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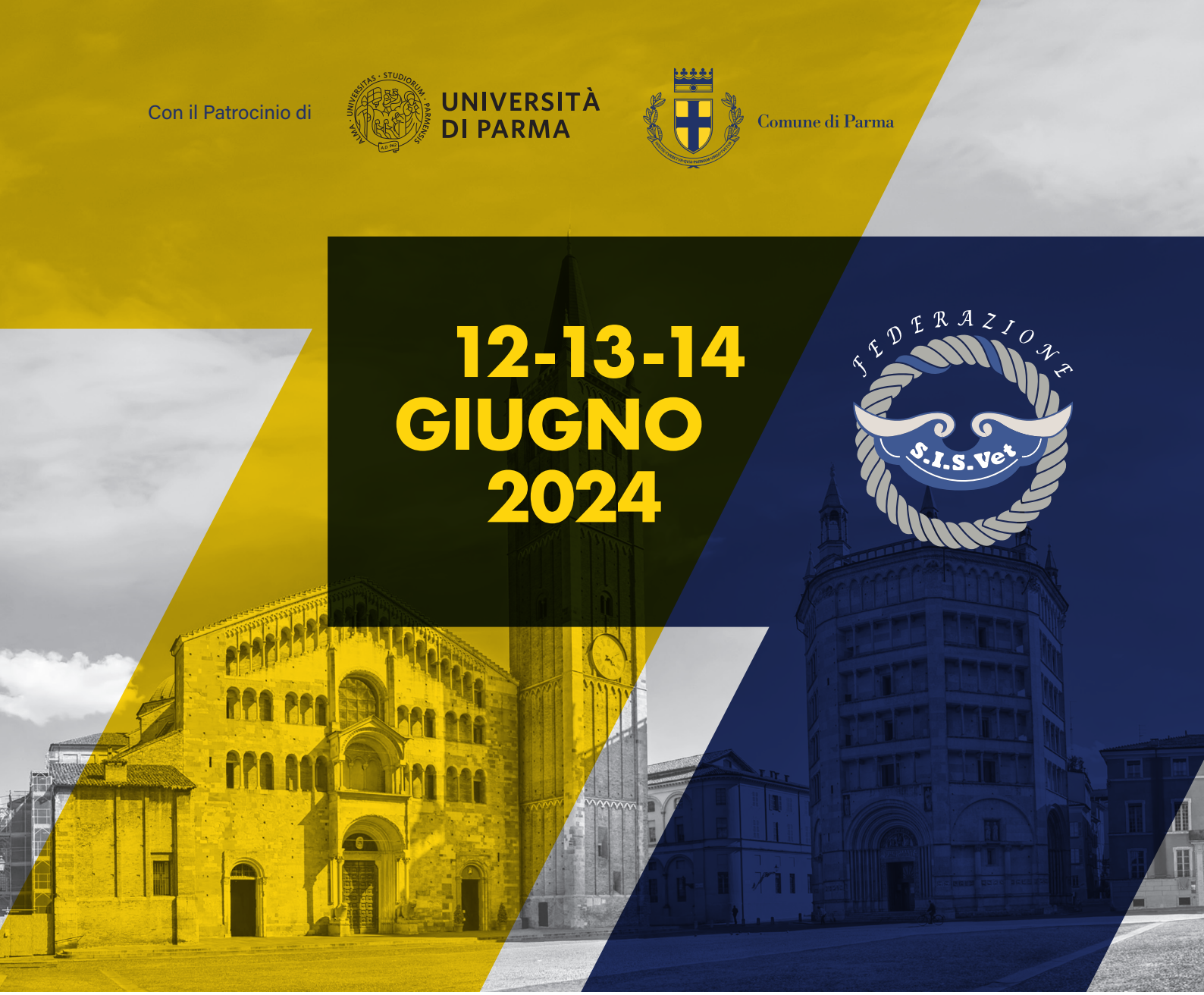

12-13-14 GIUGNO 2024

FEDERAZIONE S.I.S.Vet

77° CONVEGNO SISVET

Campus Universitario
Plesso Aule delle Scienze (ex Q02)

PARMA

ATTI 77° CONVEGNO SISVET

CODICE ISBN 9788890909269

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Lafarmacia.

PARMIGIANO REGGIANO

PreMix

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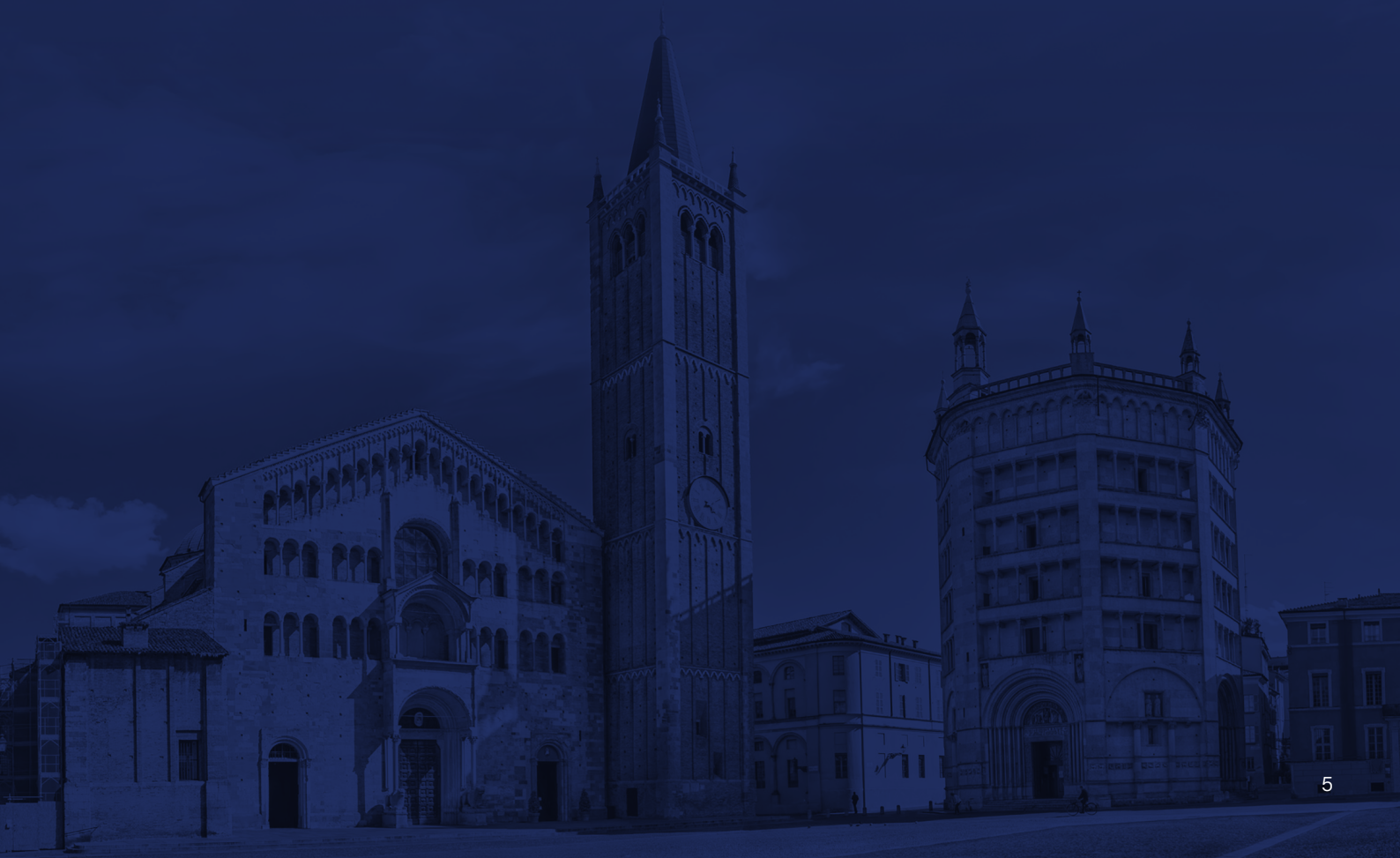
ABSTRACT

Workshop, Tavola Rotonda e Simposio Federale

Di seguito vengono riportati i programmi
e i relativi contributi pervenuti



WORKSHOP | **Mercoledì 12 Giugno**, 11.00 - 13.30

INNOVATIVE THERAPIES IN PRECISION MEDICINE: RNA DRUGS, NANOPATICLES AND GENOMIC EDITING

Moderatori

Tiziana Cannizzo - Università degli Studi di Torino

Katia Cappelli - Università degli Studi di Perugia

Maurizio Mazzei - Università degli Studi di Pisa

Programma

11.00 – 11.30
PNRR National Center for development of gene therapy and drugs with RNA technology: prospective for research in Veterinary Medicine
Laura Rinaldi – Università degli Studi di Napoli

11.30 – 12.00
Stimuli-responsive nanoparticles as an innovative strategy for cancer treatment
Giulia Mesiano – Politecnico di Torino

12.00 – 12.30
Extracellular vesicles as theranostic drug delivery vehicles
Roberta Tasso – Università degli Studi di Genova

12.30 – 13.00
Innovative vaccines: new opportunities for Veterinary Medicine
Caterina Lupini – Università degli Studi di Bologna

13.00 – 13.30
Genome editing: applications on new breeding tools in livestock
Tad Sonstegard – President & CEO Acceligen

77° CONVEGNO SISVET

SOCIETA´ SCIENTIFICA DI RIFERIMENTO | **SOCIETA' ITALIANA DI PARASSITOLOGIA (SOIPA)**

TITOLO | **PNRR National Center for development of gene therapy and drugs with RNA technology: prospective for research in Veterinary Medicine**

Autori | L. Rinaldi[1], E. Ciccone[1], P. Pepe[1], F. Sepe[2], C. Campanile[3], A. Bosco[1], M.P. Maurelli[1], O. Petillo[2], J. Guccione[1], G. Oliva[1], N.A. Cacciola[1,2]

Affiliazioni | *1 Dept. of Veterinary Medicine and Animal Production, University of Naples "Federico II", Naples – Italy*
2 Research Institute on Terrestrial Ecosystem, National Research Council (IRET-CNR) Naples–Italy
3 Institute of Genetics and Biophysics "A. Buzzati-Traverso", National Research Council (IGB-CNR), Naples - Italy

Testo e Riferimenti bibliografici

Ribonucleic acids (RNAs), including messenger RNA (mRNA), transfer RNA (tRNA), and ribosomal RNA (rRNA), play an important role in living organisms. In recent years, RNA-based technologies have shown remarkable efficacy in the prevention and treatment of infectious diseases in humans and animals [1]. In particular, mRNA vaccines are highly effective, inexpensive to produce, rapid to develop, and safe to administer, suggesting promising applications also in the veterinary field. Currently, most mRNA vaccines have been developed to protect against zoonotic diseases such as Ebola, influenza, rabies and Zika virus [1].
Accordingly, in 2022, the National Center for Gene Therapy and Drugs based on RNA Technology, funded by the EU National Recovery and Resilience Plan (NRRP Mission 4 – Component 2, Investment 1.4), and spearheaded by the University of Padua, was established with the aim to support Key Enabling Technologies related to the "Development of Gene Therapy and Drugs based on RNA Technology".
The National Center brings together 46 Italian and international partners from the public, private and corporate sectors and has two main objectives: (*i*) increasing the technological know-how necessary to design and deliver RNA-based and gene therapy medicinal products and (*ii*) identifying promising candidate drugs/genes for genetic diseases, cancer, metabolic/cardiovascular diseases, neurodegenerative disorders and inflammatory/infectious diseases. The National Center is structured along a Hub and Spoke dissemination model. The central Hub manages and coordinates research program activities, while the 10 Spokes focus on translating research results into the development and manufacturing of personalized medicines. Specifically, Spoke 5 focuses on coding and non-coding RNA as therapeutic targets, vehicles, or biomarkers for inflammatory and infectious diseases.
Among parasitic diseases, cystic echinococcosis (CE) has been selected to be investigated within the National Center. CE is a worldwide zoonotic parasitic disease caused by the larval stages of the tapeworm *Echinococcus granulosus*. To date, the control of CE has been a public health priority due to the difficulties in diagnosis and treatment. Despite the availability of therapeutic agents, e.g. benzimidazoles, to treat intermediate hosts, these approaches have proven to be ineffective. Therefore, novel chemotherapy alternatives and drug targets are urgently needed to improve control programs against CE. Non-coding RNAs have been identified as potential diagnostic targets and therapeutic candidates for metacestode infections. In particular, some miRNAs, including mir-71, have been shown to be involved in parasite development [2]. In this sense, the main objectives of this task are: (*i*) identifying and characterizing non-coding RNA involved in different parasite stages and (*ii*) discover potential new drug targets against CE. The research results could form the basis for the development of innovative control strategies against CE based on RNA technology. Finally, the novel approaches developed using *Echinococcus* as a "parasite model" could also be used to control other parasitic infections.

[1] Phan et al. RNA therapeutics for infectious diseases. Prog Mol Biol Transl Sci., 204, 109–132, 2024.
[2] He et al. miRNAs and lncRNAs in *Echinococcus* and Echinococcosis, Int J Mol Sci 21(3): 730. 2020.

This research was funded by EU funding within the MUR PNRR National Center for Gene Therapy and Drugs based on RNA Technology (Project no. CN00000041, RNA).

77° CONVEGNO SISVET

SOCIETA´ SCIENTIFICA DI RIFERIMENTO **Politecnico di Torino**

TITOLO **Ultrasound and smart biomimetic nanoparticles: innovative therapeutic approach against cancer.**

Autori Giulia Mesiano [a], Marco Carofiglio [a], Giada Rosso [a], Veronica Vighetto [a], Valentina Alice Cauda [a].

Affiliazioni *[a].Department of Applied Science and Technology, Politecnico di Torino, C.so Duca degli Abruzzi 24, 10129 Turin, Italy.*

Testo e Riferimenti bibliografici

Cancer represents the primary cause of morbidity and mortality worldwide. Over the past few decades, there have been significant advances in cancer treatment, including surgery, chemotherapy, radiotherapy, immunotherapy, targeted therapy, and precision medicine approaches. These advances have led to improvements in survival rates and quality of life for many cancer patients, but numerous limitations such as systemic toxicity, drug resistance, limited drug penetration, on set of metastasis and excessive costs, remain unsolved. Adress to these restrictions requires continuous research and innovation in the field of oncology.
Recently, nanomedicine has been the subject of various research efforts, offering new perspectives and promising breakthroughs for cancer patients thanks to the capability of enhance the effectiveness and safety of therapeutic strategies. One of the key areas where nanomedicine therapies are making significant steps is that of targeted drug delivery using nanoparticles (NPs). NPs can be engineered to target specific molecular markers or receptors expressed by tumor cells, enabling precise delivery of drugs to tumor sites, and consequently minimizing exposure to healthy tissues and systemic side effects and enhancing the efficacy of the treatments. Moreover, NPs found applications in the enhancement of the imaging techniques as contrast agents. In fact, NPs can improve the resolution and sensitivity of imaging modalities, allowing for earlier detection and more accurate diagnosis of cancer.
Theranostics, the integration of therapy and diagnosis, represents a rapidly evolving field in nanomedicine. Theranostic NPs can simultaneously deliver therapeutic agents and imaging probes, enabling real-time monitoring of treatment response.
In our laboratory at Politecnico of Turin, we focus our attention on a new groundbreaking branch of nanomedical therapeutic approach: the opportunity to remotely activated NPs to induce selective tumor toxicity when exposed to different external stimuli. By fine-tuning the properties of both the nanomaterial and the triggering stimulus, therapeutic outcomes are achieved while creating a powerful platform for simultaneous imaging, resulting in a nano-theranostic application.
The main objective of our research is the development of advanced biomimetic and targeted NPs capable of responsive and localized therapy, activated by the safe application of acoustic shockwaves for deep tissue stimulation.
With this aim, we synthesize iron-doped zinc oxide nanocrystals encased in a biomimetic lipid bilayer shell, incorporating a peptide (YSA) for selective targeting of colorectal cancer (CRC) and osteosarcoma (OS) cells. According to the literature, the ephrin-mimetic peptide YSA specifically targets the EphrinA2 (EphA2) receptor, which is good expressed by several types of solid tumors, for example CRC and OS. Then we developed such a kind of targeted NPs which are specifically directed against tumor cells without affecting healthy tissues.
Our in vitro work was conducted both in 2D and 3D models of CRC and OS models and compared the efficacy between non-targeted (L-ZnO) and targeted (YSA-L-ZnO) nanoparticles, highlighting the better ability of the latter to adhere and be internalized by tumor cells. YSA-L-ZnO NPs demonstrate high biocompatibility and hemocompatibility and can induce selective damage upon activation by safe shockwaves. This mechanism synergistically eliminates tumor cells in both 2D and 3D models, validating the concept of an innovative stimuli-responsive nanomedicine with targeted and biomimetic strategies, offering promising prospects for cancer treatment.

References

L. Racca, G. Rosso, M. Carofiglio, S. Fagonee, G. Mesiano, F. Altruda, V. Cauda CANCER NANOTECHNOL., 2023, 14, 37.

77° CONVEGNO SISVET

SOCIETA´ SCIENTIFICA DI RIFERIMENTO **ANIV**

TITOLO **Innovative vaccines: new opportunities in veterinary medicine**

Autori C. Lupini

Affiliazioni *Dept. of Veterinary Medical Sciences, University of Bologna, Ozzano dell'Emilia (BO) - Italy*

Testo e Riferimenti bibliografici

The success of inactivated and live-attenuated vaccines has enhanced livestock productivity, promoted food security and attenuated the morbility and mortality of several animal and zoonotic diseases. However, traditional vaccine technologies are not without fault. Within the last three decades, veterinary medicine has spearheaded the advancement in innovative vaccine development to circumvent several of the flaws associated with classical vaccines [1]. Innovative vaccines include (but are not limited to):

- **Vectored Vaccines**: Vectored vaccines are based on the use of a living, non-pathogenic microorganism to express one or more heterologous antigens. Viral vectors, for instance, are modified viruses that carry foreign genes or sequences integrated in positions that are not essential for viral replication or infectivity. The expression of the integrated transgene *in vivo* stimulates the development of a specific cellular and humoral immune response against the pathogen for which the vaccine is designed.
- **Recombinant Subunit Vaccines**: Subunit vaccines contain non-infectious short specific protein of a given pathogen, produced by *in vitro* plasmid expression vectors harbouring genes of protective antigens.
- **Nucleic Acid Vaccines**: Nucleic acid vaccines work by administering genetic material (such as DNA or messenger-RNA) encoding pathogen's antigens into the host. The host's cells then produce the antigens, stimulating a specific immune response. Recently, self-amplifying RNA vaccines, able to encode not only the target antigen but also the machinery necessary for RNA replication (derived from alphaviruses viral vectors), have been designed. This technology allows the production of multiple copies of RNA, leading to a more robust and prolonged antigen expression. While RNA vaccines are not yet widely available in veterinary medicine, they hold promise for control of relevant diseases such avian influenza.

Innovative vaccines offer several advantages, including rapid development, flexibility in antigen selection, potential scalability in manufacturing; they induce both humoral and cellular immune response, and can be utilized to differentiate infected from vaccinated animals. In general, these vaccines do not cause adverse effects or local reactions after vaccination.

Furthermore, major technology advantages owned by innovative vaccines recently lead the inclusion of "vaccine platform" in veterinary regulatory legislation. Vaccine platforms are defined as technologies that utilize a common backbone or vector to deliver specific antigens [2]. This regulatory strategy has the potential to accelerate registration process of innovative veterinary vaccines based on a well-defined platform technology and offers new opportunities to the prompt control of emerging animal diseases.

[1] Aida et al (2021) Novel Vaccine Technologies in Veterinary Medicine: A Herald to Human Medicine Vaccines. Front. Vet. Sci. 8:654289.

[2] Entrican and Francis (2022) Applications of platform technologies in veterinary vaccinology and the benefits for one health. Vaccine 40: 2833-2840

77° CONVEGNO SISVET

SOCIETA´ SCIENTIFICA DI RIFERIMENTO | **INNOVATIVE THERAPIES IN PRECISION MEDICINE: RNA DRUGS, NANOPARTICLES AND GENOMIC EDITING**

TITOLO | **Genome editing: applications of new breeding tools in livestock**

Autori | T. Sonstegard[1]

Affiliazioni | *1 Acceligen, a Recombinetics Company, Eagan, MN – U.S.A.*

Testo e Riferimenti bibliografici

Genome editing is the latest addition to a series of breeding tools, capable of accelerating the rate of genetic improvement in food animals. This technology enables the introduction of only the beneficial alleles within a single generation, including those of low frequency or entirely absent in animals under selection; and therefore, offers precise and transgene-free methods for improving resilience and health compared to alternative conventional breeding methods. Crossbreeding is already proven to be costly and time-consuming even when combined with genomic selection. Acceligen's precision breeding platform based on genome editing has proven to provide heat stress and disease resilience in breeds that traditionally did not have these phenotypes. Maximizing the value chain benefits of genome editing also depends on commercialization in the face of rapidly evolving regulatory statutes for risk assessment. The breeding of some gene-edited founder animals has resulted in commercialization of genetics to only require following standards used for conventional animal breeding. In these countries, new breeding technologies like genome editing are now poised to have significant impact in better equipping cattle to combat diseases and climate change without the loss of production gains obtained from decades of selection.

BIODIVERSITY AND ECOTOXICOLOGY: OPPORTUNITIES FOR SUSTAINABLE DEVELOPMENT

Moderatori

Paolo De Girolamo - Università degli Studi di Napoli

Pierluigi Di Ciccio - Università degli Studi di Torino

Programma

14.30 – 15.00

Can the veterinarian mitigate the impact of livestock farming on the enviroment?

Giuseppe Argiolas – Presidente OMV Cagliari, Sementusa

15.00 – 15.30

Innovation for Sustainable Development in Bioarchives: the International Mouse Mutant Archive and Centre for Phenogenomics (EMMA/Infrafrontier/IMPC)

Marcello Raspa – Italian National Research Council, Institute of Cellular Biology and Neurobiology, Monterotondo (Roma)

15.30 – 16.00

A sea of hazards: effects of exposure to emerging pollutants in aquatic organisms, focus on microplastics

Maria Carmela Ferrante – Università degli Studi di Napoli

16.00 – 16.30

Animal welfare and sustainability of dairy cow farming

Angelo Peli – Università degli Studi di Bologna

77° CONVEGNO SISVET

SOCIETA´ SCIENTIFICA DI RIFERIMENTO **SIRA**

TITOLO **CAN THE VETERINARIAN MITIGATE THE IMPACT OF LIVESTOCK FARMING ON THE ENVIRONMENT?**

Autori Giuseppe Argiolas

Affiliazioni

Testo e Riferimenti bibliografici

Sementusa protocol is the name given to the specific technical activities that are applied in sheep and goat dairy and beef farms. This involves standardized veterinary medical activities that are to be systematically applied in livestock activities.[1] The aim of the application of the Sementusa protocol method is to bring the fertility of the flock above 97%, the farms that apply the protocol in the majority achieve above 99%. Another very important priority for livestock systems that involve part of the productive life in grazing activities is to coincide meat and milk production with the periods when natural pastures are present. The structure of the protocol includes at least one checkup per year on males, with a complete health examination, ultrasound inspection of the reproductive system, blood sampling, and evaluation of diseases circulating in the herd. Being males a stable population, we also evaluate over the years the serological evolution of diseases. [2] On the females, 4 ultrasound checks are scheduled, the first at 50-60 days after the entrance of the males, the subsequent ones at 45 days apart. In these checks, the days of pregnancy, the number of fetuses, and the BCS, for pregnant animals are recorded. In non-pregnant animals, the physiological status of the ovaries (presence of follicles or corpus luteum), possible pathologies, and BCS are recorded. [3] The protocols provided by Sementusa to manage non-pregnant ewes, take into consideration ovarian physiology and fattening status of the ewes. The application of this protocol allows a marked improvement in the efficiency of sheep and goat husbandry systems, and over 5 years of application, with a result that can reach 100% productivity. All the data collected from individual animals are entered into an application “Sementusa Mobile” which is specially designed to record all the relative health information of each animal and produces a graph of farm efficiency.

[1] Argiolas G., Boi R., Corso S., Sale S., Spezzigu A. La gestione riproduttiva nell’allevamento ovini e caprino, corso teorico pratico sulla tecnica ecografica nella gestione della attività riproduttiva & Alimentazione. https://www.sementusa.it
[2] Chemineau P., Pellicer-Rubio M.T., Lassoued N., Khaldi G., Monniaux D. Male-induced short oestrous and ovarian cycles in sheep and goats: a working hypothesis. Reprod Nutr Dev. 2006 Jul-Aug; vol. 46, n. 4: pp. 417-29. Epub n2006 Jul 7.
[3] Spezzigu A. Utilizzo dell’ultrasonografia nei piccoli ruminanti per la caratterizzazione della dinamica follicolare al fine di identificare fattori ovarici in grado di influenzare la qualità dell’oocita. Tesi di Dottorato, Università degli Studi di Sassari, 2010.

A SEA OF HAZARDS: EFFECTS OF EXPOSURE TO EMERGING POLLUTANTS IN AQUATIC ORGANISMS, FOCUS ON MICROPLASTICS

M.C. Ferrante[1]

1 Dept. of Veterinary Medicine and Animal Productions, University of Naples, Naples – Italy

Abstract

Emerging pollutants are mostly anthropogenic chemicals which include pharmaceuticals, industrial additives, personal care products, brominated flame retardants and plastic particles among others. They have recently been recognized as dangerous for the ecosystems and the total environment, including the health of all living organisms. In addition, many of these pollutants have not been regulated under European or national legislation, hence posing a potential greater risk for environmental and public health. Plastics are a group of synthetic materials made of organic polymers and some additives with special characteristics that is gaining increasing scientific and public attention during the last decade. Indeed, since the 1950s, there has been a sharp rise in the production of plastics. As of 2015, it is estimated that approximately 6,300 million metric tons of plastic waste had been generated and, if current trends continue, roughly 12,000 million metric tons of plastic waste will accumulate by 2050 in landfills or the natural environment. Once in the natural environment, due to their non-biodegradable nature, plastics may take several thousands of years to partly break down in the wild. Plastics undergo fragmentation and biodegradation into smaller particles named microplastics (MPs) (100nm-5mm) and nanoplastics (1-100nm).

Among emerging pollutants, MPs are those on which the scientific world has particularly focused in recent years. Massive occurrence of MPs in the environment implies the exposure of animals and human beings to them with health consequences not yet well understood. Although MPs are frequently found in the atmosphere and the terrestrial ecosystems, they are mostly detected in an aqueous environment, in both water and sediment phases, even in remote areas with scarce or no human activities. Aquatic ecosystems are considered the final sinks, building up over time, undergoing bioaccumulation processes along the trophic chain and resulting in alterations of fauna and flora wild species.

MPs potentially induce several adverse effects on multiple target organs in aquatic vertebrate species. Neuronal disorders, behavioral changes and impairment of reproductive function, growth, glucose and lipid metabolism, among others, have been observed in experimental and natural environmental conditions. Due to their hydrophobicity MPs are also important carriers of organic and inorganic xenobiotics adsorbed onto their surface leading to potential additive, synergistic or antagonistic effects. Xenobiotics adsorption may be augmented by biofilms, microorganisms concentrated on the surface of MPs, causing further negative consequences for biodiversity and ecosystem functioning as well as living organisms' health.

The presentation will be an overview of the sources, fate and impact of MPs on marine and freshwater ecosystems. The main adverse health outcomes induced by MPs on aquatic organisms will be discussed, focusing on gastrointestinal homeostasis investigated in a teleost fish as fairly novel experimental model.

77° CONVEGNO SISVET

SOCIETA´ SCIENTIFICA DI RIFERIMENTO **SICLIMVET**

TITOLO **Animal welfare and sustainability of dairy cattle farming**

Autori A. Peli[1], M. Roccaro[1]

Affiliazioni *1 Dept. for Life Quality Studies, University of Bologna, Rimini - Italy*

Testo e Riferimenti bibliografici

The livestock sector is considered to play an important role in climate change as it is estimated to produce 14.5% of anthropogenic emissions, with beef and cattle milk production accounting for the majority of them [1]. Besides GHG emissions, the livestock sector is responsible for several negative environmental impacts (EIs), e.g., particulate emissions, freshwater consumption, soil acidification, water eutrophication, fossil fuel consumption, waste output.
Life Cycle Assessment (LCA) is currently considered the best tool to quantify the EI of products. Despite being an internationally standardized and widely recognized methodology, LCA application to agricultural systems has received criticism as being fallacious in capturing the complexity of farming systems. Indeed, ruminant production systems are often multi-functional, providing ecosystem services (ES), including pasture, landscape, and biodiversity preservation, carbon storage, wildfire prevention. However, in studies on the EI of ruminant products ES are often ignored; as a result, intensive farming systems generally have lower impacts than extensive systems [2].
Sustainability is a multifaceted concept that encompasses environmental, economic, and social aspects. Animal welfare (AW) is a key component of livestock farming sustainability. Besides ethical considerations, it is well known that good husbandry practices, AW and health are beneficial to production efficiency, which is a major asset for reducing EI. Specifically, as the growing body of research on this topic has shown, the increases in GHG emissions from diseases in dairy cattle originate from discarded milk, reduced milk production, prolonged calving interval and culling [3]. However, this evidence is still underused.
EI mitigation strategies of livestock systems might sometimes conflict with AW. Enhancing animal productivity can lead to improved resource efficiency and the consequent reduction in the number of animals required to generate a given quantity of product, hence lowering emission intensity (kg GHG/kg of output). Production intensification, however, can come at a high prize for animal health and welfare, as it might increase the risk for diseases such as acidosis, laminitis, ketosis, mastitis, infertility, and reduced longevity. On the other hand, some AW improvement measures, e.g., increased space allowance and cleanliness, temperature management, carry potential EIs [4].
Given these interdependencies, research on the combined AW and LCA evaluation and a quantification of trade-offs in highly needed. As recently reviewed, AW assessment is highly inhomogeneous compared to LCA and the two evaluations are rarely integrated in an overall sustainability score [5]. Before an effective integration can be achieved, several limitations of LCA and AW evaluation need to be addressed. To support the sustainability and resilience of the livestock farming systems worldwide, a comprehensive on-farm sustainability evaluation that takes into account both EI, without overlooking ES, and AW is required.

[1] Gerber et al. Tackling climate change through livestock – A global assessment of emissions and mitigation opportunities, FAO, 2013.
[2] von Greyerz et al. A large share of climate impacts of beef and dairy can be attributed to ecosystem services other than food production, Journal of Environmental Management, 325:116400, 2023.
[3] Özkan et al. The role of animal health in national climate commitments, FAO, 2022.
[4] Herzog et al. In pursuit of sustainability in dairy farming: A review of interdependent effects of animal welfare improvement and environmental impact mitigation, Agriculture, Ecosystems and Environment, 267:174-187, 2018
[5] Lanzoni et al. Review: The challenge to integrate animal welfare indicators into the Life Cycle Assessment, animal, 17:100794, 2023.

ASSISTENTE VETERINARIO: IL PUNTO SULLA SITUAZIONE E LE PROSPETTIVE FUTURE

Coordinatore

Domenico Bergero - Università degli Studi di Torino

Moderatori

Giuseppe Crescenzo - Università degli Studi di Bari

Stefania Lauzi - Università degli Studi di Milano

Interventi

La formazione in ambito veterinario: novità e prospettive
Paolo Vincenzo Pedone - Presidente CUN

Classe L38: il giusto inquadramento della figura professionale
Emiliano Lasagna - Presidente FIDSPA

Il punto di vista della FNOVI: definizione delle competenze
Gaetano Penocchio - Presidente FNOVI

La figura tecnico o assistente veterinario: esperienza di un cds in Italia
Antonio Mollo – Università degli Studi di Padova

Gestione di un ospedale didattico veterinario: un modello integrato di competenze
Serena Ceriotti - Auburn University, USA

THE MEAT SUPPLY CHAIN BETWEEN HISTORY, TRADITION AND INNOVATION

Moderatori

Ugo Della Marta - Ministero della Salute
Adriana Ianieri - Università degli Studi di Parma
Luciano Pinotti - Università degli Studi di Milano

Programma

08.30 – 09.00
The development of "artificial" refrigeration and its role on the meat trade in the late 19th and early 20th century
Ivo Zoccarato – Università degli Studi di Torino

09.00 – 09.30
European Policies and Challenges for the Livestock Sector
Paolo De Castro – Università degli Studi di Bologna

09.30 – 10.00
Health and welfare risk-based farm categorization: the role of slaughterhouse
Giovanni Loris Alborali – Istituto Zooprofilattico Sperimentale della Lombardia e dell'Emilia Romagna

10.00 – 10.30
Veterinary challenges in exports: addressing health barriers and managing African swine fever
Davide Calderone – ASSICA, Associazione Industriali delle Carni e dei Salumi

10.30 – 11.00
***Toxoplasma gondii* in meat: what is the risk?**
Laura Kramer – Università degli Studi di Parma

77° CONVEGNO SISVET

SOCIETA´ SCIENTIFICA DI RIFERIMENTO | **ASSOCIAZIONE ITALIANA DI STORIA DELLA MEDICINA VETERINARIA E DELLA MASCALCIA**

TITOLO | **THE DEVELOPMENT OF "ARTIFICIAL" REFRIGERATION AND ITS ROLE ON THE MEAT TRADE IN THE LATE 19TH AND EARLY 20TH CENTURY**

Autori | I. Zoccarato

Affiliazioni | *Italian Association for the History of Veterinary Medicine and Farriery*
c/o Veterinary Sciences Museum, Dept. of Veterinary Science, University of Turin, Grugliasco - Italy

Testo e Riferimenti bibliografici

The urbanization, which manifested itself from the beginning of the 19th century, led to an increasing demand for foodstuffs, including meat products. This demand was periodically further increased trough meat hording by the military authority's for war necessities, which characterized both the 19th and 20th century. The increased demand encouraged the trading in live animals for slaughter, not only between rural areas and cities, but also among different regions and states. The transport of live animals became a widespread practice both by land, thanks to the railway network, and by sea for all the exchanges that also took place among different continents and between the islands and the continent, as in the case of Sardinia from where, in the first two years of the WWI, as many as 26,000 head of cattle shipped from Sardinian ports disembarked in Civitavecchia. However, the exchange of live animals has always risen problems of sanitary nature – as the great epizootics that followed armies and others – as well as welfare of animals and economic and logistics aspects. With the development of the refrigeration industry, however, food preservation methods changed and with them those of transport: no longer live animals (standing meat) but meat preserved by cold. The development of the "cold chain", therefore, represents an essential achievement for the evolution of the commercial meat sector, not only considering the improvement of its hygienic and organoleptic characteristics. Until then, preservation was based almost exclusively on drying, smoking and packaging in brine or dry salt to preserve the integrity of the meat. In United States, the first attempts at "artificial refrigeration" were realized in the first half of the 19th century, this was followed by the first chilled transport around 1860. In Europe and Italy, where the first industrial refrigeration plant was inaugurated in Milan in 1887, the advent of the refrigeration industry in general came later. It was between the end of the 19th century that the role of "artificial" refrigeration received more attention and the first systems were installed in slaughterhouses. Edoardo Perroncito report on a visit he made to Geneva in 1895, where he visited the refrigeration plants of the city's municipal slaughterhouse. In particular, he described how part of the installations ware intended to receive frozen meat from Argentina and Oceania. He also recalled that German authorities, in 1903, had defined the refrigerator as a necessary appliance and an indispensable part of the slaughterhouses intended for the preparation of the most important food for man [1].
The success of "artificial" refrigeration was determined by the advantages it provided over cooling with natural ice: stability in storage, transport over long distances, absence of the negative effects of climatic conditions, better hygienic and qualitative meat characteristics. At the outbreak of the WW, artificial refrigeration was the standard for meat preservation. From this point of view, the fear of a wartime food shortage played a key role in the spread refrigerated storage facilities in order to continuously supply US soldiers and the European allies with meat.
The presentation addresses the development and the effects of this industry from a hygienic and socio-economic perspective between the late 19th century and the early 20th century.

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77° CONVEGNO SISVET

SOCIETA´ SCIENTIFICA DI RIFERIMENTO **SOIPA**

TITOLO ***TOXOPLASMA GONDII* IN MEAT: WHAT IS THE RISK?**

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Testo e Riferimenti bibliografici

Toxoplasma gondii is a protozoan that infects virtually all warm-blooded animals, including humans, livestock, marine mammals, birds and reptiles. In Europe, *T. gondii* is ranked second out of 23 foodborne parasites (1). Consumption of undercooked infected meat is considered a major risk factor for humans, especially in Europe, where it has been associated with 30–63% of infections (2). The current prevalence of infection in food-producing animals in Italy varies and depends on numerous factors, including: presence of cats on farms, farm size, geographic location of farm and intensive vs extensive farming. When considering the relative proportion of the different species in the overall meat consumption, the proportion of meats eaten raw or undercooked, the exposure and susceptibility of different species to infection and the survival of parasites in their tissues, meat from pigs and small ruminants are likely the main sources of infection (farm to fork). Meat from wildlife, horses, poultry and cattle are less common sources. Grazing ruminants acquire infection by ingestion of oocysts, therefore prevalence in sheep tends to be high (27.8%-87.4%). Interestingly, recent studies suggest that while primary infection in sheep protects against subsequent abortion, it does not necessarily inhibit vertical transmission and lamb should be considered a high-risk meat (3). Cattle, on the other hand, are susceptible to infection, but resistant to the formation and persistence of tissue cysts (4). Pigs are infected by ingesting either oocysts or meat containing tissue cysts (rodents or kitchen waste). Prevalence is therefore low (0–2%) in pig farms with controlled housing conditions, rodent control, protection of feed from cats and restriction of farm access. Outdoor access of pigs considerably increases the risk for *T. gondii* infection such as in free-range organic farms (5). Cured ham and salami have been suggested as possible sources of human infection. A study carried out on Prosciutto di Parma obtained from experimentally infected pigs reported that the curing process and aging time of 12 months duration eliminates viable tissue cysts (6).
Even though *T. gondii* is a high priority foodborne zoonotic parasite, there are no specific regulations and no standardized methods for the detection it food products. Tissue cysts cannot be detected during routine meat inspection and most infected carcasses pass meat inspection and enter the food chain. Future research priorities include development of effective vaccines for cats and/or food-producing animals and low cost, sensitive detection methods of the parasites in meat.

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THE ROLE OF VETERINARY PROFESSION IN PET FOOD MARKET: OPPORTUNITIES AND CHALLENGES

Moderatori

Giuseppe Cringoli - Università degli Studi di Napoli

Antonio Crovace - Università degli Studi di Bari

Interventi

Evolution of pet animal nutrition in Italy: scientific, technological and professional aspects
Pier Paolo Mussa – Università degli Studi di Torino

AMR and BARF diets for companion animals: a real threat?
Piera Anna Maria Martino – Università degli Studi di Milano

Feeding the orphaned newborn puppy and kitten
Cristina Veronesi – Università degli Studi di Milano

Pets food and immune response: "fiends or foe"?
Elisabetta Razzuoli – Istituto Zooprofilattico Sperimentale del Piemonte, Liguria e Valle d'Aosta

Radiographic assessment of skeletal pathologies in growing dog
Mauro Di Giancamillo – Università degli Studi di Milano
Anna Zurlo – Università degli Studi di Milano

Dog and cat nutrition for "healthy aging"
Alessandro Gramenzi – Università degli Studi di Teramo

From the Bowl to the Biome: The Macrobiota's Role in Pet Physiology and Health
Paolo Mongillo – Università degli Studi di Padova

Petfood: the clinicopathological perspective
Alessia Giordano – Università degli Studi di Milano

Neurochemical chemosensing in the gastrointestinal tract
Maurizio Mazzoni – Università degli Studi di Bologna

Reality and fiction on Pet Food and parasitic risk
Emanuele Brianti – Università degli Studi di Messina

BARF Diet: Food Safety Pillars
Gianluigi Ferri – Università degli Studi di Teramo

Pet Food: between toxicological and nutraceutical aspects
Alessandro Di Cerbo – Università degli Studi di Camerino

Dietary interventions and intestinal microbiota modulation as treatment strategies for the management of chronic inflammatory enteropathies in dogs and cats
Carla Vecchiato – Università degli Studi di Bologna

77° CONVEGNO SISVET

Stato: INVIATO - ID: 12959

Evolution of pet animal nutrition in Italy: scientific, technological and professional aspects

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The first studies on dog nutrition date back to 1700: Francois Magendie (1783-1855) identified the role of nitrogen in dogs; Max Josef Pettenkofer (1818-1901) published one of the first works on dog nutrition in 1864. Later, Casimir Funk (1884-1967) introduced the term “vitamins” in 1912 and in 1932 Max Kleiber (1893-1976) identified the first equation for determining energy requirements as a function of weight: MR=aMˆ0.75. Since the second half of the last century, many gaps have been filled by works such as those of the National Research Council (publications of 1974, 1985, 2006), books such as “Small animal clinical nutrition” (1983, 1984, 1987, 2000, 2007), AAFCO publications. In Europe, FEDIAF has been updating the nutritional requirements of dogs, cats and, lately, pet rabbits since 2008. In Italy, the most recent book “Nutrizione e alimentazione del cane e del gatto” is dated 2021 and published by Edagricole.Towards the end of the last century, courses on pet nutrition were introduced in Italian Faculties of Veterinary Medicine and dedicated scientific societies were founded: SCIVAC, AIVPA, SIANA in Italy and ESVCN in Europe. Alongside the specific studies on animals, those on feedstuffs processing technologies have emerged, which have favoured the birth of industries specializing in the production of wet food by sterilisation and dry food by extrusion. Research has investigated the effect of technologies on the elimination of pathogenic organisms and anti-nutritional factors, as well as their impact on the persistence of vitamins, with more than satisfactory but often unknown results. Gradually, pre-packaged foods have attracted new admirers with increasing in market shares: from 254 million (1985) to 1,000 million (early 2000s) to 2,419 million of euros (2022) million in revenue from the sale of dog and cat food plus 12 million for other animals (birds, fish, small rodents...). There are 12 million Italian families interested in shopping (46.9% of the total). The number of pets in the family has also risen, reaching a total of 64.8 million, distributed as follows: dogs 8.7 million, cats 10.1, small mammals 1.8, reptiles 1.4, birds 12.9 fish 29.9. More than the number, the care for these animals, which have become true members of the family, has increased. This complex of phenomena had a strong impact on the veterinary professional who, in the meantime, had an increment in the number of graduates. Between 1880 and 1980 the number of members of the professional order had grown from 1972 to 6300, but with a substantially stable relationship with the Italian population (1:10,000). Since then there has been an exponential increase in the number of members to 35,350 with a ratio of 6:10,000 and a progressive increase in women (37.4% in 2008 and 49.17% in 2024). Pets have contributed to limiting the crisis in professional practice and increasing the activity in nutritional counselling.The recruitment of veterinarians by the feed industry has contributed substantially to the spread of scientific knowledge about nutrition, playing a fundamental role in the prevention and treatment of many diseases. These are often the work of veterinarians working in the feed and supplement industry and find their way into many areas: Research and Development, Registration and Regulation, Marketing, “Technical” Service, Promotion/Information, Sales. Compared to the past, much has been lost at the professional level, but much has also been gained.

77° CONVEGNO SISVET

SOCIETA´ SCIENTIFICA DI RIFERIMENTO **Associazione Nazionale Infettivologi Veterinari (ANIV)**

TITOLO **AMR and BARF diets for companion animals: a real threat?**

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Testo e Riferimenti bibliografici

While the number of pets continues to rise globally, it remained relatively stable in Italy in 2022. Euromonitor estimates that there are 64.95 million of them, of which approximately 19 million are dogs and cats, with the latter consistently exceeding 10 million at present. A greater focus on animal care, including the quest for "healthier" but sometimes "natural" and "alternative" pet food, is influenced by these trends and by the strong bonds that exist between people and their companion animals. As was previously mentioned, pet owners can choose from a wide variety of diets these days, and raw meat has grown in popularity as a way to give their animals a natural and healthy diet. Raw meat-based diets (RMBD), also known as Biologically Appropriate Raw Food (BARF), comprise raw muscle meats, organ meats, and meaty bones of livestock that are either meant for human consumption but are rejected for commercial reasons (unfit) or passed fit for human consumption at the abattoir [1]. Numerous bacterial pathogens, including *Escherichia coli*, *Salmonella* spp., *Campylobacter* spp., *Listeria* spp., and *Clostridium* spp., have been identified from raw pet food globally, even if these materials follow microbiological controls (e.g., for *Enterobacteriaceae* and *Salmonella*) [2]. A study conducted in Italy identified *E. coli* in 93% of beef samples and 100% of poultry and pork samples. The pet diet products in question were of human grade but had been rendered unfit for human consumption due to defects, manufacturing issues, or commercial considerations [2]. Concerns about zoonotic diseases are not the only subject attracting attention: raw pet food may include bacteria that are resistant to antibiotics (AMRs). In a One Health perspective, this worry is connected to the worldwide rise in the transmission of AMR not only in the human (and hospital) domain but also in the veterinary and environmental fields. It has been shown that RMBD-feeding increases the risk of canine faecal shedding of AMR bacteria [3]. Previous research conducted in Europe and other countries has provided evidence for the faecal carriage of ESBL-producing *E. coli* and *Enterobacteriaceae* strains that exhibit resistance to third generation cephalosporins. Additionally, these studies have identified Enterococci that are resistant to phenolics and oxazolidinones in both raw meat and the faeces of dogs and cats [2].
Therefore, the threat posed using RMBD in dogs and in cats (to a lesser extent) is real for themselves, for humans particularly during the handling of these products and for the environment due to the spreading of AMR bacteria excreted in faeces of pets feed with BARF diets.
The knowledge and the respect of simple hygiene procedures may help to control this “bad” relationship between AMR and BARF diet.

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77° CONVEGNO SISVET

SOCIETA´ SCIENTIFICA DI RIFERIMENTO **SIRA**

TITOLO **Feeding the orphaned newborn puppy and kitten**

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Testo e Riferimenti bibliografici

Dogs and cats are considered altricial species, in which offspring are born relatively immature compared to other domestic species and depend entirely on the mother for survival during the neonatal period, which means the first 3-4 weeks of age [1]. Feeding the newborn puppy and kitten is provided by the mammary secretion including colostrum in the 1st-2nd day after parturition, followed by milk, with species-specific characteristics adapted to the newborns nutritional requirements, for their growth and development early in life, but also with long-term effects [2]. According to the type of placenta, in both species, the passive immune transfer is provided by the soon-after-birth intake and absorption of good-quality colostrum, containing immunoglobulins produced by the mother [3]. However, colostrum is also an important source of nutrients such as carbohydrates and lipids, bioactive compounds, growth factors, etc, and allows the expulsion of meconium. Colostrum is followed by the secretion of milk, whose characteristics change over time from parturition. Artificial feeding could be requested for many reasons in newborn dogs and cats, and at different ages after birth. The condition of orphans is the more frequent cause of artificial feeding requests and can concern rescue catteries, breeding catteries, or veterinary patients. Artificial feeding must therefore respect the maternal mammary secretion composition, in agreement with changes occurring with the time after parturition, providing the correct energy requirement, all the nutritional needs, and the immunological defenses. Many species-specific milk replacers are available on the market and must be preferred to homemade formulae. The feeding management must rely on the calculated energy requirements and the calories provided by the formulae. The feeding schedule frequency, amount, and administration modality must be adapted to the newborn age, body weight, stomach capacity, and health conditions. In all cases, enteral feeding is only allowed for orphans with body temperatures higher than 35°C, with good sucking reflex and without malformations such as cleft palate. In these cases, the patient must be stabilized in body temperature before feeding, and milk must be provided by tube feeding. Errors in feeding could lead to failure to thrive, constipation, or, more often diarrhea, causing delayed or arrested weight gain, intestinal prolapse, or even puppy or kitten death. In conclusion, feeding the orphan newborn puppy or kitten is a very frequent request, but underlying newborn physiologic knowledge, and practical handling procedures are needed to avoid even life-threatening consequences.

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Pets food and immune response: “fiends or foe?”

