hymenolepis*. Subsequently, Dna from isolated eggs was extracted and an end-point PCR was performed revealing the presence of *Hymenolepis diminuta*.
The results obtained in this study provided current information on the prevalence and loadings of gastrointestinal parasites of fattening pigs in the early stages of the production cycle. Particularly, *B. coli* is prevalent, and data obtained in this investigation suggest the need to evaluate its impact on animals and humans.
Further investigations will be directed to assess the circulation of gastro-intestinal parasites at the end of the fattening period and to identify, through molecular methods, the presence of zoonotic genotypes of *B. coli*.

Keywords: endoparasites, fattening period, zoonosis

References:
[1] Roepstorff, A., Mejer, H., Nejsum, P., and Thamsborg, S.M. Helminth parasites in pigs: new challenges in pig production and current research highlights. Veterinary Parasitology, 2011, 180(1-2), 72-81. DOI: 10.1016/j.vetpar.2011.05.029.
[2] Giarratana, F., Nalbone, L., Napoli, E., Lanzo, V., and Panebianco, A. Prevalence of *Balantidium coli* (Malmsten, 1857) infection in swine reared in South Italy: A widespread neglected zoonosis. Veterinary World 2021, 14(4), 1044-1049. DOI: 10.14202/vetworld.2021.1044-1049.
[3] Cringoli, G., Rinaldi, L., Maurelli, M.P., and Utzinger, J. FLOTAC: new multivalent techniques for qualitative and quantitative copromicroscopic diagnosis of parasites in animals and humans. Nature Protocols, 2010, 5(3), 503-515. DOI: 10.1038/nprot.2009.235.

VAS Days, 27-29th September 2023

Bronchoalveolar lavage fluid cytokine mRNA expression in different equine asthma phenotypes

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Equine asthma (EA) is a common respiratory syndrome in horses, classified as severe (SEA) or mild-moderate (MEA), associated with relatively increased leukocyte populations in bronchoalveolar lavage fluid (BAL) [1]. Due to the diverse EA subtypes, conflicting findings on the involved immune responses are reported [2,3]. This study aimed to examine the association between the mRNA expression of cytokines representing different immune response types (innate, Th1, Th2, and Th17) in the BAL, and EA severity, cytological phenotype, and lung function. Fifteen horses were enrolled and underwent respiratory tract examination, lung function testing, airway endoscopy, and BAL cytology. Based on the findings, horses were classified as MEA or SEA and categorized into neutrophilic EA or mixed EA. The remaining BAL was used to assess mRNA expression of IL-1β, IL-2, IFN-γ, IL-4, and IL-17 using quantitative RT-PCR. Cytokines expression was compared between groups, and their correlations with BAL cytology and lung function were analyzed. Statistical analysis revealed that IL-1β expression was higher in SEA than MEA and positively correlated with BAL neutrophil count. IL-2 expression correlated with higher respiratory resistance at 5 Hz and lower expiratory reactance at 6 Hz, while IFN-γ correlated with BAL mast cells percentage. IL-4 expression was higher in horses with mixed EA than neutrophilic EA, positively correlated with BAL mast cells count, and inversely correlated with respiratory and expiratory reactance at 4 and 5 Hz. A strong inverse correlation was observed between IL-17 expression and total and expiratory reactance at 5 Hz. These findings suggest that multiple immune response types contribute to EA pathogenesis. Innate immunity may be associated with neutrophilic inflammation and disease severity, while Th1, Th2, and Th17 responses appear involved in airway hyperreactivity and lung

function impairment, possibly due to acute bronchospasm or chronic airway remodelling.

Keywords: equine asthma; asthma immunology; bronchoalveolar lavage

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References:

[1] Couetil, L.L., Cardwell, J.M., Gerber, V., Lavoie, J.P., Leguillette, R., and Richard, E.A. Inflammatory Airway Disease of Horses—Revised Consensus Statement. Journal of Veterinary Internal Medicine, 2016, 30, 503–515. DOI: 10.1111/jvim.13824.

[2] Bond, S., Leguillette, R., Richard, E.A., Couetil, L., Lavoie, J.P., Martin, J.G, and Pirie, R.S. Equine asthma: Integrative biologic relevance of a recently proposed nomenclature. Journal of Veterinary Internal Medicine, 2018, 32, 2088–2098. DOI: 10.1111/jvim.15302.

[3] Davis, K.U., Sheats, M.K. Differential gene expression and Ingenuity Pathway Analysis of bronchoalveolar lavage cells from horses with mild/moderate neutrophilic or mastocytic inflammation on BAL cytology. Veterinary Immunology and Immunopathology, 2021, 234, 110195. DOI: 10.1016/j.vetimm.2021.110195.