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Advances in the understanding of how the immune system functions in response to diet have altered the way we think about feeding companion animals. To date we known that some compounds like short chain fatty can directly activate TLRs than immune response. Studies dedicated to understanding how immune function can be altered with diet has revealed additional functions of essential nutrients such as Zinc, vitamins D and E or omega-3 polyunsaturated fatty acids (PUFA). Furthermore, the supplementation of feed with phytogenic or probiotic additives further modifies the immunomodulatory potential of modern diets. For some nutrients such as vitamin D or PUFA, supplementation can optimize immune function and reduce inflammation, while for other molecules as zinc hight doses may inhibit immune function. Moreover, is important to consider the negative effects of over-immune modulation, where important functions such as clearance of microbial infections may be reduced when supplementation reduces the inflammatory action of the immune system. Continued studies in nutritional immunology will further enhance our understanding of the power of nutrition and diet to improve health of Pets.

77° CONVEGNO SISVET

SOCIETA´ SCIENTIFICA DI RIFERIMENTO **SICV**

TITOLO **RADIOGRAPHIC ASSESSMENT OF SKELETAL PATHOLOGIES IN GROWING DOG**

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Testo e Riferimenti bibliografici

Nutrition-related skeletal disorders can affect animals of any age, but they play a relevant role in the skeletal development of growing dogs.
They can be divided in three large categories:

1. Pathologies related to imbalances in key nutrients (calcium, phosphorus, vitamin A and vitamin D), mainly represented from secondary nutritional hyperparathyroidism, rickets and hypervitaminosis A;
2. Pathologies in which the etiology has not yet been clearly determined, but that are suspected to have a nutritional component, such as eosinophilic panosteitis and metaphyseal osteopathy;
3. Pathologies related to imbalances caused by overnutrition, which are found primarily in fast-growing, large and giant dogs, mainly represented from hip and elbow dysplasia and osteochondrosis.

Secondary nutritional hyperparathyroidism is a condition that mobilizes calcium from the bone, consequent to an insufficient absorption or intake of calcium or unbalanced absorption of phosphorus. The most susceptible patients are growing animals fed with incorrectly formulated home diets (e.g., meat-only diets) and the main radiographic signs are reduced bone opacity, thin cortex, incomplete (greenstick) fractures and compression fractures of the epiphyses and vertebrae.
Rickets is a rare condition due to hypovitaminosis D that can be found in dogs subjected to improperly balanced home feeds (e.g., vegetarian diets). Due to the defect of mineralization, on radiographs, osteopenia, thin cortex of the long bones and thickening of the physis creating large lacunar areas including the adjacent metaphyses and epiphyses are visible.
Hypervitaminosis A is a condition most frequently associated with high dietary consumption of liver. Radiographs show premature closure of the physis, new bone deposition and ankylosis of the vertebrae, enthesiopathy and degenerative joint disease.
Panosteitis is a condition with unknown etiology, in which high calcium intake is supposedly involved, which may interfere in the remodeling of vascular channels in bone during growth. An early radiographic manifestation of the pathology consists of the appearance of circumscribed radiopaque nodular areas within the medullary cavity of the diaphysis of the long bones (especially near the nutritious foramen) that can become more diffuse and homogeneous and associated to cortical thickening.
Pathogenesis of metaphyseal osteopathy is unknown and the role of the nutrients is discussed and actually disproven. Bone lesions, usually bilateral and symmetrical, are comparable to those observed on vitamin C deficiency in man and involve the metaphyses of the long bones where is possible to detect a radiolucent line parallel to the physis (double physis sign).
Hip and elbow dysplasia represent a common clinical condition in dog and they are related to a combination of genetic and environmental factors. Overnutrition and oversupplementation can cause disharmonious development in the growth curve, in which muscle masses grow too rapidly compared with the skeletal system.
Radiographically articular osteophytes, remodeling of bone epiphyses, increased subchondral sclerosis and soft tissues mineralization are observed.
Osteochondrosis occurs from necrosis of epiphyseal cartilage, which results in failure of normal endochondral ossification. Various nutritional deficiencies/excesses have been proposed as contributing causes. The main radiographic signs are flattening of the surface of the affected subchondral bone, subchondral sclerosis and mineralization of cartilaginous flap.
Although investigations by ultrasonography, computed tomography, magnetic resonance imaging and nuclear medicine have been proposed in the diagnosis of these conditions, radiology remains the first step in the diagnostic protocol in the skeletal development abnormalities in growing dogs.

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77° CONVEGNO SISVET

SOCIETA´ SCIENTIFICA DI RIFERIMENTO **SiSVET**

TITOLO **Dog and cat nutrition for"healthy aging"**

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Testo e Riferimenti bibliografici

In pets, like humans, there is an extension of the average lifespan as a result of improvements in diet and quality of life as well as in the diagnostic and therapeutic systems of the main pathologies of aging, therefore an adaptation of the nutritional aspects is also necessary precisely in order to meet needs that change according to the age of the subjects. Aging is a complex biological process that consists of a progressive reduction in individuals' ability to maintain homeostasis in the event of both internal physiological and external environmental stress and a consequent increase in susceptibility to disease. In particular, various systems and organs are involved which are more involved in pathological processes which can find in nutrition a valid aid for prevention or therapy. The aims of geriatric dietetics are therefore to: improve the quality of life and life expectancy, slow down the progression of metabolic changes with age and control the development of pathologies linked to ageing. The speed of the aging process is influenced by many factors, in particular genetic, environmental and nutritional ones, but also by lifestyle, emotional state, pathologies and traumas. The common feature of aging is a progressive and irreversible change that can be accelerated by the effects of disease, stress, malnutrition, decreased exercise and environment. Older animals rarely have a single pathology, but more often a multi-organ syndrome with various levels of dysfunction which therefore involves various systems. The effect of aging can affect the digestive system's ability to consume, digest or metabolize food. With senescence, the motility of the colon decreases, with the very frequent appearance of constipation in elderly subjects. Older animals have greater difficulty assimilating food and therefore need high-quality ingredients to improve digestion. To these problems must be added the pathologies linked to senescence which are often prevented or improved by specific dietary regimes: reduction of maintenance energy requirements, periodontal problems, reduction of colon motility, reduction of renal function, joint problems, reduction of cardiac function, reduced efficiency of the immune system, tumors, reduction of hearing, sight and taste and behavioral changes. From a nutritional point of view we can divide the geriatric population into categories according to the effects of aging: animals that maintain a good weight and health conditions (physiologically young), animals with a predisposition to weight gain (metabolically efficient), animals with predisposition to lose weight (metabolically inefficient) and those with clinically manifest pathologies (true geriatric). To determine the group to which it belongs, each animal must undergo a thorough clinical examination together with the determination of the BCS (body condition score). For animals that have maintained their actual weight and body condition and show little age-related changes, the diet they are eating should not change; those who tend to gain weight (BCS > 6), but are otherwise in good health, must follow a low-calorie diet that will help them lose weight and prevent further weight gain. Animals that struggle to maintain a good body weight require foods with high caloric density and high nutritional value, while subjects with typically manifest pathologies respond well to specific diets. Therapeutic strategies that may slow the progression and improve signs of ageing include drugs, functional foods, and nutritional supplementation oriented to reduce damage caused by oxidative stress, correct metabolic changes associated with cognitive decline, reduce inflammation, and ameliorate mitochondrial function, neuronal health, and signaling. Dietary supplements, more adequately known as nutraceuticals, have shown a sharp increase in veterinary medicine in recent years. There is a reasonable body of literature confirming the positive effects of nutraceutical supplementation on neuronal damage and cognition in ageing animals.

77° CONVEGNO SISVET

SOCIETA´ SCIENTIFICA DI RIFERIMENTO

TITOLO **From the Bowl to the Biome: The Microbiota's Role in Dog Physiology and Health**

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Testo e Riferimenti bibliografici

It is well known that nutrition plays a pivotal role in the health and well-being of animals. What is becoming more evident in recent years is that many of these effects are mediated or modified by the gut microbiota (GM), i.e. the ensemble of bacteria, archaea, viruses, and eukaryotic organisms inhabiting the gastrointestinal tract. The GM is highly responsive to nutrition, and while large changes in dietary macronutirents are generally required to alter GM composition, more subtle changes in nutritional components are sufficient to impact on the production of microbial metabolites, in turn affecting the host physiology and health.
In fact, the microbiota intervenes in host metabolism by participating in the breakdown and fermentation of dietary substrates and the synthesis of essential nutrients and vitamins. Microbial fermentation of dietary fibers produces SCFAs, which serve as an energy source for intestinal epithelial cells and contribute to the maintenance of gut barrier function. Additionally, microbial metabolism of bile acids, amino acids, and lipids can impact host energy homeostasis, lipid metabolism, and glucose regulation. However, the role of the GM extends beyond its' contribution to digestive function. One of the key mechanisms by which the microbiota influences host physiology is through modulation of the host immune system. Commensal microbes residing in the gut stimulate the development and maturation of the host immune system, promoting immune tolerance to harmless antigens while enhancing immune responses to pathogens. Microbial-derived molecules, such as short-chain fatty acids (SCFAs), polysaccharide A (PSA), and various metabolites, can directly interact with immune cells and modulate their function, influencing inflammatory responses, immune cell differentiation, and cytokine production. Moreover, emerging evidence suggests that the gut microbiota communicates bidirectionally with the central nervous system (CNS) through the gut-brain axis, influencing neurological function and behavior. Microbial-derived metabolites, can cross the gut epithelium and enter systemic circulation, modulating neuronal activity and neurotransmitter signaling within the CNS. Moreover, GM can influence the production of neurotrophic factors, inflammatory mediators, and other molecules that regulate brain development, synaptic plasticity, and cognitive function.
A paradigmatic example of the complex relationship between GM and the host physiology and health is represented by changes in GM composition observed through ageing and their effect on the CNS, largely studied in humans. Indeed, age-related loss of GM richness and diversity and altered ratios among populations are thought to play a role in the development of age-related neurodegeneration and cognitive impairment associated with Alzheimer's Disease.
Uniquely among mammals, ageing dogs can spontaneously develop a progressive neurodegenerative disease that results in behavioral abnormalities and impairment in cognitive functions, clinically known as Canine Cognitive Dysfunction Syndrome which shares many pathological features with human's Alzheimer's Disease, to the extent that dogs are recognized as an excellent species for comparative investigations on the impact of ageing on brain physiopathology. Work by the author's laboratory has looked at the effects of ageing on both cognition [1] and behavior [2] in dogs. Moreover, we have explored relationship between age-related cognitive decline and immune system, showing alterations in leukocyte populations in cognitively impaired aged dogs [3]. Dogs' GI hosts a highly biodiverse microbial ecosystem, which richness is undermined by agein, and suggesting that a role of the GM may be played in age-related conditions. Current studies by the author's group are looking into exploring the relationship between GM and cognitive decline across ageing in dogs.
In summary, the microbiota exerts multifaceted effects on host physiology through intricate interactions with the metabolic, immune, and neurological systems. Understanding the mechanisms underlying microbiota-host interactions and the role of factors, including genetics, ageing and diet, is essential for elucidating the etiology of disease and developing strategies aimed at preventing dysbiosis or restoring microbial balance and promoting health.

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77° CONVEGNO SISVET

SOCIETA´ SCIENTIFICA DI RIFERIMENTO **AIPVET**

TITOLO **Petfood: the clinicopathological perspective.**

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Testo e Riferimenti bibliografici

The assessment of pet food effects on the health of dogs and cats requires a comprehensive understanding of their physiological responses. Clinical pathology plays a crucial role in evaluating the impact of pet food on the well-being of companion animals. By analyzing various biochemical, hematological, and cytological parameters, clinical pathology provides valuable insights into the nutritional adequacy, tolerability, and potential adverse effects of toxic compounds or spoiled and contaminated food.
Biochemical analysis of blood and urine samples allows for the assessment of metabolic processes influenced by dietary intake. Parameters such as glucose, total protein, cholesterol, triglycerides, liver and pancreas enzymes, creatinine and electrolytes provide information about energy metabolism, lipid metabolism, and liver, pancreas and renal function. Alterations in these parameters may indicate nutritional imbalances, organ dysfunction, or metabolic disorders attributable to pet food consumption.
Pet food ingredients significantly impact renal function, necessitating the evaluation of blood renal biomarkers such as urea nitrogen (BUN), creatinine and electrolytes. Alterations in these parameters may suggest renal insufficiency, dehydration, or electrolyte imbalances possibly associated with dietary factors. The assessment of urine parameters as proteinuria, specific gravity and urine crystals may help detecting kidney injuries and failure.
Liver enzymes, including alanine aminotransferase (ALT) and alkaline phosphatase (ALP), and markers of functionality as bile acids, bilirubin, hemostasis factors and albumin, serve as indicators of hepatic health and function. Changes in these parameters may indicate hepatic injury, cholestasis, or liver failure induced by dietary factors.
Similarly, the evaluation of amylase, lipase and trypsinogen like immunoreactivity (TLI) may help detecting injuries or dysfunction at pancreatic level and together with other markers as folate and cobalamin may help understanding the causes of malabsorption.
Hormonal imbalances can also arise from dietary factors, necessitating the assessment of endocrine parameters such as thyroid hormones, cortisol, and insulin. Dysregulation of endocrine function may lead to metabolic disorders, obesity, or diabetes mellitus associated with pet food formulations.
Changes in hematological parameters, in turn, reflect the body's response to dietary factors and may indicate nutritional deficiencies, immune dysfunction, or systemic inflammation. For example, anemia characterized by decreased red blood cell count or hemoglobin concentration can result from inadequate intake of essential nutrients such as iron, vitamin B12, or folic acid present in pet food. Likewise, alterations in white blood cell counts or differential leukocyte counts may signify immune system modulation or inflammatory responses triggered by dietary antigens or contaminants. Cytological investigations involve the microscopic analysis of cells obtained from various tissues or body fluids. In companion animals, cytology can help identify inflammatory reactions, neoplastic processes, or infectious agents associated with dietary factors. For instance, gastrointestinal cytology may reveal changes indicative of food intolerance, allergic reactions, or microbial overgrowth in response to specific ingredients or contaminants present in pet food. Similarly, cytological evaluation of skin lesions or ear discharge can aid in diagnosing dermatological conditions aggravated by dietary allergens or toxins.
Clinical pathology represents a fundamental tool for evaluating the effects of pet food on the health of dogs and cats. By means of biochemical, hematological, and cytological changes associated with dietary intake, clinical pathology contributes to the identification of nutritional deficiencies, metabolic disorders, and adverse reactions or toxic effects attributable to pet food formulations. Through interdisciplinary collaboration between clinicians and nutritionists, clinical pathology may contribute to understand the relationship between diet and disease in dogs and cats.

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Neurochemical chemosensing in the gastrointestinal tract

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The gastrointestinal (GI) tract is the largest interface between the animal's body and the external environment. Because of its strategic function, the GI tract exerts a constant monitoring and an accurate discrimination between beneficial nutrients (to be absorbed) and harmful substances (to be eliminated). A variety of highly specialized cells distributed along the GI epithelial lining are thus equipped with complex chemosensory systems that convey sensory information to various effector systems involved in the regulation of appetite, immune responses and GI motility. In this context, taste receptors (TR1 and TR2 family) coupled to G-proteins (GPCRs) have been identified that respond to a variety of nutrients, non-nutrients and other food components. These GPCRs are localized in GI chemosensory cells and elicit hormonal and neuronal signalling to the brain and periphery in the so-called gut-brain axis. Among the GPCRs, two α-subunits of the G-protein, i.e. α-transducin and α-gustducin, emerged as key players in the endoluminal chemosensing. Consistent evidence grown during the last ten years indicates that changes in nutrient intake, such as fasting and refeeding, as well as unbalanced (high protein, hyperlipidic and high carbohydrate) diets can be able to modify the expression of α-transducin, α-gustducin and their respective TRs. Disturbances or adaptations in the transmission of this sensory information may contribute to the development or maintenance of disease. This is an emerging field of research in which endoluminal chemosensing can be considered as a novel mechanism involved in the regulation of GI function. Specific diets or agonists targeting these chemosensory pathways may be considered as new therapeutic options tuning the appropriate physiological processes in GI health and disease.

77° CONVEGNO SISVET

SOCIETA´ SCIENTIFICA DI RIFERIMENTO **PARASSITOLOGIA E MALATTIE PARASSITARIE DEGLI ANIMALI (VET/06)**

TITOLO **REALITY AND FINCTION ON PET FOOD AND PARASITIC RISK**

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Testo e Riferimenti bibliografici

Current parasites of dogs and cats are results of adaptation from wild hosts to domesticated animals. Because of the predatory habit of dog and cat ancestors many of their parasites including protozoa, tapeworms and nematodes display indirect life cycles and use intermediate or paratenic hosts (preys) for their transmission; the life cycle is completed when the final host eats the tissues (e.g., mussels, organs, or brain) of the infected preys. Some of these parasitic infections are still present at high prevalence especially among pastoral or free ranging animals because of the higher likelihood of eating animal preys and/or raw meat and organs. It is noteworthy that humans may be infected and serve as intermediate hosts for many of these parasites being some of them of great zoonotic concern.
Most dog and cat owners feed their animals commercial food, but also raw meat. In recent years, the trend of feeding raw meat-based diets (RMBD) to domestic cats and dogs has significantly grown among pet keepers. Approximately, 60% of pet owners feed their cats and dogs completely or partially raw meat-based diet, and this practice is popular in several European countries. RMBDs consist of raw ingredients such as organs, muscle tissues and bones of slaughtered animals which may be prepared and offered as home-made diets, or purchased, either refrigerated, frozen or dried from the market likely complemented by cooked carbohydrate premix. These diets are also referred as BARF (Biologically Appropriate Raw Food or Bones and Raw Food) when feeding regimen is completely based on raw ingredients including carbohydrate part.
It is a common belief that raw diet is a natural and healthy way to advance pet health, because respectful of ancestral feeding habits. However, such opinions are not supported by scientific evidence of beneficial effects on pet health, most of times empirically supported by owner persuasion. As a matter of facts, while commercial food does not pose risk for dogs and cats, feeding raw ingredients may pose some concerns either on the safe handling at home and on the tangible risks of parasite infections to companion animals. Main parasite species associated with raw meat use in dogs and cats include protozoa such as *Hammondia*, *Neospora*, *Sarcocystis*, and *Toxoplasma*; nematodes such as *Toxocara* spp., and *Trichinella*; and tapeworms such as *Echinococcus* spp. and *Taenia* spp. [1]. In addition, the origin of the raw meat fed to companion animals is often insufficiently known and freezing does not inactivate all parasite species. The limited availability of prevalence data makes difficult a valuable risk analysis. However, the life cycles demonstrate that eating raw meat-based diets and prey animals, can be a route of infection. These infections can lead to disease in the animals themselves, but also in humans and other species of animals, while contamination of the environment can take place with eggs or oocysts. The safest advice is therefore to give complete commercial food or to cook raw meat before feeding.
The speech would provide the current understanding on parasitic risk linked with raw meat-based diet in dogs and cats and provide evidence-based advice to prevent parasitic infections in animals and humans.

[1] Ahmed et al. Raw Meat Based Diet (RMBD) for household pets as potential door opener to parasitic load of domestic and urban environment. Revival of understated zoonotic hazards? A review. One Health 13 (2021) 100327.

77° CONVEGNO SISVET

SOCIETA´ SCIENTIFICA DI RIFERIMENTO **AIVI (Associazione Italiana Veterinari Igienisti)**

TITOLO **BARF DIET: FOOD SAFETY PILLARS**

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Testo e Riferimenti bibliografici

The so-called biologically appropriate raw food diet, commonly known with the acronym BARF, has increasingly gained a wide applicability for pets feeding (defined as “ancestral feeding habit”) with special regard in the developed countries [1]. It means that the main used ingredients are raw animal origin tissues (i.e., muscle, viscera, and skeletal ones) which are obtained from different species. These matrices should be managed as animal by-products belonging to the Category-3, as reported in the EU Reg. N. 1069/2009. Among the different microbiological and zoonotic foodborne pathogens, EFSA and FDA have indicated *Salmonella* spp., *Listeria monocytogenes*, *Escherichia coli* O157:H7, and *Campylobacter* species as the main bacterial concerns isolated from raw pet foods, and also resulting responsible for confirmed cases of infections. From a hygienic point of view, the absence of the thermal treatments, before ingestion, does not permit a certain microbiological stabilization (bactericidal effect) of any quantitative parameters i.e., total mesophilic and psychrophilic counts. Indeed, the used matrices result commercialized in refrigerated or frozen forms. Possible cross-contaminations are mainly correlated to the pet owner improper food handling and/or to the usage of same kitchen utensils both for human and animal feeding. In the European and Italian scenarios, many studies discovered quantitative bacterial amounts conform to the acceptability limit of 5×10^6 cfu/g, in accordance with the EU Reg. N. 2073/2005 – hygienic criteria for mechanically separated meat intended for human consumption. On the other hand, qualitative and selective studies have generally discovered many zoonotic pathogenic species as *E. coli* O157:H7 from 23% of BARF products, *Salmonella* species from 71%, *L. monocytogenes* from 90%, and *Campylobacter* spp. from 29% ones, as reported by Bottari et al. [1]. Their detection was also associated with the identification of antibiotic resistant profiles against antimicrobials normally administrated in veterinary medicine and to the so-called Critical Important Antimicrobials (CIA) which usage is restricted for humans only. This condition represents the environmental pollution of mobile genetic determinants which are horizontally transmitted among commensal and pathogenic bacteria.

Raw animal tissues could also provide possible chances to complete parasitic life cycles which result involved as definite or intermediate hosts (i.e., *Echinococcus granulosus*, *Sarcocystis* spp., *Cryptosporidium* spp., etc.) [2]. Therefore, possible bacterial and parasitic issues should be considered as risk both for animal and human species, as One-health concerns. The selection of highly qualified feed suppliers, detailed instructions for usage of BARF products on labels, and formative courses on the so-called “good hygiene practices” for pet-owners, organized by veterinary practitioners, will represent substantial and critical points to prevent and/or reduce possible cross-species infections with special regard during the food handling and preparation steps. In conclusion, innovative food technologies, such as high-pressure pasteurization and ultrasonication [3], have produced promising results on the bacterial and parasitic inactivation and stabilization as valid and sustainable alternatives.

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77° CONVEGNO SISVET

SOCIETA´ SCIENTIFICA DI RIFERIMENTO **SiftVet**

TITOLO **Pet food: between toxicological and nutraceutical aspects**

Autori Alessandro Di Cerbo

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Testo e Riferimenti bibliografici

Nowadays, pets are considered family members whose food is of great importance for their health in terms of safety and efficacy. As observed in the human food supply chain, pet food is subjected to direct (e.g., foreign materials or microbial colonization) or indirect (e.g., drugs and chemicals residues or microplastics) contaminations throughout the production process, which unavoidably impacts the pet's health. For instance, antibiotic use and abuse worldwide in food-producing animals, particularly chicken and turkey, has demonstrated the ability of such drugs to induce cytotoxic and proinflammatory phenomena *in vitro* and *in vivo* [1-3]. Moreover, the presence of environmental pollutants in red meat and fish-based pet diets [4], and the recent detection of polyethylene terephthalate and polycarbonate in wet and dry pet food [5] have posed a serious concern about multiple threats to which pets are daily exposed to during their meal. Nevertheless, the growing awareness of the efficacy of nutraceutical substances (e.g., herbal extracts, probiotics) in human medicine has prompted their use in the veterinary counterpart as a tool to mitigate contaminants impact and, at the same time, enhance health-related molecular mechanisms [6-11]. In this sense, the use of nanotechnological applications (e.g., inorganic or organic micro-capsules) [12] might represent a great opportunity for the pet food industry to deliver such substances and ensure the delivery of the right amount required to exert their beneficial effects, giving light to functional pet food.

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77° CONVEGNO SISVET

SOCIETA´ SCIENTIFICA DI RIFERIMENTO **Siclimvet**

TITOLO **Dietary interventions and intestinal microbiota modulation as treatment strategies for the management of chronic inflammatory enteropathies in dogs and cats**

Autori C.G. Vecchiato[1], M. Pietra[1], G. Biagi[1]

Affiliazioni [1]*Dept. of Veterinary Medical Sciences, University of Bologna, Ozzano dell'Emilia – Italy*

Testo e Riferimenti bibliografici

Chronic inflammatory enteropathies (CE) are recognized in dogs and cats as a common clinical syndrome leading to chronic intestinal signs. Canine and feline CE have a multifactorial pathophysiology and involve a loss of tolerance to diet with subsequent changes in the immune system, as well as changes in intestinal function and microbiome composition. In cats, the disease shares common mechanisms with human inflammatory bowel disease (IBD). CE is currently subclassified based on the response to treatment. Dogs and cats are categorized as food-responsive enteropathy (FRE) if the achievement of clinical resolution of symptoms occurs after dietary therapy. FRE comprises the majority of CE cases, with 50-65% of dogs and cats responding to dietary therapy alone. Commercial hydrolyzed protein, limited ingredient novel protein, and highly digestible or high fiber diets have all been used successfully for canine and feline CE [1]. However, FRE might be difficult to manage due to the lack of specific markers for response to diet therapy, therefore the current approach is based on a trial-and-error method. In CE, intestinal inflammation and mucosal damage lead to changes in intestinal functions, with a negative influence on nutrient absorption and intestinal microbiota composition. Diet represents an important factor in influencing the composition of the microbiome and its metabolism, as it is in close contact with dietary substrates crossing the luminal environment. Dietary factors that seem to have a great influence on the intestinal microbiota include the proportion among macronutrients (protein, fat and fiber), the digestibility of protein sources, the characteristics of indigestible carbohydrates (soluble vs. insoluble fractions) and the type of diet (extruded vs. wet products) [2]. The extent of changes on the microbiome exerted by diet can be limited in healthy animals, while in those with CE, major effects on the richness of composition (biodiversity) and production of postbiotics (such as short-chain fatty acids, SCFA), might occur. SCFA, secondary bile acids, and other metabolites deriving from the bacterial digestion of different nutrients are involved in the communication between intestinal bacteria and the host via signaling molecules targeting intestinal function, motility and gut barrier integrity. In addition, bacterial-deriving compounds produced in the intestinal tract can affect the functionality of other organs, e.g. SCFA receptors are involved in the gut-brain axis signaling. The perturbations of the intestinal microbiome that can happen secondary to CE are defined as dysbiosis; the degree of changes in both the compositional and functional status of the microbiome can be assessed by different metrics, which take into consideration the relative abundance of specific bacterial populations known for beneficially influence the host health. For instance, *C. hiranonis,* which is decreased in dogs and cats with CE, is identified as the major convertor of primary bile acids into secondary bile acids. Microbiota changes can persist long-term and might not correlate with clinical remission achieved after dietary therapy [3].
In conclusion, in addition to the effects derived from compositional changes in the intestinal microbiome, it is of great interest for future research to know how functional changes driven by nutritional interventions might improve the health of dogs and cats with CE.

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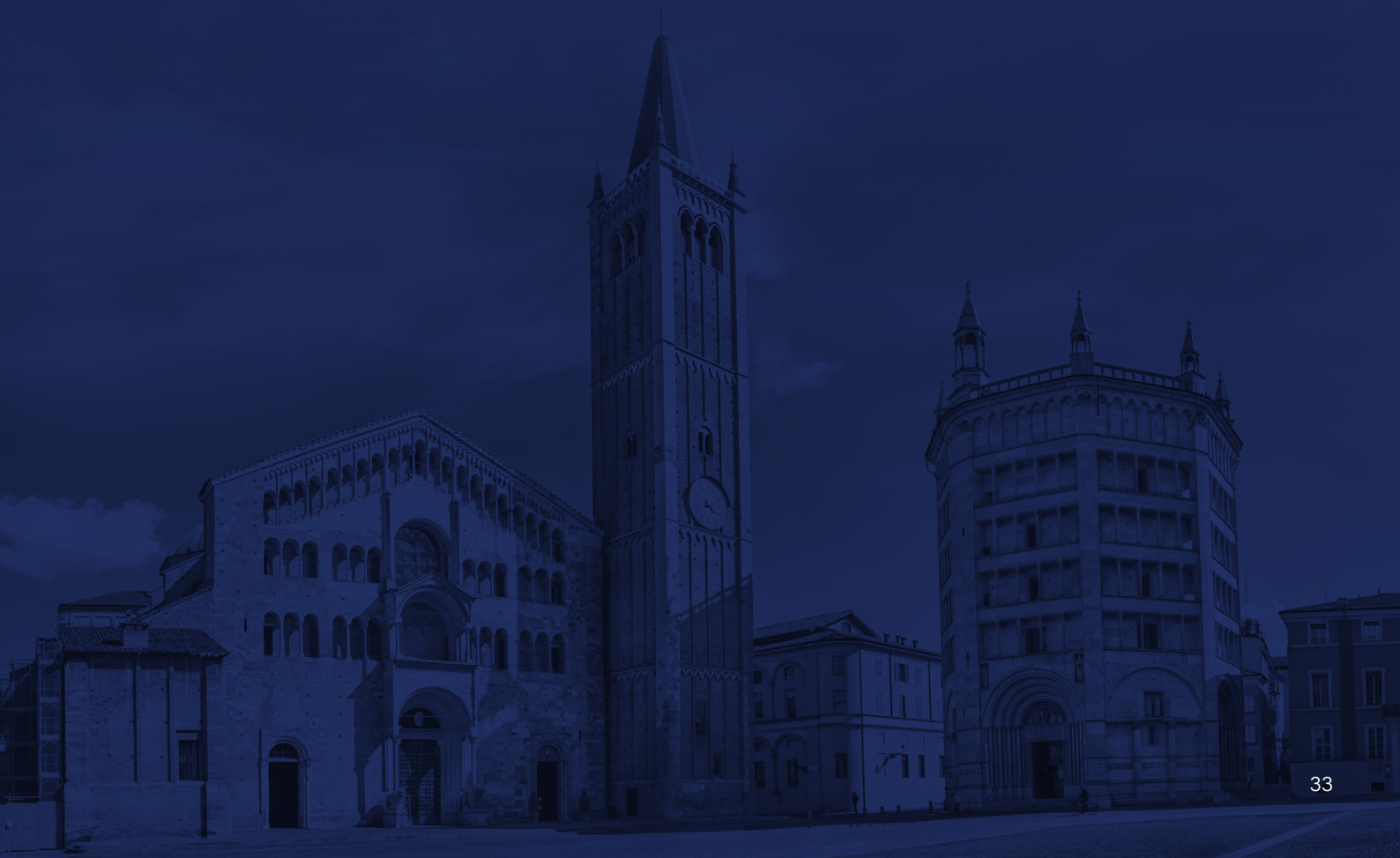
ABSTRACT

Main Lecture

Di seguito vengono riportati i programmi
e i relativi contributi pervenuti



AIPVET

Mercoledì 12 Giugno, 16.00 – 17.30

COME INSEGNARE LA PATOLOGIA VETERINARIA: LA PROSPETTIVA ITALIANA

Massimo Castagnaro

AIVI

Venerdì 14 Giugno, 09.30 – 10.00

WEBINAR

Avelino Alvarez Ordonez, University of Leon

AMV

Mercoledì 12 Giugno, 17.30 – 18.00

MENISCAL MYSTERIES: CURRENT INSIGHTS INTO KNEE HEALTH

Alessia Di Giancamillo

SICV

Venerdì 14 Giugno, 14.30 – 16.00

HOLE IN THE HEAD-DEALING WITH HEAD FRACTURES AND SINOCUTANEOUS FISTULA

Dylan Gorvy

SIRA

Mercoledì 12 Giugno, 14.30 – 15.00

THE WSAVA GUIDELINES ON SMALL ANIMAL REPRODUCTION CONTROL: SUMMARY AND IMPLICATIONS

Stefano Romagnoli

77° CONVEGNO SISVET

SOCIETA´ SCIENTIFICA DI RIFERIMENTO | **ASSOCIAZIONE ITALIANA DI PATOLOGIA VETERINARIA**

TITOLO | **Teaching Veterinary Pathology to Undergraduate Veterinary Medicine Students: an Italian perspective.**

Autori | M. Castagnaro[1], L. Aresu[2], C. Brachelente[3], B. Restucci[4], P. Roccabianca[5], A.Sfacteria[6]

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Testo e Riferimenti bibliografici

The COVID-19 pandemic and its consequences on teaching methodologies and activities have provided an unexpected opportunity to reconsider what constitutes essential, relevant or unnecessary knowledge and skills in Veterinary Pathology (VP), redefining the Intended Learning Outcomes (ILOs) for the undergraduate students in the Italian Veterinary Medicine (VM) programmes.
VP is a large and complex area of knowledge (Kn), skills (Sk) and competences (Co) encompassing the teaching of a wide range of subjects including General Pathology, General and Special Anatomical Pathology, Pathophysiology, Post-Mortem Techniques, Molecular Pathology, as well as several other specialized fields such as Ultrastructural Pathology, Histopathology, Cytopathology, and Organ Pathology, among others. Furthermore, in Italy some of these subjects are regularly taught in several Veterinary Science-related programmes such as Veterinary Assistant/Nurse and Animal Production. However, the focus of the Round Table will be on VM programmes, where the goal of teaching VP is to allow students to reach a level of ILOs (Kn, Sk and Co) adequate to the needs of the veterinary profession.
Setting realistic and appropriate ILOs in all pertinent subjects for VM undergraduates requires a clear understanding and definition of Kn (content of technical information), Sk (abilities/techniques) and Co (applied Kn and Sk in a variety of professional contexts) across relevant subjects. Valuable guidance in this endeavor is provided by the Standard Operating Procedures (SOPs) of the European Association of Establishment for Veterinary Education (EAEVE)[1] that embodies Kn, Sk and Co within the framework of the so-called Day-One Competences (DOCs). The latter categorize VP into two main areas: General Pathology (GP) and Diagnostic Pathology (DP). While DP has a direct professional impact in Clinical and Public Health settings, GP is essential for establishing and advancing Kn across all professional subjects.
In addition to an adequate definition of ILOs, another important critical factor in the teaching of VP has been identified by the Standards and Guidelines for Quality Assurance in the European Higher Education Area (ESG, 2015)[2] where in section 1.3 it is stated that “Institutions should ensure that the programmes are delivered in a way that encourages students to take active role in creating the learning process, and that the assessment of students reflects this approach”. As a matter of fact, it is an integral aspect of academic teaching experience to acknowledge the continuous and rapid evolution of cognitive functions and specific learning abilities along different student cohorts[3].
These factors collectively pose significant challenges to the efficacy and efficiency of teaching VP.
The Round Table discussion aims to address these challenges and to inform participants about the primary pathways for acquiring intended ILOs in VP within the Italian Academic System, ii. to compare the efficacy and pinpoint specific hurdles and iii. to discuss future perspectives. Additionally, the Round Table will explore innovative teaching methodologies that can enhance the teaching and learning experience in VP.

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Meniscal Mysteries: Current Insights into Knee Health

Alessia Di Giancamillo, Università di Milano

The knee is a highly intricate joint prone to injury and harbors several critical components, with the menisci standing out as particularly vulnerable structures. Acting as shock absorbers, these menisci resemble two fibrocartilage "pads" with a semicircular shape and triangular cross-section, strategically located on the inner and outer sides of the knee joint. Nestled between the femoral condyles and the tibia, they are pivotal in cushioning impact forces and bolstering joint stability [1]. Meniscal tears represent a prevalent clinical issue, with injuries frequently affecting the medial meniscus in both human and veterinary practice. Each meniscus fulfills distinct roles: the medial meniscus is crucial in preserving anterior-posterior knee stability, while the lateral meniscus contributes significantly to rotational stability. Given the variations in biomechanical function, tibial attachment biology, stress distribution, and contact areas, repair techniques, especially in the horn and root regions, must be tailored to the specific characteristics of each attachment. In routine activities like walking, the knee joint encounters mechanical forces reaching up to 5 times the normal body weight. During knee extension, approximately 40–60% of these forces are directly transferred to the meniscus, escalating to 90% during knee flexion. Notably, the posterior horn bears the brunt of this increased load, making it one of the most frequently affected areas in medial meniscus tears [2]. Understanding the intricate macro and microstructure of the meniscus demands a multidisciplinary approach to enhance our current understanding of its developmental biology and achieve favorable outcomes in regeneration efforts. In the adult meniscus, three distinct zones can be identified based on vascularization, biochemical composition, and regenerative capacities: i) the outer zone, fully vascularized, and characterized by a fibrous-like matrix and robust regenerative capabilities; ii) an intermediate zone exhibiting transitional characteristics; iii) the inner zone, devoid of vascularization, featuring a cartilaginous-like matrix with limited healing potential due to inadequate blood supply [3]. Hence, meniscal tissue engineering aims to bridge the gap between tissue's structural and functional aspects under normal conditions. The goal is to create a compound capable of repairing injuries within the inner zone, reinstating the tissue's physiological characteristics while preventing degenerative processes. This endeavor involves a comprehensive exploration of both endogenous and exogenous factors influencing meniscal development.
At the core of tissue engineering lies a thorough understanding of the tissue's anatomical structure and its responses to various stimuli. This knowledge forms the foundation for devising effective strategies to address meniscal injuries and promote tissue regeneration.

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77° CONVEGNO SISVET

Stato: INVIATO - ID: 13568

The WSAVA guidelines on small animal reproduction control: summary and implications

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The WSAVA guidelines on small animal reproduction control: summary and implications
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The presence of pets in our families and the emotional value our clients put on them has made clients' requests for small animal practitioners increasingly more challenging. Reproduction control as a presenting complaint has gone from the very simple "my pet should be spayed/neutered" to a very elaborate set of questions the most intriguing of which are "how and at what age should it be done" and most importanty "should we do it or not"?" The amount of knowledge on the effects of gonadectomy has increased enormously over the last few decades thanks to studies (mostly in dogs but also in cats) addressing advantages and disadvantages of the surgical approach. These lead to the discovery of many negative effects for the health of gonadectomized animals - most notably a significant contribution to the development of certain types of tumors in some breeds of dogs. As a consequence, the question is there any alternative to surgical removal of gonads is becoming more and more common in small animal practice.

These guidelines (1) provide evidence-based scientific information on a) how to achieve control of reproduction in dogs and cats with (spay-neuter, vasectomy, ovary sparing techniques) or without surgery (through the use of hormones, vaccines, locally necrotizing agents); b) what are the benefits and the detriments of both approaches for the health of dogs and cats; c) what are the ethics criteria veterinarians should consider when making decisions and advising owners, municipalities or constituencies on what is the better strategy to control reproduction in a pet vs a shelter animal or a colony cat.

There is not a single technique which is ideal in all situations or one which should never be used. Every approach to reproduction control has advantages and disadvantages depending on the practical/financial situation of the owner, the animal's genetic component, age, health and living conditions as well as the purpose for which the animal is kept. Surgical gonadectomy has long been regarded as being devoid of disadvantages but this concept has gradually changed over the last few decades as a number of metabolic and neoplastic conditions have emerged as potential consequences. However, in spite of how many of these gonadectomy-related conditions have been discovered, they are currently well known only in a selected number of breeds and there are many concurrent factors whose importance is still unknown (i.e. the age at gonadectomy, frequency of the condition within a given breed etc.) which may play a role in modulating the risk of their development. Therefore, surgical gonadectomy remains a valid option in many situations, and there may even be cases in which advising its adoption in a breed at risk of developing a gonadectomy-induced condition may be conceivable because of specific constraints, provided that the owner has been well informed about all risks connected to this decision.

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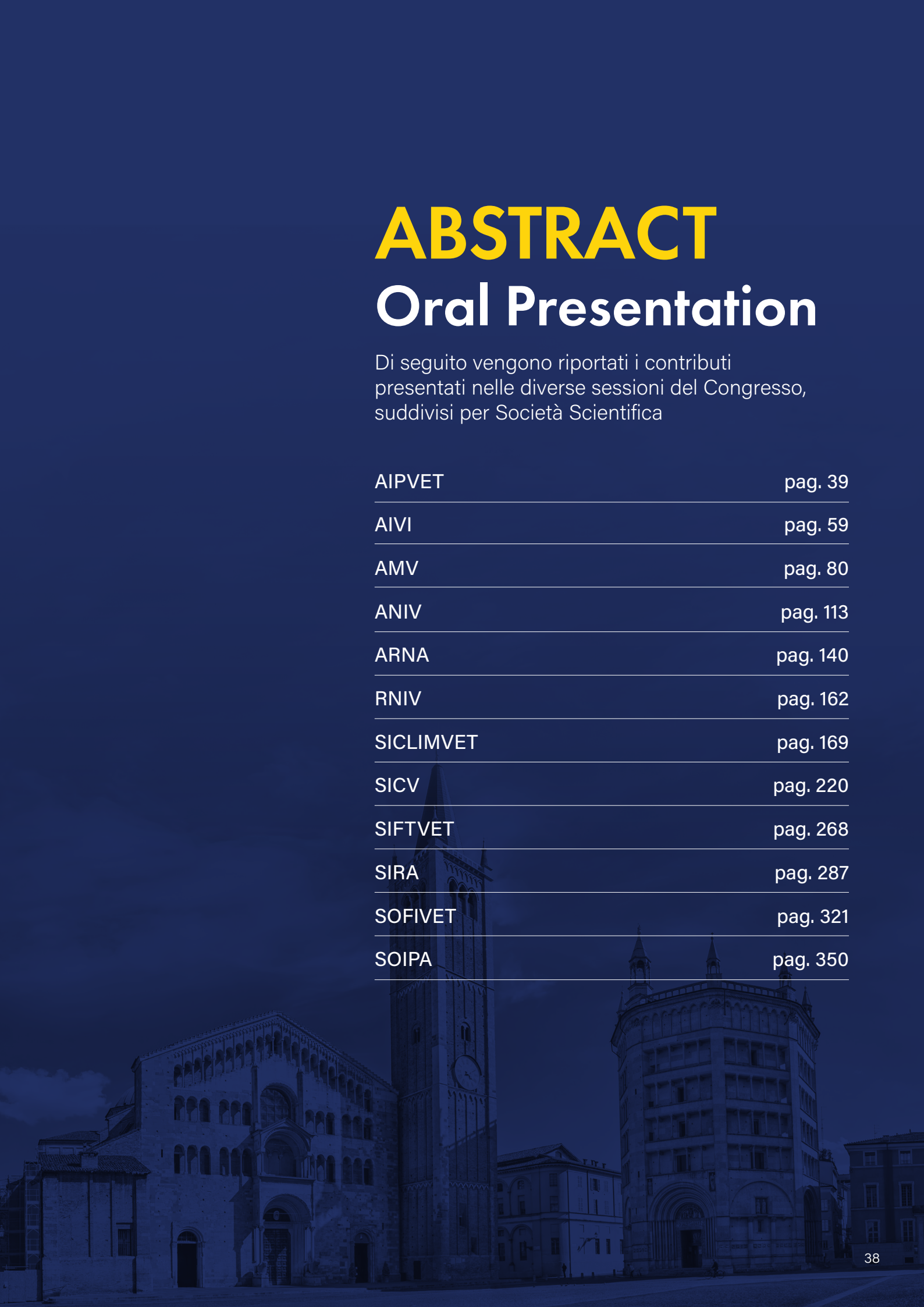
ABSTRACT
Oral Presentation

Di seguito vengono riportati i contributi presentati nelle diverse sessioni del Congresso, suddivisi per Società Scientifica



AIPVET

77° CONVEGNO SISVET

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The K9 Pan-Cancer Genome Atlas to improve understanding of canine cancer genetics

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Canine cancers are highly prevalent, affecting nearly 50% of dogs over ten years old. They are primarily driven by complex genetic aberrations resulting from spontaneous mutations, environmental influences, and human selection pressure via breeding practices. Notably, canine cancers serve as potent comparative oncology models, providing valuable insights into the mechanisms underlying human cancers and new drug development.In human medicine, the Cancer Genome Atlas has been a pivotal effort in elucidating the genetic landscape of most tumor histotypes, providing a comprehensive repository of genetic knowledge about the disease [1]. Inspired by its success and recognizing the absence of a comparable resource for dogs, we have developed a canine-specific counterpart called the K9 Pan-Cancer Genome Atlas.
This initiative utilizes existing cancer genetic data from 20 studies conducted over the last seven years. To ensure future collaborations and the inclusion of selectively high-quality data, we have implemented stringent criteria for analysis. Only data derived from whole exome sequencing (WES) experiments were considered, requiring paired whole exome data from matched normal DNA. Additionally, a quality threshold was enforced. These rigorous standards ensured robust and reproducible results across key genomic coding regions, facilitating the removal of sequencing artifacts and filtering out non-pathogenic variants. The bioinformatic analysis pipeline followed the best practices in human studies, utilizing well-established tools such as GATK.
We have successfully retrieved, processed, and analyzed data from over 700 canine tumors, encompassing ten distinct tumor histotypes. This comprehensive dataset is now easily accessible through a user-friendly website for consultation. Single Nucleotide Variants (SNV) and Indels were obtained using three mutation callers and then combined through majority voting. Copy Number Aberrations (CNA) were also analyzed using ASCAT segmenter and scored by GISTIC2. We derived several information from these data, including Tumor Mutational Burden (TMB), the number of recurrently mutated genes within each histotype, tumor heterogeneity, and altered pathways.
We observed significant variation in TMB when comparing different histotypes; glioma and mammary tumors exhibited the lowest (0.2-0.24), while mast cell tumors displayed the highest (3.54). Similar to humans, canine cancers potentially resulting from chronic exposure to mutagens, such as pulmonary and urinary carcinomas, showed elevated levels of TMB. A total of 36,746 mutations were identified among 11,506 altered genes. TP53, FAT4, USH2A, CSMD3, PIK3CA, and PTEN emerged as the most frequently mutated genes among histotypes. Additionally, a modest positive correlation was observed between the number of mutated genes and TMB. Finally, driver mutations were retrieved using dNdScv, revealing 17 driver genes, including TP53, PIK3CA, SETD2, POT1, KRAS, and MYC.
The K9 Pan-Cancer Genome Atlas represents a resource dedicated to fighting canine cancers, and it also provides a tool for the broader comparative and veterinary oncology community. We encourage all interested researchers and potential contributors to visit the website and explore its potential.

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77° CONVEGNO SISVET

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The mycotoxin Enniatin B alters bovine PMN phagocytosis and extracellular ROS production in vitro

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Enniatins (ENN) are a class of mycotoxins produced by the fusarium genus, which are widely distributed as contaminants of food and feed, they often pose a risk to human and animal health.There are several analogues of enniatins, among which enniatin B (ENN B) is considered one of the most emerging, whose effects on human and animal health are not yet completely defined.

This study investigated for the first time the effect of increasing concentrations (0.625, 1.25, 2.5, 5 and 10 μM) of ENN B on polymorphonuclear leucocytes (PMN) in dairy cows. Dimethyl sulfoxide (DMSO) was used as a vehicle to solubilize ENN B and included as a negative control.

After isolating from the peripheral blood of healthy multiparous cows, PMNs were incubated with different concentrations of ENN B and the following activities were assessed: 1) cell viability by the water-soluble tetrazolium salt cell proliferation test kit (WST-1); 2) chemotactic function, using a transwell plate using zymosan-activated serum (ZAS) as a chemotactic molecule; 3) the ability to phagocytize fluorescein-labelled S. aureus and E.coli; 4) the extracellular production of reactive oxygen species (ROS), using the cytochrome c reduction assay, with and without phorbol myristate acetate (PMA) to simulate pro-inflammatory action. Data were analyzed by GraphPad Prism and normality was assessed by Shapiro–Wilk test. Repeated measures of 1-way ANOVA for matched or paired data and Tukey's multiple comparison test were then applied on the considered parameters.

PMNs viability and chemotactic activity were not affected by ENN B at all tested concentrations ($p=0.952$; $p= 0.218$, respectively), while E. coli and S. aureus phagocytosis ability were reduced by the highest concentrations (ENN B 5 and 10 μM) compared to DMSO ($p\leq0.001$; $p=0.001$, respectively). Extracellular ROS production was increased by ENN B under normal and pro-inflammatory conditions ($p=0.014$; $p<0.001$, respectively). In conclusion, ENN B did not exert cytotoxic effects on bovine PMNs, while it reduced the phagocytic ability and increased the production of extracellular ROS highlighting its potential role on bovine innate immune response.

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Expression of TfR1, TfR2 and Ferritin in canine neoplastic testis

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Cell proliferation and spermatogenesis are iron-dependent processes, and alterations in iron metabolism have already been described in human testicular tumors (1). On the contrary, very little is known on this topic in the canine species. Cells acquire iron via endocytosis mediated by the Transferrin Receptor 1 (TfR1) (2) and Transferrin Receptor 2 (TfR2), it is then stored in cells by Ferritin (Ft) to guarantee iron bioavailability. Overexpression of TfR1 is often observed in tumoral cells compared to normal cells (3), on the contrary Ft shows a lower expression in tumors compared to normal tissues, while little is known about TfR2 expression in neoplastic tissues. The aim of this study was to investigate by immunohistochemistry (IHC) and western blotting (WB) the expression of TfR1, TfR2 and Ferritin (FTH1) in normal and tumoral samples of canine testis.
26 canine testicular samples were retrieved from the Departmental archive and subjected to histological analysis. Subsequently, samples were routinely processed for IHC and WB. Histological analysis revealed that 3/26 samples were non-neoplastic, 16/26 samples were Seminomas (SEM), 5/26 samples were Sertoli Cell Tumors (SCT) and 2/26 samples were Interstitial Cell Tumors (ICT).
Immunohistochemical analysis revealed that in non-neoplastic samples 30/40% spermatogonia and Sertoli cells showed strong cytoplasmic TfR1 immunostaining, while anti-TfR2 signal was observe in 10/20% of spermatogonia, Sertoli cells and spermatozoa with membrane localization. In non-neoplastic samples 10% spermatogonia showed weak cytoplasmatic staining for FTH1 and more interestingly 30% of spermatocytes I/spermatocytes II presented a labelled perinuclear dot, probably localized at mitochondrial level. In SEM samples 60-70% of spermatogonia showed cytoplasmic immunostaining for TFR1, 20% of spermatogonia were positively labelled by anti-TfR2 antibody at cytoplasmatic level, 40-50% of spermatogonia and Sertoli cells showed weak cytoplasmatic immunostaining for FTH1. In SCT samples, 40% of Sertoli cells presented membrane immunostaining for TfR1, meanwhile only 5% of Sertoli cells were labelled by anti-TfR2 antibody at cytoplasmatic level, while no signal was detected for FTH1. In ICT samples, 50-60% of Leydig cells showed cytoplasmatic immunostaining for TfR1, 30-40% of Leydig cells were positively labelled by anti-TfR2 antibody, and 50-60% of Leydig cells showed a strong cytoplasmatic staining for FTH1. WB analysis confirmed the cross-reactivity of anti-TfR1, anti-TfR2 and anti-FTH1 antibody.
According to our results an increased expression of TfR1 was observed in all tumoral samples compared to controls, suggesting a greater need for iron also in canine neoplastic cells to support the increased rate of proliferation, as already reported in humans. On the other hand, compared to non-neoplastic samples, only ICT samples showed an increased expression of TfR2, suggesting a predominant role of TfR1 in iron up-take in every type of canine testicular tumors. Moreover, increased expression of FTH1 in ICT and SEM samples could indicate higher iron storage compared to SCT samples where iron is constantly used for proliferation.
Understanding iron metabolism in different canine testicular tumors could help to better define its role in carcinogenesis and help find new tumor targets and treatment strategies.

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Adreno-hepatic fusion in two dogs associated with tumor development

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Embryological organ fusion of organs is considered a rare congenital anomaly. It must be distinguished from an adhesion, which represent a secondary attachment between two organs without the fusion of their parenchymas. These phenomena may involve, among others, the liver and the adrenal gland, resulting in an adreno-hepatic fusion (AHF), characterized by the merging of hepatic and adrenal tissues, or in an adreno-hepatic adhesion (AHA), wherein the two parenchymas remain distinctly separated by a capsular structure[1]. Although common in humans, AHF/AHA are scarcely reported in veterinary literature, with merely five cases documented across baboons, ferrets, and monkeys[2–3]. We describe histologically two cases of canine AHF in two adult dogs (11 and 7 years), specifically with the fusion of the right adrenal gland and the caudate hepatic lobe. Histologically, the region showed cords and large clusters of adrenal cortical cells intermingled with the hepatic parenchyma without any capsular separation. One dog exhibited disorganization within the hepatic parenchyma, including loss of lobular architecture, fibrosis, and hepatocyte atrophy. Conversely, the other subject displayed significant architectural and diffuse hepatocyte atypia, consistent with a hepatocellular tumor. Both cases presented multifocal thrombosis in the fused region. Additionally, each dog had a cortical adenoma within one lobe of adrenal gland. Immunohistochemistry for vimentin and cytokeratin clearly showed the mixture of the two cell populations. A final diagnosis of adreno-hepatic fusion with cortical adenoma was made in both dogs with a presumptive hepatocellular carcinoma developing only in one individual. Given its rarity and the associated clinical challenges, including thrombosis risk and diagnostic difficulty, this entity warrants further characterization and documentation.

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77° CONVEGNO SISVET

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Age at tumor diagnosis in 14,636 canine cases from the pathology-based UNIPI Animal Cancer Registry, Italy: One size doesn't fit all

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Cancer is one of the leading causes of death in adult dogs [1]. All dogs, regardless of breed, would benefit from routine preventive cancer screening, as early cancer detection is essential for effective therapy and improved clinical outcomes for many malignancies. An important aspect pertains to the appropriate age at which to start screening [2]. However, to date, there are no specific guidelines for cancer screening in veterinary medicine. The main goal of this study was to retrospectively assess the age at diagnosis by sex, breed, size, and most common cancer types in a sample of tumor-diagnosed Italian canine patients. A total of 14,636 canine histologically-confirmed neoplastic cases collected from the pathology-based Animal Cancer Registry of the University of Pisa from 2008 to 2023 were analyzed. Data were coded according to the Vet-ICD-O [3], and stratified by malignancy (benign vs malignant), sex, neutering status, breed (breeds with more than 50 tumor cases), cephalic index (brachycephalic, mesocephalic, dolichocephalic), body size (small, medium, large), and cancer type. The Wilcoxon and Kruskall-Wallis Rank-Sum tests were used to assess age differences among the subsets, with statistical significance set at $p<0.05$. In addition, a survival model was applied to assess the influence of the previously mentioned variables on the timing of malignancy diagnosis. The median age at diagnosis was 9 and 10 years for benign and malignant tumors, respectively. Intact females were diagnosed 1.6 years earlier than males, while neutered dogs, especially the females, were diagnosed later than intact dogs. Compared to mixed-breed dogs, purebred dogs were diagnosed at a younger age, but the median age at diagnosis for each breed with more than 50 individuals ranged from 8 years for American Staffordshire Terriers to 11 years for Yorkshire Terriers. Among malignant tumor types, the earliest age at cancer diagnosis was recorded for lymphomas [959-972] and mast cell tumors [974]. Skin adnexal neoplasms [839-842], squamous cell neoplasms [805-808], melanomas [872-879], and blood vessel tumors [912-916] were diagnosed in eldest patients. Finally, the model showed that malignancies in large-sized, brachy- and dolichocephalic, and intact female dogs were diagnosed in advance, with an accelerating effect of these variables on age at diagnosis. Our results regarding the influence of sex diverge from previously published findings on the optimal age for cancer screening in dogs [2]. This might be due to differences in demographics and cancer distribution between the Italian and American canine populations. Nevertheless, our results regarding the age by breed, based on a larger subset of individuals, confirm that a "one size fits all" approach to cancer screening is not the most effective one in the dog population and provide relevant data that could drive more individualized screening schedules.

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77° CONVEGNO SISVET

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p53 immunohistochemistry, rather than TP53 in situ hybridization, predicts TP53 mutation in canine Diffuse Large B-Cell Lymphoma and correlates with worse outcome

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Diffuse large B-cell lymphoma (DLBCL) is the most frequent hematopoietic tumor in dogs, characterized by high mortality and clinical heterogeneity. Both exome and Sanger sequencing approaches revealed recurrent mutations in several genes, including TRAF3, SETD2, POT1, and TP53 [1]. TP53 aberrations, primarily missense mutations affecting the DNA-binding domain, have emerged as critical factors associated with shortened survival times and limited response to therapy [1]. TP53, recognized as a key tumor suppressor gene encoding p53 protein, stands as the most recurrently mutated gene both in human and canine cancers. The immunohistochemical identification of p53 is employed in routine clinical practice as an indicator for mutations in different human tumors [2]. In canine DLBCL (cDLBCL), no studies have been conducted to correlate the TP53 mutational status, mRNA, and protein expression.
This study aimed to investigate the expression of TP53 using RNAscope® in situ hybridization and of p53 by immunohistochemistry (IHC) in FFPE cDLBCL samples. Results were correlated with the TP53 mutational status and clinico-pathologic features to assess the reliability of these techniques in predicting TP53 mutations and to evaluate the prognostic value of each technique.
A total of 37 cDLBCL samples were subjected to RNAscope® using a specific probe for canine TP53, and to IHC with a p53 monoclonal antibody [2], performed on the Ventana Discovery Ultra autostainer (Roche). A semi-quantitative scoring system was applied for RNAscope®. Conversely, results for IHC were scored as positive or negative. TP53 mutational status was previously assessed [1].
TP53 expression was detected by RNAscope® in all samples. With IHC, 10/37 (27%) cDLBCLs tested positive for p53. The density of p53-positive tumor cells varied greatly among cases. No correlations were identified between TP53 RNAscope® scores and p53 IHC expression. Furthermore, the expression of TP53 detected by RNAscope® was not influenced by its mutational status. Conversely, a significant association was observed between TP53 mutations and p53 expression by IHC ($p<0.001$). Specifically, all mutated samples except for one expressed p53, while all wild-type samples did not. The accuracy of p53 IHC testing in detecting TP53 mutation was 97.3% (95% CI 85.84-99.93), with robust sensitivity and specificity. Moreover, cases expressing p53 had significantly shorter time to progression (TTP) and lymphoma specific survival (LSS) compared to negative cases (median TTP: 52 vs 196 days, $p=0.002$; median LSS: 66 vs 278 days, $p=0.002$).
Our findings indicate that the p53 IHC assay accurately predicts TP53 genetic aberrations, unlike TP53 RNAscope®, highlighting its utility as a cost-effective tool in routine clinical diagnostics. Moreover, positive IHC results were associated with poorer survival outcomes, indicating the prognostic value of this finding. The positive IHC staining in cases with mutated TP53 is indicative of p53 protein accumulation and suggests the acquisition of ex-novo oncogenic functions by mutated p53. The absence of correlations between TP53 in situ gene expression both with IHC and with TP53 mutational status raises questions about TP53 transcriptional and post-transcriptional regulatory mechanisms, warranting further investigation.
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Hazelnut skin and linseed as partial replacements for maize in lamb diet: impact on growth, ruminal histomorphometry, and gut-health

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Agro-industrial by-products represent sustainable and cost-effective resources for animal nutrition, enhancing their gut-health and performance [1]. Bioactive compounds in plant-based feeds could exert beneficial effects on rumen and abomasum health [2, 3]. To evaluate the potential effects of hazelnut skin (HS) and linseed (LS), 40 male lambs were assigned to the following groups: control (C), LS (8%), HS (15%), and HS+LS (7.5%, 4%, respectively); the dietary modifications were carried replacing maize. After a 60-day growth trial, lambs were slaughtered and ruminal/gut histomorphometry was evaluated. Regarding rumen, ten intact papillae from both the ventral and the dorsal sac were measured macroscopically and microscopically. Additionally, the gastrointestinal tract segments underwent evaluation using a histological scoring system. Inflammatory infiltrates were assessed based on type (lymphoplasmacytic = 1, mixed = 2, eosinophilic = 3), severity (absent = 0, mild = 1, moderate = 2, severe = 3), and extension (focal = 1, multifocal = 2, disseminated = 3, diffused = 4). Vacuolar degeneration in the liver and the forestomaches, as well as pigmentation and hyperkeratosis in the forestomaches, were also examined using a semi quantitative scoring system. Statistical analysis was performed using SPSS software, with dietary treatment and animal ID as fixed and random factors, respectively. Significance was determined at $p < 0.05$, and mean differences were assessed using Tukey's and Dunn's post hoc tests. Growth performance remained unaffected by the dietary treatments ($p > 0.05$). Regarding ruminal morphometry, papillae length from the ventral sac was notably higher in LS and HS+LS groups (0.521 ± 0.064 cm, 0.428 ± 0.158 cm, respectively) compared to the control group (0.379 ± 0.814 cm, $p = 0.028$). Notably, significant differences were observed between LS-C ($p = 0.047$) and LS-HS ($p = 0.038$) groups. Similarly, the LS group exhibited higher ruminal pigmentation severity, particularly in the cornified squamous cell layer (ventral: $p = 0.001$; dorsal: $p = 0.014$). The hyperpigmentation was associated with longer papillae. The intestinal inflammation scores were higher ($p < 0.05$) in HS treatment. No significant histological differences were observed in other organs. The eventual pro-inflammatory effect of hazelnut skin warrants further investigation. Ongoing analyses of rumen microbiota and volatile fatty acids aim to provide further insights. Overall, HS+LS, LS diets improved gut health, particularly rumen papillae morphology and thus enhancing nutrient absorption. These findings emphasize evaluating novel feed components to optimize animal welfare.
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Prevalence of Staphylococcus aureus, Methicillin-resistant Staphylococcus, and Enterobacteria producing extended-spectrum beta-lactamases in Water buffalo farms in the Salerno Province

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The water buffalo (Bubalus bubalis) holds an important economic role in the dairy industry in Italy, the top producer of buffalo milk in Europe, accounting for 97% of the continent's production. With the rise of the global health concern of antimicrobial resistance, our study aimed to highlight the prevalence and genotypic diversity of *Staphylococcus aureus* (*S.aureus*) isolated from milk of water buffaloes sampled in the Campania region in southern Italy. Methicillin-resistant Staphylococcus (MRS) and Extended-Spectrum Beta-Lactamase (ESBL) producing Enterobacteria have also been assessed. Bulk tank milk samples from 59 different farms were collected between June and November 2023 and subjected microbiological analyses on selective media, with and without enrichment, plus molecular analysis. Results revealed a high presence of *S. aureus* as 71.18% of samples tested positive, indicating variable bacterial load. Up to six colonies for each positive farm were characterized by molecular analyses, totaling 202 isolates. According to the primary results of the RNA template-specific polymerase chain reaction (RS-PCR), the isolates belonged to 8 different genotypes with a genotype B predominance (71.80% of farms). Analysis using selective media demonstrated that 45.76% of samples were positive for MRS. Using MALDI-TOF the most frequently identified species were *S. aureus* (44.44%), *S. epidermidis* (18.51%), *S. sciuri* (18.51%), *S. haemolyticus* (3.70%), *S. saprophyticus* (3.70%). On the other hand, 23.72% tested positive for ESBL-producing enterobacteria. The identified species were *E. coli* (64.28%), *Enterobacter spp* (21.42%), and *Klebsiella pneumoniae* (14.28%). Several isolates were resistant to antibiotics. The results demonstrate the high prevalence of *S. aureus* but also MRS and ESBL bacteria in water buffalo farms. Given their impact on animal and human health, this reaffirms the relevance of monitoring and characterizing antibiotic resistance.

Morphological and Morphometric Analysis of Hypopharyngeal Glands (HPGs) and Vitellogenin Transcription Level in caged honey bees at different ages fed with different diets.

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Honey bee well-being depends on accessing a variety of nutrients, especially pollen, which they obtain through pollination (1). Pollen proteins are a fundamental component of the honey bee diet and are necessary for appropriate formation and functioning of the hypopharyngeal glands (HPGs). The HPGs are paired organs in the honey bee's head (2), responsible for producing royal jelly used to feed future queen, drone and worker larvae, for synthesizing enzymes for the transformation of nectar into honey and for storing glycogen for intense flight activity. The aim of this study was to evaluate the morphological aspects, morphometric parameters of HPGs and vitellogenin transcription level (an important protein involved in royal jelly syntesis and honey bee aging) in caged honey bee at different ages fed with only fondant or fondant and pollen. Morphological and morphometrical analysis were performed on HE stained honey bee samples. Additionally, samples were stained with Periodic Acid Schiff (PAS) and PAS-Alcian blue (PAS-BA) to detect glycogen and mucins; while vitellogenin transcription level was evaluated with qRT-PCR approach.
Histologically honey bees aged from 7 to 21 days and fed with only fondant showed a marked reduction of HPGs size. The acini appeared underdeveloped in 7 day old honey bees, degenerated in 14 day old honey bees and completely atrophied in 21 day old honey bees, indicating scarse/absent secretory activity. On the contrary, honey bees aged 7 to 21 days fed with fondant and pollen showed an increase of HPGs size. The acini increased in number and size due to the abundant foamy and clear material, indicating high and constant secretory activity, mainly mucosal (PAS + and BA -). The morphometric results confirmed the histological results. The HPGs were always larger in honey bees fed with fondant and pollen (mean area (mm^2) and standard deviation (SD) values ranging from 3.27±1.04 to 6.60±4.04) than in those fed with only fondant (mean area (mm^2) and standard deviation (SD) values ranging from 0.69±0.41 to 5.45±1.54). Interestingly, qRT-PCR results show that the quantity of vitellogenin is dependent on the age of the honey bee and not on type of food source. Collectively, our results confirm that a proteic diet (pollen) is necessary for the normal development and function of the HPGs and that the amount of secretion is positively correlated with glandular activity and acinar size (3). Therefore, using integrated diets with pollen could be a useful strategy to improve honey bee health and productivity, contrasting the decline of this species.

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77° CONVEGNO SISVET

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Iron-related molecular pathways in Canine Osteosarcoma: preliminary results from an immunohistochemical study

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Osteosarcomas (OS) are the most common bone tumor in dogs, characterized by high metastatic rates and poor prognosis. Treatment includes surgery, radiotherapy and chemotherapy; however, tumor recurrence and metastasis are frequent and survival time remains short. Thus, the identification of new possible therapeutical targets is needed. Furthermore, Canine osteosarcoma (COS), is a well-recognized model for human OS.As with most cancers, previous studies have evidenced dysregulation of iron metabolism in OS human patients, suggesting similar condition also in dogs. Iron is an essential trace element as it is involved in a wide variety of cellular processes both in normal and neoplastic cells.In human oncology Transferrin Receptor 1(TfR1) and Transferrin Receptor 2(TfR2) appear upregulated, while Ferritin (FTH1) appears down-regulated. Increased expression of TfR1 is effectively related to increased rates of iron uptake by OS cells, supporting OS proliferation, while TfR2 represents a less effective iron receptor and it is only activated following TfR1 saturation. Nuclear-receptor-coactivator-4(NCOA4) promotes autophagic Ferritin degradation to mobilize iron from deposits, making it available for cell proliferation, but also for ferroptosis, a regulated intracellular non-apoptotic iron-dependent form of cell death.The aim of our study was to evaluate by immunohistochemistry(IHC) and Western Blotting (WB), in osteoblastic COS, the expression of proteins involved in iron pathways, namely TfR1, TfR2, FTH1, NCOA4, and PCNA to assess proliferation.3 normal bone samples and 20 OS samples were retrieved from the Departmental archives and routinely processed for IHC and WB. Immunoreactivity was evaluated by counting immunoreactive cells in 1000 cells in 10 fields at 400× magnification.Immunoistochemical analysis of normal samples revealed no immunostaining for TfR1 and TfR2, while moderate cytoplasmic positivity for anti-FTH1 was observed in 40% of osteocytes, and weak nuclear immunostaining for NCOA4 was observed in 30% of osteocytes.In OS samples 85-95% of tumoral cells showed strong cytoplasmic immunostaining for TfR1, while 60-70% were immunostained for TfR2; 10-30% of tumoral cells were moderately immunolabeled by anti-FTH1, and 60% of tumoral cells presented weak/moderate immunostaining at the nuclear or perinuclear site for anti-NCOA4. 80-90% of tumoral cells showed immunolabelling for PCNA. WB analysis confirmed the cross-reactivity of all antibodies.Results reinforce data of previous studies suggesting an increased demand of iron by COS cells as highlighted from the over-expression of TfR1 and TfR2 in OS samples compared to controls. Interestingly, the expression of TfR2, usually only exhibited in normal liver and bone marrow tissues, is here reported for the first time in canine tumors, and could underline the activation of atypical molecular pathways in canine OS as a results of increased iron demands by cancer cells. On the same line, the decreased expression of FTH1, probably mediated by NCOA4, could indicate an increase in iron availability and consumption to sustain higher levels of proliferation in OS samples, as suggested by the higher PCNA index.In conclusion, our results further support the hypothesis that OS may be more sensitive to iron metabolism dysregulation, thus suggesting the possibility of targeting iron pathways as a new therapeutic strategy.

77° CONVEGNO SISVET

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Ex Vivo Expansion and Characterization of Canine Monocyte-Derived Dendritic Cells: A Gateway for Delivering Cancer Neoantigens

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Adoptive cell therapy (ACT) using cancer neoantigen-reactive T-cells holds promise for developing new therapies in dogs with malignant tumors. Within this context, dendritic cells (DC), which are specific and unique antigen-presenting cells activating naive T-cells, play an essential role in innate and adaptive immune responses. Indeed, DC are deputated in initiating both helper and cytotoxic T cells acting as a 'nature's adjuvant'. However, the use of canine DC in cancer therapy is hampered by the lack of comprehensive information regarding their functional properties and phenotyping, alongside the absence of an established in vitro protocol for obtaining mature DC ex vivo. These gaps significantly impede the development of ACT technologies.
The aim of the present study was evaluating the ex vivo expansion efficiency of DC obtained by monocytes circulating cells from healthy dogs (hdDC) and tumor bearing dogs (tbDC) affected by fibrosarcoma, oral melanoma and B-cell lymphoma.CD14 positive cells were immunomagnetically selected from peripheral mononuclear blood cells of healthy and tumor bearing dogs, and the adherent monocytes were cultured for 6 days with recombinant Canine GM-CFS, Canine IL-4, Canine IL-1beta/IL-1F2, Canine TNF alpha to obtain mature DCs. Flow cytometry analysis conducted after CD14 isolation (day 0) showed that both hdDC and tbDC were positive to CD14, CD40, CD90, CD86, CD11c and DLA class II antibodies, while negative for the expression of CD83, CD80, and CD1a. At day 0, tbDC exhibited a higher expression of CD40 (median 44%, range 41-58%) compared to hdDC (median 16%, range 12-21%), and a lower expression of DLA class II (median 6%, range 3-10% in tbDC versus median 91%, range 87-94% in hdDC). At the end of the culture period (day 6), tbDC showed an increase of DLA class II expression (median 78%, range 65-84%). Additionally, both populations displayed de novo expression of CD1a, CD80, and CD83.
These preliminary data suggest that canine DC can be expanded ex vivo using methods comparable to those used in humans, with a similar growth efficiency. Moreover, after 6 days of in vitro maturation, both hdDC and tbDC express several mature dendritic markers (CD1a, CD80, CD83), indicating their ability to differentiate starting from circulating monocytes. Interestingly, the low expression of DLA class II in tbDC at day 0 suggests a distinct phenotype of circulating monocytes in tumor bearing dogs compared to healthy dogs. However, the constitutive expression of DC can be restored after in vitro cytokine stimulation, indicating the potential for reversing the phenotype and enhancing the functionality of DC in a cancer scenario.

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77° CONVEGNO SISVET

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EFFICACY OF A BACTERIAL COMPOUND APPLIED ON THE LITTER IN REDUCING SEVERITY OF FOOTPAD LESIONS IN BROILERS

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Footpad dermatitis (FPD) is a condition that causes necrotic lesions on the plantar surface in growing broilers, affecting their welfare, and representing a risk factor, since it is one of the main entry points for pathogenic microorganisms [1]. The lesion is characterised by multiple histological features, starting from subepidermal heterophilic infiltration to necrobiotic-degenerative changes which affect first the superficial epidermal layers and, as the lesion progresses from erosion to ulcer, involve all the epidermidis penetrating deep into the dermis [2]. In this trial, a total of 89,200 Ross 308 chickens (39±3g) were housed in two sheds (C, control; T, treated) at the same environmental conditions. Females were housed in the first part of each shed, males in the second and third part. A bacterial bedding conditioner (EazyBed Pro, Lallemand, France) was applied in T: a pre-treatment (30g/m2) was carried out on the floor before placing the litter and, from week 1 to the end of the cycle, the conditioner was applied weekly on the litter (90g/m2). In C no treatments were carried out. Females were slaughtered at 36d (T: 1528±195g; C: 1562±188g) and males utilized the whole space until 43d (T: 2696±296; C: 2737±364). At slaughtering, 12 legs for each group were randomly selected for histological examination. Using a scoring system, several parameters were analysed: keratinization, epidermal layer structure, inflammation, leukocytes (heterophiles, macrophages, and lympho-plasmacytes), neoangiogenesis, dermal and hypodermal involvement. In addition, to investigate the mechanism of lesion development, the number of apoptotic cells (DeadEnd™Colorimetric TUNEL System, Promega Italia Srl, Italy) and the expression of Hypoxia Inducible Factor (HIF-1α, MA1-516, Invitrogen, USA) were also assessed by immunohistochemistry. Ordinal and cardinal variables were analysed by Mann-Whitney test and Student t-test, respectively. In relation to the variables, median (minimum-maximum), and mean ± standard deviation, were considered. Chickens farmed in T showed a total histological score significantly lower than in C (18 vs 28.5; p=0.0002), especially in males (19.5 vs 30; p<0.0001). Considering the single parameters, several differences were noted, except for the heterophiles count (3 vs 3; p=0.251). Treatment significantly reduced inflammation (2 vs 4; p<0.0001) and hypodermal involvement (1 vs 2.5; p=0.004), both in females and males. Only in males, keratinization (3 vs 4; p=0.018), epidermal layer structure (2.5 vs 5; p=0.012), leukocytes [macrophages (2 vs 3; p=0.016) and lympho-plasmacytes (1 vs 2; p=0.021)], neoangiogenesis (2 vs 3; p=0.027), dermal involvement of the lesions (2 vs 5; p=0.0004), were significantly reduced. HIF and TUNEL positive cells counts resulted significantly reduced in chickens farmed in T (HIF: 136.4±24.59 vs 210.5±38.67, p<0.0001; TUNEL: 137.8±34.30 vs 215.0±57.08, p<0.0001) both for females (HIF: 143.5±23.15 vs 191.7±21.12, p<0.0001; TUNEL: 113.5±22.94 vs 167.8±23.93, p<0.0001) and males (HIF: 129.3±24.87 vs 229.4±43.73, p<0.0001; TUNEL: 162.2±25.22 vs 262.2±36.97, p<0.0001). Results suggest that concomitant factors may lead to reduction in the bioavailability of oxygen at footpad level, and therefore that lesions may be induced by activation of the HIF gene and increased apoptosis that favours its onset, expansion, and aggravation, while preventing structural regeneration. The use of bacterial bedding conditioner positively affects the severity of footpad lesions, improving broilers' welfare.

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Hemosiderin-laden macrophages in canine mammary carcinomas: EPO, EPOR and HIF-1α expression

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Tumour-associated macrophages (TAMs) are involved in all stages of cancer from tumorigenesis to metastasis outgrowth. Classically divided into two opposite phenotypes, today the spectrum of macrophage subtypes is recognized to be much broader and with a division not so clear-cut. In inflammatory contexts, macrophages can modulate the immune response thanks to their central function in iron recycling [1]. Different studies are focusing on connections between cancer, TAMs, and iron metabolism. Hemosiderin-laden macrophages (HLMs) are described in both human and canine mammary tumours, however, their function is still not completely understood [1, 2]. This study aims to evaluate erythropoietin (EPO), its receptor (EPOR) and the hypoxia-inducible transcription factor-1α (HIF-1α) in HLMs inhabiting the stroma of canine mammary carcinomas.

HLM-rich canine mammary carcinomas were selected and evaluated through haematoxylin-eosin (H&E), Prussian blue, and Meguro stain. Immunohistochemistry was carried out for EPO, EPOR and HIF-1α.

HLMs showed granular cytoplasmatic hemosiderin deposits and peritumoral or stromal localization. They were also located near to tumour-infiltrating lymphocytes (TILs) and tertiary lymphoid structures (TLSs) or necrotic areas. Prussian blue and Meguro stain revealed the iron deposits in HLMs with blue and brown colour, respectively. HLMs, along with macrophages and mast cells, were variably positive for EPO, EPOR and HIF-1α. EPOR and HIF-1α were also expressed in epithelial neoplastic cells with greater intensity in foci of solid growth pattern and close to the margins of necrosis. EPO was mainly expressed by endothelial cells and variably by neoplastic cells.

Considering its proliferative, antiapoptotic and angiogenic roles, the colocalization of EPO and EPOR in HLMs suggests the possibility that these may generate a loop of autocrine and paracrine stimulation. In the inflammatory context, EPO is directly involved in macrophage polarization to M2 phenotype and enhances efferocytosis contributing to the elimination and clearance of apoptotic cells and cellular debris [3]. Knowing the similarities between the inflammatory process and the tumour context, EPO could trigger the M2 switch of HLMs leading to the production of protumoral substances and stimulating phagocytosis. The latter hypothesis would also explain the HLMs localization near necrotic areas. In addition, HLMs could be directly involved in neoangiogenesis, considering their already demonstrated positivity for VEGF and its receptor [2]. The expression of neoangiogenic molecules is known to be strictly linked to the hypoxic environment. M2 macrophages express high levels of HIF leading to the production of substances favouring tumour proliferation and survival [2, 3]. HLMs are supposed to sequester iron to prevent its depletion and maintain adequate levels of cytotoxic free iron. Therefore, they could act as iron reservoirs that can be exploited by tumour cells to promote their growth [1]. In addition, tissue iron low levels can stimulate HIF-1α activation leading to the production of angiogenic molecules [2]. Then HLMs could represent a novel macrophagic subtype worth to be better investigated for its potential role in cancer survival and progression.

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MHC-II gene overexpression in canine Mast Cell Tumor: can it be a potential therapeutic target for immunotherapy?

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Canine mast cell tumor (MCT) is one of the most common cutaneous tumors in dogs characterized by a very variable biological behavior [1]. Gene expression profiling is nowadays considered a great instrument in cancer research for its immense potential to fully explore the pathophysiological process leading to cancer development and progression and to discover new prognostic markers and therapeutic targets [2]. Up to date, numerous studies have been dedicated on the investigation of predictive and theragnostic markers in canine MCT [1], but research focusing on genetic changes are still lacking. With these premises, the aim of the present study was to identify aberrantly regulated genes in canine cutaneous MCT that could possibly represent unexplored markers of tumor biological behavior and/or represent targets for a new therapeutic approach. The gene expression profile of Kiupel low (group 1, n. 6) and high (group 2, n. 6) histologic grade canine MCTs and mastocytic dermatitis (control group, n. 4) was analyzed with the NanoString nCounter Canine IO Panel (NanoString Technologies, Seattle, Washington) using RNA extracted from selected tumor tissue areas from paraffin blocks. Immunohistochemical analysis for MHC II expression was also performed on MCTs. Thirteen genes were differentially expressed between the 2 groups, whereas no significant change in expression was found for control group. Gene set analysis highlighted an upregulation of KIT and genes involved in antigen processing such as MHC II (DLA-DQA1) and mast cell activation (FCER1A) in high grade MCT compared with low grade MCT. Neoplastic mast cells showed immunoreaction to anti-MHC II antibody. MHC-class II (MHC-II) molecules are predominantly expressed by professional antigen presenting cells (APC) with the primary function of presenting exogenously derived peptide antigens to CD4+ T cells. Unlike professional APCs, mast cells do not constitutively express MHC-II on the cell surface in their resting state, but its expression can also be induced by activation independent of degranulation. A growing body of scientific literature suggest that a subset of tumors originating from a variety of tissues also express MHC-II [3]. To our knowledge, this is the first report to describe an increase of MHC II gene expression and anti-MHC II antibody immunoreaction in canine MCTs. Although this finding must be further investigated, in our opinion it may potentially pave the way for a better understanding of the pathophysiology of canine MCT and, above all, for future therapeutic approaches. An enhanced tumor antigen recognition suggests an increase recognition of a tumor by the immune system, hence an increase of cytotoxic activity and a crucial role in immunotherapy [3].

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Genotoxic effects and oral pathology in cats exposed to indirect cigarette smoke.

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Cigarette smoke contains a high concentration of carcinogenic substances to which smokers are regularly exposed periodically. Secondhand smoking (SHS) is a mixture of the side stream smoke, and the mainstream smoke exhaled from the lungs of smokers. SHS exposure results from the involuntary inhalation of sidestream and exhaled mainstream smoke and it reaches higher concentrations indoors. Passive smoking is seriously harmful to the health of non-smoking humans and animals. Indeed, in human medicine, it has been shown that smoking, even if passive, can lead to precancerous and cancerous oral lesions [1]. However, only a few reports have investigated effects of nicotine oral ingestion during grooming in cats [2]. Cats spend 30 to 50 % of their day in grooming activities which make them more susceptible to effects of indoor environmental smoke [3]. Several techniques have been validated to investigate exposure to tobacco smoke; among them, cotinine level evaluation has been used as a reliable marker for smoking studies due to its long half-life. Considering these observations, this study aimed to correlate the morphological and genotoxic alterations of the oral cavity to cotinine levels in the urine of cats exposed to SHS. Urine samples and cytologic smears from the oral cavity were collected from 30 cats: 20 with smoker owners (10 male and 10 female, age range 1-2 years) and 10 with non-smoker owners (5 male and 5 female, age range 1-8 years). Urinary cotinine concentration was measured using two tests, NicAlert® rapid test kit by Nymox and Coitinine ELISA Kit by Abnova. Cytologic smears from the oral mucosa were stained with MGG Quick stain (Bioptica) and Papanicolaou stain (Bioptica) for the evaluation of oral inflammation, with a score from 0 to 3, and for Micronuclei (MNi) evaluation, that was scored in 1000 cells with well-preserved cytoplasm. Smears were also stained with Papanicolaou stain for the evaluation of epithelial dysplasia. Our results showed statistically significant differences between exposed and non-exposed cats in terms of urine cotinine levels with both tests. Oral brush smears showed a low to moderate number of inflammatory cells consisting mostly of neutrophils, macrophages, rare lymphocytes, and plasma cells in 15 out of 20 of the exposed cats. Among exposed cats, MNi were seen only in 5 out of 20 cases. Papanicolaou stain showed a moderate grade of dysplasia of the oral cavity such as binucleated cells, green/pink cytoplasm, and a higher N/C ratio in 10 out of the 20 exposed cats. Our results suggest that Coitinine ELISA Kit may be a useful tool to evaluate cotinine levels in cats and that the exposure to second-hand tobacco smoke can determine chronic and time-dependent morphological and genotoxic alterations in cats. Further study will be needed to better investigate the correlation between smoke exposure duration, genetic and morphologic alterations.

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Testicular cancer and environmental pollution: a comparative onco-epidemiology study in Campania Region

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Comparative biological analysis between testicular cancer in dogs and humans, considering space sharing and exposure to environmental pollutants, highlights, similarities in clinical manifestation, genomic instability, and metastatic potential. The ubiquitous distribution of toxic substances, capable of interacting with the animal and human organism, conditions the development of cancers. Diethylhexyl phthalate (**DEHP**), polybrominated diphenyl ethers (**PBDE**), polychlorinated biphenyls (**PCB**), dichlorodiphenyltrichloroethane (**DDT**), perfluoroalkyl substances (**PFAS**), perfluorooctane sulfonate (**PFOS**), perfluorooctanoic acid (**PFOA**), perfluorohexane sulfonate (**PFHxS**), phthalates, bisphenol A (**BPA**), xenoestrogens (like **ZEA**) and dioxins fall into the group of persistent organic pollutants (**POPs**). These toxic analytes act as endocrine disruptors, heterogeneous substances capable of mimicking the action of hormones and interacting with their receptors, altering their proper stimulation. Retrospective analysis of canine testicular oncology data from the **Campania Animal Cancer Registry**, about the years 2020-2021-2022-2023, produced the following results: 61 testicular tumors in 2020, 68 testicular tumors in 2021, 41 testicular tumours in 2022, and 68 testicular tumors in 2023, includes Leydigomas, Sertoliomas, and Seminomas. From the management system of the National Cancer Institute “Fondazione G. Pascale-IRCCS”, we examined the cases of human testicular cancer evaluating 30 cases from 2020 and 50 cases from each year 2021-2022-2023 (total number 180 cases) including a high incidence of mixed tumours and seminomas, with a smaller number of Leydigomas and Sertoliomas. For environmental analysis, we extrapolated the data from the Ministry of Environment and Energy Security website and the Campania Regional Agency for Environmental Protection (ARPAC) site. The cities with the highest incidence of testicular cancer in humans and dogs were Naples, Salerno, Ercolano, Portici, Torre del Greco, Somma Vesuviana, San Giuseppe, San Giorgio a Cremano, Cava de’ Tirreni, Castel San Giorgio and Pagani. In the same areas were recorded severe contamination of soil and water by metals and metalloids, hydrocarbons, dioxins furans, PAHs, PCBs, aromatics, carcinogenic and non-carcinogenic chlorinated aliphatic, organic compounds, inorganic compounds, chlorinated and non-chlorinated phenols, phytochemicals. This association highlights a strong correlation between environmental pollution and the incidence of cancers related to them. The biological behavior of testicular cancers in dogs and the possible role of environmental risk factors may provide useful indications for preventing neoplasms affecting the human species. Risk assessment coupled with strategic prevention are essential to reduce cancer mortality in dogs and humans. The short latency period for cancer in animals compared with humans creates an advantage in studying spontaneous disease in animal models by being able to consider them as "sentinels."

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SPONTANEOUSLY OCCURRING TUMORS IN ASIAN ELEPHANTS (*ELEPHAS MAXIMUS)*: A RETROSPECTIVE CASE SERIES

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Elephants are rarely affected by tumors, this is thought to be due in part to their extra TP53 tumor suppressor genes, which code for the p53 protein [1]. The elephants have 20 copies, that is, 40 alleles of the TP53 genes, compared with the typical number of one copy found in all other mammals. According to Peto's Paradox referring to why larger animals with a higher number of cells and cell divisions do not also have a higher tumor incidence the elephant's multiple copies are proposed to have evolved to defuse enhanced DNA damage response to promote neoplasm suppression [2].

Our study aimed to describe the neoplastic and non-neoplastic diseases in *Elephas Maximus* examined in the period 2004 to 2021. Five female Asian elephants, two from the circus and three from zoos, with ages ranging from 40 to 56 years, were necropsied. After a complete macroscopic examination, tissue samples were collected and processed for routine histopathology and immunohistochemistry. After a microscopic examination, the tumor was diagnosed in 4 out of the 5 elephants (seven tumors and a herpesvirus infection). Based on histopathological features, tumors were classified as follows: four leiomyomas, a leiomyosarcoma, a uterine adenocarcinoma [3], and a lymphoma. Animals with neoplasms were older than animals without neoplasms (mean age 55,25 vs 40 years). The malignant and benign tumors were 43% and 57%, respectively. Among malignant tumors, metastasis was observed in 1 out of the 3 cases. Based on our findings, leiomyoma was the most common tumor identified and the female reproductive system was the most frequent primary tumor site. Moreover, this case series shows a higher incidence of neoplastic disease compared to non-neoplastic disease among elephants in southern Italy considering this data extremely important also for epidemiological purposes. Furthermore, in human medicine, subjects with TP53 gene mutations show a high risk of developing neoplasm. Genetic analyses of three elephants with tumors are ongoing to assess the presence or absence of possible mutations. Such mutations would explain tumor development in the assessed elephants in Southern Italy.

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Detection of *Vibrio aestuarianus* associated with tissue lesions in farmed oysters (*Magallana gigas*)

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Vibrio aestuarianus subsp. *francensis* has been identified as a pathogenic agent responsible for periodic mortality in adult Pacific oysters (*Magallana gigas*) [1]. However, studies attribute these episodes to the interaction of environmental factors and pathogens. Little information available in the literature emphasizes the need to clarify the pathogenetic mechanisms of *V. aestuarianus* in *M. gigas* [2]. *Magallana gigas* subjects (n=358) were collected during a mortality episode in Sardinian oyster farming areas, with a water temperature of 14°C. Samples (n=28) were deeply investigated by molecular, histopathological, and in situ hybridization (ISH) techniques with a system (RNAscope®) to detect the presence of *Vibrio* spp. in *M. gigas* tissues. The mantle of oysters (29%) exhibited a moderate to severe, nodular to multifocal hemocytic inflammatory infiltrate associated with a concentration of *V. aestuarianus* greater than 10^4 copies/µl in qPCR. ISH demonstrated the presence of *Vibrio* spp. in almost all the oysters associated with the mantle and gills inflammation, with a stronger and diffuse signal in oysters displaying a moderate to severe degree (r=0.66, p 66, p<0.05). Our results suggested that *V. aestuarianus* is the cause of mortality in oysters probably in association with changes in environmental conditions.

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77° CONVEGNO SISVET

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Investigating immunohistochemistry in fish: western immunoblotting validation of the most common antibodies used in mammals.

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Recently fish research has significantly progressed, especially in aquaculture1. Immunohistochemistry (IHC) has proved as a necessary tool for supporting investigations in fish anatomical and pathological research. However, very little is known about a possible cross-reactivity and specificity in fish of the antibodies developed for mammals2. This study aims to evaluate the IHC reactivity of commonly used antibodies in mammals, such as pan-cytokeratin (CKAE1/AE3), vimentin (V9), S-100, glial fibrillary acidic protein (GFAP), and desmin (D33), validated by the Western immunoblotting (WB), in the tissues of the main aquaculture fish species (Sparus aurata, Dicentrarchus labrax, Oncorhynchus mykiss). The results revealed that the pan-cytokeratin and GFAP antibodies cross-react with all tested fish species, while S-100 demonstrated specific staining only in the sea bream and rainbow trout tissues. Regrettably, both mouse monoclonal anti-desmin and anti-vimentin clones didn't show any reactivity. In conclusion, our results emphasize the need to create specific antibodies for aquaculture research purposes, where available antibodies used in mammals did not cross-react with fish tissues.
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AIVI

77° CONVEGNO SISVET

Stato: INVIATO - ID: 12972

Hepatitis E virus in ovine raw milk from herds farmed in Central Italy

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Among viral foodborne pathogens, hepatitis E virus (HEV) has been widely detected in different animal origin foodstuffs. Milk, obtained from large and small ruminants, have demonstrated the possibility to detect infectious amounts of virions. Indeed, HEV transmission from blood to the mammary gland, during viremia step, has been observed in different mammalian species (including humans). Small ruminants result receptive to HEV and result also involved in its environmental diffusion through feces and raw milk [1]. In Abruzzo region (Central Italy), the ovine species (Ovis aries) are traditionally farmed following the transhumance method. It means the involvement of many geographical areas (including National Parks) used for grazing also usually shared with wild ruminants. This study aimed to amplify specific HEV RNA regions from 220 ovine unpasteurized milk specimens collected from 3 herds farmed in three provinces (located in Abruzzo region): Teramo, Pescara, and L'Aquila. Successively to the pre-dipping procedures, a volume of 50 mL was individually sampled from each animal. Prior to the RNA extraction, the milk fat layer was removed after centrifugation at 3000X g for 15 minutes at 4°C. The adding of 300 μL of HCl 1M solution was used to facilitate the proteins and nucleic acids precipitation, as described by Dziedzinska et al. [2]. The Trizol LS method was successively used for the viral RNA extraction. Nested RT-PCR and RT-qPCR were performed to amplify specific genetic regions belonging to the HEV ORF-1, ORF-2, and ORF-3. Sanger sequencing and phylogenetic analysis were performed. The IBM® SPSS® Statistics Software was used for the statistical data analysis calculating the chi-square value (with Yates's correction). Results showed HEV RNA fragments amplification from 5/220 or 2.27% (both ORF-1 and ORF-2 amplicons) milk/subject specimens, and the RT-qPCR detected on average 102 copies/μL from positive specimens. From a geographical perspective, among the discovered positive subjects, 3 animals were farmed in Teramo province and 2 in Pescara one. Sequence analyses and the phylogenetic assays permitted to determine high nucleotide similarities with HEV genotype 3 which is the most diffused one in Central Italy. This investigation discovered for the first time HEV RNA fragments from raw ovine milk in Italy. The scientific explanation of HEV RNA detection can be justified by the transhumance farming method and the environmental sharing with wild animal species, which are natural reservoirs, [3] providing environmental conditions for possible cross-species infections. Basing on the One-health approach, HEV environmental surveillance represents a crucial public health concern.

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Metabarcoding based on Next Generation Sequencing (NGS) for the authentication of mussel products (*Mytilus* spp.): a preliminary study.

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The genus Mytilus includes species of commercial relevance, namely *M. chilensis* (MC), *M. edulis* (ME), *M. galloprovincialis* (MG), and *M. trossulus* (MT). Hybridization has been reported in areas where more species coexist. Hybrids MExMG and MExMT are reported in Europe, and MCxMG, MCxME, and MCxMT in Chile. The difficulties in differentiating Mytilus species, also increased by hybridizations, may favor deceptive practices misleading consumers, such as species substitution. The use of DNA based methods is encouraged in the EU to counter such type of food fraud. The PCR Restriction Fragment Length Polymorphism (PCR-RFLP) of the Adhesive Protein (PAP) gene is one of the most applied molecular techniques for *Mytilus* spp. identification. We recently observed that the Sanger sequencing has some practical advantages over PCR-RFLP, and it was proposed as a valid alternative. The Next Generation Sequencing Technologies (NGS) have become increasingly attractive in the food authentication field. In this preliminary study, metabarcoding based on NGS was tested on 19 DNA samples obtained from market products made of *Mytilus* spp. (10 hybrids and 9 pure), already authenticated by both PCR-RFLP and Sanger sequencing. The PAP gene was amplified with primers added to Illumina adapters, and sequenced on a Miseq Illumina (150-bp paired-end mode). Raw files were processed using DADA2 R package to generate amplicon sequence variants (ASVs), which were taxonomically assigned by Blastn against GenBank. Overall, 20 ASVs were taxonomically assigned to MC (123 bp), MG (123 bp), ME (177 bp), or MT (165 bp). Sixteen DNA samples (84.2%) were found to be composed by pure species (n=5 ME; n=2 MC; n=1 MT) or hybrids (n=4 MCxMG; n=3 MExMG; n=1 MGxMT), in line with previous analyses. In these samples, sequences of pure species were found in percentages 96.9%-100.0% (average 99.3%, SD 1.05%), with the sample having the lowest percentage (96.9%) composed for the remaining part with not assigned ASVs. In the case of hybrids, the highest represented species (in terms of sequences number) was found in percentages 57.1%-97.7% (average 82.3%; SD 14.7%) and the less represented 2.1%-41.9% (average 17.5%; SD 16.8%). In hybrids MCxMG, sequences of MC were generally less represented (2.1%-3.0%) respect to MG (97.1%-97.7%). In the remaining three DNA samples, sequences of ME, not detected by PCR-RFLP and Sanger sequencing, were found. Since all the ASVs assigned to ME showed a typical 177 bp in length, erroneous results due to comparison with sequences from mis-identified specimens on Genbank can be excluded. In details, one sample previously identified as pure MT was found to be a hybrid MExMT (3.5% ME and 96.1% MT sequences), and two samples previously identified as MCxMT were found to be composed as follows: MT (80.4%-84.8%), ME (13.1%-14.0%), and MC (2.1% - 5.7%). Also in this case, sequences of MC were less represented. Thus, assuming that hybrids involve two parental species, and sequences of MC can be under-estimated in the samples (e. g. due to lower primer binding efficiency), a possible cross-contamination with ME DNA should be investigated, especially considering that the DNA samples have been highly handled during the previous analyses. Indeed, NGS is a very sensitive analysis. A further method optimization and validation, involving a significative sample number and including DNA from voucher specimens, positive controls, and replicates, is therefore needed. Once validated, NGS can be assumed as efficient approach to authenticate mussel-based products.

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Insects-based products (Novel Food) sold by e-commerce: assessment of the information to consumers in the light of the EU legislation.