VAS Days, 27-29th September 2023

Computed tomography and clinical findings in 44 foals diagnosed with osteomyelitis

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Bacterial sepsis is a common reason for neonatal mortality in foals. Systemic sepsis is caused by spreading of bacteria through the hematogenous route with localized foci of infection such as osteomyelitis (OM) [1]. Computed tomography (CT) has been suggested to be superior in the diagnosis of OM compared to radiography, but comprehensive studies are lacking [2]. The aim of this study was to investigate clinical cases and establish correlations between CT findings, prognosis for survival to discharge and long-term.
Clinical data were collected retrospectively, and diagnostic imaging were re-evaluated for lesion characteristics. Statistical calculations were performed using SPSS 29.
Twenty-six (59%) of the foals were male and 18 (41%) were female. The most common breed was thoroughbred (45%). Seventy-one (71%) percent of foals had lesions detected on radiography and 64% by ultrasonography. In 81% of cases the diagnosis was modified after CT examination by; improved localization (38%), extension of the lesion (68%) or diagnosis of additional lesions (62%). Twenty-nine (66%) of foals survived to discharge and 23 foals (55%) long-term (0.5-3 years). Variables associated with decreased survival, to discharge and long-term, on univariable analysis included joint collapse (p=0.004 & 0.002), joint luxation (p=<0.001 & 0.005), physeal (p=0.026 & 0.02) and articular involvement (p=0.018 & 0.027). Additional significant variables for long-term survival were coxo-femoral joint (p=0.014) and bone sclerosis (p=0.004). In multivariable analysis for survival to discharge, joint collapse remained significant (p=0.011, OR=0.054 95%CI 0.006-0.506), and for long term survival, bone sclerosis (p=0.006,OR=0.142 95%CI 0.035-0.575) remained significant.
In conclusion, only variables from examination of the CT images were significantly associated with decreased survival and none of the clinical variables. This highlights the importance of performing CT examination in foals suffering from OM.

Keywords: computed tomography, osteomyelitis, foal

References:
[1] Giguère, S., Weber, E. J., and Sanchez, L. C.. Factors associated with outcome and gradual improvement in survival over time in 1065 equine neonates admitted to an intensive care unit. Equine veterinary journal, 2017, 49, 45-50. https://doi.org/10.1111/evj.12536.
[2] Lean, N., Perkins, N. and Ahern, B. Comparison of conventional radiography and computed tomography CT as aids in the diagnosis of osteomyelitis in 11 foals. Australian Veterinary Journal, 2018, 96(7), 257-261. https://doi.org/10.1111/avj.12710.

VAS Days, 27-29th September 2023

Investigation of the prevalence of ESBL-producing bacteria isolated from cattle farms in northern Italy and risk factors associated with the spread of antimicrobial resistance. Preliminary results.

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Antimicrobial resistance is increasingly a global public health issue for animal and human medicine. In other livestock systems, such as on poultry or pig farms, antimicrobial use (AMU) and AMR have been reduced due to improved biosecurity levels on farms [1]. Improved biosecurity leads to better health status of the herd and reduces the need for antibiotic drugs, consequently reducing AMR risk. In cattle, the studies concerning AMR and biosecurity are lower [2,3].
The main aim of this project is to evaluate the prevalence of ESBL-producing bacteria in healthy and diarrhoeic calves in northern Italy and potentially correlate them with risk factors related to biosecurity and calf management.
On farm samplings will consist in faecal sampling of healthy calves and calves affected by neonatal calf diarrhoea (NCD) and submit a checklist to the farmers in order to evaluate potential risk factors related to the spreading of AMR among neonatal calves. For each farm, at the beginning of the trial (T0) and, following the clinical examination (vitality score, faecal score, and dehydration score), 5 with neonatal calf diarrhoea and 5 healthy calves will be enrolled.
The microbiological analysis will be performed on fresh refrigerated faecal samples. After an overnight pre-enrichment of the feces in Mueller-Hinton broth, the 100 microliters of each sample will be plated onto McConkey and ESBL selective agar for 24h at 37°C. The identification of the colonies will be performed using the direct transfer method of MALDI-TOF MS. The double disc (DD) test will be performed with amoxicillin clavulanic acid, cefotaxime, and ceftazidime test for the resistance ESBL for each identified colony. To

meropenem to also screen the presence of carbapenemases-producing bacteria. Kirby-Bauer disk diffusion test will also be performed testing amoxicillin, ceftiofur, enrofloxacin, florfenicol, oxytetracycline, penicillin, trhimetropim+sulphametazol for checking the presence of multidrug resistance bacteria and if there are similar resistance patterns between bacterial colonies from healthy and sick calf.
A statistical analysis will be applied to assess the prevalence of ESBL/carbapenemases producing bacteria on each farm and the correlation with risk factors using χ^2 test and multinomial logistic regression.