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Edible insects are today considered one of the most promising alternative protein sources due to their nutritional properties and the environmental benefits correlated with their farming [1]. Although the insect industry sector has grown worldwide in the last decade. the acceptance rates of EU consumers towards edible insects are still low. Indeed, their integration into the EU countries' diets appear a distant perspective [3]. Currently, according to European legislation, insect-based products (IBPs) are considered as Novel Foods (Reg. 2015/2283). These kinds of products are sold in the EU mainly through e-commerce [2]. In this study, the current situation of the EU IBPs market through e-commerce was characterized by i) identifying Food Business Operators involved in the selling and ii) assessing the IBPs' compliance to the EU labelling regulation (Reg. 1169/2011) and to the "additional specific labelling requirements" imposed by Implementing Regulations authorizing their placing on the EU market (T. molitor, L. migratoria, A. domesticus and A. diaperinus). Overall, 26 e-commerce platforms were identified, mainly with head offices located in North and Central Europe and 656 IBPs proposed to be sold online were found. Most of IBPs consisted of whole insects (54.9%), followed by protein products (9.5%) and powder insects (8.1%). IBPs made with House cricket (A. domesticus) (50.2%) were the most represented, followed by IBPs made with yellow mealworm (T. molitor) (32.9%). On the contrary, IBPs made with lesser mealworm (A. diaperinus) (9.1%) and grasshopper (L. migratoria) (7.2%) represented a marginal share of the analyzed IPBs. Overall, only 3.4% of the IBPs were fully compliant with the EU labeling requirements. The high level of non-compliance was mainly related to the absence, incompleteness, or inaccuracy of the "additional specific labelling requirements" imposed by Implementing Regulations authorizing the placing on the EU market of IBPs, probably due to their recent entrance in force. Among these, issues related to allergens' declaration observed in many IBPs reflected possible safety implication for consumers. This study, by providing useful data on the FBOs involved in e-commerce of IBPs, namely a list of the main FBOs active in this sector, and describing the main IBPs categories sold on the EU online market, could also support the Competent Authorities towards more targeted official control activities. Finally, by sharing information about IBPs, our results could increase consumers' acceptance.

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Comparison of official non-compliances and internal non-conformities in a pig slaughterhouse

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In the European Union, primary responsibility for food safety rests with the food business operator (FBO). In a slaughterhouse, food safety evaluation depends on the maintenance of Good Manufacturing Practices (GMP) and Good Hygiene Practices (GHP), as well as on product and process controls. For these reasons, FBOs must implement a self-checking system (SCS)), to ensure safe meat for consumers, secure animal welfare, prevent transmissible animal diseases, and verify proper implementation of SCS. Previous studies have shown similarities and overlap in official controls and Food Safety Management System (FSMS). The aims of the present study were to compare the non-compliances assigned by the CA during official controls carried out in a high-throughput abattoir (with a daily output of about 3,000 heavy pigs) located in Lombardy region with the non-conformities registered by the FBO in the SCS during a ten-year period (2012-2021), and to examine potential overlapping of SCS and official controls.

Overall, 451 non-compliances/non-conformities (NCs) were recorded, and the majority (52.3%) were registered by the FBO. Among all the NCs detected by the FBO and the CA, the majority were assigned to Housekeeping and Hygiene (26.5%), Personal hygiene (13.7%), Maintenance (12.3%), Control of operations (12.1%), and Training (5.6%). Significant differences were observed in the type of NCs recorded among FBO and CA ($p < 0.01$ Chi-Square Test for independence). For the FBO, the control of the personnel is certainly important, being carried out through the verification of Personal hygiene. The CA was focused, instead, on the Control of Operations. Significant differences were observed in the distribution of NCs in the working areas (hot deboning room, slaughtering room, cold deboning room, cold rooms and changing rooms) based on the origin of the NCs (FBO, CA) ($p < 0.01$ Chi-Square Test for independence). In the slaughter area, cold rooms, lairage, and quality area (food safety and quality manual, documentation and record-keeping), NCs were assigned mainly by the CA. On the contrary, in the hot and cold deboning rooms, as well as in the changing rooms, the majority of NCs were assigned by the FBO. These differences underline the different point of views between CA and FBO and their purpose. It is important to investigate the comparability of official inspection and self-checking control. Discrepancies in the type of NCs between FBO and CA can be due to different reasons, such as the inherent conflict of interest for the FBOs when assigning NCs to their own company, the likelihood that some NCs are addressed without leaving any documented evidence, the ability of the auditors to recognize them, their sensitivity on specific topics or even their training background.

In the present study, the types of remarks varied among the two groups with different areas of focus. Although there could be an overlap between CA and FBO controls in slaughterhouse, it can be concluded that they have different objectives and are both crucial. Due to this overlapping, proposals to reduce the frequency of official inspections should not be considered unless similarity in NCs by FBO and CA is reached over the time.

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77° CONVEGNO SISVET

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A novel verification method of high-pressure processing (HPP) treatment of meat products based on near infrared spectroscopy

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Within the meat industry, high-pressure processing (HPP) is primarily used as a post-packaging decontamination technology for ready-to-eat (RTE) products. By inactivating prevalent vegetative spoilage and pathogenic microorganisms, HPP extends shelf-life and enhances food safety [1]. Additionally, HPP enables European companies to meet stringent safety regulations in foreign markets like the USA and Japan, which have a zero-tolerance policy for Listeria monocytogenes in RTE foods, facilitating the export of meat products also to third countries [2]. Given that the traceability of HPP products currently relies solely on documentation, integrating analytical techniques to confirm the treatment would greatly reinforce regular controls.
This work presents, for the first time, the application of near-infrared (NIR) spectroscopy as a rapid and non-destructive verification method of the HPP treatment of meat products.
Three types of meat products were examined: i) N=25 pressed hams (dry-cured, boneless, in rectangular parallelepiped blocks, approx. 5 kg each); ii) N=26 entire hams (dry-cured, boneless, in the classic leg-shape, approx. 9 kg each); iii) N= 20 salami (dry fermented sausages, approx. 8 kg each). All the products were wrapped under vacuum in a plastic film and subjected to HPP treatment (isostatic pressures of 600 MPa for 6 min) using a commercial scale unit (Hiperbaric, Spain) located in the production facility of Certosa Salumi - SterilParma –S.p.A. (Collecchio, Italy). NIR analyses were conducted before (“C”), immediately following (“T0”), and 48 hours after (“T48”) the HPP treatment using a portable MicroNIR spectrometer (908–1676 nm range, Viavi Solutions, USA). Samples were scanned through their packaging, targeting specific portions of the sample surface. The data analysis, employing orthogonal partial least square discriminant analysis, aimed to distinguish C from T (T0/T48) spectra of meat products through the development of discriminant models, whose reliability and validity were assessed through cross-validation.
The results indicated that spectral changes occurred both in hams and salami after HPP treatment, likely due to alterations in protein structure and water state. The discriminant models, based on different spectral fingerprints of C and T samples, exhibited all high fitting (R^2X >0.98) and predictive abilities (R^2Y and Q^2 >0.84), with salami globally outperforming ham datasets. A perfect recognition rate (100%) of C and T entire ham and salami samples was achieved, while one T pressed ham sample was misclassified neither as a T nor as a C sample. This was likely due to higher variability in the scanned surfaces of the pressed hams. The distribution of the samples in the score plot allowed to verify that the primary modifications induced by HPP treatment represented the major source of variability within the data. Minor spectral changes related to the storage time of T salami samples also occurred, but these were of a minor impact and did not cause overlap between the T0/T48 and the C samples, thereby suggesting that modifications induced by the HPP-treatment and enclosed within the NIR spectra were retained even after storage.
In conclusion, this method offers promise for improving control procedures, ensuring the traceability of HPP treatment, and maintaining strict food safety standards in the meat industry.
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Salmonella in wild boars: prevalence and antimicrobial resistance in Parma province, northern Italy

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Wild boars are the most widespread large wild mammals in the world, accounting for more than 1.5 million in Italy. Following their invasiveness, wild boar hunting is urged resulting in an increase in wild boar meat and derivatives production and consumption. Wild boar meat is a health, sustainable and environmentally friendly food which supports local economies. However, wild boars are known reservoir of zoonotic pathogens including *Salmonella* spp. Salmonellosis is the second most reported foodborne zoonoses in the EU and *Salmonella* spp. is considered of high priority in wild boar meat safety assurance. Additionally, as consumer safety risk is increased by antimicrobial resistance in foodborne pathogens, a One Health control strategy needs an integrate surveillance.
The aim of the study is focused on the prevalence and antimicrobial resistance of *Salmonella* spp. in wild boar carcasses in relation to some variables linked to the hunting activity.
During the 2023-2024 hunting season, 64 wild boars were shot (dog - driven hunted) in two territorial hunting areas (ATC PR04 and PR07) of Parma province. Information on sex, age, killing mode, times between killing, evisceration and skinning, and storage temperatures of the carcasses was acquired. The carcasses were sampled by using sterile sponges, according to ISO 17604:2015 for pigs. The samples were analyzed for *Salmonella* spp. detection and typing according to ISO 6579-1:2020 and ISO/TR 6579-3:2014, respectively. In the isolates, the Minimal Inhibitory Concentration to fifteen antimicrobials was tested.
The sampled carcasses belonged to 37 young (22 females and 15 males) and 27 adult animals (13 females and 14 males). *Salmonella* spp. was detected in 6.25% (4/64) of the samples: three *S. enterica* subsp. *enterica* (two *S.* Coeln and one *S.* Typhimurium) and one *S. enterica* subsp. *diarizonae* O:50. *S. enterica* strains were detected in both young and adult males, whose time between killing and evisceration was ≥ 3h, time between evisceration and skinning ≥ 15 minutes and storage temperature of carcasses was 4°C. *S. diarizonae* was detected in an adult female, whose time between killing and evisceration was ≥ 3h, time between evisceration and skinning was immediate and carcass storage temperature was 2°C. Statistically significant difference in time between killing and evisceration was found (Fisher's exact test).
The three *S. enterica* isolates were susceptible to all the antimicrobials tested, and the isolate of *S. diarizonae* was resistant to sulfamethoxazole.
Since wild boars don't have territorial boundaries and homogeneous diet, their microbial population can differ considerably between geographical areas. According to different Italian studies, *Salmonella* prevalence in wild boars can vary from 1.9% to 35%. The isolates detected in this study are of concern, especially regarding *S.* Typhimurium and *S.* Coeln, which ranked second and ninth, respectively, among the serovars responsible for the human cases of salmonellosis notified in the EU in 2022. *S. diarizonae* is detect commonly in wild boars in Italy, as well as its resistance to sulfamethoxazole.

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Occurrence, antibiotic resistance and pathotype of Escherichia coli from hunted wild boars in Sardinia

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Unlike the numerous studies investigating *E. coli* presence in pigs, there is not much data related to the role of wild boars as reservoir of this enteric bacteria which include commensal and pathogenic isolates. Poor hygiene during the processing phases, particularly incorrect evisceration practices can lead to contamination of wild boar meat or other carcasses. Therefore, the aim of this study was to evaluate *E. coli* carrier status and carcass contamination of 66 wild boars hunted during two seasons and to further characterize the isolates. At the end of 15 hunting days, after evisceration and before chilling, samples of lymph nodes, colon contents and carcass surface were collected from each animal. *E. coli* was determined on each sample, colonies with typical characteristics were isolated from each positive sample and submitted to the species confirmation by MALDI–TOF. Sixty-one isolates were identified and characterized in order to asses antimicrobial resistance (AMR) with the Kirby Bauer disk diffusion method. Moreover, the pathotype was determined with whole genome sequencing. Overall, strains referable to *E. coli* species were detected in 100% (66/66) of sampled wild boars. *E. coli* was detected in 54/66 lymph nodes samples (81.8%), 60/66 colon content samples (90.9%) and 40/49 (81.6%) carcass surface samples. The three kind of tested samples were simultaneously positive in 36/66 (54.5%) wild boars, while 24/66 (36.4%) animals tested simultaneously positive in two samples analysed, and 6/66 (9.1%) were positive in only one out of three types of tested samples. As regard AMR, phenotypic susceptibility testing showed the presence of 3/56 (5.3%) resistant *E. coli* strains, with two different patterns: 1/56 strain (1.8%) showed resistance to sulfonamide, tetracycline, doxycycline and streptomycin, while 2/56 (3.6%) strains showed resistance to fosfomycin. Most *E. coli* isolates (44/54, 81.5%) did not possess any defined pathotype, 7/54 (13%) isolates were classified as UPEC, 3/54 (5.6%) as ExPEC-UPEC and 2/54 (3.7%) as ETEC. Results confirm that wild boars in Sardinia can act as reservoirs and spreaders of *E. coli*. Specifically, the finding of carcass contamination by potentially pathogenic isolates represents a risk to public health, since the consumption of undercooked wild boar meat or fermented meat products with a short ripening period, can lead to food-borne infections. Adequate training of hunters and application of good hygiene and slaughtering practices are essential for wild boar meat safe production. Overall, limited antimicrobial resistance was observed among isolates, presumably attributable to the low selective pressure related to low exposure to antimicrobial substances of resistant microbial populations and to the scarce anthropic impact in the areas where wild boars live. However, constant monitoring of antimicrobial resistance must be carried out in wild boars, considering the continuous increase of their populations which can lead to their presence in the inhabited centres, thus enhancing a potential human-wild animal interaction.

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77° CONVEGNO SISVET

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Implementation of AI-based model using a machine learning approach to predict the type of non-compliance during official controls of food production plants

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This study aims to develop an artificial intelligence (AI)-based model using a machine learning approach able to predict the type of non-compliance (NC) that competent authorities can most likely detect during official controls of food production plants based on structural and production information. A Bayesian Network (BN) model was developed based on n.186 NCs detected by the Veterinary Service of Messina Health Authority during official controls performed on n.49 dairies in the period 2018-2022. The NCs were categorized into n. 12 different types based on the requirement not met: i) water supply; ii) cleaning and sanitizing conditions; iii) structural conditions and equipment; iv) microbiological criteria; v) labeling; vi) by-product management; vii) HACCP; viii) hygiene of staff and processing; ix) fight against pests; x) approval or registration; xi) traceability; xii) storage and transport systems. The model was constructed by relating the number and type of NC with the criteria and respective attributes established by the Veterinary Services for each dairy during plant risk categorization according to Annex 2 of the Intesa Stato-Regioni CSR 212/2016 [1]. In detail, n.8 different criteria were considered: 1) food category; 2) intended use; 3) completeness of the HACCP manual; 4) enforcement level of the HACCP manual; 5) professionalism of management and staff training; 6) general maintenance conditions; 7) date of construction or renovation; 8) size of the establishment and marketing area. The model was built using the Hugin Lite software (v.9.4) in default setup considering the NC type as the parent node and the n.8 different criteria as the child nodes. The BN nodes are random variables connected by directed arcs that reflect the dependencies between the nodes; in detail, the event occurrence of parent nodes is related to the events of child nodes by a conditional probability. The implemented model enabled the prediction of the most likely type of NCs by inserting the attributes of each criterion of the considered dairy as input data in the child nodes. A total of n.22 NCs detected on n.15 dairies during the period 2023-2024 were used to validate the model. The validation cases were not included in the learning dataset. The proposed model correctly predicted the occurrence of n.21 NCs (95.45%) while only n.1 (4.55%) was not detected. However, only n.4 NCs (19.05%) were reported as the first most probable. Machine learning has shown to be a useful technology for data analysis and modeling in several fields, and its use for monitoring and prediction in the food safety domain has been growing in recent years [2]. Although further efforts are needed to implement the model with a greater number of data, the present study has highlighted how machine learning can be a useful tool for competent authorities in organizing and performing official controls according to the request of Article 9, comma 1, letter e) of the Regulation (EU) 2017/625 [3].

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Analysis of the *Listeria monocytogenes* population structure among isolates from an Italian cold salmon plant

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ABSTRACT

Listeria monocytogenes (*L.m.*) is a foodborne pathogen responsible for human listeriosis, a severe disease acquired through the ingestion of contaminated foods. The invasive form of this infection could be fatal for susceptible individuals such as the elderly, immunocompromise patients, pregnant women and infants. Once introduced in a food plant by different ways (raw materials, food handlers etc.), *L.m.* can attach and form biofilm on surfaces and machineries, representing a source of recurrent contamination of food products. Ready-to-eat foods (RTE) are often implicated in the listeriosis transmission [1]. Among RTE foods, smoked salmon is highly vulnerable to pathogen colonization. In cold smoked salmon plants, the combination of an efficient food safety management system with a well-design environmental monitoring plan is crucial to mitigate the risk of contamination from this foodborne pathogen [2]. In regard to this, the goals of this survey were: i) to evaluate the presence of *L.m.* in an Italian cold smoked salmon plant; ii) to analyze the population structure of the isolates. The plant was visited at times when there was high, medium and low activity (three sampling visits). Environmental samples (60) and food products (9) from two production lines (I-Slicer; TT5) were collected.

Microbiological analyses were carried out according to the standard method EN ISO 11290-1:2017. Suspected colonies were identified by Maldi-TOF (Biotyper - Bruker). The screening characterization by Multi Locus Sequence Types (MLST) were performed using Pasteur [3].

Seventeen *L.m.* strains from environmental samples and one strain from food product were isolated. In particular, 11 isolates (61,1%) from I-Slicer and 6 isolates (33,3%) from line TT5. The strain from food product was isolated from I-Slicer line. The preliminary results of molecular typing showed the presence of four molecular profiles (CC5/ST5, CC6/ST6, CC7/ST7, CC9/ST9) suggesting potential multiple sources of contamination. A deeper characterization of strains will be performed by core genome MLST (cg-MLST). In addition, the analysis of Whole Genome Sequencing data will be carried out to evaluate the presence of sanitizers genes resistance and virulence traits.

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77° CONVEGNO SISVET

Stato: INVIATO - ID: 13590

ILLEGAL TREATMENT OF RED TUNA WITH NITRITE: UPDATES FROM THE IZSPB 05/21 RESEARCH PROJECT

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The illegal treatment of tuna with high concentrations of nitrite to obtain a significant improvement in appearance prolonging the shelf-life is a significant concern in food safety [1]. The food safety risk can be due not only to the high concentration of nitrite, but also to the possible increase of biogenic amines such us histamine, other than the microbial growth and possible presence of pathogens. Furthermore, the reaction of nitrites with free amines can lead to development of N-nitrosamines, pro-carcinogenic substances. The analytical aspects are also important, since the development of a rapid and non-destructive method able to detect added nitrite in tuna would be very useful for companies involved in processing and/or commercialization of red tuna. This study describes the results obtained from a research Project financed by the Italian Ministry of Health and carried out at the IZS Puglia e Basilicata. Both chemical and microbiological results obtained by simulating the treatment of red tuna (Thunnus thynnus) with nitrite solutions are presented. Moreover, the development and validation of a simple, non-destructive and user-friendly analytical tool for detecting the addition of nitrite in fresh red tuna samples, based on a hyperspectral method and chemometrics to identify the best detection wavelengths [2], is presented. Nitrite treatment was standardized in order to achieve a proper stabilization of tuna red colour in a 5-days period, under refrigeration. The samples were injected with solutions containing 0.4% (w/v) NaNO2 and 10% (w/v) NaCl, and then analysed for nitrite quantification. The following chemical parameters were determined: histamine (HIM), volatile basic nitrogen (TVBN), biogenic amines, nitrite/nitrate, ascorbic acid and sulphites, comparing the results obtained on fresh sample with those obtained on treated sample after 5 days of storage at 4 °C. Regarding microbiological aspects, the following determinations were carried out: total microbial count at 30 °C, enumeration of Enterobacteriaceae, Vibrionaceae, coagulase-positive staphylococci, Salmonella, Escherichia coli. Three samplings were carried out completing 186 analyses, composed of 114 chemical and 72 microbiological determinations. The possible effects of such treatment on samples with higher initial microbial count and in presence of pathogens were also investigated. Regarding novel analytical method by hyperspectral analysis, samples added with different levels of nitrite were analysed by both ion chromatography with conductivity detection and the presented approach, in order to define its detection capability (CC_β).The results indicate that, starting from products characterized by good hygienic and sanitary quality, the values of histamine, TVBN and total microbial load found in the treated samples, after 5 days of storage, are still abundantly within the normal ranges and such that they do not constitute a health risk. Therefore, this study confirmed that no other food safety concern is highlighted so far, apart from nitrite amount, and the possible development of N-nitrosamines. Some interesting results, such as the increase of nitrite level, were obtained when the starting products was intentionally contaminated with Salmonella. Regarding novel analytical tool under development, at least five wavelengths in the visible region were identified as able to detect the illegal treatment of tuna with nitrite solutions. This points out the potential of image analysis methods to identify nitrites at concentration of 50 ppm or more. This first result could be the way to develop and optimize an industrial on-line application to inspect tuna in objective and non-contact manner.

Thanks to the Italian Ministry of Health who financed the Research Project IZS PB 05/21 RC.

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77° CONVEGNO SISVET

Stato: INVIATO - ID: 13613

Use of new silver generation nanoparticles during cleaning in a seafood processing plant

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Biocides are antimicrobial chemical substances widely used for food industry environmental and equipment surfaces disinfection. Nowadays, they are carefully considered because they can have an important role in bacterial survival and resistance. Infact, an inappropriate use of biocides at different ranges of concentrations in various industrial applications, can result in the development (or selection) and spread of pathogenic bacteria, resistant to both the biocides themselves and antibiotics [1]. Thus, finding innovative and alternative substances represents one of the most important global challenge of this era. In this regard, nanotechnology may offer potential solutions for this challenge. The Argirium Silver Ultra Nano Clusters (Argirium SUNc®) are a new generation silver nanoparticles with a size < 2 nm (the smallest of all nanoparticles so far studied), generated using a reproducible electrochemical method [2], that have shown their antimicrobial effectiveness on either microbial cells (sensitive and resistant strains) and bacterial biofilm structures at a concentration much lower than that reported for other silver formulations (< 1 ppm) [3]. For this reason, they could have the great potential for use as innovative biocidal compound. The aim of the present work was to evaluate the antibacterial power of Argirium SUNc® nanoparticles used as a biocide in an Italian seafood processing plant located in Campania Region. In this regard, two sanitizing solutions (spray and drop formulation) containing only Argirium SUNc® with sizes ranging from 0.5 nm to 3 nm at a concentration of 2 ml, were tested for a time period of 10 minutes on 9 different surfaces (3 environmental, 3 equipment and 3 tools). These innovative solutions were then compared with the disinfectant used for the routine cleaning of the interest food industry. 6 swabs per surface were performed (before and after using the three different sanitizing solutions) and for each swab hygiene indicator microorganisms were searched (Coagulase-positive Staphylococci, Escherichia coli, Enterobacteria, Total Bacterial Count, Yeasts and Molds). Data shown that Argirium SUNc® used both as spray and drop formulation were able to reduce (50-70%) or inhibit bacterial growth on all tested parameters, proving to have a wide spectrum of action (in particular the spray formulation). Furthermore, it is important to underline that in most cases, the innovative solutions had a greater effectiveness compared to the disinfectant currently used in the seafood processing farm. In conclusion, Argirium SUNc® nanoparticles have shown their antimicrobial effectiveness also against the pathogens and spoilage agents most commonly found in the seafood chain, harmful both to health and seafood quality. At the same time, acting at very low concentrations, they don't burden fish food industries economically. For this reason, in a near-future, these innovative solutions could be used as new generation disinfectants, alternative to those currently used which favor the spread of antibiotic resistance.

IZSME RC 01/2021

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Metabolomic approach to support food safety toward innovative milk processes

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Milk is considered a staple food and raw milk placed on the market for human consumption must be heat treated before packaging. The heat treatments that guarantee safety are mainly the pasteurization and sterilization. Considering the nutritional and organoleptic variations after these treatments, innovative nonthermal technology as Infrared (IR) technology can offer a promising solution for reducing microbiological loads while preserving quality traits, such as flavour [1]. The advantages of IR application are also energy efficiency, effective process control, shortening process time, uniform product temperature, high heat transfer coefficient, and to be environmentally friendly. Considering that milk processing is one of the most water-intensive industries in the agro-food system these peculiarities meet the needs of Sustainable Development Goals of the 2030 agenda in finding new technologies to reduce the environmental impact while maintaining high levels of safety and environmentally friendly food systems. However, the use of infrared radiation for milk and dairy products is still limited. Thermal processes are currently assessed by controls on the thermal inactivation kinetics of the endogenous milk enzyme, alkaline phosphatase, which is not applicable to IR treatment because it remains active despite being subjected to sufficient treatment to inactivate the pathogens. So, metabolomics could become a potential support for controls as well as secondarily investigating changes in milk. Metabolomics is a golden standard to identify chemical composition and quantify metabolites from products driven by various biochemical or treatment processes. Given the absence in literature of metabolomic studies on infrared radiation on milk, the aim of this work was to compare, through a metabolomic approach, the milk treated with IR radiation at 2 different energies with the starting raw milk, to identify possible treatment markers that can potentially be adopted at an inspection level. The milk of 3 different batches was sampled directly from the plant tank (100.000 L capacity, refrigerated at 4 °C), transported to the laboratory at 4 °C until the IR treatment at 2 energies (IR80 and IR85) on the basis of our recent preliminary study [1]. Then 2mL of raw milk, IR 80 and IR 85 milk samples were extracted with 4 mL of acetonitrile with 3% formic acid and then analysed by LC-HRMS analyses to investigate the metabolomic profile with subsequent data processing using Compound Discoverer software. Overall, 48 of 3005 items were confirmed in the negative electrospray ionization mode and 49 of 5240 in the positive one. IR-treated milk samples showed only one up-regulated compound (P2) compared to raw milk: hypoxanthine. In the presence of oxygen, xanthine oxidase catalyses the oxidation of hypoxanthine to xanthine, further oxidized to uric acid, which has been shown, in literature, to display antioxidative activity in milk [2]. Moreover, from the differential analysis we noted a statistically significant increase of oleic acid, linoleic acid, mesaconic acid, arachidonic acid, dihomo-γ-linolenic acid, uridine, uric acid, uracil, vitamin B2, vitamin B4, carnitine, betaine, acetylcholine, cytidine, 7-Methylguanosine, L-tyrosine, β-alanine, L-glutamic acid, proline and a decrease of adenosine, citric acid, decanoylcarnitine, glucose, lactose, pyruvic acid, (±)9(10)-DiHOME, (±)12(13)-DiHOME. Currently, hypoxanthine could be a potential marker but further omic studies (e.g. lipidomic) will be conducted to discover other potential markers on this regard.

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Title: Animal welfare indicators in dairy cattle farms in the context of the ClassyFarm system

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Abstract

The farm veterinarian has the task of assisting the Food Business Operator (FBO) in the process of drawing up and implementing farming management plans to guarantee the health quality and sustainability of livestock farming [1]. The voluntary subscription of the farm to the ClassyFarm system, that is an integrated risk analysis and risk rating system in Italy, fits into these management plans [2]. The aim of the study was to identify possible correlations between three different animal-based measures (ABMs), that were plasma cortisol, interleukin-6 (IL-6), and individual Somatic Cell Count (SCC) and to compare these parameters with data provided by the Classyfarm system. The study was carried out in May and June 2023 and involved three dairy cattle farms in the province of Brindisi (Apulian region) taking part in the ClassyFarm system, herein-after referred to as farms A, B and C, which respectively obtained a ClassyFarm risk rank of 10, 13 and 17 in 2022 (the latest data available from the system). The number of heads was equal to 655 on farm A with 277 lactating cows, to 383 on farm B with 116 lactating cows and to 278 on farm C with 148 lactating cows, respectively. The study involved sampling 15% of lactating cows per farm (during their 3rd or 4th lactation) which received the same commercial feed ad libitum and were managed in a loose housing system. Samples were processed to evaluate cortisol and IL-6 levels, respectively, using a Bovine Cortisol ELISA Kit and a Bovine IL-6 ELISA Kit. Plasma cortisol and IL-6 levels were measured using the protocol outlined by Ceci et al.

(2017) [3]. While, the SCC, expressed as cells/mL was determined by Fossomatic FC (Foss Analytical A/S, Foss Allé 1, DK-3400 Hillerød, Denmark).

Based on our findings, all the plasma cortisol values were highly correlated with SCC levels in the three herds ($r > 0.70$), particularly in herd B ($r = 0.92$). The observed SCC values were generally compliant with the European Commission (EC) Regulation No 853/2004. IL-6 levels did not show a statistically significant correlation with the other two ABMs ($r < 0.6$). This preliminary study indicates a good agreement between the risk analysis performed by the ClassyFarm system and the ABMs examined. These measures could serve as good real-time proxies for the ClassyFarm risk rank, released only every 12 months by the health authorities, as it could help to implement any corrective measures required on the farm in a more timely manner.

Improving animal welfare and biosecurity on-farm is essential to enhancing animal health, reducing the need/costs for drug treatments, and minimizing antimicrobial use on farms.

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Ethical Statement: The experimental procedures were approved by the Department of Veterinary Medicine - University of Bari "Aldo Moro" (Italy) (Approval Number 20/23, Prot. 2645-III/13 of 20 June 2023).

77° CONVEGNO SISVET

Stato: INVIATO - ID: 13677

Analysis of defective dry-cured Iberian ham by Multi-Amplicon Microbiome Analysis

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The production of dry-cured Iberian ham, rooted in ancient culinary traditions, faces enduring technological hurdles that often lead to defects and financial setbacks. Processing parameters and microorganism activities are closely related to the development of quality grades of dry-cured ham, and the different grades of the products could occur even though same raw materials and processing conditions due to processing susceptibility of raw hams (1). Traditionally, detecting microbial defects in aged hams relies on the "probe and sniff technique" typically performed at several steps of the aging period. These defects, categorized as deep spoilage, present significant challenges for the ham industry.

In this study, we examined three defective dry-cured Iberian hams, exhibiting noticeable defects, alongside three normal hams sourced from the same producer in Andalusia, Spain.Our aim was to explore the microbiome of these samples to ascertain whether observed defects correlated with shifts in the microbial population.

We employed 16s Multi-Amplicon Sequencing using the Ion 16S Metagenomics Kit and Ion Torrent Sequencing Platform. Raw reads underwent analysis via QIIME2 for data processing. Subsequently, we computed and visualized alpha-diversity and beta-diversity indexes, conducted ANCOM tests, Linear Discriminant Analysis (LEfSe), and co-occurrence network analysis using the “microeco” R package. Additionally, we conducted pangenomic and functional enrichment analyses of genomes available in the NCBI database.

Thanks to the Multi-Amplicon approach, our analysis encompassed six different 16S rRNA variable regions associated with the ham samples' microbiome. Results revealed a distinct association between Tetragenococcus and the defective dry-cured ham samples. Tetragenococcus, known for its prevalence in fermented fish, vegetable products, and cheese microbiota (2), was consistently linked to the defective hams across LEfSe and ANCOM tests.Further investigation into Tetragenococcus genomes through pangenomic functional analysis highlighted metabolic disparities between T. koreensis and T. halophilus. Notably, T. koreensis exhibited a unique set of genes encoding enzymes for carbohydrate metabolism, while T. halophilus demonstrated marked genomic heterogeneity and differentially abundant amino acid metabolism functions.

This study not only addressed the producer's inquiry regarding a specific microbial population's potential role in causing defects but also pointed to the likelihood of a halotolerant microorganism as the culprit.While previous research has associated halotolerant microorganisms with defects in dry-cured meat (3), this is the first instance of Tetragenococcus being implicated in such cases. This finding sheds new light on Tetragenococcus' involvement in defected dry-cured hams. Ongoing investigations will seek to identify specific Tetragenococcus strains responsible for these defects, contributing to a deeper understanding of their impact on dry-cured ham production.

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77° CONVEGNO SISVET

Stato: INVIATO - ID: 13678

A culturomic approach to understand the behaviour of specific spoilage organisms in Atlantic mackerel (Scomber scombrus) and rainbow trout (Oncorhynchus mykiss) fillets stored in ice and at refrigeration temperatures

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Fishery products are characterized by high perishability and very short shelf-life. During storage, a rapid deterioration of their organoleptic properties occurs through different mechanisms, including autolysis, oxidation, and microbial proliferation. The portion of the total microbiota mainly responsible for alterative phenomena is represented by the so-called Specific Spoilage Organisms (SSOs) [1]. SSOs reach high loads during fish storage, becoming the largest fraction of the total microbial population. These microorganisms produce specific metabolites and cause texture, odour, and flavour alterations that make fish unacceptable for consumption. Although SSOs have been extensively studied in recent years, consistent data about the evolution of single genera and species during fish storage are still lacking.
The aim of this study was to investigate the evolution of SSOs in rainbow trout and Atlantic mackerel during storage in ice (0°C) and at refrigeration temperature (4°C) by using a culturomic approach.
Fillets of rainbow trout (Oncorhynchus mykiss; freshwater fish) were provided by a local fish farm. Atlantic mackerels (Scomber scombrus; wild saltwater fish) were purchased at the fish market of Turin and filleted at our laboratory. Fillets were stored at 0°C (in ice) and 4°C. At regular intervals, SSOs and histamine-producing bacteria (HPB) (only in mackerel) were quantified on Lyngby Iron Agar and modified Niven's Agar, respectively. At each sampling point, bacteria grown in the various media were identified by MALDI-TOF (Matrix-Assisted Laser Desorption/Ionization – Time of Flight) mass spectrometry (MS). The relative abundances (AR) of each microbial genus were calculated to understand the behaviour of different populations during storage. Within each genus, the percentage of microorganisms identified at the species level (MALDI-TOF MS scores $\geq$ 2.00) was estimated.
Results showed that storage temperatures significantly influenced SSOs proliferation. In mackerel, the critical limit reported in the literature for SSOs (6 Log CFU/g) was reached after 10 days at 0°C and 3 days at 4°C, while in rainbow trout it occurred after 6.4 (0°C) and 2 (4°C) days. MALDI-TOF MS analysis revealed that, in mackerel fillets stored at 0°C, Psychrobacter was the predominant genus at the beginning (AR=66.7%), but it was progressively replaced by Pseudomonas and Shewanella, which reached an AR of 44.6% the last day of storage. A similar trend was observed at 4°C, but changes occurred more rapidly (Shewanella reached AR of 60.4% on the third day). HPB mostly belonged to the genus Pseudomonas. In rainbow trout samples stored at 0°C, Chryseobacterium (AR=37.4%) and Acinetobacter (AR=26.4%) were initially dominant, but Pseudomonas consistently increased in abundance, exceeding the 60% of AR from the fifth day. At 4°C, nineteen genera were identified, including Pseudomonas, Acinetobacter, Battiauxella, Carnobacterium, Aeromonas, and Serratia, which persisted until the end of storage. MALDI-TOF MS often identified microorganisms at genus level and reliable species-level identifications were possible only for few genera.
The present study showed the influence of storage temperatures on SSOs growth in fish fillets, highlighting the importance of keeping values close to 0°C during their entire shelf-life. The culturomic approach could be a useful tool to identify SSOs and study their evolution during fish storage. Further studies, including different culture conditions and various fish species, are needed to completely comprehend the spoilage dynamics of fishery products during storage.

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77° CONVEGNO SISVET

Stato: INVIATO - ID: 13696

Evaluation of perfluoroalkyl substances contamination in blue crabs from the Adriatic Sea: preliminary data

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The blue crab (Callinectes sapidus) is a native species along the Atlantic coasts of the Americas, but its invasive presence in the Mediterranean area poses ecological and economic challenges. Its susceptibility to accumulate environmental contaminants, coupled with its increasing market availability, raises concerns about food safety and human health. In the European market, crab meat is offered in various forms, including live, processed, or preserved options, and utilized in several products like pâtés and crab cakes. In aquatic environments, particularly in coastal areas, the presence of poly- and perfluoroalkyl substances (PFASs) poses a significant risk. PFASs, known for their persistence and bioaccumulation, can easily enter the food chain, leading to adverse human health effects [1]. The EU Commission responded to these concerns by establishing maximum residue levels for PFASs in certain foods [2], reflecting the growing awareness of their risks. Furthermore, the recent classification of PFASs as potential carcinogens highlights the urgency of monitoring their presence in food sources [3].Despite extensive research on PFAS contamination in fish and shellfish, there's a notable gap in data regarding blue crabs. The present study aims to monitor the extent of 26 PFAS contamination in 20 blue crabs from the Adriatic Sea by a UHPLC-HRMS method, analyzing both their claws and cephalothorax. The obtained preliminary results showed the occurrence of PFOA, PFOS, PFNA, PFHxS, PFDA, PFUdA and PFDoA. Their concentrations in claws ranged from 0.11 to 0.51 ng/g, while in cephalothorax from 0.10 to 2.98 ng/g. Further research and monitoring efforts are crucial to comprehensively understand PFAS contamination in aquatic environments and food sources. By prioritizing consumer safety and environmental health, stakeholders can mitigate the risks associated with PFAS exposure and ensure the sustainability of seafood consumption.

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77° CONVEGNO SISVET

SOCIETÀ SCIENTIFICA DI RIFERIMENTO	**AIVI**
TITOLO	**Control of spoilage and pathogenic bacteria with antimicrobial compounds deriving from food industry waste and investigation of potential applications in meat products**

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Testo e Riferimenti bibliografici

One of the most common cause of food spoilage and food poisoning is the microbial contamination. Its control is achieved by the use of food preservatives but this treatment may contribute to the selection and the maintenance of resistant bacteria in the food chain. For these reasons, several efforts have been focused on the use of plant extracts as antimicrobial agents to improve safety and quality of food products. This approach could represent an innovative strategy in food preservation and development of new products. In addition, it may promote the reduction of food waste and the conversion of by-products into high-value-added products [1,2]. The goals of this study were: i) to estimate in vitro the antibacterial activity of an hazelnut pericarp extract obtained with subcritical water extraction method; ii) to investigate its potential application in meat preparations in order to improve the shelf-life of these products. Estimation of phenol content was performed by spectrophotometry (Folin-Ciocalteu assay) and proanthocyanidins through the DMAC (4-dimethylaminocinnamaldehyde) colorimetric assay. Gram positive (L. innocua, L. monocytogenes, S. aureus) and Gram negative (E. coli, S. enterica) bacteria were used to screen the antibacterial activity of hazelnut extract. The minimum extract concentration with the highest antimicrobial effect was determined by testing in triplicate each strain using the broth microdilution method described by CLSI, to which some modifications have been applied to overcome pigmentation interference with spectrophotometric measures. During in vitro tests, increasing concentrations of hazelnut extract (2.5 -5 -10 -20 mg/mL) were tested. In vivo trials were performed to analyze TVC, Enterobacteriaceae and Pseudomonas spp. of bovine meat burgers during shelf-life. Microbiological analyses were carried out in triplicate on control and treated samples prepared with 2% and 4% of hazelnut extract at 0, 3 and 6 days of storage (4°C). Cooking weight loss, spectro-colorimetric and colorimetric evaluations were conducted at each time point. Hazelnut pericarp contained a considerable amount of phenol compounds (724.99 +/- 39.59 mg/g) and proanthocyanidins (121.07 +/- 12.55 mg/g). Furthermore, during in vitro tests, it revealed a different efficacy in inhibiting microbial growth, mainly against Gram positive bacteria. S. aureus was the most susceptible bacteria responding at a concentration of 5 mg/mL, meanwhile there was no effect against Gram negative bacteria except for E. coli which was inhibited at 20 mg/mL. No significant differences in microbial counts were highlighted during in vivo tests between control and treated samples at each time point analyzed (0, 3 and 6 days of storage). After cooking, high weight loss was observed in burger meats treated with 4% of hazelnut extract. Regarding the colorimetric and spectro-colorimetric evaluations, lightness (L*) values increased as the amount of hazelnut extract decreased. In conclusion, in vitro tests showed an antibacterial activity mostly at high concentration of hazelnut extract and its addition in meat burgers during in vivo trials did not prolong the shelf-life and negatively influenced color and weight loss.

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77° CONVEGNO SISVET

Stato: INVIATO - ID: 13736

From the Slaughterhouse to the Lab: meat inspection lesions unveil the presence of Sarcocystis species in pig farming.

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Sarcocystosis is a parasitic disease caused by protozoan parasites belonging to the genus Sarcocystis. As humans can be infected by some Sarcocystis species after consumption of food containing sarcocysts, getting intestinal or extra-intestinal sarcocystosis, this taxon could represent a food safety concern. In addition to the zoonotic potential, there is increasing interest around Sarcocystis spp. in the food industry due to the possible economic losses arising from their association with macroscopic lesions detectable at slaughter and leading to carcass condemnation. Out of over 200 species, at least two Sarcocystis spp. use pigs as intermediate hosts, namely Sarcocystis miescheriana, whose definitive hosts are canids, and Sarcocystis suihominis, whose definitive hosts are humans and non-human primates; therefore, the consumption of raw or undercooked meat from infected domestic or wild swine can pose a risk for the consumer. Nevertheless, data regarding the presence of Sarcocystis spp. in pigs in Italy are scarce and limited to wild boars (Sus scrofa). Here, we report i) the detection of three generalized cases of macroscopic sarcocystosis in swine carcasses and ii) the first molecular investigation of Sarcocystis spp. in fattening pigs in Italy.
Between June 2022 and June 2023, muscle samples collected from the carcasses of three pigs were sent to the Department of Veterinary Sciences of UniTO due to the unusual detection of whitish, cyst-like gross lesions in different muscles during the post-mortem meat inspection. Samples containing visible lesions were subjected to histological examination, while a subset of the lesions was isolated from each carcass and submitted to DNA extraction and PCR targeting the cox1 mtDNA gene of Sarcocystis spp. and Taenia spp.; PCR products were sequenced to achieve species identification. Concurrently, cardiac muscle samples were collected from 201 fattening pigs raised in 16 different farms in Lombardy, submitted to DNA extraction and screened for the presence of Sarcocystis spp. by PCR. Amplification products were sequenced; samples revealing the presence of S. miescheriana DNA were further molecularly characterized amplifying and sequencing the cox1 mtDNA gene.
Muscle samples collected from the condemned pig carcasses showed well demarcated, oval lesions, up to 1 cm in length. The cysts were partially calcified and surrounded by a non-suppurative inflammatory reaction. All collected samples tested negative for the presence of Taenia spp. DNA. The partial amplification and sequencing of the cox1 mtDNA gene revealed the presence of S. miescheriana DNA in all sampled lesions. Out of 201 cardiac muscle samples of healthy fattening pigs, 31 tested positive for Sarcocystis spp. DNA through the partial amplification of the 18S rRNA gene (15.42%, CI95%: 10.73-21.17); Sanger sequencing results confirmed the identification of S. miescheriana in 7 out of 31 samples. The amplification and sequencing of the cox1 gene resulted in 1043-1044 bp sequences showing 98.8-98.9% identity with S. miescheriana GenBank entries.
Our study highlights the key role of slaughterhouses as epidemiological observatories and reports the first molecular investigation into the occurrence of Sarcocystis spp. in domestic pigs in Italy. Since the zoonotic S. suihominis was not detected, a low risk for the consumer can be predicted. Nevertheless, the possible economic losses related to carcass condemnation due to macroscopic sarcocystosis point out the need to increase the data on the prevalence of Sarcocystis spp. in domestic pigs.

77° CONVEGNO SISVET

Stato: INVIATO - ID: 13907

Automated assessment of pig lung lesions: a preliminary investigation

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The role of slaughterhouses in identifying potential threats to animal and public health is of utmost importance. Lesion assessment during post-mortem inspections provide crucial feedback on the health and welfare conditions of the herds, regardless of the assurance of food safety. Compared to on-farm evaluations, abattoir scoring is more cost-effective and provides data on larger number of animals potentially reared over a wide area [1]. Several scoring methods for lesions in pig carcasses and organs have been proposed in recent years. Bites, scars or necrosis on the tail and the skin have been described as 'iceberg indicators' of welfare problems on pig farms. Evaluation of lung lesions is also of great interest, as respiratory diseases severely impair pig health worldwide [2]. Although commonly used, operator-dependent scoring systems are difficult to implement continuously due to the increasing speed of slaughter lines and lack of human resources. Method subjectivity can also reduce data quality and comparability. Artificial Intelligence (AI)-based technologies may offer promising alternatives. Among these, Computer Vision Systems (CVSs) are suitable to perform highly repetitive visual tasks such as lesion detection at the slaughter line. Thus, their application within abattoirs would allow for standardized, objective, and systematic data collection, avoiding the handling of carcasses and organs [3]. The present study aimed to train an AI-based model to automatically detect and quantify lung lesions on digital images. This is the first step of a broader research project that will lead to the application of such a system in pig slaughterhouses.Overall, 1000 pig lungs were collected at the slaughterhouse and transported to the diagnostic laboratory for post-mortem examination. A standardized coding system to define lung anatomy and lesions was developed. The most prevalent patterns of pneumonia (bronchopneumonia, pleuropneumonia, interstitial pneumonia, embolic pneumonia) and artefacts related to slaughtering procedures (bronchoinhalation of blood) were considered. Pleuritis and pericarditis were also included. For each case, a morphological diagnosis was formulated, and two images were taken showing the entire left and right lung surfaces, respectively. A total of 2000 images were selected and labelled with annotation polygons by three trained veterinarians based on the proposed coding system using an open-source image segmentation tool. Whole lungs, lung lobes and heart silhouettes were also outlined. Accurate image labelling is essential to ensure strong model performance. Indeed, annotated pictures will be used to train CVS model to identify specific lung patterns. The preliminary results of this exploratory study will serve as starting point for developing automate systems to assess skin, tail, ear, and pluck lesion in slaughtered pigs. The information flow gathered would be extremely useful for the ClassyFarm system, optimising risk-based categorisation of Italian pig farms, at the same time returning valuable information to farmers as an expanded Food Chain Information (FCI).

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AMV

77° CONVEGNO SISVET

Stato: INVIATO - ID: 12958

Anatomical topics and tools to sensitize citizen towards the research social utility

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Here an observational study is presented with the aim to explore children's knowledge and level of awareness to three general concepts representing the project's pillars: the biodiversity of the hilly and mountain landscape, animal welfare, and the social utility of research.
The study (Approved PG University Bioethics Committee 10/20/2021) was performed through a series of educational activities and the administration of tests to assess the effectiveness of the method used; it involved 252 students of both genders, specifically 138 males and 114 females, aged between 9 and 11.
The activities were structured to provide children with a progressive understanding of topics ranging from biodiversity preservation by means of sheep grazing activity, the recognition of sheep breeds by anatomical differences, and culminating in the knowledge of anatomical parameters and marker molecules that can be used to assess the welfare status of animals.
At each meeting an interactive ppt presentation about the project topics was proposed, followed by a ludic activity to enable the children to review the concepts discussed during the theoretical part [1].
Two different types of tests referred to as "attitude questionnaire" [2] and "maximum performance test" were administered during the project.
The attitude questionnaire was administered before the start and at the end of the project to have an idea of children's sensitivity to the treated topics and to assess their awareness toward the project's pillars. The test consisted of 15 items (5 for any pillar); for each item children had to express their degree of agreement/disagreement by choosing from five response modes which were assigned a score from 1 to 5 to obtain useful data for statistical processing. The Cumulative Link Mixed Models were used to test for the effects of the various explanatory variables on the children's responses. Data were analysed in R Studio (Version 1.2.5042) using the "lme4" package. Children ID code and school were included as random effects, while the fixed effects tested were before/after the project and gender of the children. Significance was set at $p < 0.05$.
The maximum performance test aim was to evaluate if children had understood the topics explained during the meetings in the immediate term. Each test contained sentences about the specific meeting topics, respondents had to choose between "true" or "false" options. The correlation between maximum performance test score and school evaluations in Science of each child was analysed.
Among the 5 items for each pillar, 4 items related to biodiversity, 3 items related to animal welfare and 2 items related to social utility of research, showed a significant difference in the second attitude test's responses.
The analysis of data from the maximum performance test showed that the didactic methodology used was effective and improved the position of the majority of children bringing them into higher Science evaluation groups.
The children's awareness of the topics increased and showed the effectiveness of dissemination in Citizen science activities. The children expressed their thoughts and appreciation through pictures and nursery rhymes, and someone expressed their intention to care for animals, protect biodiversity and to become a scientist.

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77° CONVEGNO SISVET

Stato: INVIATO - ID: 13200

Characterization of taste and smell sense organs in the African turquoise killifish

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Taste and smell are evolutionary conserved senses in all vertebrates and contribute to the regulations of many vital functions, such as food intake, reproduction, and social interactions [1]. Although smell and taste are largely studied in zebrafish, there is a lack of information on the phenotypic characterization of those systems in the African turquoise killfish (Nothobranchius furzeri), the short-lived fish used mainly as experimental model for ageing research. In the present study, the whole heads of adult specimens were fixed in Bouin's solution, embedded in paraffin and cutted into serial transverse sections of 7 μm. For the general morphology, sections were stained with hematoxylin/eosin. Immunohistochemical stainings were performed by using the following antibodies: calbindin, transient receptor potential cation channel subfamily M member 5 (TRPM5), phospholipase C beta 2 (PLCB2), Glial fibrillary acidic protein (GFAP), tubulin, neuropeptide Y (NPY) and Ki-67, a marker of cellular proliferation. The morphological measurement of the olfactory epithelium was carried out using the methodology outlined by Hu et al. [2]. To quantify the number of taste buds, we considered the skin, mouth, pharynx, gill arches and oesophagus, where taste buds were present. We estimated the diameter of taste buds and then counted them every four sections to ensure that each one was counted only once without any being missed. Subsequently, we calculated the total number of taste buds for each region considered. Additionally, transmission electron microscopy was utilized to characterize the ultrastructure.The analysis of the olfactory epithelium indicates a lack of organized structures seen in other species like zebrafish, such as olfactory rosettes. In the turquoise killifish, folds or lamellae structures are absent. The average dimensions of the olfactory epithelium are approximately 600 μm in length, 300 μm in width, and 70 μm in thickness. In terms of taste bud distribution, the anterior system (mouth and head skin) averages 720 taste buds, while the posterior system (gills, pharynx, and oesophagus) averages 1,610 taste buds.In the olfactory epithelium, sensory cells were calbindin positive, whereas sustentacular cells were immunopositive to anti-tubulin antibody. In the taste buds, receptor cells resulted positive to Calbindin, PLCB2, TPRM5, Tubulin and NPY. Supporting and basal cells respectively showed only GFAP and Ki-67 immunoreactivity. This morphological characterization contributes to a deeper understanding of the sensory organization of taste and smell in Nothobranchius furzeri. This research could help in developing a rearing protocol for turquoise killifish, as well as understanding their food preferences.[1] Hara Olfaction and gustation in fish: an overview, Acta Physiol Scand, 152, 207-217, 1994 [2] Hu et al. Potential roles of smell and taste in the orientation behaviour of coral-reef fish larvae: insights from morphology, the Journal of Fish Biology, 95:311–323, 2018.

77° CONVEGNO SISVET

Stato: INVIATO - ID: 13216

Enhancing Tendon Regeneration: The Role of 3D PLGA Scaffolds in Modulating Ovine Amniotic Epithelial Stem Cell Paracrine Activity

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Tendon disorders present an arduous challenge for regenerative medicine, mostly due to the inherently poor healing capabilities of the tissue. Achieving successful tendon healing necessitates a harmonious integration of angiogenesis, immune modulation, and tenogenesis. Thus, an efficient tendon engineering (TE) strategy must carefully regulate the interactions among these systems to reach tissue regeneration. In this context, ovine amniotic epithelial stem cells (oAECs) represent an attractive stem cell source to accelerate tendon regeneration due to their low immunogenicity, high immunomodulatory properties, and tenogenic differentiative ability, as demonstrated through in vitro [1] and in vivo[2] studies. Moreover, these properties can be modulated and boosted when oAECs are engineered on validated tendon biomimetic PLGA 3D electrospun scaffolds with highly aligned #bers, resembling tendon macrostructure, hierarchical microarchitecture and biomechanics [3].The present research represents a step forward, delving into the paracrine effects exerted by oAECs cultured on 3D scaffolds on the target cell types involved in tendon repair: HUVECs for angiogenesis, PBMCs/Jurkat for immune response, and oAECs for tenogenic differentiation.Findings from the study highlight the significant impact of scaffolds' topography and topology on the paracrine signaling of oAECs. Specifically, taking advantage of a protein microarray analysis, it was shown that cells engineered on 3D scaffolds enhanced their basal secretion of key bioactive molecules, notably VEGF-D, b-FGF, RANTES, and PDGF-BB, among other 40 cytokines, indicating a marked increase compared to control media ($p<0.0001$). Furthermore, biological assays demonstrated the 3D scaffolds' ability to amplify the paracrine-mediated suppression of PBMCs proliferation ($p<0.001$ vs. CTR) and to mitigate LPS-induced Jurkat activation ($p<0.01$ vs. CTR) without promoting pro-angiogenic activities in HUVECs. Moreover, the paracrine teno-inductive ability of oAECs seeded on 3D scaffolds was evaluated on co-cultured ones, which formed tendon-like structures. These newly formed structures exhibited the expression of tendon-specific genes (SCX, THBS4, COL1, and TNMD) and proteins (TNMD and COL1) with respect to naïve AECs, which normally do not express these markers, underscoring the potential of this approach for tendon regeneration.Overall, this research emphasizes the crucial role of PLGA 3D scaffolds' topography and topology in influencing oAECs behavior, underscoring the strategic significance of in vitro models in predicting the interplay between engineered scaffolds and somatic/immune/blood vessels, essential for tendon regeneration. Additionally, the study suggests that the secreted molecules could serve as a valuable source of factors for potential cell-free therapy in tendon repair.

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77° CONVEGNO SISVET

Stato: INVIATO - ID: 13231

Histological study of the effects of recombinant Fsh and Lh on spermatogenesis in pre-pubertal meagre (Argyrosomus regius)

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Shortening generation time by anticipating puberty may help economic sustainability of fish aquaculture production. We recently reported the effectiveness of recombinant follicle stimulating hormone (rFsh) in stimulating spermatogenesis in pre-pubertal meagre (Argyrosomus regius) [1]. Aim of this study was to evaluate the effects of the administration of a combination of rFsh and recombinant luteinising hormone (rLh) on spermatogenesis in pre-pubertal meagre. The study was approved by IRTA's Committee of Ethics and Experimental Animal (CEEA) and the Catalan Government (authorization number FUE-2020-01809522).After a six-week treatment with rFsh injections [1], 18-months old meagre were treated with a combination of rFsh and rLh for five weeks. Control fish were administered injections of saline solution and euthanized at week 0 (CONTROL 0; N = 5), 12 (CONTROL 12; N = 5) and 21 (CONTROL 21; N = 3). Hormone-treated fish were then euthanized at week 12 (TREATED 12; N = 8) and 21 (TREATED 21; N = 6). The gonadosomatic index (GSI) was calculated as 100 x gonad mass/body mass; one-cm thick gonad slices were cut and fixed in Bouin's solution. Four-μm thick sections were stained with haematoxylin-eosin or destined to the detection of proliferating (anti-PCNA immunohistochemistry) or apoptotic (TUNEL method) germ cells. The density of anti-PCNA–positive germ cells and the surface occupied by TUNEL-positive germ cells were measured on randomly selected fields of testicular sections. Statistical differences were evaluated by ANOVA followed by Duncan's new multiple range post hoc test.No significant differences in GSI among the three control groups or between the two treated groups was observed; treated fish had higher GSI compared with the respective control group. Fish from the control groups showed: seminiferous tubules containing mainly spermatogonia and spermatocytes with no or a very small lumen (CONTROL 0); all stages of spermatogenesis, larger lumina and small amount of luminal spermatozoa (CONTROL 12); arrested spermatogenesis in seminiferous tubules devoid of spermatocysts and containing few luminal spermatozoa (CONTROL 21). Fish of the TREATED 12 group were in active spermatogenesis, showing all stages of spermatogenesis in the peripheral testis region and accumulation of large amounts of luminal spermatozoa, whereas testes of the TREATED 21 group showed arrested spermatogenesis and large amount of spermatozoa. No differences in the density of proliferating single type A spermatogonia were observed among the three control groups or between the two treated groups. The two treated groups showed significantly lower density of single type A spermatogonia compared to respective control group. The surface occupied by apoptotic germ cells was significantly higher in the CONTROL 0 group compared with the other control groups. After a decline at week 12, a significant increase of germ cell apoptosis was observed in the TREATED 21 group.The present data showed that the combined rFsh and rLh treatment was effective in stimulating testicular growth, spermatogenesis and spermiation in pre-pubertal meagre through a mechanism involving disruption of the physiological apoptotic block that prevented spermatogonia to proceed towards spermatogenesis.

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77° CONVEGNO SISVET

Stato: INVIATO - ID: 13235

Short- and mid-term fasting impacts ribosomal protein S6 phosphorylation in zebrafish brain

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Fasting triggers metabolic responses in organisms and influences several cellular pathways [1]. There is a growing number of experimental designs addressed to expose living organisms to the fasting condition and evaluate the responses at cellular levels. In the central nervous system (CNS) hypothalamic neurons respond promptly to metabolic signals by phosphorylating proteins and/or synthesizing appetite-regulating neuropeptides. A marker of neuronal response to stimuli, including fasting, is represented by pS6, which corresponds to the phosphorylation of ribosomal protein S6 [2].
The goal of our work is to evaluate the activation of central neurons induced by the short- and mid-term fasting conditions in the model organism Danio rerio, commonly known as zebrafish. Zebrafish is largely used as model in biomedicine as well as in aquaculture field [3]. This study was approved by the Italian Ministry of Health n° 291/2022-PR. Adult fishes (n=10/group – mixed sexes) were divided in three experimental groups: control, four and seven days of fasting. Control group was feed twice/day with SDS (Special Diets Service) 400, a specific aquatic diet for regular maintenance. The four- and seven-day fasting groups were completely food deprived. At the end of the experimental period, animals were euthanized, brains were sampled for western blotting and immunohistochemical analyses.
Western blot results revealed a significant increase in phosphorylation of S6 protein in animals exposed to mid-term fasting compared to the control, whereas, surprisingly, the expression decreased in brain homogenates of animals exposed to short time fasting. Immunostaining experiments confirm that the highest immunoreactivity occurred upon seven days of fasting in the zebrafish brain with a strong positivity in the neuronal cells of the dorsal telencephalic areas and in the preoptic area. Less numerous and weakly stained neurons were seen in the hypothalamic area, near the hypothalamic recess. In addition, we detected immunoreactivity to pS6 in the taste buds lining the mouth epithelium in the three experimental groups. pS6 immunopositive cells were identified as sensory cells type, by immunofluorescence stainings.
Our data demonstrate that four days of fasting does not result in neuronal activation in zebrafish, while seven days of fasting does. These results may have valuable consequences on the animal welfare, especially in particularly stressful conditions, such as transportation.

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Where do the atypical long-range somatostatin projections go in the brain? A neuroanatomical study

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The atypical long-range somatostatin projections belong to a subtype of somatostatin-expressing (SOM) inhibitory interneurons in the cerebral cortex (1,2). These long-range interneurons are characterized by axon trees that span two or more cortical and sub-cortical regions, providing fast communication between the innervated areas (3). Little is known about the circuits that extend throughout the brain starting from the anterior cingulate cortex (ACC). The aim of the present study was to characterize the SOM long-range neurons innervated areas, studying the once starting from ACC. To achieve this goal, brains from three SOM-Cre line mice in which the virus AAV5-EF1a-DIO-eYFP.WPRE.hGH was injected in ACC, were processed using immunofluorescence. We performed the rostrocaudal evaluation by processing 40µm thickness coronal sections of the whole brain and mapping the areas in which the long-range projections spread. We found different innerveted nuclei and areas. In general the interested areas were: telencephalon (in particular neocortex, olfactory system, septum, the accumbens nuclei,ventral pallidum,caudate putamen and amygdaloid complex) and diencephalon (in particular hypothalamus). Our results suggest that the SOM long-range neurons reaching these areas can be involved in the modulation of emotional responses and cognitive and homeostatic functions. Further studies are needed to confirm these hypothesis, however this anatomical study represents the first suitable starting point for future analysis.

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Exploring Spirulina's Lifelong Impact: A study on Zebrafish Fitness and Reproductive Performance

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In aquaculture, the pursuit of environmental and economic sustainability is spurring the search for alternative fish feed and supplements. Spirulina stands out as a highly promising option due to its excellent nutritional profile, providing essential amino acids, polyunsaturated fatty acids, vitamins, and minerals required for fish health. Literature indicates that the effects of spirulina on fish growth vary with the supplemented amount and are species-specific [1]. Considering this, we conducted a 32-week longitudinal study on adult zebrafish daily fed with a food/spirulina ration in the following groups:

- 1: 100% standard feed
- 2: 100% spirulina
- 3: 50% standard feed and 50% spirulina
- 4: 75% standard feed and 25% spirulina
- 5: 25% standard feed and 75% spirulina
- 6: 95% standard feed and 5% spirulina

Starting from the second week of supplementation, we evaluated the biostimulant effects on fitness and reproductive performance of all groups. At the end of the period, we conducted a morphological study on male and female gonads.

Regarding fitness, we evaluated survival, BCI (body condition index), and SGR (Specific Growth Rate). The survival analysis indicates that group 6 with group 4 show higher percentage in comparison with the other groups. Moreover, Group2 has obtained the worst result, as 0% of the fish reached the 10th week of the study. This result was predictable, as a diet solely based on spirulina is not sustainable in the long term, not providing alone an adequate amounts of full macro and micronutrients required for fish. The evaluation of the BCI has revealed that, compared to the other groups, group 6 has the highest BCI from the 10th week onwards.

To analyze reproductive fitness, we considered 1) the number of eggs produced per mating, 2) the number of fertilized eggs, and 3) the total number of hatched larvae. Only group 4 gave results comparable with the control group, differently from the other groups.

In morphological study, we identified and counted the number of pre-vitellogenic and vitellogenic eggs, with groups 1 and 4 having similar quantities of pre-vitellogenic and vitellogenic eggs, in the other groups, more post-vitellogenic eggs were detected, particularly in group 6, where 83% of the eggs were vitellogenic. In the male gonads, we counted the number of spermatogonia, spermatocytes, and spermatozoa, observing that in group 6 more spermatogonia out of the total identified cell types compared to other groups were observed, thus indicating that more precursors of germ cells are present in this group and that could represent an advantage in long lasting reproductive capabilities. In groups 4 and 5 spermatocytes were the largest number of identified cells, while in groups 1 and 3 more spermatozoa over the other cell populations were counted.

These preliminary results shed light on the potential effects of lifelong supplementation of spirulina, generally used as biostimulant and healthy improvement for a very restricted period of time [2] as feed-supplement in diet and can be translated into aquaculture studies to improve animal health and welfare.

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77° CONVEGNO SISVET

Stato: INVIATO - ID: 13291

Histological alterations in Mytilus galloprovincialis as early-warning signals under environmental changes: effects of 4-methylbenzylidenecamphor and temperature variations.

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The Mediterranean mussel Mytilus galloprovincialis serves as a crucial biomonitoring species, and its histological examination offers valuable insights into overall organism and ecosystem health. However, despite evidence of pollutant-induced toxic effects, histomorphological changes in mussels have often been overlooked [1]. In this context, the present study aimed to understand how thermal and pollutant stressors affect the status of M. galloprovincialis male gonad and digestive gland by inducing morphophysiological changes. Specifically, mature mussels were chronically exposed for 28 days to environmentally relevant concentrations of 4-methylbenzylidenecamphor (4-MBC), alone or in combination with temperature variations. At the end of the exposure period, a subset of male gonads (n=4) underwent biometric measurements to determine the gonadal index [2], while a second subset (n=4) was used to obtain a cross-section of approximately 1 cm² of their central part for histological examination and evaluation of gametogenesis stage. Analogously, the entire digestive gland (DG) (n=4) was used for the histological determination of tubular and interstitial changes integrated by a semi-quantitative weighted approach to define a DG condition index (IDG) [3]. For histological analyses, tissues were carefully excised, immersed in buffered 4% formalin for fixation, and processed for paraffin embedding and Hematoxylin and Eosin staining. Gonad observations revealed that both temperature and 4-MBC induced the spawning process. While the temperature rise also induced a prolonged resting phase, 4-MBC treatment seemed to result into an abnormal conformation of germinal follicles. Histological alterations worsened when the stressors were combined. These outcomes confirmed the role of temperature in influencing mussels' reproductive biology, while highlighting 4-MBC potential reproductive endocrine-disrupting effects. With regards to DG, results showed that both temperature and 4-MBC significantly compromised the histological integrity of tubules, as evidenced by atrophy, increased lipofuscin aggregates, and hemocyte infiltration. The combined exposure to 4-MBC and high temperature led to elevated IDG values and histological abnormalities, including necrosis. Given the pivotal role of the digestive gland in food digestion and homeostasis in marine mussels, such morphological changes suggested a rapid and non-adaptive response to the combined stressors as well as interactive effects of temperature in modulating 4-MBC toxicity.This study pointed out the use of histological analysis as a powerful and sensitive integrated approach in environmental biomonitoring to detect early-warning signals in aquatic environments. The vulnerability of M. galloprovincialis to anthropogenic disturbances demands effective conservation strategies to mitigate mussel population declines and their cascading effects on ecological communities, human welfare, and socio-economic well-being in a One Health approach.

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77° CONVEGNO SISVET

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Histological and transcriptome analysis of the ovaries of wild and hatchery-produced greater amberjack (Seriola dumerili)

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The greater amberjack, an emerging aquaculture species, undergoes severe gametogenesis impairment when reared in captivity [1]. In the present study, a comparative histological and transcriptome analysis of the ovaries of wild and hatchery-produced greater amberjack is reported. During the reproductive season 2022, wild (N = 3) and hatchery-produced (N = 7) females were sampled on board a fishing vessel and in a fish farm in Salamina (Greece), respectively. For the reproductive state assessment, ovary samples were fixed in Bouin's solution, dehydrated in ethanol, embedded in paraffin wax, sectioned and stained with basic histological methods. For transcriptome analysis, ovary samples were preserved in RNA later®, total RNA extraction was performed by RNeasy® Plus Micro kit (Qiagen, Germany), mRNA libraries were prepared by SureSelect Strand Specific RNA Library Preparation kit (Agilent Technologies, California, U.S.) and paired-end sequencing was performed on the Illumina NextSeq platform (Illumina Inc., California, U.S.). Differential gene expression analysis was carried out using DESeq2. DAVID (https://david.ncifcrf.gov/tools.jsp) and ShinyGO (http://bioinformatics.sdstate.edu/go) were used to perform the functional annotation of Differently Expressed Genes (DEGs) and the Gene Ontology enrichment analysis. A protein-protein interaction (PPI) network based on DEGs associated with each comparison was built using STRING (https://string-db.org). KEGG Search tool was used for direct mapping of genes in KEGG pathway maps (https://www.genome.jp/kegg/mapper/search.html).The ovaries of the three wild (WILD) and of four hatchery-produced fish (non-dysfunctional farmed group; NormalF) showed advanced vitellogenesis follicles, i.e. the expected stage at the time of sampling. The other three farmed individuals were affected by atresia of vitellogenic follicles, characterised by zona radiata fragmentation and yolk coalescence. These animals were classified as affected by reproductive dysfunction (dysfunctional farmed group, DysF). RNA-seq data evidenced 1,166 and 755 differentially expressed genes (DEGs) in the comparisons DysF vs WILD and DysF vs NormalF, respectively. DysF showed significant gene enrichment in the biological categories secreted, ECM-receptor interaction and focal adhesion. Proteins involved in several pathways, such as ECM-receptor interaction, Enzyme-linked receptor protein signalling and Wnt signal transduction, and in ovulation cycle were found in DysF. The KEGG analysis showed DEGs involved in 111 pathways, including Neuroactive ligand-receptor interaction, Steroid hormone biosynthesis, Cell cycle, Oocyte meiosis, Necroptosis, Ferroptosis, Apoptosis, Autophagy, Progesterone-mediated oocyte maturation, Endocytosis and Phagosome, as well as Hedgehog, Apelin, PPAR, Notch, and GnRH signalling pathways. The present study showed a widespread gene dysregulation in the ovaries of reproductively dysfunctional greater amberjack born and reared in captivity. This dysfunction involved alterations in neuroendocrine signaling pathways and several factors controlling pituitary secretion feedback mechanisms. Consequently, cell cycle and meiosis pathways were disrupted along with a decrease in gonad morphogenesis factors, resulting in ovarian follicle destructuration. Further analyses are in progress in order to characterize gene expression in the brain and pituitary of reproductively dysfunctional greater amberjack, with the aim of optimising the existing protocols for the reproduction control in a promising species for the aquaculture industry.

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In vitro Differentiation of Definitive Endoderm from Sheep Epiblast

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The gastrulation process is a pivotal event in mammalian embryo development, marking the emergence of three primary somatic germ layers: ectoderm, mesoderm, and definitive endoderm. Investigations into embryos with a typical flat embryonic disc, such as those of humans, rabbits, and ungulates, remain scarce (1). Expanding on previous findings by Simpson et al. in 2023 (2), which highlighted the critical balance between WNT and Activin/NODAL signaling for endoderm fate acquisition in pigs, our study aims to elucidate the role of these pathways in driving the in vitro differentiation of definitive endoderm (DE) from sheep epiblast cells (sEpiSCs).
Blastocysts obtained from in vitro matured and fertilized oocytes underwent immunosurgery to remove the trophoblast layers, resulting in isolated inner cell masses (ICMs). These ICMs were cultured on Matrix Laminin 511-coated wells in N2B27 medium supplemented with Activin, FGF, and a WNT inhibitor to induce epiblast formation. While 80% of ICMs adhered to the plate and proliferated in vitro, only 25% successfully established the epiblast in culture.
Upon reaching the epiblast stage, cells were cultured with a higher concentration of Activin for varying durations, and DE cells were identified through immunofluorescence, specifically targeting Sox17 and FoxA2 markers while excluding other mesoderm markers. This confirmed the direct differentiation of sEpiSCs into DE cells without a transition through a mesendodermal progenitor.
The DE layer represents a crucial initial germ cell layer in the development of vital visceral organs such as the gut tube, liver, lungs, and pancreas (3). Our study contributes to bridging gaps in gastrulation research, particularly in species with mammotypical flat embryonic discs, and provides insights into the early stages of visceral organ development, with potential future applications in regenerative medicine.

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77° CONVEGNO SISVET

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The application of marine bioactive compounds to treat skin wounds

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Skin wound healing is a dynamic process orchestrated by different cell types and biological factors that aims to restore the lost skin integrity. However, it usually leads to wound closure by scar formation, hence the partial loss of tissue structure and function. Furthermore, it might result in the development of a chronic wound, i.e., a permanent condition of inflammation causing discomfort to the patient. For this reason, wound care management has been considered a crucial point for healthcare both in Human and Veterinary medicine, making the development of innovative wound healing tools a growing field in biomedical research. The application of wound dressings allows the protection of the wound from external stimuli while sustaining healing. In addition, they can be blended with bioactive molecules to give them an added-value and generate a composite wound dressing that might further improve healing outcomes. Herein, we describe the application of a marine collagen-based wound dressing enriched with antioxidant molecules to treat skin wounds.
Collagen and antioxidant molecules, namely polyhydroxynapthoquinones (PHNQs), were extracted from sea urchin (Paracentrotus lividus) food-waste. First, the biological activity of PHNQs was assessed in vitro: they were tested for cytotoxicity on dermal fibroblasts, then cells were exposed to non-cytotoxic concentrations to evaluate their antioxidant properties in an oxidative stress environment by measuring ROS production and the mitochondrial membrane potential ($\Delta\Psi m$), along with the protein expression of antioxidants enzymes. Secondly, PHNQs were blended in a collagen-based membrane (MCDT) and tested in an experimental wound healing model. Two circular lesions (diameter = 1cm) were created on the back of 32 rats (authorization n°57/2022-PR): one wound was left untreated, and one treated with the MCDT or MCDT added with pigments (A-MCDT, n = 16 per group). Wounds were monitored daily, and at 5- and 10-days post-wounding samples were collected for histopathological and gene expression analysis.
PHNQs did not show cytotoxicity at low concentrations (1-10 μg/mL) and showed to act as scavengers by reducing ROS levels and protecting cells against oxidative stress. Additionally, they allow the maintenance of physiological $\Delta\Psi m$ levels and upregulated the expression of SOD2. Histologically, after 5 days untreated wounds showed mild inflammation; both treatments showed a similar level of inflammatory infiltration, but it was lower in A-MCDT-treated wounds. At 10 days, inflammation levels were similar among wounds. Concomitantly, the application of the wound dressing led to a higher abundant deposition of granulation tissue (GT) at day 5 compared to untreated wounds; nonetheless, at day 10 an opposite tendency was observed: treated wounds showed an improved maturation of the GT along with a more mature dermis; this observation might be linked to the up-regulation gene expression of TIMP-2, especially in A-MCDT group. Also, in A-MCDT-treated wounds a more prominent presence of neo-epithelium was observed in the healing skin compared to untreated and MCDT-treated lesions. The higher gene expression of MMP-9 might be the main reason as it has an important role in keratinocyte migration.
Overall, an improved wound healing pattern was observed in treated wounds as a better development of GT into mature dermis and higher levels of re-epithelialization were observed: both results are supported by histological and molecular observations. The addition of antioxidants led to an enhanced skin reparation as lower levels of ROS in the wound might have supported proper and faster healing.

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Breakthrough in Histo-Morphological Analysis: The Role of Artificial Intelligence

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Histo-morphological analysis traditionally relied on classical light microscope observation and histomorphometric quantifications were classically based on Regions of Interest (ROIs) sampling. The rise of digital imaging technologies has transformed this landscape. Microscope scanners able to generate high-resolution pyramidal images have become widely used, greatly increasing the information power. The integration of artificial intelligence (AI) techniques, even in the morphology field, has further accelerated this translation.

AI algorithms can be used to classify and segment tissue structures based on predefined characteristics, as well as training models to recognize patterns and associations between image features and corresponding histological labels [1]. Overall, AI has the potential to revolutionize morphological analysis enhancing the ability to simultaneously extract multiple and meaningful insights from digitalized images. This advancement enables the precise quantification, classification and stratification of samples with heightened accuracy and reliability. In the context of experimental mouse model of Bleomycin induced lung fibrosis, we developed an AI-based predictive model involving multiple morphological features and parenchyma-related alterations to assign Ashcroft score, the gold standard in lung fibrosis grading [2].The experimental design involves 10 healthy and 10 fibrotic mice lungs. After histological processing, Masson's trichrome and Sirius Red staining were performed. Lung whole slide sections were digitized using a scanning microscope and AI-based histomorphometric analyses were performed employing open-source software for pyramidal image management [3]. Image processing consists of different steps: first, each digitalized section was divided into ROIs of user-determined sizes; subsequently, the software was appropriately trained by an experienced operator to identify in each ROI parenchymal features relevant to the prediction of the Ashcroft score, including tissue component, collagen fibers and alveolar patterns in terms of shape, size and numerosity. Finally, high-specificity cell segmentation was used to identify the cellular component. The quantitative histomorphometric parameters were then combined as predictors to develop a linear regression-based model to automatically determine the Ashcroft score of the animal samples under investigation.The instruction sequences employed for histomorphometric analysis resulted functional and reliable in both tissue detection and data collection, regardless of the stainings and experimental conditions tested. To evaluate the data set's efficiency in discriminating the Ashcroft score, the analysis was performed on two different experimental conditions: healthy lungs and lungs with induced pulmonary fibrosis. Statistically significant differences were detected in tissue and air content, collagen area fraction and confluence, cell surface and density, alveolar number and geometry. Finally, we compared the reference Ashcroft score method with the linear regression-based prediction to evaluate the accuracy of the developed AI-based morphological analysis. The correlation between the two methods resulted in a R2 of 0.93, validating the proposed methods.Considering the selected morphological parenchyma-related alterations as predictors, our study demonstrates the effectiveness and reliability of AI-based image analysis tool in predicting the Ashcroft score, a reference method in the grading of pulmonary fibrosis severity. Moreover, this AI-based workflow can be applied to different histological stainings demonstrating its versatility and applicability in several experimental contexts. Successful application of AI algorithms in Ashcroft score prediction is an excellent example of exploiting advanced computational techniques to improve morphological analysis.

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Could a chitosan-based thermosensitive hydrogel be used in clinical applications for healing meniscal lesions?

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The menisci are two semilunar-shaped tissues located in the knee. In the newborn pigs, it appears completely vascularized, cells have a fibroblast-like phenotype and secrete prevalently type I collagen. But in the adult animal, it is possible to identify different areas depending on its vascularization, cell type, and matrix. There is an "outer zone" still vascularized, with fibroblast-like cells which still secret type I collagen. There is an "inner zone" with no vascularization, where cells acquired a chondrocyte-like phenotype and secret prevalently type II collagen and glycosaminoglycans. Hence, when a lesion occurs in the inner zone, repair or regenerative mechanisms are rarely activated, and meniscectomy is the gold standard. Long-term osteoarthritic phenomena occur when meniscectomy is performed, affecting both the patient's quality of life and the healthcare system [1]. Therefore, it would be a matter of great clinical relevance if meniscectomy could be avoided with new tissue engineering methods. For this reason, this study aims to evaluate the biocompatibility of a thermosensitive hydrogel that is well-suited to clinical practices and normal knee physiology. A thermosensitive injectable hydrogel was prepared by mixing a solution containing chitosan (Ch), beta-glycerol phosphate (bGP) and sodium hydrogen carbonate (SHC) [2-3]. The neonatal porcine infrapatellar fat-derived cells (IFPCs) were extracted from 6 samples of infrapatellar adipose tissue of the knee of newborn piglet that died immediately after birth, under the weight of the sow or due to natural causes. The IFPCs were expanded till passage 5 and encapsulated considering $3x10^6$ cells for 1ml of hydrogel. Live/dead assay was performed to assess cell viability at four different time points (24 hours, 4 days, 7 days, and 14 days in culture), and the *in vivo* trial on nude mice 086NU/Nu CD1 (Health's Ministry authorization: Animals (Scientific Procedures) Act C28/2022-PR) was performed to investigate the biocompatibility and safety of the hydrogel. Cellularized hydrogel and blank hydrogel were placed in a dermal pouch, and animals were postoperatively monitored for 4 and 12 weeks. Hence, histological, immunohistochemical and statistical analyses were carried out. As concerning cell viability, it significantly increased over time ($p<0,05$). Haematoxylin & eosin staining was used to evaluate the morphology of the implants, and physiological degradation were observed after 4 weeks. After 12 weeks, cells were interspersed across the matrix. Anti-PCNA immunohistochemical staining showed negativity of the sample after 4 weeks, but significant positivity after 12 weeks ($p<0,01$). The cells had good viability in the hydrogel *in vitro* and this was confirmed by *in vivo* trial after 12 weeks. Furthermore, the hydrogel did not activate the host's immune response, suggesting good healthiness and biocompatibility. Moreover, this hydrogel could represent excellent cellular support in the field of tissue engineering and regenerative medicine for meniscal lesions.

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Gut health and barrier in weanling piglets fed with a blend composed of carvacrol, tannic acid, and medium chain fatty acids; a nutritional strategy to shape the gastrointestinal tracts

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During the weaning of piglets, the intestine undergoes changes, such as morphometry and digestive enzyme production which may lead to chronic and acute inflammatory status [1]. Active compounds from natural extracts could prevent inflammation and oxidative stress demonstrating positive effects on gut morphology and function. The intestinal epithelium is a single layer of absorptive enterocytes that are bound together by junctional complexes that regulate cellular permeability and are crucial for the integrity of the epithelial barrier. Two of the most important junctional complexes consist of the Tight Junctions (TJ) and, adherens junctions, [2]. TJ includes a series of transmembrane proteins, such as Occludin and Zonulin, while adherens junctions, like E-cadherin, are located beneath the TJ and are involved in cell-cell adhesion, and all together they regulate the gut barrier.
This study aimed to assess the dietary administration of a blend composed of essential oils from natural extracts (carvacrol), tannic acid (TA), and medium chain fatty acids (MCFAs) on post-weanling piglets' growth, gut morphology, and tight junctions' expression.
A total of 210 weanling piglets were randomly assigned to two experimental treatments, with 7 replicates each. The control group (CTR) was fed the basal diet, and the treated group (T) was fed the basal diet mixed with a dosage of blend, corresponding to 1.5 kg/ton of complete feed. Growth performances were registered on days 0, 14, and 35. At day 35, 7 animals per group were slaughtered and duodenum and jejunum were sampled to evaluate the small intestine morphology as well as the expression of E-Cadherin, Zonulin 1, and Occludin. Small intestine samples were fixed in buffered formalin until further analyses, according to [3]. Even if there was no significant interaction between treatment and time, body weight and average daily gain of T piglets were positively influenced by the treatment (16.67 ± 3.13 kg *vs* 15.82 ± 2.79, $P<0.05$ and 0.25 ± 0.087 kg *vs* 0.23 ± 0.078, $P<0.01$, respectively). The increased fecal score was outlined in T compared to CTR at 6, 7, and 8 d after weaning ($P<0.05$) thus revealing a positive influence on PWD. Histometry of the duodenum and jejunum of piglets of the T group showed higher villi ($P<0.05$), deeper crypts ($P<0.01$), and increased V/C ratio ($P<0.01$), indicating a higher absorptive and surface area. Regarding the gut barrier, CTR animals exhibited a higher expression of duodenal Occludin ($P<0.05$). Jejunal E-cadherin and Occludin were more expressed in the T-animals jejunum sections ($P<0.05$). In conclusion, the administration of a blend containing carvacrol, tannic acid, and MCFAs produced a positive effect on the gut health of weaned piglets, even if, further research is needed to elucidate the synergistic effect of these blended substances when applied in commercial farm conditions.

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MYOGENIC POTENTIAL AND MUSCLE ASSESSMENT OF GILTHEAD SEABREAM (*Sparus aurata*) FOLLOWING REPLACEMENT OF SOYBEAN MEAL WITH A BLEND OF ALTERNATIVE PROTEIN MEALS

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The environmental and economic sustainability of the aquaculture industry are closely linked to the reduction of food protein content deriving from fishmeal and the use of more sustainable ingredients to produce aquafeed. Soybean-meal is commonly used in aquaculture as a substitute for the expensive and impactful fishmeal, but it has an inadequate amino acid profile and many anti-nutritional factors that, in the long run, can cause intestinal disorders in carnivorous fish [1]. In this trial, the effects of substitution of soybean meal were evaluated in gilthead seabream (*Sparus aurata*). Soybean alternative flours have been incorporated into feed formulations as follows: 5% or 10% of a mixture of insect meal (*Hermetia illucens*), water lentil (*Lemna minor*), microalgae (*Nannochloropsis gaditana*), and macroalgae (*Alaria esculenta*). The fish were reared at the Spallanzani Institute (Italy), divided into three groups, and fed three isoenergetic and isoproteic diets: the control group (CTR) with a standard commercial diet, an experimental group (L5) with 5 % inclusion of the alternative protein mentioned above, and the other experimental group (H10) with 10% inclusion. The trial lasted 5 months. The zootechnical parameters like weight and total length were used to calculate the K condition factor as described in [2].

White muscle was studied using morpho-functional analyses such as: hematoxylin-eosin (HE) to evaluate structural aspects of the muscle; succinate dehydrogenase (SDH) to highlight oxidative and low-oxidative muscle fibers/red fibers and periodic acid Shiff's assay (PAS) to reveal glycogen content in muscle fibers with glycolytic metabolism/white fibers. The HE stained sections were also used to perform histometrical analysis to evaluate the presence of hyperplastic and hypertrophic fibers as already perfomed by [3]. Moreover, a whole-mount muscle immunofluorescence was performed to assess the expression of actin (Alpha-actin). Finally, molecular analyses were carried out to monitor the expression of genes involved in the regulation of myogenesis and in the specification and maintenance of satellite cells.

The zootechnical parameters did not highlight significant differences between the experimental groups and the same was observed for the histometric analyses of the muscle, where no differences were seen in the area and number of the muscle fibers. Moreover, the SDH-negative and PAS-positive stainings confirmed that the muscle fibers have a glycolytic metabolism type (white fibers). Molecular analyses highlighted that MyoD1 expression, a key master regulator of skeletal-muscle differentiation, was significantly higher in the H10 group compared to CTR. It also showed a trend towards a significance in H10 compared to L5 (H10 vs CTR, $p<0.05$; H10 vs L5, $p=0.0736$). These data indicate that replacing soybean-meal with a blend of alternative protein meals had no negative effect on growth performance and muscle characteristics and development. Results suggest that the experimental diets H10 could be an alternative to soybean-meal, thus contributing to the aquaculture sustainability.

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"Exploring the effects of aging on olfactory and gustatory senses in *Nothobranchius furzeri and Mus musculus*."

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Taste and smell allow vertebrates to detect and distinguish chemicals in their surroundings, such as food, predators or sexual partners. Sensory perception changes over the course of aging. Animals can perceive different scents and flavors, which may indicate the presence of nutrients or toxic substances.
The aim of our research is to broaden knowledge on the interplay of chemical senses in the metabolic control via nutrient-sensing mechanism in vertebrate aging. We will try to achieve this objective through *in vivo* (behavioural tests) and *ex vivo* studies in two different species: *Mus musculus* outbred strain CD1 and *Nothobranchius furzeri* strain MZM0410 (Autoriz. n° 291/2022-PR + 1177/2020-PR). In particular, the study aims to i) analyze the differences in olfactory and gustatory responses *in vivo* among young, adult and elderly individuals; ii) investigate the morphological and molecular changes in smell and taste associated with aging; iii) examine the possible correlations between olfactory and taste abilities over aging. We considered three age points, corresponding to 1) young animals at sexual maturity, 2) adults in full reproductive activity, 3) reproductive and phenotypic senescence.
For behavioural analyses, we tested CD1 mice in the habituation/dishabituation test (smell) and the preference 2 bottle test (taste). These tests were adapted for aquatic environment [1], in which the fishes were subjected to a preference test for 2 different types of food.
From the two species, olfactory epithelium (OE) and taste buds (TBs) were collected as target tissues for *ex vivo* analyses.
CD1 mice at all ages showed habituation to the odors presented and were able to discriminate a new odor, geraniol or citralva, presented to them after the last trial of mineral oil or geraniol, respectively (dishabituation). However, we observed an age-dependent reduction in the total time spent sniffing. In the taste preference test, CD1 mice at all ages preferred saccharin and umami over water and avoided citric acid and quinine solutions, while NaCl solution was equally preferred as water. With aging we also observed an increase of total (water+taste solution) fluid intake and a slight change of preference.
In fish, *Chironomus spp.* is confirmed as the preferred food choice at all ages compared to dry food, although slight differences in food nibbling were observed over the three age stages. Old animals swam less and only for feeding.
Histological analyses revealed differences in the morphology and distribution of olfactory and gustatory receptors [2], suggesting evolutionary adaptations to optimize sensory perception and food assimilation [3].
This study provides important insights into the relationship between smell, taste, feeding behavior, and sensory perception in both models, highlighting the importance of integrated approaches to understand the complexity of sensory and behavioral systems in phylogenetically distant species.

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77° CONVEGNO SISVET

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Methodology development for use of 3D scanners in education and anatomical research

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In recent years there has been an increasing demand for digitisation of veterinary content, especially in the field of anatomy (1). This is mainly due to ethical and biosafety concerns, as well as the lack of usable cadavers and the inability to replicate the preparations obtained from them. In addition, the past health emergency resulting from COVID-19 has highlighted the lack of usable digital content to support distance learning (2-3). The aim of this study was to create a virtual resource that would be useful for both students and teachers, to enhance learning and teaching. Moreover, the file format should be compatible with free software and feasible for online sharing.This research focuses on the fetlock, pastern, and hoof regions of the horse, which are crucial areas that have been extensively investigated. The model should accurately depict the relationships between the regions, their stratigraphy and their anatomical components, such as muscles, joints, bones, vessels and nerves.The equine proximal interphalangeal joint received particular attention, including an evaluation of the potential benefits of 3D scanning for assessing new surgical approaches and improving existing techniques through digital analysis. This will aid future biomechanical studies to provide surgeons with anatomical information as well as preparatory or auxiliary information to simulate and perform orthopaedic surgical techniques.

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Nucleoside diphosphate kinases 1 and 2 regulate a protective liver response to a high-fat diet

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Deregulated De novo Lipogenesis (DNL), especially in the liver, is implicated in diverse pathologies. Increased rates of DNL are associated with nonalcoholic fatty liver disease (1, 2), insulin resistance and type 2 diabetes (3, 4), cardiovascular disease (5), incident heart failure (6), and cancer (7). We reported in vitro and structural data showing that NME1/2 binds cytoplasmic Acetyl-CoA, the main carbon source for DNL. In this work, we investigated the implication of NME ½ in liver of mice fed a high-fat diet focusing on a mouse knockout (KO) model with drastically reduced NME1/2 levels. Histological section analyses show, Nme2 knockout mice fed a high-fat diet (HFD) exhibit liver steatosis with excessive triglyceride synthesis. Here, we report that fatty acid accumulation is negatively regulated by nucleoside diphosphate kinases 1 and 2 (NME1/2), housekeeping enzymes involved in nucleotide homeostasis, hence potentially sensitive to the cellular energy status. At the same time, NME1/2 mediate an increased targeted histone H3K9 acetylation, activating a gene signature known to protect liver cells during regeneration. These observations identify NME1/2 among a select group of metabolic enzymes with a crucial function in and protection from liver steatosis. In conclusion, the work reported here highlights the function of NME1/2 as a major regulator responsible for a liver protective gene expression program. In agreement with this finding, a previous study investigating genetic factors controlling liver injury susceptibility identified NME1/2 as important determinants in protecting the liver against an injury-inducing treatment (8). Therefore, NME1/2 may be a general regulator of a protective liver response, as well as a general regulator of DNL in various pathophysiological contexts.

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Mediterranean Sheep Breeds: histological investigation of the merinization grade in the Gentile di Puglia sheep

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Wool fibre is the most important product of the sheep economy for the textile industry and for a wide range of consumer goods. Furthermore, the sheep can be considered an important animal model for studying hair follicle biology. This complex structure is characterised by excellent flexibility, moisture absorption, fire resistance, warmth, biodegradability, recyclability, breathability, resilience softness, noise absorption (1). The quality and commercial value of sheep's wool is determined macroscopically by fibre diameter, staple characteristics, comfort factor, spinning fineness, fibre curvature and clean fleece yield (2). Delving into the histological structure of the wool fibre through research aids in comprehending the efficiency of wool growth and enhances the assessment of product quality (3).

Fine wool is soft, curly and white and characterizes Merinos Sheep, which are considered one of the oldest and most economically influential breeds in the world (3), and the top three producers are China, Australia and New Zealand. Differently, coarse wool is thicker and characterizes non-Merinos sheep, such as the Sarda and many others. There are many breeds that have not been classified because of lack of studies or because of their own mixed characteristics.

The Gentile di Puglia is a sheep widespread mainly in Apulia and Basilicata regions, which produces fine wool of the highest quality but is not considered Merinos. For this reason, we decided to conduct an extensive morphological analysis of the Gentile di Puglia breed, comparing it in particular with the non-Merinos Sarda breed, and evaluating histological differences between adult animal and lamb.

From the evaluations of the number, size, distribution, and type of follicles, we can show that the Gentile di Puglia presents a merinized but not completely merino wool; also, it is interesting to note that lambs present for some characteristics intermediate values, indicating a progression to merinization during growth. Gland analysis shows a high presence of sebaceous glands in the Gentile di Puglia breed, and this correlates with wool quality and could also be associated with lanolin production.

Our histological work demonstrates how the Gentile di Puglia breed is partially merinized and, consequently corroborates its economic potential for use in the textile industry.

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ZEBRAFISH SPINAL CORD INJURY: A MODEL FOR SCREENING NATURAL COMPOUNDS ENHANCING RECOVERY

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Spinal cord injury (SCI) is a permanent and chronic condition that results in a wide range of sensorimotor and autonomic nerve impairments. Activation of innate immune responses following injury plays a critical role in the pathogenesis of spinal cord injury, leading to an inflammatory reaction that causes further immune cell-mediated apoptosis [1]. Most molecular natural and chemical therapies for SCI aim to promote axonal regrowth and protect neurons from secondary cell death through anti-inflammatory and antioxidant actions. In recent years, zebrafish have been consolidated as a SCI model to better understand regeneration mechanisms and test molecules that can accelerate regeneration and improve recovery [2].

The present study, therefore, aims to test the neuroprotective and neuroregenerative activity of a flavonoid-rich lemon peel extract and β-caryophyllene (BCP), on an established zebrafish model of spinal cord injury. First of all, the LD_{50} for both substances was determined for zebrafish embryos. The experiment was conducted at two times using the transgenic line (huc:gfp) or the wild-type zebrafish embryos. At each trial the embryos have been divided into two batches. One of them was exposed to the BCP or to the lemon peel extract while the second was a sham. The exposure was extended for all the experiment period (until 120hpf). To assess the regenerative effect of both natural substances, the spinal cord injury was carried out at 48 and 72 hours post fertilization on BCP and lemon peel extract respectively. By the end of the experiment the behavioral test was also performed using the Danio Vision system, to assess functional recovery. The treated groups showed a higher survival rate, a reestablished motility and more glial bridge formation compared to sham groups. Two days after injury, the cell proliferation rate was assessed using EdU (5-ethynyl-2′-deoxyuridine) and immunofluorescent specific marker of regeneration. The fluorescence quantification showed an increased cell proliferation around the injury site, reflecting a higher healing and regeneration capacity compared to sham groups. These findings highlight the potential role of natural compounds as adjuvants in the recovery of spinal cord injuries and similar conditions due to their remarkable anti-inflammatory properties, although further research will better explain their mechanisms and potential applications in human treatments.

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ROLE OF ION CHANNELS IN THE GENITAL TRACT OF PREPUBERTAL BITCH

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Extensive studies have revealed a variety of ion channels in eukaryotic cells that can sense various forms of mechanical forces. These ion channels include transient receptor potential (TRP) channels, voltage-gated Na+, K+ and Ca2+ channels (e.g., DEG/ENaC/ASIC), and a family of mechanically activated (MA) cation channels Piezo1 and Piezo2. Furthermore, most of these ion channels are activated not only by mechanical stimuli but also by chemicals, temperature and osmolarity. Several members of these families have been described in gonads and adjacent tissues of different species [1]. This would suggest that such ion channels may be involved in spermatogenesis, oogenesis, and/or early embryonic development [2]. The focus of the current study was to assess, for the first time, the expression of ASIC2, ASIC4 and PIEZO2 in the genital tract of prepubertal bitches. Ovary, uterine tubes and uterus were taken from prepubertal bitches of various breeds and ages, during sterilization at the OVUD (Ospedale Veterinario Universitario Didattico, UNIME). The samples were processed for western blot and immunohistochemistry. Our studies demonstrated the immunolocalization and expression of ASIC2, ASIC4 and PIEZO2 in all the genital tracts examined. In particular, immunoreactivity was observed in the ovarian follicles, in the oocytes of primordial and primary follicles, in the vessels and in the uterine tubes and uterus. These findings show that such ion channels, in prepubertal age, could be involved in the primordial ovarian follicles development regulation and in follicular migration.

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77° CONVEGNO SISVET

Stato: INVIATO - ID: 13489

Morphological and molecular features of the fimbrial epithelium of the baboon Papio hamadryas oviduct during the menstrual cycle.

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The oviductal fimbria is proximal to the ovary and picks up the ovulated oocytes in the ostium of the infundibulum [1] and could be involved in several pathological conditions including tumors [2]. Although several reports have been published on the cyclic changes of the fimbrial epithelium of some non-human primate oviduct, a sole study reports the effect of the menstrual cycle on the oviductal fimbriae of the baboon Papio hamadryas [3]. This study aimed to investigate more in-depth the fimbrial epithelium of Papio hamadryas oviduct during the menstrual cycle. The fimbriae obtained by laparoscopic salpingectomized adult and healthy Papio hamadryas females bred in the Zoo Safari (Fasano, Italy) were used. The tissues were processed for the study of 1) the morphology, using histological and scanning electron microscopy (SEM) approaches, 2) the glycopattern through conventional (PAS, HID/Alcian Blue) and lectin (AAL, GNL, LCA, RCA120) histochemistry, 3) the immunolocalization of the chaperone heat shock protein 70 (HSP70), estrogen (ERα) and progesterone (PR) receptors. The histomorphological analysis demonstrated that the fimbrial epithelium reached the maximum height and differentiation status during the preovulatory phase when the ciliated and non-ciliated cells were well distinguishable. SEM observations revealed that non-ciliated cells of the preovulatory phase contained small apical protrusions covered by thin microvilli and that ciliated cells were unevenly distributed along the fimbrial epithelium. The glycohistochemistry displayed the presence of glycopeptides mainly in the apical surface of the epithelium, which displayed 1) a low presence of non-sulfated acidic (Alcian Blue positivity) and complex (Man/GlcNAc core with α-1,6Fuc) Nglycans (LCA reattivity) during the follicular phase, 2) the appearance of sulfated (HID positivity), fucosylated (AAL affinity), terminal βGalalactose-terminating glycans (RCA120 labeling), as well as increase of complex mannosilated Nglycopepites (GNL and LCA affinity) during the preovulatory phase, 3) low presence of AAL, LCA, and RCA120 binders during the luteal phase. Immunohistochemistry evidenced the presence of HSP70 1) in the cytoplasm and nucleus of all epithelial cells during the follicular phase, 2) mainly in the cilia and the cytoplasm of the ciliated cells during the preovulatory phase, 3) in the cytoplasm of some epithelial cells during the luteal phase. ERα and PR were constitutively expressed in the fimbriae during the menstrual cycle and were localised to the cell nuclei of the epithelium. The percentage of ER positive cells was higher in the follicular phase than the preovulatory and luteal phases. ERα staining intensity did not change during the menstrual cycle. PR-immunostaining was detected in the nuclei of all epithelial cells and the immunostaining intensity was low in the preovulatory phase and increased from the luteal to the follicular phases. Overall, this study adds further information on morphological and chemical changes occurring in the fimbrial epithelium of the baboon Papio hamadryas oviduct during the menstrual cycle.