Keywords: ESBL. Calves, Antimicrobial resistance

References:
[1] Laanen, M., Persoons, D., Ribbens, S., de Jong, E., Callens, B., Strubbe, M., Maes, D., & Dewulf, J.. Relationship between biosecurity and production/antimicrobial treatment characteristics in pig herds. Veterinary journal, 2013, 198(2), 508–512. https://doi.org/10.1016/j.tvjl.2013.08.029
[2] Weber, L.P.; Dreyer, S.; Heppelmann, M.; Schaufler, K.; Homeier-Bachmann, T.; Bachmann, L. Prevalence and Risk Factors for ESBL/AmpC-E. coli in Pre-Weaned Dairy Calves on Dairy Farms in Germany. Microorganisms 2021, 9, 2135. https://doi.org/10.3390/microorganisms9102135
[3] Ferroni, L.; Lovito, C.; Scoccia, E.; Dalmonte, G.; Sargenti, M.; Pezzotti, G.; Maresca, C.; Forte, C.; Magistrali, C.F. Antibiotic Consumption on Dairy and Beef Cattle Farms of Central Italy Based on Paper Registers. Antibiotics 2020, 9, 273. https://doi.org/10.3390/antibiotics9050273

VAS Days, 27-29th September 2023

Pulmonary artery stiffness in racehorses affected by Exercise-Induced Pulmonary Hemorrhage (EIPH)

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Exercise-induced pulmonary hemorrhage (EIPH) occurs commonly in horses performing high-intensity exercise. It is characterized by bleeding in the alveoli, consequent airways inflammation, and pulmonary hypertension, which leads to pulmonary vessel wall remodeling, thickening and a reduction of venous lumen [1]. The diagnosis is based on bronchoalveolar lavage fluid (BALf) cytology. In literature it has been reported that racehorses may be all potentially affected by EIPH [2]. Total Hemosiderin Score (THS) is used to distinguish EIPH-positive horses (THS > 75) from those negative (THS < 75). Pulmonary Artery Stiffness (PAS) is a non-invasive echocardiographic index of pulmonary artery elasticity that allows to assess the structural and functional features of the pulmonary vascular bed. PAS has been evaluated in asthmatic horses and correlated with an indirect index of pulmonary hypertension [3]. In the present study the differences of PAS between EIPH-horses and healthy horses and between EIPH-positive and EIPH-negative horses according to the THS were evaluated. PAS was measured in 4 EIPH-positive horses, 11 EIPH-negative horses and in 15 healthy horses. Since a limited number of horses were evaluated, data were studied by a non-parametric test such as Mann-Whitney U Test. When compared to healthy horses, EIPH-horses had a significantly higher PAS value ($p=0.041$). However, no significant differences were observed between EIPH-positive and EIPH-negative horses. The absence of statistically significant differences observed could be related to the small number of horses that may have induced a type II statistical bias. These are only preliminary results, however PAS could be useful for the evaluation of EIPH-horses and the severity of clinical condition.

Keywords: racehorses, pulmonary artery stiffness, EIPH

References:
[1] Derksen FJ, Williams KJ, Pannirselvam RR, de Feijter-Rupp H, Steel CM, Robinson NE. Regional distribution of collagen and haemosiderin in the lungs of horses with exercise-induced pulmonary haemorrhage. *Equine Vet J*. 2009;41(6):586-591. doi:10.2746/042516409X429419.

[2] Doucet MY, Viel L. Alveolar macrophage graded hemosiderin score from bronchoalveolar lavage in horses with exercise-induced pulmonary hemorrhage and controls. *J Vet Intern Med*. 2002;16(3):281-286. doi:10.1111/j.1939-1676.2002.tb02370.
[3] Alberti E. Evaluation of Pulmonary Artery Stiffness in Asthma Affected Horses. Tutor: Zucca E.; Coordinator: Ceciliani F.; DIVAS of University of Milan; 2022 March 31 (34° cycle PhD in VAS; academic year 2020/2021); http://hdl.handle.net/2434/916663.