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77° CONVEGNO SISVET

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Unraveling lung epithelial cell dynamics through integrated morphological and omics analysis

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Lung epithelial cells play a pivotal role in maintaining respiratory homeostasis and defending against environmental insults. Understanding processes underlying lung regeneration after injury is crucial to elucidate disease mechanisms and develop effective therapeutic strategies. This study investigates the role of murine lung epithelial cells under normal condition and after BLM-induced injury, integrating high-throughput technologies such as multiplex immunofluorescence (mIF) (1) and transcriptomics. Combining these technologies allows for molecular and spatial profiling of the lung epithelial microenvironment, which is often missed using tissue bulk methods. Epithelial antibodies panels (Panck, Hopx, Tubulin, Nkx2-1, Pdpn, Foxj1, Abca3, Aqp5, Epcam, Scgb1a1) were used for sequential reactions. Whole slide images (WSI) were digitally acquired for each round using a slide scanner (VS200, Evident). Following image acquisition, single-cell analysis was performed using dimensionality reduction algorithms to obtain different phenoclusters, which were identified and compared in normal and BLM-treated mice at different time points (7, 14, 21 and 28 days). Coupled with mIF data, the Nanostring GeoMX Digital SPAtial Profiler (DSP) platform was utilized to measure the expression of compartment-specific proteins (2). Through hybridization with oligonucleotide barcodes and subsequent imaging, spatially resolved gene expression data were obtained, capturing the intricate molecular heterogeneity within the tissue microenvironment. The GeoMx DSP allows for the detection of specific regions of interest (ROI), through spatially profiled digital mask based on pan-cytokeratin signal, thus detecting epithelial cell populations within the lung. After data acquisition, advanced computational analyses were performed to quantify gene expression levels within defined ROI. Analysis of single cells from BLM-treated groups revealed qualitative and quantitative changes in epithelial cell populations compared to controls. Notably, a transient decrease in alveolar pneumocytes type 1 (AT1), peaking at 14 days, and a progressive decline in alveolar pneumocytes type 2 (AT2) were observed, supported by downregulated gene expression. Specifically, genes encoding markers for AT1 (Pdpn, Aqp5) and AT2 (Sp-C, Nkx2-1) exhibited reduced expression post-injury. Additionally, a transitional cell phenotype expressing markers for both AT1 and AT2 (Hopx, Tubulin, Pdpn, Nkx2-1) emerged. Their presence is further demonstrated by the reduced expression of Tfcp2l1, 14 days after injury, a key gene for AT2-AT1 cell differentiation. A slight increase in Club cell subpopulation and related gene (Scgb1a1) was also noted across all time points compared to controls. In the context of BLM-induced lung injury, cells undergo dynamic alterations and function pivotal for tissue repair and remodeling, demonstrating their central role in alveolar regeneration and homeostasis (3). Thus, it becomes essential to assess the modulation of molecular factors, such as of Tfcp2l1, which plays a role in promoting AT2-AT1 differentiation in a spatiotemporally specific manner after acute injury and Sp-c marker while that resulted decreased influencing the AT2 maintenance. miF technology responds to the growing need to study the spatial organization of molecular targets, the relationship between multiple cell types and morphology. Integrating immunofluorescence and transcriptomic data provides a comprehensive understanding of cellular changes post-injury, aiding in identifying therapeutic targets and elucidating repair mechanisms.

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Neurogenic Marker Expression in Ovine Achilles Tendon Regeneration: The Modulatory Role of Amniotic Epithelial Cells

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Emerging evidence shows that tendon innervation participates actively in tissue healing involving nerve growth factor (NGF), nerve ingrowth (neurofilament-200-NF200) and the expression of various neuromodulators from both autonomic (neuropeptide Y-NPY) and sensory innervation (galanin-GAL, calcitonin-gene-related peptide-CGRP). These molecules may play a significant role in controlling tissue homeostasis by modulating inflammation, pain, and cell proliferation [1]. Furthermore, an active role in tissue regeneration is exerted by ovine amniotic epithelial cells (oAECs) [2]. Thus, oAECs pro-regenerative influence may extend to the expression of neurogenic markers.

Thus, this study aims to investigate in an ovine Achilles tendon injury model the differential expression of the neurogenic markers involved in tissue recovery, comparing spontaneous healing (CTR) with oAECs allotransplantation-induced regeneration.

Twenty sheep were randomly assigned to two experimental groups: control (CTR) and oAEC-treated, with assessments at 14- and 28-days post-injury (Ministry of Health approval ID 1205/2015-PR.18.11.2015). Under general anesthesia, a unilateral injury was induced in the Achilles tendon. In the CTR group, surgical lesions were sealed with fibrin glue, while in the oAEC-treated group, lesions received oAECs transplants sealed with fibrin glue. Immunohistochemistry (IHC) was employed to evaluate the expression of neuromarkers. Fluorescence intensity was quantified for statistics.

An increased expression of the analyzed markers was observed in all experimental groups compared to healthy tendons ($p<0.0001$). Moreover, it was verified that nerve endings and NGF expression were influenced by oAECs transplantation. At 14 days only CTR tendons showed significant nerve ingrowth, while NF-200 expression always remained lower in oAEC-treated groups at all time points ($p<0.05$) suggesting oAEC-mediated regulation of nerve ingrowth during the early stages of tendon healing. Furthermore, while in CTR NGF expression increased over time (14 vs. 28 days, $p<0.05$), it remained constantly expressed at high levels in oAEC-treated group, particularly increasing at day 14 with respect to CTR ($p<0.05$), confirming its pro-regenerative role.

Neuromodulators were modified in oAECs-treated groups, whereas in CTR these were always expressed at similar levels independently of the time points considered. NPY significantly increased in oAEC-treated groups over time (14 vs. 28 days, $p<0.01$). Interestingly, 28 days post-injury, oAEC-treated groups tendons showed also remarkable extracellular matrix remodeling, characterized by a favorable COLI/COLIII ratio; indeed NPY could influence collagen production and deposition due to its vasoconstrictive role[1,3]. Both CGRP and GAL levels in the oAEC-treated groups significantly decreased over time (14 vs. 28 days $p<0.0001$), and at 14 days were expressed at higher levels compared to CTR ($p<0.05$). CGRP and GAL play roles in inflammation and pain regulation, respectively. The inflections observed in treated groups suggest that oAECs modulate inflammation and accelerate tendon regeneration by influencing both neuromodulators.

In conclusion, this research investigates the possible regenerative mechanism stimulated by oAECs in modulating neurogenic markers, highlighting their role in fostering regeneration, finely regulating tendon innervation and autonomic and sensory neuromodulators.

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77° CONVEGNO SISVET

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GUT MICROBIOTA ANALYSIS IN NOTHOBRANCHIUS FURZERI MODEL OF DIET-INDUCED OBESITY

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Obesity, a chronic metabolic disorder caused by an energy imbalance, is a major global health problem. In recent years, increasing evidence linking obesity to the gut microbiota has been reported. The gut microbiota, which is composed mainly of anaerobic bacteria, facultative anaerobic bacteria and aerobic bacteria residing in the gastrointestinal tract, is a dynamic ecosystem that coevolves with its host. As an important and potential "organ," gut microbiota on one side is known playing a notable role in regulating intestinal epithelial cell differentiation, proliferation, metabolic function and immune response, angiogenesis and host growth while on the other affecting systemic inflammation, immune response, energy harvest and also the gut–host interface. New animal models are required for prospective evaluation of causative factors in obesity initiation or progression linked to gut microbiota [1-2]. In the last years, the African turquoise killifish Nothobranchius furzeri has emerged as an important model system for the study of vertebrate biology. N. furzeri is an annual fish that inhabits seasonal freshwater ponds in the southeast of Africa and is characterized by rapid growth and early sexual maturation. Importantly, despite its short lifespan, in fact it is currently considered the shortest-lived vertebrate that can be bred in captivity, N. furzeri recapitulates typical age-dependent hallmarks that make it a suitable model for aging research [3]. The goal of our work is to characterize a N. furzeri model of diet-induced obesity (DIO). The research was approved with protocol 77/2023-PR by the Ministry of Health. The objective is to typify gut microbiota in old obese animal analyzing the hypervariable region V3-V4 16S rRNA by 16S miseq Illumina. To this aim, fishes belonging to MZM 04/10 strain, both male and female, at 6 weeks of age were divided into two different group feed with Chironomus spp. as follow: a) 300mg/die (ctrl) and b) 780mg/die (obese). At 27 weeks post hatching, fishes were suppressed to perform microbiota profile of single gut for each group included breeding water samples. Our results show that females exhibit greater biodiversity in microbial populations than males while in obese animals of both sexes biodiversity decreases when compared with their respective controls. In control females, Plesiomonas prevails while Areomonas takes over in the obese. There are no significant differences in abundance rates between control and obese males. Next experiments will be carried out to understand how microbiota regulates the functions and inflammatory status of the gut: to this aim, we will perform real-time qPCR and immunohistochemistry of some markers of inflammation and food-intake peptides. Our study represents a pilot step for understanding the appetite regulation during vertebrate ageing process.

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77° CONVEGNO SISVET

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Ex vivo differentiation/maturation of hippocampal neurons and neural stem cell-derived oligodendrocyte precursor cells is altered by a short-chain PFAS pre-natal exposure

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PFASs (per-/poly-fluoroalkyl substances) are a class of emerging persistent organic pollutants characterized by a chemical structure based on fully/partially fluorinated carbon chain, high thermal/chemical/biochemical stability, and hydrophobic/oleophobic properties. Despite the wide use in a variety of products and applications, health risks of the dietary exposure are unknown, as well as the potential molecular/cellular targets.
Epidemiological and experimental data suggest exposure of human fetuses to a mixture of PFASs throughout gestation and association with different pathologies. In this view, the interference with the thyroid hormone action and central nervous system (CNS) development is of particular interest. To fill this gap of knowledge, the effect of pre-natal/neonatal exposure of two PFASs (GenX, PFBA) on neurodevelopment has been investigated in rats, demonstrating a clear deficit on hippocampal-dependent neurological features and integrated cortical-hippocampal signals. Thus, the present ex vivo study has been designed to deeply investigate the underlying cellular and molecular mechanisms.

Female animals were exposed to PFBA (5 mg/kg/p.c./day) or vehicle, for a period covering one month before the mating and the 21 days of gestation until birth. Newborn animals were euthanized, and brains dissected to set up specific cultures: neurons/astrocytes i) cortical and ii) hippocampal cultures, iii) sub-ventricular zone-derived neural stem cells (NSCs). Cultures were analyzed at specific time points using immunocytochemistry coupled with High Content Screening (HCS) technology, allowing the automated acquisition and analysis of the whole culture (> 5'000 cells/well), avoiding the operator-dependent bias on choosing the representative field to analyze, through morphology-based high-informative outcome. NSCs were cultured using neurosphere protocol, and cells obtained from primary spheres were seeded as monolayer and exposed to vehicle, retinoic acid or thyroid hormone (T3) to induce spontaneous, neuronal or oligodendroglial differentiation, respectively. Specific markers were used to identify neural precursors (nestin), astrocytes (GFAP), neurons (beta-III-tubulin, MAP2), oligodendrocyte precursors (OPCs; NG2) and mature oligodendrocytes (MBP). Moreover, synaptic markers (PSD95, VGLU1, VGAT) were included to quantify neuronal maturation.

In short-term hippocampal cultures (7 DIV) we described an increase in beta-III-tubulin neurite net complexity (neurite total count, average length, ramification index), and a decrease in MAP2-positive neurites followed, at 21 DIV, by a decreased percentage of cells positive for synaptic markers, measured by confocal microscope imaging.
The NSC-derived cultures spontaneously differentiated resulted enriched in OPCs, but non-responsive to T3 action. Moreover, using the IMARIS 3D analysis on confocal images, we described a PFBA-dependent maturation block of MBP-positive oligodendrocytes (Sholl analysis).

The in vivo PFAS treatment mimics differentiation induction mechanisms, preserved in the ex vivo cultures. However, the same cells are then unable to properly maturate, leading to a strong impairment in the differentiation/maturation yield. This experiment suggests the impact of the PFAS pollution on the development of the CNS, highlighting also the fundamental role of the ex vivo approach, coupled with advanced microscopy/HCS technologies, to investigate the cellular/molecular mechanisms.

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F. Mutinelli and L. Calzà contributed equally to this work.

77° CONVEGNO SISVET

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CHALLENGES IN LIGHT SHEET IMAGING FOR QUANTITATIVE ANALYSYS OF WHOLE BRAIN VASCULAR NET

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Neuronal function relies on a complex network of blood vessels that deliver oxygen and nutrients essential for metabolism. However, our understanding of the intricate capillary network allowing substance and metabolite exchange remains incomplete. While structural cerebral vasculature and molecular analyses hold promise for offering crucial insights into cerebral circulation and cerebrovascular diseases, technical limitations make difficult to obtain detailed 3D structural information on vascular networks, from large vessels to capillaries. Mapping the anatomy of capillaries throughout the entire mouse brain presents challenges due to the need for micrometer-scale resolution and rapid acquisition rates to cover the sample volume efficiently. Light sheet microscopy (LSM) coupled with tissue clarification, very high calculation power and computer vision algorithms, has emerged as a powerful tool in neuroscience research, offering unique advantages for imaging biological large samples at cellular resolution. A thin sheet of laser light illuminates a cleared specimen, reducing photodamage and enabling long-term, three-dimensional (3D) imaging of live tissues with minimal phototoxicity. Fluorescence signals are generated by chemical fluorophores, viral vector-GFP-tagged genes or antibody staining. Utilizing LSFM for vascular network imaging yields vast datasets of 1–2 TB per brain, underscoring the necessity for automated analysis driven by machine learning and artificial intelligence algorithms to extract valuable morphological insights and offer quantitative analysis. Amidst the vast potential, the pivotal challenge lies in achieving quantitative imaging at single-cell resolution.To tackle this challenge, we addressed two methodological hurdles in our laboratory: optimizing antibody-stained section imaging employing non-toxic clearing protocols, and validate quantitative measure using different approaches to visualize the vascular bed also in 2D confocal microscopy. Mice were euthanized and perfused with phosphate-buffered saline (PBS) followed by dextran-FITC conjugate dissolved in porcine skin gelatin or processed with anti-CD31 VioB515 immunostaining. Brains were carefully dissected and post-fixed in the fixative solution at 4°C. The high molecular weight of dextran prevents the marker leaking from blood vessel walls. Then, the fixed tissues underwent dehydration through a series of increasing ethanol concentrations and then were cleared using the clearing protocol of Miltenyi MACS® Clearing Kit. Miltenyi UltraMicroscope Blaze™ was used for imaging. We acquire images from the entire hemibrain at 4 and 12x mag using three different wavelengths (561nm for autofluorescence, 488nm for FITC-dextran, 640nm for VioB515). Three-dimensional sampling was performed from lateral to medial sides (442 × 442 × 500 μm at 12x of magnification). Miltenyi stitcher software was used for large areas sampling. Three-dimensional, voxel-based images were analyzed using IMARIS software (v. 9.6.2; Oxford Instruments), by the "surface" algorithms to construct the three-dimensional vascular network.Experiments revealed the capability to label and detect until 3–5μm diameter microvessels, to quantify capillary density, branching, and total occupied volume. We also evaluated inter-subject variability attributable to the clearing and staining methodology by possible decrease in fluorescence signal intensity, also comparing intravascular labelling by FITC-dextran and endothelial cells visualization was by CD31. While LSM offers great advantages in imaging large volumes of tissue with high resolution and minimal photobleaching, standardized and optimized protocols for clearing, imaging, and post-processing analysis are a crucial step toward the use LSM for image quantification.

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Imaging Hydrogen Peroxide in bovine oocytes and early embryos using time lapse microscopy: challenges and opportunities

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Reactive Oxygen Species (ROS), particularly hydrogen peroxide (H_2O_2), are recognized for their role in inducing oxidative damage in oocytes and early embryos, especially when oocyte maturation, fertilization, and early embryogenesis are recapitulated in vitro. However, H_2O_2 is starting to be recognized as an essential signaling molecule in biological processes, including early development in model organisms [1] . However, the dynamics of H_2O_2 fluctuation and its role in mammalian oocytes and embryos remain largely unexplored owing to the absence of imaging techniques to monitor H_2O_2 fluctuation in living oocytes and embryos and the challenge of experimentally suppressing its production during culture. This study aims to bridge this gap by taking advantage of a novel sensor named hyper 7, an ultrasensitive genetically encoded fluorescent ratiometric sensor capable of monitoring H_2O_2 fluctuations in real-time and in different cellular sub-compartments, surpassing traditional detection methods. In our experiments, mRNAs encoding for the mitochondrial and the cytoplasmic-targeted Hyper7 sensors were obtained by in vitro transcription of the respective plasmids and microinjected into immature oocytes or in vitro matured ones. The microinjected oocytes were then subjected to either in vitro maturation or fertilization and analysed under control culture conditions or in the presence of 100 mM tert-Butyl hydroperoxide (t-BOOH), which served as a prooxidant challenge to validate the probe ability to detect increasing levels of H_2O_2 in real-time. Imaging was conducted at the NOLIMITS microscopy facility of the University of Milan, using a Nikon ECLIPSE Ti2-E microscope with a Yokogawa W1-SoRa spinning disk, 405 and 488 nm lasers, and a multi-band filter. A high-resolution camera and a temperature-controlled CO_2 chamber enabled time-lapse imaging. Exposures were set at 3 and 2 seconds for 405 and 488 nm, respectively, capturing emissions at 516 nm. Image analysis was performed using NIS ELEMENTS AR software. Both sensors demonstrated their ability to detect H_2O_2 levels within oocytes and zygotes, revealing distinct fluorescence patterns. However, under control conditions, a significant increase in the sensor signal was observed after around 20 minutes of imaging in both oocytes and zygotes. This suggests phototoxicity as an exogenous causal factor during prolonged imaging. Nevertheless, both oocytes and zygotes treated with tBOOH exhibited significantly higher values than those in the control group, confirming the sensors' capacity to quantify elevated H_2O_2 levels. This validation step marks the first application of Hyper7 probes in mammalian oocytes and zygotes, promising a novel tool for investigating the role of H_2O_2-mediated mechanisms in oocyte biology while posing the critical need to manage phototoxicity in subsequent research.
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77° CONVEGNO SISVET

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Effects of diet supplemented with bioactive molecules from *Olea europaea* L. in Casertana x Large White (Neroametà) hybrid pigs in extensive farming

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The use of by-products from the olive oil supply chain in livestock farming is an important resource due to their high content of biomolecules with strong antioxidant properties and beneficial effects on animal welfare, growth performance and production quality [1,2]. The effect of olive leaves (*Olea europaea L.*) extract rich in polyphenols, mainly represented by oleuropein and verbascoside, on morpho-functional characteristics and antioxidant capacity in adult hybrid pigs (*Casertana* X *Large White*) in extensive farming was tested in a feeding trial. The study was conducted for 60 days on 10 pigs at the end of fattening (finishing) phase raised with the same extensive rearing techniques. The pigs were divided into two groups of 5 of which the first control group fed a standard diet and the second experimental group fed a diet supplemented with 400 mg/head/day of bioactive molecules from *Olea europaea L.* The nutritional and functional quality of the standard diet, the polyphenolic extract and the enriched diet supplemented with bioactive molecules was evaluated. For the study, liver and duodenum samples were collected at 1 hour post-slaughter, and at 48 hours post-mortem, *Psoas major* and *Longissums dorsi* muscles were sampled.
The histo-morphological characteristics of the muscle fibres of two muscles and "tunica mucosa" of the duodenum were evaluated in order to assess the morpho-structural effects of the diet supplemented with polyphenols.
The muscle samples were processed for the evaluation of the rheological characteristics of the meat by chemical analysis; the effects of the supplemented diet on the acid profile of the intramuscular fat were evaluated by gas chromatography, while the endogenous and exogenous functional activity in the muscles was evaluated by DPPH, Folin Ciocalteu, aluminium chloride, MDA and SOD assay. Finally, for the direct assessment of animal welfare, hormone assays of ACTH and cortisol levels were performed on blood and muscle.
The results showed that the group of pigs fed a diet enriched with bioactive molecules from *Olea europaea* L. showed an increase in anti-radical activity in all muscles; the fatty acid profile showed a significant increase in total unsaturated fatty acids with a decrease in omega 6 and an increase in omega 3 (PUFA) and omega 9 (MUFA), improving the omega 3-omega 6 ratio. Hormonal assays showed no statistically significant difference between the two groups of pigs.
In conclusion, it was shown that feeding pigs with feed enriched with *Olea europaea* L. extracts positively influences the intramuscular lipid composition and improves the oxidative stress response of the muscle. In view of the circular economy, precision feeding techniques developed with a targeted recovery of molecules extracted from the co-products of the olive-oil chain and their use in feedstuffs can be a useful tool both for improving animal welfare and for enhancing the functional and nutraceutical quality of animal products. According to the One Health approach, our results can contribute to expanding knowledge about the improvement of the global health status, human-animal-environment.

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77° CONVEGNO SISVET
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Neuron distribution and neurochemical characterization of the enteric submucosal plexuses in the pig vs. human colon

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The pig is a valuable pre-clinical model to investigate gastrointestinal (GI) physiopathology for its homologies with humans. These include: i) weight and organ structural similarities (i.e. tenia and sacculation in colons); ii) both are omnivores and colon fermenters with similar microbiota; and iii) have equivalent structures of the enteric nervous system (ENS), the major control system of GI functions. In the GI wall, enteric neurons are organized in two ganglionated plexuses: the myenteric plexus (MP), between the longitudinal and circular muscle; and the submucosal plexus (SMP), located in the submucosa, with morphological features differing among species. In large mammals, like pigs and humans, the SMP is multilayered and subdivided into inner (ISP) close to the mucosa, and outer (OSP) SMP, near the circular muscle. We have shown that density of enteric neurons in pigs is significantly higher in ISP than OSP in ascending (AC) and descending colon (DC) and that neurons in both SMPs contained choline acetyltransferase (ChAT) immunoreactivity (IR), neuronal nitric oxide synthase (nNOS)-IR (markers for excitatory and inhibitory neurons, respectively) and substance P (SP) IR [1,2]. In this study we compared the distribution and neurochemical profiles of ISP and OSP neurons in the AC and DC specimens from pigs (18 Yucatan pigs, 3 F, 25-30 kg) and humans (14 patients; 6 F, age: 48-86; clean margin from resected colonic adenocarcinoma). In whole-mount submucosal preparations, IR was assessed with HuC/D (pan-neuronal marker), ChAT, nNOS and SP antibodies with confocal imaging and Imaris software for quantification. In both pig and human colons, the highest density of neurons was observed in the ISP of AC, although the overall HuC/D-IR/mm2 was nearly 4 times greater in pig vs. human. The density of HuC/D-IR neurons in ISP of the AC was significantly higher than in the DC in both pigs and humans ($P<0.01$), whereas was comparable in OSP of AC and DC in both species. The percentage of ChAT-IR neurons in human (36-43%) and pig (31-44%) colon was comparable in ISP and OSP in AC and DC. Conversely, the percentage of nNOS-IR neurons, which was similar in ISP and OSP in the human AC and DC (16-22%), differed in the pig, where the percentage of nNOS-IR neurons in the ISP of AC was significantly lower than OSP (15% vs. 45%; $P<0.001$) and both ISP and OSP of DC (38-42%, $P<0.001$). The percentage of SP-IR neurons was smaller in human (15-20%) vs. pig (23-26%) in both plexuses in AC and DC. SP-IR varicosities were detected through the ganglia of both pig and human colon. This study, showing quantitative and neurochemical similarities between pig and human submucosal neurons, provides robust evidence to use this animal model to explore neuromodulation in patients with colonic disorders.

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77° CONVEGNO SISVET

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Transcript trends of leptin system and selected fertility markers in post-spawning zebrafish ovary

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Leptin is an adipocytokine produced by various organs, with an evident role on reproduction in mammals. In most teleosts, including zebrafish (Danio rerio), leptin has two paralogues, leptin-a (lepa) and leptin-b (lepb), and a single cognate receptor, leptin receptor (lepr). In these taxa, the leptin system may influence puberty and later sexual maturation stages, but its role in regulating female zebrafish reproduction remains poorly understood. In wild-type zebrafish (WT), the ovary lepb displays peak expression levels over the other organs, while the expression of lepa and lepr is minimal. Subfertility — lower eggs per spawning and fertilisation rate, partial anovulation, slower oocyte maturation, and increased atresia — was shown in female lepr -/- knockdown (loss of function) strains, while various genes related to steroidogenesis, oocyte maturation, ovulation, and atresia were differentially expressed compared to WT [1; 2].
Our study aims to explore the trends and localization of leptin system transcripts in WT ovaries to better characterise their role in late stages of female reproduction.
Six-month old female AB strain WT were synchronised (purging by natural mating) and selected when laid > 50 eggs. Latter individuals mated again after 7 days, and were re-selected for the quality and quantity of spawned eggs. Five to 8 individuals were anaesthetized and euthanized at 0, 2, 4, 6, 8, 10, 12, and 14 days post spawning (dps). The full excised left ovary was collected for RNA extraction and the rest of the animal was FFPE for histology. RT-qPCR was conducted for lepa, lepb, lepr, and for selected transcripts related to steroidogenesis (cyp19a1a, star, hsd3b1), oocyte maturation (pgr, pgrmc2) or ovulation (cpla2) previously found to be up- or down-regulated in female lepr -/- [1]. ef1a and rpl13a were used as housekeeping genes. Animal experiments followed Swedish Ethical Committee guidelines and approval in Uppsala (permit C10/16). Spearman's rho correlation analysis was performed to assess the relationship between different targets across all time-points collectively, as well as parametric to non-parametric tests to compare single genes at different time points post-spawning.
The leptin system 2^-(ΔCt) is one to two orders of magnitude smaller compared to other targets. lepr is significantly and positively correlated with lepb (0.467, p: 0.001), cpla2 (0.466, p: 0.001), and pgr (0.409, p: 0.005). Overall star, hsd3b1, pgr, pgrmc2, and cpla2 show a significant positive correlation between them and against the GSI (gonado-somatic index), while cyp19a1a has a different correlation trend being negatively correlated with the GSI (#0.475, $p < 0.001$). In a less-than-significant manner, lepa shows its peak at 0 dps, lepb tends to be higher at 12 dps compared to the other time points, while lepr fluctuates peaking at 0 and 6 dps. hsd3b1, pgr, pgrmc2, and cpla have an increasing trend and are significantly upregulated at 8-12 dps, while cyp19a1a has a significant peak at 0 dps and, successively, drops sharply. star remains stable over time.
In conclusion, while the leptin system appears crucial in zebrafish reproduction, its dynamic temporal trends post-spawning revealed in this study need collateral investigation through different methods. ISH, histological characterization of the follicular population, and further statistical analysis are ongoing.

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Protein localization and gene expression of the nerve growth factor and its cognate receptors in different testicle morphotypes of the grey squirrel (*Sciurus carolinensis*) living in the Umbria region.

Mercati F.[1]; Anipchenko P.[1]; Guelfi G.[1]; Capaccia C.[1]; Suvieri C.[2]; Palmioli E.[1]; Dall'Aglio C.[1]; Bufalari A.[1]; Palermo F.[3]; Cocci P.[3]; Paoloni D.[4]; Zerani M.[1]; Maranesi M[1]

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The gray squirrel (Sciurus carolinensis) is an invasive species that constitutes a serious threat to the native Eurasian red squirrel conservation due to its ability to adapt well to new environments such as the Umbria region (1). The safeguarding of native species is implemented from diversified actions including knowledge of the reproductive characteristics of the alien species to limit its reproductive success. To this end, the nerve growth factor (NGF) and its high- and low-affinity receptors (NTRK1, NGFR) were studied in the testis of gray squirrels since several studies describe NGF as an important factor involved in the regulation of mammalian reproduction. The NGF system was already described in the ovaries of female gray squirrels living in the Umbria region (2) while there is no information on male squirrels. In humans and laboratory animals, NGF affects the process of testicular morphogenesis and spermiogenesis as demonstrated by the presence of NGF receptors in both Sertoli and germ cells in prepubertal and adult subjects. In this study, seventeen male squirrels were captured in a natural area close to Perugia city. The animals were anesthetized with dexmedetomidine ketamine, maintained with sevoflurane in 100% O2, and underwent external or internal orchiectomies (3). Squirrels were captured and treated in compliance with regulations regarding wildlife control laid down in Art. 19 of the Italian Law 157/92, “Rules for the protection of wild animals and homeotherms and for hunting”, Habitat Directive 92/43/CEE, and European Parliament Regulation n. 1143/2014. Collected testis were classified into 3 different morphotypes by histological procedures: active spermatogenesis, pubertal, and immature (1). NGF, NTRK1, and NGFR were evaluated in the testis of the three different morphotypes by immunohistochemistry, western blotting, and Real-Time PCR. Significant differences among group means were calculated by ANOVA followed by Tukey post hoc test. Western Blot revealed the presence of bands matching the expected size for NGF and its receptors. Immunohistochemistry showed the NGF localization in the Leydig cells in all morphotypes with more intense cytoplasmic staining in pubertal and spermatogenesis ones. NTRK1 was localized in the Leydig cells. NGFR immunostaining was observed in Sertoli cells of the pubertal morphotype and both the Sertoli cells and Leydig cells of the spermatogenesis morphotype. By Real-Time PCR, NGF significantly increased in pubertal with respect to both active spermatogenesis and immature morphotypes. The presence of NGF receptors indicates that the testis of gray squirrels is sensitive to NGF action while the overlapping localization of the molecule and its receptors indicate that NGF, produced by Leydig cells, can exert its function on the testis through an autocrine and paracrine mechanism. The results obtained suggest that in the grey squirrel population here investigated the NGF system is involved in the testicle development and function, probably contributing to the reproductive success of this species.

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ANIV

77° CONVEGNO SISVET

Stato: INVIATO - ID: 13060

Detection of extended-spectrum β-lactamase and metallo-β-lactamase genes in Pseudomonas aeruginosa strains isolated from canine otitis externa.

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Detection of extended-spectrum β-lactamase and metallo-β-lactamase genes in Pseudomonas aeruginosa strains isolated from canine otitis externa.

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Otitis externa represents the main reason for dogs' presentation in small animal practice, and it can affect dogs of any age or gender. Pseudomonas aeruginosa is considered the second major causative agent of otitis externa in dogs, after Staphylococcus pseudintermedius [1]. Currently, Pseudomonas aeruginosa has become a challenging and worrisome pathogen, since it is often associated with chronic otitis externa, poorly responding to antimicrobial treatments, due to its ability to form biofilm and its intrinsic and acquired antimicrobial resistance [2]. Furthermore, the growing dissemination of extended-spectrum β-lactamase producing Pseudomonas aeruginosa has developed into a relevant threat both in veterinary and public health. This study aimed to evaluate the antimicrobial resistance profiles and to detect the extended-spectrum β-lactamase (ESBL) and metallo-β-lactamase (MBL) genes in Pseudomonas aeruginosa. Precisely, seventeen Pseudomonas aeruginosa strains, recovered from auricular specimens of dogs affected by otitis externa attending the University Veterinary Teaching Hospital of Naples between 2020 and 2022, were identified by MALDI-TOF MS. Antimicrobial susceptibility testing was carried out against ten clinically relevant antimicrobials using the Kirby Bauer disk diffusion method on Mueller Hinton agar plates. PCR assay was performed to detect ESBL blaCTX-M, blaTEM, blaSHV, blaPER, and MBL blaIMP, blaOXA-48, blaVIM, blaNDM, blaGES genes, using specific primers. The results showed Pseudomonas aeruginosa isolates had a phenotypic resistance value of 100% to ceftazidime, imipenem and meropenem, followed by sulfamethoxazole-trimethoprim (94%) and ceftriaxone (88%). The highest susceptibility level of 100% was recorded for both ciprofloxacin and tobramycin (100%), followed by amikacin and marbofloxacin both with a value of 94% and by gentamicin (88%). An alarming result was represented by the high prevalence of multidrug-resistant strains with 94% of the total isolates, showing resistance to at least three different antimicrobial classes. The ESBL genotypic resistance was driven by blaPER (100%; 17/17), followed by blaSHV (29.4%; 5/17), blaTEM (23.5%; 4/17), and lastly by blaCTX-M (17.6%; 3/17). The most common ESBL-genotype combination was blaPER + blaSHV (23.5%). Referring to MBL-genotypic resistance, blaVIM was detected in all 17 Pseudomonas aeruginosa isolates (100%), followed by blaGES (76.5%; 13/17), blaOXA-48 (23.5%; 4/17), blaNDM (23.5%; 4/17) and blaIMP (17.6%; 3/17). Nine isolates (52.9%) carried together blaVIM + blaGES genes, which resulted to be the most common combination. Furthermore, the simultaneous presence of all five MBLs blaIMP, blaOXA-48, blaVIM, blaNDM, blaGES was detected in two strains. The findings of this study revealed worrying antimicrobial resistance profiles of Pseudomonas aeruginosa- associated canine otitis externa. Furthermore, to the best our knowledge this is the first investigation carried out to detect extended-spectrum β-lactamase and metallo-β-lactamase genes in Pseudomonas aeruginosa of animal origin in Italy.

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Evaluation of seasonal dynamics for surveillance and management in buffalo milking parlors of the Campania Region, Italy: a preliminary study by use of adenosine triphosphate luminometry and bacteriological investigation.

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It is essential to employ proper sterilization and disinfection techniques, practice hand hygiene, wear protective clothing such as gloves, use infection control techniques during milking procedures. Careful cleaning of milking parlour and its equipment is fundamental to guarantee a good raw milk quality and to prevent the dissemination of bacteria and improve animal welfare [1, 2].
This study aims to investigate, by adenosine triphosphate (ATP) bioluminescence assay and bacteriological analysis (bacterial isolation and identification), the bacterial contamination of the milking parlour, evaluating the seasonal dynamics during the year 2022, on milking parlour surfaces of buffalo farms in Campania Region. The farms were selected by Italian ClassyFarm system, which assesses the level of animal welfare and biosecurity according to risk analysis. During the sampling process, all dairy farm proprietors completed a questionnaire concerning milking practices, animal hygiene, and health. The surveys revealed comparable cleaning procedures yet highlighted the lack of a uniform cleaning protocol across various farms.
The ATP bioluminescence findings indicated similar contamination levels across all surveyed buffalo farms, with the highest levels observed in the internal milking area during summer compared to other seasons. There was a fluctuation in the percentages of bacterial isolates observed across different seasons. During the autumn, *Bacillaceae* exhibited a higher prevalence (64%), whereas in the summer, *Bacillaceae* accounted for 41%, with a concurrent high proportion of *Enterobacteriaceae* observed at 38%. A few samples showed no bacterial growth. While identifying bacteria remains crucial for comprehending the microorganisms present in the milking parlour, employing ATP luminometry could provide extensive and precise applications in buffalo milking parlours.
In conclusion, utilizing ATP bioluminescence to assess the hygiene of a buffalo milking parlour could represent a significant advancement in dairy farming technology, introducing an innovative and promising on-farm method for evaluating the quality of buffalo milk.

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Megalocytivirus infection in ornamental fish imported to Italy

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The trade in marine ornamental fish is a multi-billion-dollar industry involving over 50 exporting and importing countries. Europe is one of the three major importers of marine ornamental fish, along with the United States and Japan [1]. Megalocytiviruses are associated with severe mortality in ornamental fish aquaculture, including both freshwater and marine species. The international trade in live ornamental fish has aided the spread of these viruses. So far, megalocytiviruses have been detected mainly in Asia and South America and in few European countries (Belgium and Germany) [2,3], but, to the best of our knowledge, they have never been detected in Italy.
In the context of a health surveillance programme on imported ornamental fish, histological lesions consistent with megalocytivirus infection were pointed out in two fish dyed during the quarantine period. Case 1# referred to one neon damselfish (*Pomacentrus coelestis*) out of 10 subjects involved in a 100% mortality event in June 2018, whereas Case 2# was a prickly leatherjacket (*Chaetodermis penicilligerus*) dead after showing lethargic behaviour in June 2021. To investigate the presence of the megalocytivirus genome, FFPE tissue samples, including the areas with enlarged cells, were subjected to DNA extraction and PCR/real-time PCR analysis using two protocols previously described. PCR products were sequenced and subjected to phylogenetic analysis.
The histological investigation showed in both species the presence in the kidney, spleen, liver, heart, gills, intestine of multiple hypertrophic cells containing granular to smudgy basophilic intracytoplasmic inclusions. Often, these cells were clearly endothelial cells. The presence of megalocytivirus DNA was pointed out in the two fish samples resulting positive to the real-time PCR and being identified as megalocytiviruses. Phylogenetic analysis of a fragment of the MCP gene from Case 2# showed its clustering within the RSIV-like group. Further ornamental fish (*Chromis retrofasciata*, *Calloplesiops altivelis, Acreichthys tomentosus, Chaetodon kleinii*) dyed during the transport or quarantine period between 2021 and 2022 were screened for megalocytivirus presence and some of them showed positivity as well. The positive specimens belong to marine fish species typically leaving in Indo-Pacific waters, however, no data are available about the country of origin of the tested specimens.
In conclusion, the presence of megalocytivirus was pointed out in imported marine ornamental fish for the first time in Italy. The viral DNA was detected in five marine ornamental species (*Pomacentrus coelestis, Chaetodermis penicilligerus, Chromis retrofasciata, Calloplesiops altivelis*) that, so far, have never been associated with megalocytivirus infection. Due to the high risk of importing megalocytiviruses through the ornamental fish trade, a strengthening of the surveillance programmes and quarantine measures is recommended.

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Antimicrobial activity of natural compounds: preliminary study on hazelnut skins

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Antimicrobial resistance (AMR) is a growing concern worldwide. For this reason, the discovery of novel compounds with antimicrobial activity appears crucial. Natural products are still one of the major sources of new drug molecules, although it is difficult to measure their antimicrobial activity because of the lack of standardized techniques [1]. Two by-products originate from the hazelnut industry during the post-harvesting processes: hazelnut shells, used as heating source, and hazelnut skins (HS) usually managed as waste material in the nut processing industry. However, they represent a rich source of bioactive compounds [2]. This study aimed to investigate the antimicrobial activity of HS extracts obtained by conventional and green extraction methods, against a panel of food-borne and clinically relevant microorganisms. Minimum inhibitory concentration (MIC) and minimum bactericidal concentration (MBC) were measured according to the official guidelines [3] against *Escherichia coli* (ATCC 25922), *Pseudomonas aeruginosa* (ATCC 27853), *Staphylococcus aureus* (ATCC 29213), *Salmonella enteritidis* (ATCC 13076), *Bacillus cereus* (ATCC 11778), *Listeria monocytogenes* (ATCC 13932), and *Campylobacter jejuni* (ATCC 33291). Bacterial suspension used for the assay was 5 × 10^5 CFU/mL in Mueller Hinton Broth. An aliquot of each suspension (100 µL) was added to a 96-well microplate containing the same volumes of two-fold serial dilution of the hazelnut extracts, ranging from 0.08 to 40 mg/mL. All the experiments were performed in triplicate, for three independent experiments. Both the conventional and green extracts revealed antimicrobial effects against the tested strains, with major efficacy on Gram-positive bacteria and depending by the extraction method. In particular, *B. cereus*, *L. monocytogenes,* and *S. aureus* were the most susceptible bacteria to both the HS extracts with a MIC ranging between 0.156 and 0.312 mg/mL. On the other hand, the extracts revealed poor antimicrobial activity against Gram-negative. *S. enteritidis* growth was inhibited by the concentration of 5 and 1.25 mg/mL for HS extracts obtained by the conventional and green methods, respectively. For *P. aeruginosa* the HS extracts obtained by the conventional method are active at the concentration of 10 mg/mL and by the green method 5 mg/mL. *C. jejuni* MIC was 2.5 mg/mL and 1.25 mg/mL for conventional and green extractions, respectively. *E. coli* appears as the less sensitive strain, with a MIC value of 40 mg/mL for the conventional methods and 2.5 mg/mL for the green extracts. Notably, it was observed that MIC and MBC values were the same or one dilution more for MBC for each bacterial strain tested.

Certainly, further researches are needed to elucidate the exact antimicrobial mechanisms of HS, including synergistic studies with traditional antibiotics. HS antimicrobial effects could be employed for topical preparations and the development of natural preservatives in food industry. Valorizing HS as a renewable source of antimicrobial compounds together with a green extraction method contributes to waste valorization, according to a circular economy approach.

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Schmallenberg virus modulates Wtn/β-catenin signaling and autophagy during its replication

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Schmallenberg virus (SBV) is an enveloped virus belonging to the genus Orthobunyavirus, in the Peribunyaviridae family. This virus was first isolated in Germany in 2011 during a report of severe malformations described in ruminants [1]. Due to its teratogenic potential and decreased milk production, SBV causes economic losses and has a negative impact on the cattle trade. Infected animals may show non-specific signs and symptoms such as fever, reduced milk production, and diarrhea. However, infection in pregnant females can cause reproductive abnormalities and fetal deformities (particularly the arthrogryposis-hydranencephaly complex) [2].
β-catenin is a highly conserved protein that has several roles. It regulates the actin cytoskeleton by mediating the interaction between E-cadherin and α-catenin. On the other hand, it is an important modulator of the canonical Wnt signaling pathway, that controls cell proliferation, differentiation, autophagy (another conserved cellular process that crosstalks with Wnt/β-catenin signaling), and cell death [3]. β-catenin is known to have a key role in the replication of several viruses, although the relationship between this pathway and SBV is still unknown. The aim of this work was to evaluate the modifications that SBV infection causes in the expression of autophagy and Wtn/β-catenin signaling markers on permissive cells and to verify their proviral role. Baby Hamster Kidney-21 (BHK-21) cell monolayers were infected with SBV at different time points. Proteins were extracted from each sample, and once run in an electrophoretic run, they were analyzed for the expression of specific autophagy (mTOR, phospho-mTOR, PI3K, AKT, LC3, Beclin) and Wnt/β-catenin signaling markers (β-catenin, GSK, and phospho-GSK) by western blot assay. No noteworthy changes were observed during the first 12 hours of infection. Starting at 24 hours, however, a progressive reduction of all the markers described above was observed (that completely disappeared after 48 hours of infection). In particular, Beclin and β-catenin were not expressed after 24 hours.
In the second part of the study, the effects that autophagy inhibitors and inducers cause on viral replication (calculating TCID50), viability (MTT assay), and expression of the previously evaluated markers were evaluated. The use of late autophagy inhibitors (chloroquine and especially bafilomycin) has been shown to reduce viral replication and consequently increase the expression of proteins degraded during the SBV cycle (such as β-catenin and GSK). Early autophagy inhibitors or inducers did not cause any significant effect on viral replication. The results of this study provide the basis for understanding SBV's pathogenetic processes (as well as Peribunyaviridae in general) and its connection to specific cellular pathways.

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77° CONVEGNO SISVET

Stato: INVIATO - ID: 13139

Antibacterial evaluation of cell free supernatant from probiotic Lactobacillus species against Shiga toxin-producing E. coli (STEC): an in vitro study

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Shiga toxin-producing Escherichia coli (STEC) are of great importance in epidemiology due to their impact on public health. According to ECDC 2021 report, the five most frequently reported serogroups were O157 (15.1%), O26 (14.7%), O103 (8.4%), O145 (4.6%), and O146 (3.7%) [1]. STEC strains are notorious for causing serious foodborne diseases, which may manifest as minor gastrointestinal discomfort or progress to life-threatening disorders such as hemolytic uremic syndrome. In veterinary medicine, they can colonize bovine intestine, making cattle and other ruminants important reservoirs for strains associated with human diseases. The primary goal of this study was to evaluate the activity of cell free supernatants (CFS) of four commercially available probiotic lactobacilli (LAB) against eight STEC strains using three assays, Kinetic killing assay, Agar well diffusion assay and Minimum Inhibitory Concentration (MIC). The CFS producing probiotic strains were L rhamnosus GG, L. paracasei CNCM I-1572, L lactis W58 and L. plantarum Lp-115. The challenged reference STEC strains were O45:H2, O145:NM, O121:H19, O104:H4, O103:H11, O157:H7, O26, O111. The CFSs were prepared by centrifuging the LAB cultured overnight in MRS broth and filtering through a 0.22μm pore size filter. The Kinetic killing ability of each CFSs was measured over four timepoint, T0 (contact), T1 (1 hour), T2 (2 hours) and T3 (4 hours) against standard STEC inocula (10^8 CFU/mL). Negative control consisted of PBS at the same pH of CFS. Agar spot assay was performed following a protocol available in liteature with minor modifications [2]. Briefly, one-hundred μL of fresh LAB were loaded on four wells on MRS agar and incubated anaerobically overnight. The next day, new MRS agar was used to seal the wells, and the plates were filled with STEC that had been diluted 1:100 in soft BHI agar (0.7%). The plates were then left to sit for 24 hours so that inhibition halos could be measured. MIC was determined following microdilution assay in duplicates for each STEC. All the LAB were able to kill each STEC in 4 hours with minor differences. CFS from L. lactis W58 killed O111, O145, and O104 in 2 hours compared to other CFS. The most susceptible serogroup was O157:H7, which killed all the LAB in 1 hour. Moreover, as found in the literature, STEC could grow even in an acidic environment (pH 3.6-4) as shown by the control groups in kinetic assay. The CFS from L. lactis W58 had the highest antibacterial activity, with an inhibition halo on an agar spot assay (42.4±0.8 mm). It was followed by the CFS from L. rhamnosus GG (40.6±2.5 mm), L. plantarum Lp-115 (39.9±2.19 mm), and L. paracasei CNCM I-1572 (37.5±2.31 mm). MIC values ranged from 1:8 to 1:4. To shed light on the antibacterial activities a label free proteomic analysis is underway to identify the proteic and peptidic composition of each LAB CFS. Overall, these results indicate that probiotics could have the potential to combat Shiga toxin-producing E. coli strains and suggest a possible use of probiotic cell-free supernatants as an additional approach to infections caused by STEC.

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77° CONVEGNO SISVET

SOCIETA´ SCIENTIFICA DI RIFERIMENTO **ANIV**

Involvement of Aryl Hydrocarbon Receptor during Feline Coronavirus Infection In Vitro

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Feline coronavirus (FCoV) is an alphacoronavirus (αCoV) generally responsible for moderate or chronic asymptomatic infection in cats. Due to the genomic mutations that can develop in a single infected cat, FCoV can modify its cellular tropism arising the aptitude to infect macrophages resulting in the development of feline infectious peritonitis (FIP). The emergence of a new, highly pathogenic FCoV-canine coronavirus (CCoV) recombinant responsible for a fast-spreading outbreak of FIP in cats from Cyprus was recently reported [1]. Interestingly, it has been hypothesized that the recombination at the S gene level, due to a deletion together to amino acid changes shows 97% identity with the pantropic CCoV-CB/05. These features could mainly modify the receptor binding domain, leading to changes in receptor binding and cell tropism, compared to FCoVs [1]. In this context, to control the impact of FCoV infection, research has focused on the development of antiviral therapies involving original mechanisms of action. Recent studies have demonstrated that the aryl hydrocarbon receptor (AhR), a transcription factor stimulated by various substrates, both endogenous and exogenous, is also involved in natural protective immune responses to various microorganisms. Indeed, through the study of virus-cell host interaction, AhR has been recognized as a pro-viral host factor of both αCoVs and βCoVs, like murine coronavirus (MCoV), Middle East respiratory syndrome coronavirus (MERS-CoV), human coronavirus (HCoV) 229E, severe acute respiratory syndrome CoV type 1 (SARS-CoV-1), SARS-CoV-2, and CCoV-II [2,3]. Based on this evidence, here, we focused on the study of FCoV infection in vitro in order to improve the knowledge about AhR mechanisms of action. Our results showed that the infection of feline enteric CoV (FECV), isolate “München”, on Crandell Feline Kidney (CRFK) cells results in a major activation of AhR, a protein expressed in CRFK cells. In addition, the selective AhR antagonist CH223191 caused a decrease in FECV replication as well as in the expression of NP nucleoprotein levels. Moreover, the AhR-inhibitor acted on the acidity of lysosomes in infected cells. Overall, AhR could be a possible drug target to explore for the development of new antivirals against FCoV.

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77° CONVEGNO SISVET

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Canine coronavirus type 1 and bacterial mixed infection in a Boston Terrier breed puppy: detection and antimicrobial susceptibility evaluation

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Different infectious enteric pathogens like viruses, bacteria, and parasites, sometimes in co-infections, are often responsible of acute and severe gastroenteric disease in young dogs. Although some of these cases goes undiagnosed, the early identification of causative agents is essential [1]. The most prevalent are canine parvovirus type 2 (CPV-2) followed by canine coronavirus (CCoV), while bacteria like Escherichia coli, Clostridium perfringens and Enterococcus spp. are commonly involved in co-infections [2]. This study reports a case of viral and bacterial co-infection in a 3-month-old Boston Terrier breed puppy, died after showing signs of vomit and diarrhoea. Puppy was then submitted for necropsy. Gross lesions as dark-brown haematic fluid in abdominal cavity, congestion of lungs and liver and segmental enteritis were observed. Tissue samples were collected for bacteriological, virological, and histological assays. Bacterial isolation was performed on selective and differential agar media, and the identification was carried out with the biochemical API method. Multiple PCR for the detection of ESBL genes in Enterobacteriaceae, and toxin genes in Clostridium spp. were performed. A set of different antimicrobial molecules were tested to evaluate antimicrobial resistance profiles by disk-diffusion method. A wide panel for viral screening by molecular assays was performed, including genotyping and sequence analysis for positive samples. A strain of Clostridium perfringens was isolated from intestine and peritoneal fluid, Enterococcus faecium strains were isolated from almost all tested samples, and a strain of E. coli was isolated from intestine. E. coli tested positive for the presence of blaTEM gene and showed resistance to 9 of the 12 tested antimicrobials; strains of E. faecium had similar antimicrobial susceptibility profiles, showing resistance to 6 of the 11 tested molecules. Only intestine sample tested positive to CCoV type I, with a high nucleotide identity (96.52%) with CCoV-I strains previously detected in the region. The histological exam of intestine revealed diffuse monocytic infiltrates in the mucosa, with fusion of the intestinal villi and partial disintegration; monocytic infiltrates were observed in the submucosa. Acute and severe infectious gastroenteritis is still one of the main causes of morbidity and mortality in young dogs [2]. Among causative agents, some are little described or underestimated. Among these, CCoV-I commonly causes mild enteritis with low mortality, but co-infection with other enteric pathogens could led to the worsening of signs or even to the death [1]. Among bacteria, enterotoxins producer Clostridium perfringens is also reported as the most common species found in intestine of dogs with acute diarrhoea [3]. This study highlights the need to elucidate and evaluate the role of different infectious enteropathogens for consequent appropriate supportive and/or antimicrobial therapies.

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Evaluation of serological cross-reactivity between different herpesviruses in calves.

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Bovine alphaherpesvirus 1 (BoAHV-1) is associated with Infectious Bovine Rhinotracheitis (IBR), a disease mainly characterized by acute upper respiratory tract inflammation that causes significant economic losses to cattle producers. The serology is used to detect antibodies to BoAHV-1, but Enzyme-linked Immunosorbent Assay (ELISA) includes limitations for cross serological reactions between different herpesviruses [1]. This study aimed to evaluate the cross-reactivity of *Bovine gammaherpesvirus 4* (BoGHV-4), *Bubaline alphaherpesvirus 1* (BuAHV-1) to BoAHV-1.Two groups (A and B) of four three-month-old calves each, devoid of BoAHV-1, BoGHV-4, and BuAHV-1 neutralizing antibodies (NAs), were subjected to two different challenge infections with the 85/16 TV of the BoGHV-4 strain (Group A) and the wild-type SAM 1/2020 TN of the BuAHV-1 strain (Group B), respectively. In addition, one uninfected calf for each group was added and served as a control. Serum samples were collected from the A and B groups on 0, 14, 21, 28 and 35 post-challenge days (PCDs). The samples collected from group A were tested for antibodies to BoGHV-4 and BoAHV-1, while those from group B were examined for antibodies to BuAHV-1 and BoAHV-1.In particular, in group A, we used twelve different commercial ELISA tests, of which nine against IBR (three competitive IBR gB-ELISA; three competitive IBR gE-ELISA; three indirect IBR ELISA) and three against BoGHV-4 (Indirect BoGHV-4 ELISA). In addition, the VN tests, using BoAHV-1 and BoGHV-4, were carried out in separate testing sessions. In group B, we applied eleven different commercial ELISA tests, of which ten against IBR (three competitive IBR gB-ELISA; four competitive IBR gE-ELISA; three indirect IBR ELISA) and one against BuAHV-1 (Indirect BuAHV-1 ELISA). We also performed the VN tests in separate testing sessions using BoAHV-1 and BuAHV-1.The study employed the VN tests previously described [2] and commercial ELISA tests, following the manufacturer's instructions and protocols. In group A, serologic cross-reactions did not occur in ELISA and VN tests. Otherwise, in group B, on 14, 21, 28 and 35 PCDs, all ELISA tests, except two IBR competitive gE-ELISA and one IBR competitive gE-ELISA on PCD 14, indicated serologic cross-reactivity between BuAHV-1 and BoAHV-1. However, the VN test for BuAHV-1 showed higher average NAs titers than the VN test for BoAHV-1. These results are in agreement with previous studies' findings [1]. The serological cross-reactivity, evidenced in group B, could be attributed to the homology of glycoprotein gB between different ruminant *alphaherpesviruses* [3]. In conclusion, cross-reactions must be carefully considered to avoid removing animals of high genetic and economic value that are erroneously positive for BoAHV-1, especially in breeding stables or in compliance with IBR control and eradication programmes.

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77° CONVEGNO SISVET

Stato: INVIATO - ID: 13410

Salmonella Kentucky outbreak in the Veterinary Teaching Hospital of the University of Turin

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Salmonella enterica subspecies enterica includes thousands of non-typhoidal Salmonella (NTS) serovars, which circulate within/between animal and human populations. Salmonella serovar Kentucky, firstly isolated from poultry, has been increasingly isolated from several animal species and humans worldwide. Despite being the cause of self-limiting gastroenteritis in most cases, it is of public health interest as severe consequences may occur in more fragile patients. Sequence type (ST) 198 is of particular concern because it is an ESBL+ multi-drug resistant (MDR) strain. Recently, it has been described as the principal serovar responsible for human cases.
Healthy carriers and, above all, infected subjects, shed large quantities of NTS through feces and body fluids. Salmonella proliferates in wet and dark places, but it can persist even in dry and dusty environments if organic material is not completely removed. This study summarises the genetic and phenotypic evidence of a Salmonella Kentucky outbreak. From September 2022 to May 2023, Salmonella spp. was identified from more than ten canine, feline and equine specimens received by the clinical bacteriology laboratory of the Veterinary Teaching Hospital (VTH) of the University of Turin. In addition to clinical cases, Salmonella was isolated from environmental samplings of VTH premises. The isolates were identified at the genus level employing selective agars, biochemical reactions, and MALDI-ToF mass spectroscopy. All isolates were sent to the Zooprophilaptic Institute (IZS) of Turin for sero-typing. In the meantime, these strains were evaluated for antimicrobial resistance (AMR) through agar disk diffusion (ADD) and minimum inhibitory concentration (MIC). Ten isolates were whole-genome sequenced at the National Reference Center for Salmonellosis through Illumina short-read sequencing. As a typing method, core-genome Multi Locus Sequence Typing (cgMLST) was performed to evaluate the allelic profile of more than 3,000 loci. Besides the unusually high number of cases, the first evidence that an outbreak was ongoing came from the AMR profile, which was qualitatively (ADD) and quantitatively (MIC) identical in all isolates. All strains were resistant to aminopenicillins, cephalosporins, fluoroquinolones, tetracyclines, and gentamycin while being susceptible to carbapenems, amikacin, chloramphenicol, and sulfamethoxazole-trimethoprim. An additional phenotypic evidence was that, among more than 2,600 possible serovars of Salmonella, all isolates were S. Kentucky.
The WGS results confirmed the phenotypic evidence, as all isolates were MDR, ESBL+ ST198 strains. Moreover, based on cgMLST, there was ≤ 2 allelic distance between neighbouring isolates, and a 5 alleles maximum distance. In conclusion, we gathered evidence that a S. Kentucky outbreak was occurring at the VTH. We noticed a high environmental resistance of S. Kentucky on wooden stable walls and other surfaces that are not easily disinfected due to the persistence of organic material. Also, there likely was a back-and-forth passage between the small animal hospitalization area and the stables. Since S. Kentucky is a pathogen of concern, VTH staff were promptly informed and measures were taken to resolve the epidemic and limit the risk of spread.

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77° CONVEGNO SISVET

SOCIETA´ SCIENTIFICA DI RIFERIMENTO | **SOCIETA' ITALIANA DI MICROBIOLOGIA (SIM)**

TITOLO | **FROG-DERIVED PEPTIDES AGAINST HUMAN AND ANIMAL VIRUSES**

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Emerging and re-emerging viruses represent a major challenge to global public health and timely action is needed to control their spread. Even in non-pandemic era, respiratory viruses have caused a vast global burden of diseases. However, COVID-19 has mostly put the spotlight on the risk of zoonoses. Animals play a pivotal role not only for SARS- CoV-2 infection, but they can represent both intermediate hosts and/or vectors for several other diseases. Currently, more than 60% of viral infections in humans have an animal origin. So, to date, it is clear that there is an urgent need to discover new antiviral agents with a broad spectrum of action. In this context, antimicrobial peptides (AMPs) derived from amphibian skin secretions could represent good antiviral candidates [1]. In the present study, we evaluated the antiviral potential of three frog-derived peptides, i.e., RV-23, AR-23 and Hylin-a1, against respiratory viruses including, respiratory syncytial virus (RSV), human parainfluenza virus type 3 (HPIV-3), influenza virus H1N1 and a wide range of animal viruses, such as bovine (BoHV-1) and caprine herpesviruses (CpHV-1), canine distemper virus (CDV), bovine viral diarrhea virus (BVDV), Schmallenberg virus (SBV), and the vector-borne Sandfly Fever Naples virus (SFNV) [2,3]. The toxicity of peptides has been measured *in vitro* by the 3-(4,5-Dimethylthiazol-2-yl)-2,5-Diphenyltetrazolium Bromide (MTT) assay on cellular models; then, to analyze whether peptides were endowed with antiviral activity, and, specifically, what stage of the infection was affected, different experimental procedures were carried out: i) co-treatment: simultaneous exposure of peptide and virus on cells; ii) virus pre-treatment: treatment of virus with peptide and then inoculation of the mixture on cells; iii) cell pre-treatment: cells were pre-treated with peptide and then infected; iv) post-treatment: cells were infected with virus and then treated by peptide. Post-infection, cythopathic effect (CPE) was observed and cells were stained with 0.5% crystal violet. The inhibition rate of the infectivity was evaluated by CPE observed in the wells treated with the peptides to CPE cells infected with virus. Data were further validated by Real-Time PCR and Western-blot to quantify the gene and protein expression levels of genes and proteins involved in the viral infection. Results demonstrated that peptides exhibited a very strong antiviral activity by targeting the viral particles in the early phase of viral infection. In addition, peptides also affected the viral adsorption on host cells by interfering with the binding of glycosaminoglycan receptors (GAGs). Our results expand our understanding of the activity of these peptides as antivirals and stimulate further investigations in the direction of novel strategies against human and animal infections.

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CANINE MALASSEZIA PACHYDERMATIS FIELD STRAINS SUSCEPTIBILITY TO AZOLES: A SURVEY

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Malassezia genus count 18 species, of which *Malassezia pachydermatis* (MP) is the most represented yeast in cutaneous mycobiome of animals, both as commensal and pathogen. MP is responsible of severe cutaneous infections in companion animals - particularly in dogs - causing dermatitis (MD) and otitis externa (MO). Therapeutic protocols for MP infections in companion animals involve azoles derivatives (miconazole, ketoconazole, itraconazole, fluconazole) - both topical and systemic - followed by terbinafine, chlorhexidine, and selenium disulfide (SeS2). Antifungal treatments are effective to control MP overgrowth when primary causes and predisposing factors are resolved firstly, but the onset of antifungal resistance can lead to failure. In human medicine the antifungal resistance increased, leading the WHO to draft the Fungal Priority Pathogens List (FPPL) of public health importance, which should include MP due to zoonotic and resistance potential [1].

Aim of this work was to evaluate the antifungal susceptibility of MP field strains to a small library of azole compounds, isolated from canine patients with dermatitis and/or otitis externa.

Fifty samples were collected from auricular (35) or cutaneous swabs (15) taken from canine patients with a suspected yeast etiology. Each sample was firstly cultured onto Sabouraud agar then isolates were identified by CHROM-agar® *Malassezia*. 36 strains were isolated: 26 from auricular and 10 from cutaneous swabs. Confirmation of the ID was performed with a nested PCR for internal transcribed spacer region of rRNA gene; all the 36 strains were identified as MP. Then, each isolate was tested with MIC assay for susceptibility to ketoconazole (KTZ), miconazole (MCZ) and fluconazole (FCZ), following CLSI guidelines for yeasts, using modified RPMI 1640 medium [2]. Since no breakpoints were established for susceptibility of MP to KTZ, MCZ and FCZ, criteria suggested by other authors were followed: susceptible (S) = MIC sample $\leq$ MIC50; intermediary susceptible (I) = MIC50 < MIC sample $\leq$ MIC90; resistant (R) = MIC sample > MIC90 [3].

Lowest MIC50 and MIC90 values were found with KTZ (0,016 µg/ml and 5 µg/ml, respectively), while MIC50 of MCZ and FCZ were 16 and 24 µg/ml, respectively, and MIC90 was 64 µg/ml for both. On 36 strains, 3 (8,33%) were resistant to KTZ, 1 (2,78%) was resistant to MCZ and 16 (2,78%) was resistant to FCZ. None of the strains were resistant to more than one azole tested compounds. Intermediary susceptibilities were found for 13/36 (36,11%) strains to KTZ, 11/36 (30,55%) to MCZ and 17/36 (47,22%) to FCZ. All the resistant strains to azoles were from auricular swabs from patients with otitis externa (5/26) and none of these were previously treated with azoles (only one was treated with florfenicol + terbinafine). Limitations of the study were represented by both the low sample size, as well as the difficulty of interpreting susceptibility in absence of breakpoints. Furthermore, a molecular investigation of the resistance mechanism needs to be carried out.

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A simple and versatile method for ex vivo monitoring of goat vaginal mucosa transduction by viral vectors

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Caprine represents a large animal model for human pathogens inducing genital diseases, such as genital herpes, exploitable for pathogenic studies, antiviral molecules, and new vaccines testing. Appropriate vaccine administration is a critical component of a successful immunization program. The recommended route and site of inoculation for each vaccine are based on clinical trials, practical experience an theoretical considerations as a wrong route of administration may reduce the efficacy of the vaccine, whereas the right route can increase the efficacy [1, 2, 3]. Although adenoviral (Ad) vectors have been employed successfully for goats and sheep immunization, no data concerning the vaginal route are available. The first concern is related to the capability of the viral vector to transduce the site of inoculation; therefore, it was of interest to develop a fast and reliable ex vivo assay for testing the transduction capability of Ad5- based vector when intravaginal administered. An Ad5 vector delivering an expression cassette with a bicistronic reporter gene, Ad5-CMV- turboGFP-IRES-Luc2, was constructed. The bicistronic TurboGFP-IRES-Luc2 was then placed under the transcriptional control of the CMV immediate early promoter and the bovine growth hormone polyadenylation signal to generate CMV-turbo-IRES-Luc2. Both turbo GFP and Luc2, were well expressed when tested by transient transfection assay in different cell lines. Ad5-CMV-turboGFP-IRES-Luc2 transfer vector was linearized with PacI restriction enzyme, transfected in HEK293 cells and infectious viral particles were reconstituted. Ad5-CMV-turboGFP-IRES-Luc2 showed replication competence in HEK293 cells, as shown by progressive cytopathic effect (CPE) and the increase of the viral titer during time post transfection. This because HEK293 cells contain Ad E1a and B genes, which are missing in the viral vector genome. Whereas transducing competence, as well as replication incompetence, was evaluated on caprine cells defective for E1A and E1B genes. Taking advantage by Ad5-CMV-turboGFP-IRES-Luc2 delivering luciferase (Luc2), it was possible to show Ad5-CMV-turboGFP-IRES-Luc2 ability to transduce caprine vaginal mucosa by ex-vivo bioluminescent imaging (BLI) employing a simple CCD camera apparatus for chemiluminescence western immunoblotting. Vaginas were collected in sterile conditions from slaughtered goats at the abattoir, transported to the lab on ice and immediately processed. From the middle third of the organ ~2cm square pieces of tissues were obtained and posed in a 6 multiwell plate with the mucosal surface facing upwards. Wells were filled with complete medium containing 10^6 transducing Units (T.U.) of Ad5-CMV-turboGFP-IRES-Luc2 and incubated at 37 °C/5% CO2. Seventy-two hours post infection luciferin substrate was added to each well and the plate exposed to the CCD camera apparatus (ChemiDoc XRS+, BioRad). Indeed, it was possible to ex-vivo visualize Ad5-CMV-turboGFP-IRES-Luc2 gene delivery in the vaginal mucosa surface. These data, although simple, are very informative in terms of immunization strategy through the vaginal route, for pathogens inducing genital diseases, when a viral vector-based vaccine is going to be employed.

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77° CONVEGNO SISVET

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Bovine coronavirus spike protein induces cross-reactive cellular immune response against SARS-CoV-2.

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Bovine Coronavirus (BCoV) is an enveloped, non-segmented, single stranded, positive sense RNA virus which causes (neonatal) calf diarrhea [(N)CD)], winter dysentery (WD) and respiratory infections in cattle as a part of the bovine respiratory disease complex (BRDC). BCoV causes serious economic losses in the global cattle industry and its interspecies transmission can generate recombinant strains potentially able to escape immune response and capable to spread to other species including humans, potentially causing a zoonotic infection [1]. Bovine coronavirus spike glycoprotein (BcoV-S) is responsible for virus' attachment to a modified sialic acid residue (N-acetyl9-O-acetylneuraminic acid) that acts as a receptor on the host cell membrane and induces fusion of the virus envelope with the cell membrane leading to formation of syncytia. Bovine coronavirus infection leads to infectivity-neutralizing (IN) and hemagglutinin-inhibiting (HAI) antibodies production and, since BcoV-S protein contains epitopes that can be recognized by monoclonal neutralizing antibodies (mAbs), virus clearance and protection against the virus infections is permitted. Additionally, BcoV shares epitopes with SARS-CoV-2 demonstrating the possible existence of cross-immunity [2]. Bovine herpesvirus 4 (BoHV-4) is a double stranded DNA γ-herpesvirus isolated from cattle and it has been associated with symptoms like metritis, abortion, pneumonia, diarrhea, respiratory infection, or mammary dermatitis; but it was also isolated in healthy animals. Nowadays BoHV-4-based vectors have successfully been produced for vaccine therapy since BoHV-4 shows several characteristics that explain its safeness as viral vector since it is easy to be manipulated as a Bacterial Artificial Chromosome (BAC) and can be exploited for long time transgenes' expression to trigger an efficient host immune response [3]. A recombinant BoHV-4 (BoHV-4-A-CMV-BoSΔRSHAΔTK) delivering the expression cassette for Bovine coronavirus Spike glycoprotein has been generated and tested. First of all the ORF encoding BCoV-S was in silico designed and the sequence was depleted of its last 19 bp, coding for a potential endoplasmic reticulum retrieval signal and labelled with a hemagglutinin (HA) tag. After human codon usage adaptation, the sequence was chemically synthesized and inserted in a shuttle vector containing two homologous regions for BoHV-4 TK gene. After the assessment of the expression of this recombinant Spike protein, this expression cassette was inserted into the TK locus of BoHV-4, through BAC recombineering mediated homologous recombination. Vaccination on BALB/C mice (authorizations N°10/2023 from Italian Ministry of Health), through intraperitoneal administration of BoHV-4-A-CMV-BoSΔRSHAΔTK, generated a cross-specific cell-mediated immune response against SARS-CoV-2.

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Preliminary investigation on presence of resistant Enterobacteriaceae in animal present in an urban system of social agriculture in Naples

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"Social farming" refers to the set of agricultural and livestock resources put in place to promote rehabilitation activities, social and work inclusion, useful services to daily life, educational and recreational activities (Giarè, 2017). The Social Agriculture is an increasing phenomenon in Italy also but information about the role of animals involved in this context as a potential reservoir of pathogenic bacteria is limited. Therefore, the objective of this investigation was to perform a health monitoring of some environmental surfaces and different animal species present in an urban system of social agriculture in Naples, collecting preliminary data on the potential prevalence of enterobacteria and the related antimicrobial susceptibility profiles associated with them.

A total of **58** samples were collected during health monitoring from May to July 2023. A total of **46** animals were sampled, belonging to 5 different species (Donkey, Duck, Goose, Chicken, Pigeon) and **12** environmental surfaces. Cloacal and faecal samples were collected from the animals using sterile swabs and Amies transport medium, while the environmental surfaces were sampled with sterile spongebags. All collected samples were processed for the search for enterobacteria and the evaluation of their antibiotic susceptibility profiles. The colonies obtained were cryopreserved in Brain heart infusion broth supplemented with glycerol (20%) for subsequent identification by MALDI-TOF.

To test susceptibility to antibiotics, all isolated strains were subjected to an antibiogram using the disk diffusion technique (CLSI, 2020). For each genus/species, the following antibiotics provided in the manual were selected and tested: Ampicillin (10µg), Amoxicillin-clavulanic acid (20/10µg); Ceftriaxone (30µg); Aztreonam (30µg); Gentamicin (10µg); Azithromycin (15µg); Tetracycline (30µg); Ciprofloxacin (5µg), Sulfatrimetropin (30µg); Chloramphenicol (30µg); Fosfomycin (200µg), Nitrofurantoin (300µg). Isolates were classified as susceptible, intermediate, or resistant based on the criteria of the M100-CLSI manual (CLSI, 2020). Furthermore, all strains were subjected to screening tests for the possession of extended-spectrum β-lactamases (ESBL) with the aid of the combined test ESBL+AmpC screen disc kit.

From 58 samples analysed, 52 bacterial strains were isolated, with a high prevalence of the *Escherichia coli* species (38/58; 65.5%), followed by the *Klebsiella* spp genus (14/58; 24.1%). Salmonella spp. it was never detected.

Antimicrobial resistance explained rather low frequencies except for amoxicillin-clavulanic acid, against which 96.9% (51/52) of the strains analysed showed resistance. The resistance to amoxicillin-clavulanic acid recorded during our study is in line with the results of a survey conducted by Tang et al. 2022. Our result is particularly relevant since according to the World Health Organization, the association amoxicillin and clavulanic acid is considered a "Critically Important Antimicrobial". The second most recorded resistance, including intrinsic ones, concerned Ampicillin with 30.3%. No isolates tested positive in the ESBL+AmpC screening test.

In conclusion, our brief study shows that the animals involved for activities in Social Farming may act as carriers of potentially pathogenic agents. Thus, during the activities with animals, particular attention is recommended for the potential risk to humans. In addition, as antimicrobial resistance is a growing concern in social agriculture, monitoring the sensitivity of microorganisms to antibiotics could represent a measure to promptly identify problems related to the phenomenon.

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ANTIMICROBIAL ACTIVITY OF HEMP EXTRACT SEED OIL AGAINST BACTERIAL PATHOGENS IN DOGS

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Hemp (*Cannabis sativa*) and its extracts have been reported as potential candidates for alternative antimicrobial therapies due to their inhibitory activities on both Gram-positive and Gram-negative bacteria [1]. Here, we evaluated the *in vitro* antimicrobial activity of Hemp seed oil against *Staphylococcus aureus*, *Staphylococcus pseudintermedius* and *Pseudomonas aeruginosa*, which are considered the most relevant antimicrobial-resistant pathogens for companion animals, especially dogs [2]. The ability of the Hemp seed oil to potentiate topical antimicrobial agents used in small animal veterinary practice, namely gentamicin (GEN) and enrofloxacin (ENR) [3], was also explored. The Minimum Inhibitory Concentration (MIC) of THC-free Hemp seed oil was determined by broth microdilution for 110 clinical *S. pseudintermedius* isolates, 48 clinical *S. aureus* isolates and 16 *P. aeruginosa* isolates. The Hemp seed oil had a low concentration of CBD (0,285 mg/mL), was diluted in DMSO and tested at concentrations ranging to 0.4 to 0,007 % v/v. DMSO concentrations was 1.6%, and did not show any inhibitory activities on the strains tested. When tested alone, the Hemp seed oil showed a promising antimicrobial activity on both *S. aureus* (MIC range 0.025-0.2%) and *S. pseudintermedius* (MIC range 0.025-0.4%), while no activity was observed on *P. aeruginosa* strains (MIC >0.4%). When tested in combination with GEN in checkerboard assays, the oil-GEN combination exhibited synergy (fractional inhibitory concentration index <0.5), with a 4-fold decrease of the GEN MIC. No synergistic effect was recorded for oil-GEN and oil-ENR combinations against *P. aeruginosa*. In conclusion, while CBD and THC have been reported as the predominant agents responsible for the antimicrobial properties of Hemp extracts, our results show that THC-free Hemp seed oil with low CBD concentrations exerted antimicrobial activity against staphylococci clinical isolates. Further analysis are needed to unveil the mechanisms underlying this activity. Our preliminary finding on the potentiation of GEN activity on *S. pseudintermedius* by Hemp seed oil deserves further *in vivo* pharmacological investigation to establish the potential of Hemp seed oil as future adjunctive compound for the treatment of topical infections in companion animals.

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77° CONVEGNO SISVET

SOCIETA´ SCIENTIFICA DI RIFERIMENTO | **ASSOCIAZIONE NAZIONALE INFETTIVOLOGI VETERINARI - ANIV**

TITOLO | **PRELIMINARY EVALUATION OF LACTOBACILLI ISOLATED FROM AUTOCHTHONOUS ITALIAN CHICKEN BREEDS FOR THEIR USE AS PROBIOTICS: SUSCEPTIBILITY TO ANTIMICROBIALS AND PH RESISTANCE**

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Testo e Riferimenti bibliografici

Bacterial strains belonging to several species within the group of lactobacilli (formerly recognized as *Lactobacillus* spp.) are commonly used as probiotics due to their beneficial impacts on the host's health. These beneficial effects are based on various mechanisms, including i) antagonism against pathogens; ii) enhancement of the immune response; and iii) modulation of the microbiota balance [1]. The employment of probiotics in poultry has proven to decrease mortality rates during periods of stress, as well as in inhibit the intestinal colonization of pathogenic bacteria, such as *Campylobacter* spp., thus enhancing the safety of poultry products [2]. The present study was included in the project TuBAvI-2 (2021-2024) founded by Italian Ministry of agriculture, food sovereignty and forestry – PSRN 2014/2022. It was aimed at providing a preliminary assessment of the antibiotic susceptibility profile and pH resistance of lactobacilli isolates from chicken cloacal swabs. Two hundred and sixty cloacal swabs were obtained from different autochthonous Italian chicken breeds. Swabs were streaked onto MRS agar and plates were incubated for 48 h at 37°C in aerobic conditions. Two hundred and seven isolates were obtained and subjected to the evaluation of their susceptibility to ampicillin, tetracycline, erythromycin, streptomycin, linezolid, and gentamicin by the Kirby-Bauer method, subsequently, resistant isolates were evaluated for the presence of some of the main antimicrobial resistance genes (*tetM, tetL, ermA, ermB, ermC, aac(6')-aph(2'))* by PCR.
The higher resistance rates were observed for streptomycin (64.3%), followed by tetracycline, erythromycin, and gentamicin with rates of 15.9%, 8.2% and 4.8%, respectively. None of the isolates showed resistance to linezolid and ampicillin. Concerning isolates resistant to tetracycline, 33.3% harboured *tetM* and 12.1% *tetL*, while among isolates resistant to erythromycin 35.5% presented *ermB*. On the contrary, none of the isolates resistant to gentamicin presented *aac(6')-aph(2')*. Eleven isolates susceptible to all the antimicrobials tested were selected for further analysis [3] and identified at the species level through the sequencing of *16s rRNA* gene. After that, the pH resistance of isolates (*Ligilactobacillus salivarius, Ligilactobacillus agilis, Lactobacillus kitasatonis* and *Limosilactobacillus reuteri)* was evaluated to assess their ability to survive the gastric barrier. Isolates able to tolerate a pH equal to 2 for a minimum time of 120 minutes (*L. reuteri* and *L. salivarius)* were identified as good candidates for probiotic applications. They will be subjected to further analysis to evaluate their resistance against bile salts, hydrophobicity, ability to produce bacteriocins and to adhere to intestinal epithelia. The use of probiotics in animal productions is among the strategies that can be pursued in an integrated approach to improve animal welfare and at the same time obtain safer food. The evaluation of the susceptibility profiles of the isolates is crucial since commensal bacteria, such as lactobacilli, can serve as reservoirs for antimicrobial resistance genes potentially transmissible to pathogenic bacteria.

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77° CONVEGNO SISVET

Stato: INVIATO - ID: 13547

Whole-genome-sequencing and de novo assembly of Pseudomonas aeruginosa strains isolated from dogs and cats in Algeria

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Pseudomonas aeruginosa is a pathogenic microorganism that exhibits opportunistic behaviour, leading to various diseases in dogs and cats. These infections include Urinary Tract Infection (UTI) and Feline Lower Urinary Tract Disease (FLUDT), skin and soft tissue infections, as well as respiratory diseases. The organism is recognised for its capacity to produce biofilms, hence posing challenges in the administration of antibiotics for treatment. Based on a research carried out in Algeria from 2021 to 2022, the frequency of P. aeruginosa in the oral cavities of stray dogs and cats was found to be 1.15% and 2.3% respectively [1]. However, the real incidence of this pathogen in Algeria remains unclear. This study aims to elucidate this epidemiological gap related to the genetic characterization of P. aeruginosa in dogs and cats through Whole-Genome-Sequencing (WGS). For this purpose, 19 P. aeruginosa strains were isolated from 15 dogs and 4 cats. Phenotypic identification was obtained with VITEK® 2. The disk diffusion test was used to assess the resistance profile of each strain against the following antibiotics Netilmicin (NET), Gentamicin (CN), Tobramycin (TOB), Amikacin (AK), Levofloxacin (LEV), Ciprofloxacin (CIP), Cefepime (FEP), Aztreonam (ATM), Tetracycline (TC), Triclocarban (TCC), Ceftazidime (CAZ), Imipenem (IMP), Amoxicillin + clavulanic acid (AMC). DNA was extracted using the Quick-DNA™ HMW MagBead Kit (Zymo Research, Irvine, CA, USA) following manufacturer's instruction. Purity was assessed with Nanodrop 2000 spectrophotometer and quality via agarose gel electrophoresis (0.75%, 126 mA, 1h). The sequencing libraries were prepared using the rapid barcoding sequencing kit (SQK-RBK114.24; Oxford Nanopore Technologies, UK). Twelve barcoded samples were loaded into a MinION FLO-MIN114 R10.4.1 flow cell (Oxford Nanopore Technologies Ltd.) and sequenced into a MinION Mk1C for 72 h. Before starting each sequencing, a 1kb filter was applied to skip the shorter DNA fragments. The fast5 files were basecalled with Dorado Basecall Server 7.1.4 (Oxford Nanopore Technologies). Samples were de novo assembled using the Automatic Bacterial Isolate Assembly, Annotation and Analyses (ASA3P) Pipeline [2]. The antibiotics ATM, TC, TCC, and IMP exhibited greater resistance profiles of 5.2%, 36.8%, 15.8%, and 31.6%, respectively, based on their phenotypic characteristics. All the other antibiotics were active against the 19 P. aeruginosa strains. Genetically the metrics after the sequencing were as follow, the mean genome length was 6533056.7±225909.6 bp, with an average contig length of 2692465.3±762819 bp and an N50 value of 6139828.3± 764887.2bp; the GC% was 66.2±0.2. All the strains were successfully assembled in linear chromosomes with a maximum of 4 contigs. No plasmids were detected using specific pipelines. The following antibiotic resistance genes (all related to antibiotic efflux) were detected in all the strains analysed (bcr-1, MexB, MexR, OpmD, and TriB). blaOXA-50 and blaPDC-3 were found in 68.4% and 15.8% of the strains, respectively. However, antimicrobial resistance, virulence, and genotyping of P. aeruginosa in companion animals remain largely unexplored. This one health approach sheds light on companion animals as potential spreaders of high-risk zoonotic bacteria in low-income countries

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Characterization of a wild-type Bovine alphaherpesvirus 1 (BoAHV-1) strain in calves and water buffaloes°

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Bovine alphaherpesvirus 1 (BoAHV-1) is one of the main causative agents of different respiratory, genital diseases and other clinical conditions, such as conjunctivitis, encephalitis, endometritis, infertility, abortions and enteritis. To date, the characterization of BoAHV-1 circulating strains in Italy is poorly known. Therefore, this study aimed to characterize a wild-type (wt) BoAHV-1 strain in calves and water buffaloes. Two groups of 3 calves (group A) and 5 buffalo (group B) were experimentally infected with the wild-type strain 16453/07 TN of BoAHV-1 by intranasal route using a dose of 5 x $10^{6.74\text{-}7.00}$ TCID50/mL for each animal. Subsequently, rectal temperatures and clinical signs were monitored daily for 30 days post-challenge (PCDs). Nasal swabs for viral isolation were collected at 0, 1, 2, 3, 4, 7 and 14 PCDs. Serum samples for virus neutralization test (VN) were collected at 0, 7, 14, and 21 PCDs. The protocols used were described in the WOAH manual [1]. At the end of the experiments (96 PCDs for buffalo; 127 PCDs for cattle), all animals were slaughtered, and macroscopic and histopathological lesions were evaluated from: trigeminal ganglion, tracheobronchial lymph nodes, lung, trachea, liver, spleen, and intestine. In addition, the virus was characterized using whole-genome sequencing (WGS). In group A, 2 calves showed mucous exudate, dyspnea, and cough at 4 PCDs, while in group B 3 buffalo evidenced the same lesions at 7 PCDs. Moreover, they evidenced pseudomembranes in calves from 4 to 8 PCDs and buffalo at 7 PCDs, respectively. Rectal temperature increased in group A up to 41.0-41.5°C from PCD 2 to PCD 8, and in group B up to 38.7°C from PCD 2 to PCD 5. In both groups, virological investigations showed positivity from 2 to 7 PCDs. In group A, the animals seroconverted to BoAHV-1 after 7 PCDs (1.35 $\log_2$), and the NA titer progressively increased up to 21 PCDs (mean titer of 3.16 $\log_2$). Otherwise, in group B the NA titer was detected after 15 PCDs, which increased reaching a value of 1.63 $\log_2$ on PCD 30. Macroscopically, in group A, the calves showed acute tracheitis associated with lymphocytic tracheitis, sometimes with karyorrhexis. In group B, the water buffalo exhibited acute or chronic lung lesions associated with severe necrotic lymphocytic tracheitis and lymphocytic-eosinophilic enteritis. Using WGS, a complete genomic sequence was obtained and deposited in GenBank (accession Number: OR211605), and phylogenetic analysis revealed a nucleotide identity >99% with all BoAHV-1 strains belonging to subtype 1.1, highlighting the genetic stability of the virus. In this study, we characterized a wt BoAHV-1 *in vivo* in calves and buffalo. The results demonstrated that the virus was highly virulent for both animal species, inducing clinical signs and anatomopathological lesions typical of BoAHV-1 infection. The findings of this study are in accordance with other manuscripts [2-3].

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77° CONVEGNO SISVET

Stato: INVIATO - ID: 13554

Efficacy of High-Pressure Processing on different tissue homogenates African Swine Fever virus artificially contaminated.

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African Swine Fever (ASF) is a viral disease belonging to the family Asfarviridae that growing threat to the global swine industry [1]. The infection is transmitted by direct and indirect contact with infected domestic and wild pigs, ingestion of products originating from infected and/or contaminated pork, and contact with contaminated surfaces and fomites acting as mechanical carriers [2]. The disease's ability to persist and spread is therefore a function of the virus's resistance to physical (temperature), chemical (pH), and the type of biological and non-biological matrix in which it is carried. High-Pressure Processing (HPP) is an innovative sanitization technique based on the application of hydrostatic pressures significantly higher than atmospheric pressure (up to 600 MPa), achieving the inactivation of present microorganisms [3]. Currently to limit the spread of the virus, long and expensive measures and restrictions on swine movements have been placed. This study aims to determine if HPP would be effective against ASF virus (ASFV). In particular, we hypothesized that HPP could inactivate or reduce the ASFV infectivity in tissue homogenates. To test this hypothesis, we infected by Turin/83 strain of ASF, at a dose of 10 7.20 median haemadsorption dose (HAD)50/mL, 30 aliquots of each homogenate (spleen, kidney, loin). Then, we treated eight aliquots of each homogenate by HPP (600 MPa) at three different times (3, 5, and 7 min). Six untreated with HPP aliquots were used as negative controls. Subsequently, each aliquot was used for viral isolation tests using the Malmquist test and the protocol described in the WOHA manual. Virological results showed 7-log/mL reduction in the viral titer treated with ASF at 600 MPa with 3, 5 and 7 min hold times. Regarding the hypothesis, our results demonstrated that HPP treatment was effective in inactivating ASFV in artificially prepared samples and so it is a good sanitizing method for products contaminated with ASF, even with short exposure times. This preliminary research suggests the need for further investigation to verify the efficacy of HPP treatment on different ASFV-contaminated matrices, particularly in processed cured meat products.

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77° CONVEGNO SISVET

SOCIETA´ SCIENTIFICA DI RIFERIMENTO **ANIV**

TITOLO **PET TORTOISES: FRIENDS OR ENEMIES?**

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Testo e Riferimenti bibliografici

The popularity of reptiles as pets is increasing due to their diverse range of species, which includes turtles, snakes, and lizards [1]. However, it is important to consider their potential role as vectors for transmitting pathogens to humans, which represents a significant public health risk. There are multiple factors that influence this risk in a multifaceted manner. One of the main aspects is the close association between humans and their reptile pets. Exposure to these pathogens can occur when touching reptiles, cleaning their habitats, or even indirectly touching surfaces they have touched. The global trade in exotic animals, including reptiles, may introduce an additional layer of complexity that is further complicated by illegal imports that can contribute to the propagation of new pathogens and the spread of infections among animals [2]. The aim of this study was to determine the prevalence of pathogenic and zoonotic microorganisms in tortoise kept as pets and to assess their antimicrobial susceptibility. For this purpose, cloacal swabs were collected from 53 pet tortoises and analyzed by cultural methods and identified using Matrix-Assisted Laser Desorption Ionization– Time of Flight (MALDI-TOF). The disk diffusion method was used for testing antimicrobial susceptibility for the following antibiotic classes: penicillins, cephalosporins, monobactams, aminoglycosides, tetracyclines, fluoroquinolones, sulfonamides, phenicols, and nitrofurans. The results highlighted a high occurrence of *Salmonella* spp. (32.1 %), followed by *Klebsiella* spp. (28.3 %), *Escherichia coli* (18.9 %), and *Enterobacter* spp. (17.0 %). Out of the total of 17 *Salmonella* strains, n= 8 (47.0%) were identified as *Salmonella* Abony 4,12:b:e,n,x, and n=6 (35.3 %) as *Salmonella enterica* subsp. *salamae*. Additional serotypes, seldom or never reported in reptiles, such as *Salmonella* Corvallis 8:z4,z23:- (n=1, 5.9%), *Salmonella* Mikawasima 6,7:y:e,n,z15 (n=1, 5.9%,) and *Salmonella* Ferruch 8:e,h:1,5 (n= 1, 5.9%) were also detected. All isolates exhibited frequent resistance to ampicillin (56.6%), piperacillin (38.5%) and piperacillintazobactam (33.7%). This study demonstrates and confirms that pet tortoises serve as a significant reservoir of zoonotic pathogens, especially Salmonella spp., and may act as spreaders of antimicrobial-resistant bacteria. This study is not meant to limit the use of tortoises as pets but suggests following strict hygiene and biosafety measures to reduce the risk of infection when adopting a reptile.

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77° CONVEGNO SISVET

Stato: INVIATO - ID: 13582

Preliminary testing of incoming cats to evaluate the risk of virus introduction: a short-term survey on selected feline enteric viruses in an Italian municipal shelter

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The growing numbers of stray and free-roaming cats, along with the increased social and legal interests in their welfare and health in urban settlements, continuously pose new challenges. According to the current Italian regulatory framework, an ever-increasing number of stray or colony cats are introduced into public health facilities for the necessary cares. Preventing, controlling, and managing feline viral infectious diseases are thus continuous challenging tasks in animal shelters [1,2]. Limited previous studies were focused on viral outbreaks in cats already hosted in shelters but a focus in incoming cats still appears necessary to positively improve sanitary management strategies.
To evaluate the risk of introduction of feline panleukopenia virus (FPV) and feline coronavirus (FCoV) in a leading regional municipal shelter (Palermo, Sicily region, southern Italy), rectal swabs from 117 colony or stray cats were collected from March to July 2023 at their incoming and analysed. A set of classical PCR/RT-PCR assays, sequence and phylogenetic analyses were performed. To determine associated risk factors for virus introduction, background information of each cat (origin: stray/colony; age group; gender and neutering status; reasons for being admitted to the shelter; clinical signs) was evaluated. Statistical analysis was performed using Jamovi ver.2.3.28 software, Fisher's exact test as statistical significance test, and values of $p<0.05$ were considered as significant.
Overall, FPV DNA and FCoV type I RNA were detected, randomly scattered over the considered timeframe, from 26 (22%) and 42 (36%) cats, respectively, either in single (FPV: 12%, 14/117; FCoV: 26%, 30/117) or mixed (10%, 12/117) infections. Sequence and phylogenetic analyses showed a high nucleotide identity with FPV and FCoV strain sequences previously collected in Sicily and other Italian regions [3]. With only one exception, all tested FCoV positive cats did not show the amino acid substitutions M1058L and S1060A in the Spike gene sequence. Presence of FPV and FCoV was significantly ($p<0.05$) more likely in cats older than 6 months or younger than 3 months, respectively. Other considered risk factor did not differ significantly. Albeit not statistically supported, positivities were also detected in apparently healthy cats or with clinical signs not limited to the gastroenteric tract. These results show that free-roaming cats admitted to shelter for various reasons, sometimes unrelated to only clinically apparent gastroenteric signs, or apparently clinically healthy may represent a not under estimable potential carriers of virus introduction in shelters. As management of FPV and FCoV positive cats in the shelter settlings is critical, this evidence contributed with additional baseline data to implement management strategies.

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77° CONVEGNO SISVET

Stato: INVIATO - ID: 13604

Wild Birds as Potential Bioindicators of Environmental Antimicrobial Resistance: A Preliminary Investigation

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Antimicrobial resistance (AMR) is an issue of global concern that includes human, animal, and environmental health. From a One Health perspective, it is crucial to investigate this phenomenon through the involvement of all these interconnected elements [1]. The environment poses challenges for investigation, but wildlife animals, not directly exposed to antibiotic treatments and interacting with their habitats, can serve as indicators of AMR contamination [2]. Specifically, wild birds could play a significant role in dissemination of AMR, as they can acquire AMR bacteria from wildlife reservoirs and disperse them through environments [3]. This study aims to assess the prevalence of AMR in commensal bacteria isolated from wild birds and to investigate their role as bioindicators of environmental AMR. Samples collection took place between November 2022 and June 2023 in two different sites: i) the bird ringing station in Maranello, in the province of Modena, Italy; and ii) the wild animal recovery centre of the Italian League for Bird Protection in Bologna. A buccal and cloacal swab were performed on each bird. Only birds that did not receive any antibiotic treatment in the previous 90 days were sampled. Additionally, for each subject, a comprehensive form was filled out, reporting essential data relating to the bird's signalment and the sampling collection. Samples were cultured on selective media, colonies were identified using MALDI-TOF technology and antimicrobial susceptibility to different drugs was assessed using the Kirby-Bauer method. Birds' data were statistically evaluated in relation to AMR percentages. A total of 73 birds belonging to various species were sampled and 117 bacterial strains were isolated, belonging to 23 genera and 46 different bacterial species. Among the bacterial isolates, 58/117 (49.6%) showed sensitivity to all antimicrobials tested, 59/117 (50.4%) were AMR and 12/59 (20.3%) were multi-drug resistant (MDR). The highest non-susceptibility percentages were observed for tetracycline (12.2%) and enrofloxacin (8.6%) considering all bacterial isolates, as well as for oxacillin (46.8%), clindamycin (29.3%) and rifampicin (20.8%), among Gram-positive isolates. In the statistical analysis, a higher AMR percentage was correlated with isolates from birds belonging to rural/urban habitat (p=0.031). Additionally, higher oxacillin and enrofloxacin non-susceptibility percentages were found to be associated with rural/urban habitat (p=0.009; p=0.002) and with the age of birds, specifically pullets (p=0.015; p=0.011); oxacillin non-susceptibility percentages resulted associated with the sampling province of Bologna (p=0.003); while higher rifampicin non-susceptibility percentages resulted associated with isolates from migratory birds (p=0.031). In conclusion, this preliminary study explores the relationship between wild birds and environmental AMR, revealing resistance towards some antimicrobial drugs commonly use in human and veterinary medicine and highlighting a correlation with the rural/urban habitat, more exposed to sources of contamination. These findings suggest a potential role of wild birds as bioindicators for monitoring AMR contamination in the environment, but further studies will be necessary to assess the phenomenon on a larger scale, such as expanding the sample size, identifying additional patterns of antimicrobial resistance and investigating other associated risk factors.

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77° CONVEGNO SISVET

Stato: INVIATO - ID: 13695

Student competition "Microbiology can be…2023" third edition and lessons learned

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The European Association of Establishments for Veterinary Education encourages the development of communication and teamwork skills in veterinary education to enhance student success in clinical practice and scientific communication for public health. Based on this, and considering the growing need to improve health literacy, especially in effectively communicating complex concepts, particularly in infectious diseases and vaccination (as evidenced during the COVID-19 pandemic [1,2]), a project aimed at training students to develop innovative approaches to scientific and technical communication was organized. Since veterinary medicine students, even in their first years of training, possess sufficient knowledge to convey messages to society, this study reports the results and final considerations of a student competition incorporated into the veterinary curriculum to enhance health literacy in microbiology and infectious diseases. Over three events held between 2021 and 2023, third-year veterinary students voluntarily participated in groups (teams) to develop novel training and informational resources. In the third edition (2022-2023), 40 students out of 71 attending the third year (8 out of 14 teams) participated in the initiative, resulting in a participation rate of 56.3%, producing three comics, three videos, and two games. Considering all three editions, a total of 125 students created 22 projects, including drawings, comics, games, and videos, aimed at enhancing health literacy. The competition promoted creativity and innovative approaches among the participants by challenging them to develop novel resources that could deliver informative content to the public regarding microbiology and infectious diseases. This approach allowed students to interact with the content and convey foundational knowledge to others in an easily accessible manner [3].

1 Student team: Antonino Blandino, Francesca Boscaro Tenenti, Ilaria Botteri, Jacopo Capitini, Erica Capoferri, Giulia Claudia Corsin, Ylenia D'Alfonso, Francesco De Angelis Corvi, Benedetta De Luca, Miriam Del Papa, Leonardo Faccioli, Laura Falsetti, Alessia Favaro, Eliana Fernandez, Gioia Sofia, Grassi Lorenzo, Guacci Francesca, Guidi Beatrice, Ibba Melania, Chiara Liti, Gianluca Lopez, Carola Lozza, Giulia Miccioni, Edoardo Moisè, Riccardo Mori, Elena Moroni, Camilla Muzzini, Viola Nesti, Edoardo Pacini, Danilo Parolisi, Francesco Pecorari, Anita Pivato, Allegra Livia Prelazzi, Ilaria Radicchi, Sara Schiavone, Rossella Speranza, Diletta Tarquini, Alexa Tasini, Beniamino Tiriduzzi, Iris Tommassini, Beatrice Zambrano.