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Radiofrequency ablation in equine distal limbs: a cadaveric study. Preliminary results

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Radiofrequency (RF) is used to treat unresponsive chronic pain in humans. RF ablation (RFA) produces a thermal lesion at the sensory nerve with transient interruption of pain signals [1]. As the equine distal limb is innervated by sensory palmar digital nerves (PDNs), RFA represents an attractive therapeutic option in horses with chronic lameness. The study aims to evaluate the feasibility of ultrasound (US) guided positioning of RF needle to the target nerve and to assess effectiveness and to compare histological lesions of PDNs after the application of two RFA protocols. Four fresh equine cadaver forelimbs (16 samples) were used. A RF needle was inserted in craniocaudal direction with an in-plane US approach, until the tip reached the target PDN in fetlock and pastern regions. RFA was randomly performed at 60°C for 6 minutes (Group LOW) or 80°C for 8 minutes (Group HIGH) and methylene blue (0,1 ml) was injected through the injection port. Anatomical dissection was performed to localize the dye. Treated PDN were histologically examined. The median of needle tip-to-nerve proximity was 5 mm (0-10 mm) in fetlock region and 0 mm (0-5 mm) in pastern region. In pastern region, RFA was always carried out at target (8/8 cases) and the length of the stained PDN was 15 mm (13-20 mm). In fetlock region in 3/8 cases, the needle was placed at 8 to 10 mm and PDN was not stained, so RFA was performed off target (1 group LOW; 2 group HIGH); in the remaining 5/8 cases, PDN was stained for 14.6 mm (3-20 mm). The histological examination of 1/8 and 6/8 nerves for group LOW and group HIGH, respectively, was consistent with acute electrocautery injury. Overall, in 13/16 cases RFA was performed at target (81% positive outcome) demonstrating the effectiveness of RF needle placement via US-guided technique in the equine distal limb. In most cases, LOW treatment was not sufficient to result in nerve coagulation, while with HIGH treatment PDNs were injured, but arteries were often involved.

Keywords: Radiofrequency ablation; Palmar digital nerve; Equine.

References:
[1] Boesch, J.M.; Campoy, L.; Southard, T.; Dewey, C.; Erb, H.N.; Gleed, R.D. Histological, Electrophysiological and Clinical Effects of Thermal Radiofrequency Therapy of the Saphenous Nerve and Pulsed Radiofrequency Therapy of the Sciatic Nerve in Dogs. Vet. Anaesth. Analg. 2019, 46, 689–698, doi:10.1016/j.vaa.2019.05.006.

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Mechanisms of chemoresistance in mammary gland tumor cells

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Mammary tumors in female dogs are spontaneous and recurring and are considered an experimental model in human medicine. Several data are reported on the effectiveness of chemotherapy treatments in women [1], while very few investigations have been performed in canine veterinary oncology [2]. This study aims to identify genetic alterations associated with resistance to chemotherapy in canine mammary carcinoma cells, validating these alterations in an independent group of samples. Four previously established cell cultures from canine mammary gland tumors were used for analysis. The expression of the genes MDR1, BCRP, MRP1 and MRP3, related to resistance to chemotherapy, was evaluated in the cells by the RT-qPCR technique. The cellular metabolism test (MTT) was performed to define the minimum inhibitory concentration (IC_{50}), and was performed before and after the induction of therapeutic resistance with doxorubicin. An IC_{50} was determined for each cell type, being 1.67 μM for UNESP-CM4, 6.03 μM for UNESP-CM5, 12.54 μM for UNESP-CM70 and 29.42 μM for UNESP-CMR1. The RT-qPCR results show that the UNESP-CMR1 cell has higher gene expression compared to the UNESP-CM4 cell. The UNESP-CM70 cell shows higher expression of the MRP3 gene. IC_{50} determination showed that UNESP-CM4 was more sensitive to doxorubicin, while UNESP-CMR1 was more resistant. It is noteworthy that cells with low expression of the MDR1, BCRP, MRP1 and MRP3 genes are more sensitive to doxorubicin, presenting a lower IC_{50}. On the other hand, cells with high expression of these genes, such as UNESP-CMR1, are more resistant to treatment. At the moment, we can suggest that specific genes may have some relation with cellular resistance and that the differential response to doxorubicin treatment among cells shows the need for personalized therapeutic strategies.

Keywords: dog, mammary carcinoma, chemotherapy resistance

References:
[1] Lovitt, Carrie J. et al., Doxorubicin resistance in breast cancer cells is mediated by extracellular matrix proteins, BMC Cancer, 11, 2018. https://doi.org/10.1186/s12885-017-3953-6
[2] Valdivia, Guillermo et al., From conventional to precision therapy in canine mammary cancer: a comprehensive review, Frontiers in Veterinary Science, 33, 2021. https://doi.org/10.3389/fvets.2021.623800