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77° CONVEGNO SISVET

Stato: INVIATO - ID: 13712

Change in biosecurity levels in dairy buffalo farms from 2018 to 2023 assessed by ClassyFarm check list

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Biosecurity in ruminant production is often an overlooked area, instead it a key aspect of the interactions among the animal production systems, the environment, and human health in a One-Health approach. ClassyFarm is an integrated system for categorizing farms according to a risk assessment methodology. The principles relating to the provisions in terms of Biosecurity in Regulation (EU) 2016/429 of the European Parliament, require reflection on the critical issues and possible mitigation actions that can be planned and possibly implemented in this sector. ClassyFarm gathers and processes data referred to biosecurity, animal welfare (AW), health, and antimicrobial usage. It can be applied to several livestock species, including water buffalo, for which biosecurity is evaluated across 15 items. Each item can be scored as ‘insufficient’, ‘acceptable’ or ‘optimal’. For each item, a weight was determined using an expert opinion elicitation process. Total and partial scores are expressed in percentage on a scale from 0 to 100%.In Caserta province using this tool, in 2018 and in 2023 evaluation was carried out on 216 and 475 buffalo farms respectively, in order to survey the levels and main critical issues detectable through a check list and to observe the change of farm conditionsThe Value of biosecurity in the period (2018-2023) increase from levels 44.6% to 65.4%. In particular, the frequency of insufficient responses decreased from 38.72% to 11.14%. Conversely, acceptable ratings increased from 48.84% to 65.46%, and optimal ratings rose from 12.43% to 23.4%. The items on which there was the greatest reduction in the frequency of negative threshold were as follows: absence of quarantine area; potential contact with other animal species due to lack of fences; loading and unloading areas for live animals located close to the stable areas; absence of disinfection devices for foreign vehicles; possibility of contact between foreign vehicles and animals bredThe data highlights how public and private awareness-raising activities, coupled with the receptivity of the sector, have led to a clear improvement in overall biosecurity levels. It is noteworthy that the primary enhancements primarily addressed structural aspects. However, while these are necessary, they are not sufficient for achieving effectiveness unless accompanied by management improvements facilitating effective awareness and monitoring of the farm's biosecurity status.

77° CONVEGNO SISVET

Stato: INVIATO - ID: 13731

Phenotypical and genotypical characterization of piperacillin-tazobactam resistant clinical isolates from companion animals in an Italian Veterinary Teaching Hospital

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Antibiotic resistance (AMR) is a major threat to human and animal health. The role of companion animals in the spread of AMR is still largely unkwown, but potentially relevant given the close contact with humans and the high proportion of shared antibiotics [1]. Pets can become source of bacteria resistant to last-resort antibiotics, such as carbapenems or piperacillin-tazobactam (TZP), with a subsequent risk to develop multi-resistant infections and to spread AMR to humans. This work aimed to describe the resistance mechanisms involved in resistance to TZP in clinical Enterobacterales isolates from companion animals attended at the Bologna Veterinary Teaching Hospital (VTH), from 2020 to 2022, in collaboration with the Antimicrobial Resistance Unit of the Veterinary Medicine Department at the Complutense University, Madrid, Spain. Thirty-two Enterobacterales clinical isolates from different specimens of dogs and cats that showed phenotypical resistance to TZP were selected. Isolates were identified with MALDI-TOF and TZP resistance was assessed through the disc-diffusion method. Subsequenlty, the complete resistance profile towards last-resort antibiotics was evaluated using Minimum Inhibitory Concentration (MIC) with Sensititre GN7F plates. Isolates with TZP resistance levels higher than 64/4 mg/l were further evaluated with broth microdiluition. Additionally, considering epidemiological data and the resistance profile, ten isolates were selected for Whole Genome Sequencing (WGS) by Illumina and Nanopore technologies. Twenty-two out of thirty-two isolates (68.8%) exhibited levels of TZP resistance higher than 64/4 mg/l. Resistance to a wide variety of critically important antibiotics, including human-reserved potentiated penicillins such as ceftolozane-tazobactam (17/32, 53.1%), ceftazidime-avibactam (11/32, 34.4%), and carbapenems such as imipenem (9/32, 28.2%) and ertapenem (15/32, 46.9%), was observed. The ten isolates submitted to WGS revealed the presence of a wide number of various resistance genes, including blaCTX-M-15, blaOXA-1, blaTEM, blaSHV and blaCMY-2. Additionally, three isolates with TZP resistance higher than 1024 mg/ml (two Klebsiellla pneumoniae and one Escherichia coli), were found to possess the blaNDM-5 gene (pandemic high-risk carbapenemase-enconing gene) found in an IncX3 plasmid. Notably, these isolates were all from suspected healthcare-associated infections, suggesting an intra-hospital spread of the plasmid. This work highlights the potential importance of pets ar reservoirs of AMR, including for antibiotics critical for humans and not allowed in veterinary medicine. The results suggest the possibility of a selection of resistance due to the use of piperacillin-tazobactam and other potentiated penicillins, with a subsequent clonal and plasmidic spread over time. Furthermore, the hypothesis of a primary reverse zoonosis should be considered.

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ARNA

Effect of *Limosilactobacillus reuteri* DSM 32264 NBF2 supplementation on the intestinal microbiota and fecal parameters of healthy Scottish cats

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The present study evaluated the effects of *Limosilactobacillus reuteri* DSM 32264 NBF2 (formerly known as *Lactobacillus reuteri* DSM 32264) on healthy Scottish cats, focusing on the "positive effect on the intestinal microbiota" and fecal parameters. The research was conducted in accordance with the directive 2010/63/EU. Ten healthy adult Scottish cats, male and non-pregnant female, were randomly assigned to the control group (CTR; n=5) and the experimental group (LACTO; n=5). The commercial diet used in the experiment was Royal Canin FIT 32; specifically, the CTR group diet was supplemented with maltodextrin (used as a placebo) while the LACTO group diet was supplemented with *L. reuteri* DSM 32264 NBF2 (5 x 10^9 CFU/kg of food). Before starting the experimentation, an antiparasitic treatment was carried out using drugs without antibacterial effect. Cats were assessed daily by a veterinarian for any health and welfare problems throughout the experimental period (two weeks of acclimatization + 35 days of study). Body weight (BW) and Body Condition Score (BCS) [1] of each cat were assessed at 0, 7, 14, 21, 28 and 35 days after the additive administration, all analyzes were performed in double. Fecal consistency and the identification of specific gastrointestinal bacteria have been considered indicators of cat health status. The fecal score (FS) was assessed using a 7-point table, then the percentage of fecal moisture (FM) was measured. For microbiological analysis, an aliquot of fresh feces (1 g) collected from each cat was diluted in sterile saline solution and vortexed for 2 minutes to obtain a homogeneous suspension and were streaked on different culture media for bacterial identification (Lactobacilli and coliforms) and count. For statistical analysis, a mixed model with repeated measurements was used [2]. All cats remain healthy during the study, BW and BCS did not change during the study in either group, the animals maintained ideal body condition. FM was significantly lower at the end of the study in the LACTO group compared to the CTR group (p=0.0001); the beneficial effect of *L. reuteri* DSM 32264 NBF2 administration was also confirmed by the fecal score (FS) values recorded between the two groups of cats. At the end of the experimental period, there was a significant increase in Lactobacilli in the experimental group (LACTO) compared to the control group followed by a slight decrease in the total amount of coliforms (p=0.0006). The data collected in this study report the ability of the probiotic *L. reuteri* DSM 32264 NBF2 to improve fecal quality parameters such as FM and FS in healthy cats. The fecal score at the end of the treatment reported a significant decrease of approximately 0.90 points in the group treated with *L. reuteri* DSM 32264 NBF2 accompanied by a decrease in fecal moisture, making the stool more consistent and well-formed, an indication of good intestinal health. The increase in Lactobacilli once again confirms the ability of this probiotic to improve the composition of the intestinal ecosystem, favoring an increase in beneficial species.

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77° CONVEGNO SISVET

SOCIETA´ SCIENTIFICA DI RIFERIMENTO ARNA

TITOLO **HYPP and PSSM concomitance in Horses: case report of a nutritional composed approach**

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Testo e Riferimenti bibliografici

American paint and quarter horses are prone to be affected by five genetic conditions, comprehending hyperkalemic periodic paralysis (HYPP) and type 1 polysaccharide storage myopathy (PSSM) amongst others [1].
A ten-year-old Paint horse gelding, and weighting 420 kg, was referred to the Veterinary Clinical Nutrition Service of the University of Turin, for a nutritional consult. Over a three-year period, the animal exhibited mild episodes of muscular tremors, staggering, dog sitting, and recumbency. The horse was housed in a single box with straw bedding and had daily outings in the paddock during winter, while exclusively in the paddock during summer. The animal consumed roughly 6 kg of first cut mixed and 1 kg of oat a day, divided in two feeds and employed daily in trekking and equestrian school, for a total of 5-6 hours/week of workload.
Under the supervision of the attending veterinarian, the animal underwent PCR testing for HYPP (Laboklin, GmbH & Co. KG) and hematobiochemical analysis. The horse resulted heterozygous carrier for N/H genotype for HYPP, but no blood and metabolic parameters' alteration were found. Due to the non-specificity of the symptoms and the lack of clear hematobiochemical indications, PSSM could not be definitively excluded, and genetic or biopsy analysis was recommended. A specific therapeutic nutritional plan was formulated to assist and prevent the onset of clinical symptoms, considering both HYPP and PSSM. The diet was formulated to meet specific requirements (3.8 UFC and 288 g MADC) and balanced using a specific horse-formulating software, divided into three meals. The use of 8kg first-cut hay was recommended, to be fed after 40 mins of soaking to partially loose non-structural carbohydrates and minerals [2] and fed in a double hay net to prolong feeding time. A commercial feed, low in starch (8%) and sugar (6%) was prescribed as 750 g per day, along with a Vit-Min balancer rich in Vit E, selenium, and omega 3-fatty acids, as 150 g per day. The total potassium concentration of the concentrate feed was 10.8 g per day; it is suggested to limit 33 g of potassium per day in HYPP cases [3]. Additionally, 10 g per day of sodium chloride was recommended. A complete Near Infrared Spectroscopy (NIR) and potassium analysis for different forage batches were advised to avoid the use of highly potassium-rich hays.
Fresh pasture is recommended for animals with HYPP, as the potassium concentration in higher-humidity forages is lower than in dry hays; on the other hand, fresh pastures are contraindicated in PSSM due to their high level of fermentable carbohydrates and sugars. Therefore, pasture use was limited to a few hours per day, with particular attention to new fields in spring and morning hours. Daily exercise is reported to be a key factor both in HYPP and PSSM, and therefore it was suggested to maintain the same workload, while avoiding work during the postprandial hyperkalemic spike. The use of sweet nutrients was to be avoided, while ensuring ready access to a highly glycemic feed to counteract mild hyperkalemic crises. In conclusion, prescribing an appropriate diet is feasible, but management issues must be addressed to avoid exacerbating either condition.

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77° CONVEGNO SISVET

Stato: INVIATO - ID: 13206

Evaluation of the impact of the dietary inclusion of salty and sugary former food products on the liver and the plasma profile of pigs through OMICS approaches

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Replacing human-edible feedstuffs, such as cereals, with food leftovers in pig nutrition is a valid way to reduce feed-food competition between humans and animals, reduce the waste biomass, and keep nutrients and energy in the food chain. Former food products (FFP) are industrial food leftovers no more intended for humans but still legally suitable for animals as alternative and sustainable sources of nutrients and energy, especially for monogastric [1]. After observing that FFP were not detrimental for the growth performance and the feeding behaviour of piglets and pigs, in this study we aimed to evaluate the metabolic impact of FFP on pigs by replacing 30% of conventional cereals with salty or sugary FFP in the diet of adult pigs through the application of label-free mass spectrometry (MS)-based approaches (i.e. proteomics and peptidomics) on liver tissue and plasma samples. Thirty-six Swiss Large White male pigs were assigned to three dietary groups [control (CTR), 30% CTR replaced with salty FFP (SA), 30% CTR replaced with sugary FFP (SU)] from the start of the growing phase (22.4 ± 1.7 kg) until slaughtering (110 ± 3 kg), after which liver and blood samples were collected. For proteomics, a nano-liquid chromatography (LC)-High Resolution MS was performed [2], whereas for peptidomics a nano-high performance LC coupled to MS-MS analysis was performed [3]. The proteomics investigation identified 2881 proteins and showed that the SA and SU diets led to a low number of significantly modulated proteins (125), of which the most relevant were related to hepatic lipid metabolism and reorganization of the cellular structure, but the metabolic interaction among the modulated proteins was null, thus indicating a limited impact on liver function and related pathways by SA and SU diets. The peptidomics investigation identified 122 peptides showing a low intra-group variability (<10%). No relevant differences among the peptidomes of the three dietary groups were observed, but by searching for potential bioactive peptides, three peptides associated with anti-hypertension action and vascular homeostasis regulation were exclusively identified in the SA group, thus suggesting that the SA diet could have triggered an endogenous self-regulating action to counteract the damage related to the higher Na content of the SA diet. Overall, the limited impact of SA and SU FFP on the modulation of liver proteome and related pathways and plasma peptidome supported the idea of recycling FFP as alternative feed ingredients to increase the sustainability of pig production and reduce the use of human-edible feedstuffs.

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77° CONVEGNO SISVET

Stato: INVIATO - ID: 13223

Unveiling the Mechanisms Behind Methane Emission Variability in Cows: Exploring High and Low Emitters

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Enteric methane is primarily generated in ruminants during enteric fermentation to eliminate CO2 and H2 from the rumen [1]. The development and recent implementation of new smart sensors for accurately predicting CH4 emissions in precision livestock farming have opened up new scenarios in the last few years. However, these emissions are well-known for their significant variability, attributed particularly to individual production, diet, sampling time, and cows' activity. This experiment aimed to study enteric methane emissions during automatic milking (AMS) using a portable laser detector in dairy cows. Forty homogeneous Italian Holstein dairy cows were enrolled in the trial and grouped into five groups of eight cows each, fully acclimated to the tie stall area over one week. Dry Matter Intake (DMI) was monitored, and the diet was kept constant. Cows were milked at specific time points: 6 am, 2 pm, and 10 pm. As each milking group comprised only eight cows, the maximum waiting period before milking was 50 minutes. Methane was measured for each cow during the last three milkings before group change. The laser was pointed at the cow's muzzle during the milking process. Data was collected in ppm/m and converted to grams/day [2]. Milk and feces samples were collected on the last three days of the trial and analyzed for composition. Statistical analysis was performed using cluster analysis (high and low emitters, HM, n=6, LM, n=34), followed by a mixed model. Results indicated different methane production levels in HM and LM cows, 503 vs 364 g/day ($P < 0.05$). No differences were observed in body weight (647 kg), lactations (n=1.5), DIM (n=146), milk yield (39.8 kg), and components (fat 3.2%, protein 3.1%, lactose 4.9%). The PMR intake was higher in LM cows (22.68 vs 21.04 kg, $P = 0.09$), while AMS concentrate intake was constant (4.55 kg). There were differences in the milk fatty acid profile ($P < 0.05$), including a higher proportion of branched forms (C16iso and C17iso), conjugated linoleic acid (C18:1c13), eicosanoic acid (C21), and ω3 (C20:3n3; C22:5n3). Fecal analysis showed higher ($P < 0.05$) pH (6.37 vs 6.20), lower residual starch (1.17 vs 1.45%), ADL (17.4 vs 16.8), and uNDF (37.5 vs 35.0%) in HM cows. Additionally, after calculating Total Tract Digestibility (TTD), HM cows exhibited higher ($P < 0.05$) TTNDFD (+12%) and TTuNDFD (+11%). These findings confirm that fiber digestibility is crucial in ruminal methane biosynthesis, with HM cows showing greater capability in digesting the fibrous part of the diet. Moreover, higher fecal pH and changes in milk fatty acid profiles suggest a more stable ruminal condition favoring cellulolytic bacteria. Indeed, HM cows were more efficient in extracting energy from the diet's fibrous part than LM cows, which consumed more PMR to produce a similar amount of milk. These results have significant implications for the modern concept of feed efficiency and sustainability, although they require confirmation with a larger number of animals, more intensive sampling protocols, and alternative methane detectors.
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77° CONVEGNO SISVET

Stato: INVIATO - ID: 13375

VALORISING UNSOLD SUPERMARKET PRODUCTS THROUGH BLACK SOLDIER FLY LARVAE REARING

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Insects have the potential to play a significant role in food waste reduction, by utilizing resources that are currently unused. This can contribute to increasing the sustainability of the agri-food system, and Hermetia illucens (Black Soldier Fly, BSF) can be a particularly useful species. In this study, the effects of different rearing substrates derived from unsold supermarket products (fruits, vegetables, cheeses, and yoghurts) on BSF larvae were assessed. Three diets were formulated as follows: 1) 100% ingredients of plant origin (fruits + vegetables, used as control); 2) 75% plant origin 25% cheese (C25); 3) 75% plant origin 25% yoghurt (Y25). The experimental rearing of BSF larvae started by placing BSF eggs in nursery trays (control substrate). Larvae were reared on the control diet until day 10. Then the larvae were divided into three different groups based on the experimental diets (control, C25, and Y25) and reared until the prepupal stage. The larvae weights were recorded to calculate growth performances and conversion indexes (T10-T13-T15-T17-T20-T22). Substrates, larvae and frass were analysed for dry matter (DM%), crude proteins (CP%), crude fats (CF%), and ash%. Chemical composition analysis revealed that C25 substrate had the highest DM% (16.68), CP% (4.19), CF% (0.13), and ash% (1.09) contents, followed by Y25 and control substrates (DM% 12.69, CP% 2.79, CF% 0.02, ash% 0.86 and DM% 10.19, CP% 1.53, CF% 0.004, ash % 0.87, respectively). The results showed that BSF larvae reared on C25 and Y25 needed less time to reach the prepupal stage (20 days) in comparison to larvae reared on the control substrate (22 days). The final weight of the larvae was 97.58 mg, 111.54 mg and 133.36 mg respectively for control, Y25 and C25 larvae. Larvae chemical compositions showed that control larvae had the lowest ($P<0.05$) values for DM% (19.97), CP% (9.83), and CF% (1.58). C25 larvae had the higher DM% (29.59) and CP% (15.88) content followed by Y25 (DM% 23.88 and CP% 11.75). On the other hand, Y25 larvae had the higher CF% (9.52) followed by C25 (CF% 4.18). Significant small variation was also detected in larvae ash% (ranged between 2.26 to 2.36). Interestingly, the CF% of the larvae was not correlated with the substrate's CF%. Indeed, Y25 larvae showed a higher content of lipids than C25 larvae. Frass results followed the proximate composition of the respective substrate. The waste reduction index of control larvae 3.89 was lower ($P<0.005$) than Y25 and C25 larvae (4.05 for both). The efficiency of conversion of ingested food (ECI) index differed between control larvae (0.20) and C25-Y25 larvae (0.28, $P<0.05$). The feed conversion ratio (FCR) calculated on wet basis and frass corrected were lower ($P<0.05$) for Y25 (3.58) than control (4.97) and C25 (5.11) larvae. The N-ECI frass and biomass corrected statistically differed between control larvae (2.73) and C25 (1.26). Considering the high quantity of water in the substrates composed of vegetables, fruits, and dairy unsold products, BSF larvae showed a high capability to bio-convert them into valuable animal products. Noteworthy the chemical composition of the larvae and the growth performances were highly affected by the diets.

77° CONVEGNO SISVET

Stato: INVIATO - ID: 13467

Effects of Ascophyllum nodosum or Arthrospira platensis in dairy cows at the onset of lactation on performance and immunometabolism

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Algae products can have immunomodulatory effects and could improve the adaptation of dairy cows to the new lactation. In this study, the effects of feeding algae on feed intake, milk production, and immunometabolic profile were investigated. 30 Holstein multiparous cows either received during the first 14 days in milk (DIM): (i) a control diet (CTR, n=10), (ii) the control diet plus 200 g/d of Arthrospira platensis (spirulina; SPI, n=10), or (iii) 100 g/d of Ascophyllum nodosum (ASC, n=10). The study was authorized by the Italian Health Ministry (n° 1002/2023-PR). Feed intake, rumination time, and milk yield were monitored daily up to 28 DIM. Blood samples were collected to assess metabolic profile at -7 (as baseline value), 3, 14, 21, and 28 DIM. Methane emissions were monitored during (0-14 DIM) and after supplementation (15-28 DIM) with the GreenFeed system on a subset of cows. Data were analyzed with repeated measures mixed models, with prepartum data as covariates. Dry matter intake, rumination, milk yield and composition were unaltered, but SCC tended to be lower in ASC and SPI than CTR (P=0.08). Milk protein yield was reduced at 7 DIM in ASC (P=0.03). No evidence for group differences was found for methane production, yield, and intensity, likely because of the low sample size and the high variability typical of the first month of lactation. Only minor differences were noted in metabolic profile. Of note, P tended to be lower in ASC than in other groups (P=0.07). Total protein was lower in algae groups compared with CTR (P=0.04). Aspartate aminotransferase was reduced at 7 DIM in SPI (P=0.01). Nonesterified fatty acids concentration was lower at 14 DIM in ASC (P=0.02), β-hydroxybutyrate tended to be lower in CTR compared with supplemented groups, and creatinine was reduced in ASC and CTR compared with SPI (P=0.03). Overall, these results suggest limited but positive impact of algae in this phase, but studies with different doses and timing of supplementation are required to understand the mode of action of these products and the possible effects on gaseous emissions.

77° CONVEGNO SISVET

SOCIETA´ SCIENTIFICA DI RIFERIMENTO **UNIVERSITÁ DEGLI STUDI DI MILANO**

TITOLO **EVALUATION OF ANTIOXIDANT AND BACTERIAL GROWTH INHIBITORY ACTIVITIES OF NUTRACEUTICAL PRODUCT CRANBERRY BASED (KP) ON *E. COLI* ISOLATED FROM URINE OF CAT AND DOG WITH RECURRENT URINARY TRACT INFECTION**

Autori

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Testo e Riferimenti bibliografici

Bacterial infections are the main cause of urinary tract diseases in dogs and cats causing inflammatory conditions associated with clinical signs. For these reasons, antibiotic therapy is the most common treatment for bacterial urinary infections [1]. However, improper use of antibiotics exposes the health of dogs and cats to several risks: a failure to resolve the infection, recurrent episodes of urinary tract disease, such as recurrent cystitis, and a possible antibiotic resistance onset [2]. Considering that, the use and administration of nutraceuticals could play a key role in inhibiting microbial growth and limiting the misuse of antibiotics in recurrent urinary infections [3]. The aim of this study is to evaluate the antioxidant and microbial growth-inhibiting characteristics of a cranberry nutraceutical product (KP) against *Escherichia coli* strains isolated from urine of cat and dog with recurrent bacterial infections. The antioxidant activity was evaluated by ABTS Radical Cation Decolorization Assay tested at different concentrations: from 1:1 up to 1:800 of KP, from 1:1 up to 1:64 for *in vitro* digested KP, both in duplicate. The results confirmed an antioxidant activity among the different dilutions even in digested then in non-digested nutraceutical. The non-digested KP reached up to 90% of inhibition, the digested product around the 50%. To clarify the molecules responsible for antioxidant activity, Total Polyphenolic Content and Flavonoid Content Assays were performed in triplicate considering the following dilutions: from 1:1 up to 1:800. The Polyphenolic Content was 617 mg TAE/g of sample, the Flavonoid Content was 325 mg CE/g of sample. The pathogen inhibitory growth capacity was assessed by a micro-plate inhibitory microbial growth assay lasted 6 hours considering different concentration of KP (from 1:1 up to 1:128) in quadruplicate. The outcomes underlined a growth inhibitory effect of the product up to 1:4 against feline urinary *E. coli* for 4 hours ($p < 0.0001$). The results on *E. coli* from dog showed inhibitory effect of KP 1:1 ($p < 0.0440$) and 1:2 ($p < 0.0001$) for 6 hours and inhibitory effect of KP 1:4 ($p < 0.0142$) for 3 hours. Moreover, the growth inhibition for the urinary strains of *E. coli* from pet animals was assessed even in KP *in vitro* digested at different concentration (from 1:2 up to 1:16) in quadruplicate. The results attested KP, as nutraceutical product, could be a viable alternative to antibiotics, making it a good candidate for spreading and improving One Health approach.

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77° CONVEGNO SISVET

Stato: INVIATO - ID: 13475

Exploring variations of faecal microbiota composition in two pig genotypes reared under identical breeding condition

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The gut microbiota is recognized as one of the most intricate microbial communities found in mammals [1]. In pigs, it has been highlighted the important impact that microbial community structure can have on their overall physiological processes, and breeds seem to play a part in shaping its diversity [1]. Therefore, we aim to explore potential differences and interactions occurring in the bacterial composition of the faecal microbiota of two pig breeds, i.e., the autochthonous Nero Siciliano (NS) and a commercial one crossbred (CB) (Landrace × Large White). Metagenome raw data, previously deposited at the Sequence Reads Archive [2,3], from five NS and seven CB pigs collected at three time-points (i.e., for a total of 36 samples) were processed to perform bioinformatics and statistical analyses. These two sets of animals are subsets of the pigs used in previous studies carried out in our group, in which they were used as control groups in comparison to pigs co-fed with liquid whey (for more details on experimental design and sample collection, see [2] and [3] for NS and CB pigs, respectively). Sequencing data were processed using DADA2 pipeline in RStudio. Amplicon sequence variants were taxonomically assigned from the phylum to genus level using the Ribosomal Database Project for the bacterial 16S rRNA gene. PICRUSt2 software was used for the functional prediction, and the Bray-Curtis distances were calculated on the Hellinger-transformed E.C. number abundances. Although relative abundance percentage differences were detected at the phylum level for both breeds, Firmicutes, Bacteroidetes and Spirochaetes were the most abundant phyla identified. To calculate bacterial richness and diversity, alpha and beta indexes were estimated by a Kruskal-Wallis test. For alpha diversity, only Chao1 index was statistically significant ($p < 0.001$) when considering differences between the three time-points and the time correlated to breed, whereas beta diversity index was statistically significant for all variable considered, i.e., breed, time-points, and time correlated to breed ($p < 0.001$). The number of predicted E.C. numbers was similar between the breeds and the time-points, but the overall predicted functional profile of the microbial communities was different according to the breed and the time-points. The results indicates differences in faecal microbiota between NS and CB pigs, demonstrating dynamic changes over time. These findings may contribute to a better understanding of the faecal microbiota of autochthonous and commercial pigs, providing further insights for breeding management.

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77° CONVEGNO SISVET

Stato: INVIATO - ID: 13543

First detection of Shiga toxin genes in Aeromonas isolates from food and freshwater in Lombardy, Italy

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The genus Aeromonas consists of 36 bacterial species widely distributed in aquatic environments, some of these can cause diseases both in humans and aquatic animals and have been considered emerging pathogens for over 10 years. Aeromonas infections have likely been underestimated due to the lack of microbiological testing methods and, especially in the past, the use of inadequate tests for identification. Aeromonas can harbor several virulence genes, essential for establishing infection in the host. A total of 473 samples collected from Lombardy were analyzed for the presence of Aeromonas, obtained from freshwater (n=81), poultry meat (n=65), red meat (n=105), fish products (n=139), dairy products (n=68), gastronomy products (n=8), and vegetables (n=7). The samples were first identified using microbiological investigation that included pre-enrichment in alkaline peptone water, followed by inoculation onto selective media (Havelaar, Glutamate Starch Pseudomonas, McConkey Agar), then bacterial identification was performed using MALDI-TOF, and the confirmation via PCR for the gyrB gene. Furthermore, virulence genes act, ast, alt, aerA, hylA, stx-1, were also investigated, and AST was performed using agar diffusion method.Aeromonas was isolated in 211 (44%) samples, with highest prevalence in poultry meat (75%), followed by fish products (60%), freshwater (46%), and red meat (37%). All gastronomy preparations and vegetables tested negative. Among the eight bacterial species identified A. salmonicida (37%), A. veronii (15.6%) and A. media (11.8%) were the most common species. The virulence genes detected in Aeromonas strains were as follows: alt 80%, aer 59.7%, act 55.9%, hylA 42.18%, stx-1 14.69%, ast 4.26%. The highest antibiotic resistances were associated with trimethoprim/sulfamethoxazole (36.6%), followed by ceftazidime (11.5%), levofloxacin (8.9%), ciprofloxacin (4.7%), cefepime (2.6%), and aztreonam (0.5%). For more in-depth characterization, two strains of A. hydrophila and two strains of A. veronii were subjected to Nanonpore whole genome sequencing. The assembled genomes were screened against the CARD database for antibiotic resistance genes. CphA genes that confer resistance to carbapenems and OXA genes which confer resistance to ampicillins were found in all samples. Both strains of A. hydrophila showed the presence of the AQU-2 gene that gives resistance to cephalosporin. Tet(E) gene, that gives resistance to tetracyclines, was found in two samples. This study shows a high prevalence of Aeromonas in water and food, and provides the first evidence of virulence genes, such as stx-1, of significant importance for public health and never reported before in Italy. This study also allowed us to develop two testing methods for the detection and characterization of Aeromonas, useful for application in analytical procedures aimed at food safety and in cases of foodborne illnesses. References Alperi et al. Human isolates of Aeromonas possess Shiga toxin genes (stx1 and stx2) highly similar to the most virulent gene variants of Escherichia coli. Clin Microbiol Infect. 2010 Oct;16(10):1563-7. doi: 10.1111/j.1469-0691.2010.03203.x. Fernández-Bravo et al. An Update on the Genus Aeromonas: Taxonomy, Epidemiology, and Pathogenicity. Microorganisms. 2020 Jan 17;8(1):129. doi: 10.3390/microorganisms8010129. Hoel et al. The Significance of Mesophilic Aeromonas spp. in Minimally Processed Ready-to-Eat Seafood. Microorganisms. 2019; 7(3):91.

77° CONVEGNO SISVET

Stato: INVIATO - ID: 13550

A novel investigation into the impact of probiotic dietary supplementation on the metabolic activities of the gut microbiota of a healthy canine donor

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The probiotic Lactobacillus reuteri NBF 1® (NBF Lanes S.r.l., Milan, Italy) is a dietary supplementation with a proven safety and efficacy profile for dogs. L. reuteri's ability to improve the composition of beneficial species of the intestinal microflora has already been demonstrated.This study aimed to investigate the impact of L. reuteri NBF 1® also at the level of the metabolic activity of canine gut microbiota.The effect of L. reuteri supplementation was evaluated using the microbiota of a healthy ± 20 kg canine donor. The SCIMETM platform, which consists of three temperature-controlled reactors representing the canine gastrointestinal tract, was utilized. The experiment was divided into a three-week stabilization period, a three-week control period, and a two-week treatment period where the regular diet was supplemented with L.reuteri. The probiotic impact on the gut microbiota composition was evaluated using the 16S-targeted Illumina gene sequencing. The metabolic activity changes were displayed by continuously monitoring the acid/base consumption, the concentration of short-chain fatty acids (SCFA), lactate, ammonium, and branched SCFA of the luminal and mucosal gut microbiota. Shifts in metabolic activity were also investigated through LA-REIMS-based analysis (laser-assisted ambient ionization mass spectrometry) that provides metabolic fingerprints.Following LA-REIMS analysis, metabolic fingerprints were generated that comprised 1603 metabolic features. Based on these fingerprints, metabolic differences were observed between the proximal and distal colon regions. L. reuteri promoted statistically significant metabolic activity changes in the proximal colon region. The probiotic treatment stimulated lactate production while reducing acetate and propionate levels compared to controls, indicating a relevant shift in microbial fermentation patterns. With respect to markers for proteolytic fermentation, a trend towards increased ammonium and branched SCFA levels was observed in the distal colon following L. reuteri supplementation. Concerning the gut microbiota composition, the probiotic supplementation confirmed the Limosilactobacillus genus enrichment, as well as it happened for Pseudomonas, Stenotrophomonas, and Faecalibacterium genera compared to the control.This study has uncovered the significant ability of L. reuteri NBF 1® to modulate the gut microbiota metabolic activity. These findings provide insights into this probiotic's mechanisms of action and suggest potential applications in certain physiological or pathological conditions. Furthermore, they can contribute to the development of effective supplementation treatment combinations.

77° CONVEGNO SISVET

Stato: INVIATO - ID: 13569

Impact of alternative bedding materials on broilers' growth performances and efficiency in conventional and organic production systems

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The trial aimed to compare poplar (PP) and vineyard pellet (VP), largely available in the organic form on the market, as alternative to conventional wood-shavings (WS) bedding on broilers' growth performances and efficiency. A total of 252 one-day-old male Ross 308 chicks were randomly assigned to 9 pens in a 3x3 factorial arrangement. Half of them were raised for 42 days (conventional production system -CPS-), while the remaining were grown up to 84 days (organic production system – OPS-). Feed and water consumption (FC and WC respectively) were measured daily on a pen basis. The body weight (BW) was recorded weekly until 42 d and every 3 weeks until 84 d of age. Dead animals were removed daily, and their weight was recorded. Weekly, 10 subsamples of the litter were collected from each pen and pooled for the microbiological evaluation. The BW gain (BWG), the cumulative feed conversion ratio (FCR) and the water to feed ratio (W:F) were calculated at each interval and at the end of the production cycles per pen. Liveability (L) was monitored throughout the experimental period and expressed as % of initial birds' number, in each bedding group, at the end of each period (42d and 84d). Productivity was summarised by the calculation of the European Production Efficiency Factor (EPEF) using the formula: L*BW/(Age*FCR) *100. The SAS software v. 9.4 (SAS Inst. Inc., Cary, NC) procedures were adopted for the BW, BWG, FCR and W:F ANOVA and LSM estimations, while L was subsequently analysed using the Chi-squared test (FREQ procedure SAS Institute Inc., Cary, NC, USA). Broilers raised on VP showed lower BWG compared to those on PP but similar to WS in the CPS (P = 0.028). Between 0 and 63 days of age, PP group showed the lowest FCR (P = 0.008) and a reduced W:F compared to the animal reared on WS, while it did not differ from VP for this parameter (P = 0.032). The latter effect persisted in the whole OPS (P = 0.022). In the CPS, the final BW was negatively affected in the VP group compared to PP group (P=0.004). Regarding microbiological evaluation an increase in total mesophilic count was observed at 28 (P=0.006), 42 (P=0.001) days in both pelleted bedding groups in comparison to WS, and only in VP at 84 d (P=0.0018). The same differences were observed for E. coli. In the CPS the liveability was not affected by different bedding groups, while in the OPS the PP showed a 25% higher liveability compared to VP (P=0.024). In conclusion, the results showed that in a CPS of 42 days, animals reared in PP and WS had better EPEF than those in VP, while in OPS, PP had the highest EPEF. This project has received funding from the European Union's Horizon 2020 research and innovation programme under grant agreement No 774340.

77° CONVEGNO SISVET

Stato: INVIATO - ID: 13679

Evaluation of biochar as novel ingredient of weaned piglets diet.

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Biochar is a carbon-rich substance produced through the thermochemical breakdown of biomass. It has gained attention for its potential to address pressing environmental issues. Biochar is derived from lignocellulosic waste biomass and is being explored across multiple fields to combat environmental challenges [1]. Its use as a feed ingredient is also gaining interest due to its capacity to improve animal health, optimize nutrient absorption, and boost productivity. Furthermore, biochar provides a sustainable and environmentally friendly alternative, helping to reduce greenhouse gas emissions and nutrient losses in agricultural practices. Biochar is a heterogeneous compound whose quality is influenced by several factors, such as the primary biomass source, residence time, and pyrolysis temperature. These variables lead to differences in its physicochemical properties, including porosity, carbon content, elemental composition, surface area, and retention capacity [2]. The aim of this study was to evaluate, after in vitro characterization, the effect of biochar from chestnut biomass (NeraBiochar, Italy) as a novel ingredient in weaned piglet diets on growth performance, health status, and diarrhoea frequency. The biochar was characterized for its surface area (BET method), humidity, ash content (AOAC, 2019), and functional properties. In particular, the antioxidant properties, E. coli F4+ and F18 growth inhibitory activities, and metabolomic profile of a hot water extract of biochar were tested using the ABTS assay, microdilution method [3], and Q-TOF MS/MS analysis, respectively. For the in vivo study, a total of 250 weaned piglets (age: 28±1 days; body weight: 9.42±1.33 kg) were housed in 16 pens under the same environmental conditions for 28 days (ethical approval 5_2024, OPBA). The animals were randomly divided into two experimental groups: control (CON, n=125, 8 pens) and treatment (TRT, n=125, 8 pens), which received isonitrogenous and isoenergetic diets, differing only by the inclusion of 1% chestnut biochar in the TRT. Individual body weight, feed intake, and faecal score were measured weekly. After 28 days, blood samples were taken individually for serum oxidative status and antioxidant barrier assessment using colorimetric assays. The data obtained were statistically analyzed (Anova two-way, GraphPad Prism9, 2020). The biochar extracts showed a polyphenolic profile consisting of low molecular weight compounds. Biochar extracts exhibited significant inhibitory activity against both E. coli strains ($p \leq .01$). The maximum percentage of inhibition (- 23% of bacterial cells) was observed after 2 hours of incubation of biochar extract (10%). Throughout the experimental period, no significant differences were observed in average body weight (BW), average feed intake (ADFI), or average daily gain (ADG) between the groups. However, the TRT group had a lower faecal score ($p \leq .01$), indicating the potential of biochar to reduce the incidence of diarrhoea during the weaning period. Our preliminary data suggest that biochar could serve as an alternative to antibiotics in the management of gastrointestinal problems during the weaning period.

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77° CONVEGNO SISVET

Stato: INVIATO - ID: 13686

Efficiency of different solvents on the extraction of bioactive compounds, antioxidant and antimicrobial activities in Fucus vesiculosus

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Fucus vesiculosus is a rich source of bioactive substances with many biochemical functions that provide it a variety of biological effects [1-2]. Over the years, significant research efforts have been made to extract bioactive compounds by applying different methodologies for various applications. There are several solvents used for the extraction of natural products, such as hot water and organic solvents (methanol and ethanol). However, the choice of solvent must be based primarily on the characteristics of the matrices and the properties of the molecular classes to be obtained [3]. Therefore, the aim of this study was to investigate the efficiency of three different extraction solvents (methanol, ethanol, acetone) to maximize the yield of polyphenol and flavonoid content as well as the antioxidant and antimicrobial power. One gram of F. vesiculosus powder was dissolved in 20mL of i) 70% acetone, ii) 80% ethanol, iii) 50% methanol (v/v; solvent/H2O), and the solutions were put in agitation for 1 hour at RT. The different extracts were tested to evaluate the Total Polyphenol Content (TPC) and the Total Flavonoid Content (TFC). The results concerning the content of bioactive molecules disclosed that the extraction carried out with the methanol (50%) was the one that gave the highest yield in both polyphenol and flavonoid content. In fact, TPC was found to be 800.35 ± 10.97 mg TAE/g of the sample for the methanol extract, while it was equal to 767.02 ± 9.41 and 700.56 ± 20.82 mg TAE/g of the sample for the acetone and ethanol extract respectively. Also, TFC was significantly higher in the methanol extract (187.12 ± 12.86 mg CE/g of sample) compared to acetone (156.43 ± 4.17 mg CE/g of sample; $p=0.0001$) and ethanol (138.38 ± 19.35 mg CE/g of sample; $p<0.0001$) extracts. In addition to the evaluation of the bioactive molecules, the functional activities derived from them were analyzed. The antioxidant capacity was tested through the ABTS assay to assess the radical scavenging activity. The results obtained disclose that the extract of F. vesiculosus had high antioxidant activity since the inhibition percentage of ABTS Radical Scavenging Activity was around 90%. Specifically, it was observed that at the higher concentration of extract, methanol was the better solvent, while at a lower concentration level, acetone was better to highlight the antioxidant power of F. vesiculosus. Finally, even the growth inhibition capacity against Escherichia coli F18+ was tested. Results disclose that F. vesiculosus is able to reduce significantly ($p<0.05$) the growth of E. coli F18+, in particular when the alga is extracted with methanol and acetone. In conclusion, methanol was found to be the best solvent for the extraction of the bioactive components present in F. vesiculosus and effective for the evaluation of functional properties (antioxidant and antimicrobial). Similarly, acetone could be used in the evaluation of these properties, in particular at high extract dilutions where was more effective than methanol.

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77° CONVEGNO SISVET

Stato: INVIATO - ID: 13707

Cooked ham quality parameters from Nero di Lomellina pigs and commercial hybrid pigs: preliminary data.

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Nero di Lomellina (NL) is a recently recognized reconstructed pig breed of the area of Pavia by the National Pig Breeders Association. These pigs have rustic characteristics and the meat is suitable for the production of high-quality processed products. The NL represents a benefit to the local economy, but in literature, there is no information on product quality parameters. Considering that in Italy cooked ham is the most consumed cured meat (27.8% of total consumption in 2022), the present study aimed to characterize the quality parameters of cooked ham from NL pigs compared to those obtained from Commercial Hybrid (HP) pigs in the same conditions. The animals were slaughtered at about 173 ± 2 kg of live weight at a commercial slaughterhouse (Macelleria Costa, Gambolò, PV). Sixteen thighs, sampled from the left side of the carcass (8 per genetic type), were processed under commercial guidelines for cooked ham production. Physical, chemical, and sensory parameters of cooked hams were determined. The data evaluation was performed using the statistical software SPSS (SPSS/24 PC Statistics 28.0 IBM). Data related to physical and chemical parameters were analyzed by one-way Analysis of Variance (ANOVA) with breed as fixed effects. No previous data reported the quality parameters of cooked ham from NL pigs. The physical and chemical parameters of the product did not differ between commercial hybrid pigs and Nero di Lomellina pigs ($P>0.05$) and are comparable with data reported in the literature on Italian cooked ham [1-2]. Similarly, sensory attributes related to appearance, aroma, flavour, and texture were not affected ($P>0.05$) by the pig breed. The present preliminary data suggest that the physical, nutritional, and sensory quality of cooked ham did not differ between commercial hybrid pigs and Nero di Lomellina pigs. Further data on the quality of derived products from Nero di Lomellina pigs are needed to confirm the present results and to evaluate the quality parameters of other processed products.

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The authors thank “Macelleria Costa” for their kind support.

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77° CONVEGNO SISVET

Stato: INVIATO - ID: 13710

Exploring the Practical Implications of Genetic Index and Genotyping: A Case Study of University of Bologna Dairy Farm

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Traditionally, heifer breeding values rely on pedigrees, assuming that all progeny from the same parents inherited the same DNA proportion [1]. The advent of genomic (pre)selection in young bulls, along with its subsequent integration into genotyping strategies at the farm level, has introduced new and superior opportunities for enhancing the genetic merit at the farm level more accurately and rapidly [2]. Moreover, it intensifies selection, resulting in an "acceleration" of annual genetic gains for the desired breeding objective(s). Consequently, the likelihood of selecting the "best" animals for the next generation is enhanced. The aim of the present study was to evaluate the benefits of genotyping dairy cows at the herd level. This study utilized data stored in the national database of the National Association of Italian Holstein, Brown and Jersey breeders (ANAFIBJ, Cremona, Italy), including pedigree index (PI) and direct genomic value (DGV) for the main three genetic indices: productivity, functionality, and type index (PFT), economic-health index (IES), and cheesemaking and sustainability index (ICS) published by ANAFIBJ. Phenotypes and genotypes of 293 Italian Holsteins born between December 2013 and December 2023 at the experimental dairy farm of the University of Bologna were utilized. Different rankings based on PI and DGV of the three aggregate indexes (PFT, IES and ICS) were used to evaluate cows' first-lactation mature equivalent 305-day milk (MY), fat and protein yield, days open in the first lactation, and average somatic cell count. The analysis was performed through linear models using the MIXED procedure of SAS software v 9.4. Considering the results based on the IES index, the difference between the top 10% and the bottom 10% dairy cows ranked for their DGV is: +1410.47 kg for MY; in terms of fat yield, it is +30.24 kg, and in terms of protein, it is +77.05 kg. The same results can be applied to other breeding values. For example, the difference in first-lactation MY for the PFT index of the top and bottom 10% differs by +1172.24 kg (PI) or by +2515.21 kg (DGV). Regarding fertility, days open of the top 10% cows ranked for IES PI was above 120 days (117 days), which is the normally accepted threshold after which poor fertility starts to have a negative impact on herd profitability; this issue was not observed in cows ranked by IES DGV (116.67 days). Difference in profitability based on PI and DGV IES approximately amounts to a profit increase of +204€ per head. All these results, which are similar when ranking animals based on ICS-PR, suggest that the higher reliability of genomic indexes truly translates into a more precise ranking, facilitating optimal breeding decisions. In conclusion, benefits of genotyping all animals at the herd level showed a large range suggesting a feasible economic and production improvement.
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77° CONVEGNO SISVET

Stato: INVIATO - ID: 13716

Revisiting LCA: Role of Automatic Milking System in Dairy Production at the University of Bologna Experimental Farm with Updated Data

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The livestock sector plays an essential role to supply the global food resources. Although the contribution is lower in comparison with other sectors, ruminant productions emit greenhouse gas emissions (GHG) such as methane (CH4) and nitrogen dioxide (NO2). A realistic approach to manage this problem is to improve the efficiency of milk and meat production from ruminants considering the “intensity” of emissions per unit of useful product instead of the total emission per se. The dairy livestock sector significantly improved their efficiency in the last decades through the reduction of health issues, genetic selection, advanced nutritional technologies, and the use of new management tools. The automatic milking systems (AMS) can improve individual milk production and the welfare of dairy cows, and for this reason we are perceiving a fast adoption of this technology worldwide. This study updates the investigation into the environmental impact of milk production at the University of Bologna dairy farm after installing the AMS [1]. The carbon footprint (CF) of milk production was evaluated employing the Product Environmental Footprint (PEF) framework for Life Cycle Assessment (LCA), conducted via open LCA software and databases provided by ENVIRONMENTAL FOOTPRINT by the European Commission. The assessment of enteric CH4 emissions was estimated by direct measurements using a laser (Laser Methane Mini; Crowcon, Abingdon, UK) in previous studies, recalculated according to Sorg et al. [2]. A comparison was made between the year 2020 when the farm operated with two daily milkings, and data from 2022 when AMS was fully operative. The outcomes achieved by the new calculation methods have revealed remarkable differences when compared to earlier research, even though the general conclusions do not differ. The results showed a CF of 1.63 kg CO2 eq/kg of 4%FCM in 2020 and 1.50 kg CO2 eq/kg of 4%FCM in 2022 (-7.97%). This improvement was mainly attributed to the increase in animal milk yield (+15% kg). Upon comparing our data with those documented in the literature, it is noted that the CF falls within the range reported by Battini et al. [3], who found a CF of 1.35 to 1.50 kg CO2 eq/kg of 4%FCM. In conclusion, the adoption of the AMS helped to decrease CF intensity supporting the environmental sustainability of milk production. Finally, it appears very important to have more precise methods to estimate the CH4 emission on the farm for each cow considering its high impact on total CF; in our data, CH4 emissions account on average for 28% of the total CF.

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77° CONVEGNO SISVET

Stato: INVIATO - ID: 13720

Metabolic shift of nutrition-related parameters in stallions during and after stud season

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The nutritional status of stallions along with the metabolic milieu can be conditioning factors of semen quality [1] and appear to be worthy of being monitored throughout the year. The aim of this research was to assess the metabolic fingerprint of plasma parameters selected for nutritional status assessment of stallions according to circannual variation. For this purpose, hormonal profile (leptin, OB; testosterone, T) and catalase (CAT) circulating level were monitored during and after the stud season. To meet these goals, a total of ten stallions of ASVI stud center (breed, horse number: Anglo-Arabians, n.6, Arabian Thoroughbred, n. 2, English Thoroughbred, n.1, Oldenburg, n.1; age, horse number: 8 years, n.1; 10 years, n.2; 12 years, n.1; 13 years, n.2; 15 years, n.1; 16 years, n.1; 19 years, n.1; 22 years, n.1; Body weight range, BW; 496 - 625 kg; Body condition score, BCS 1-9 points scale: 4-6, at start) were enrolled in the study, following the criterion of homogeneous serving frequency per week. Stallions were housed in individual boxes and were subjected to carousel training 50 minutes per day, for 5 days a week and semen sampling alternatively twice or thrice a week. Animals were fed a diet consisting of high-quality hay and mixed compound feed, following the feeding practices of the stable to meet requirements [3], with ad libitum water offer. Horses were sampled for whole blood at the beginning (T1), in the middle (T2) and at the end (T3) of stud season (Mar-July). A follow up was carried out at the beginning of the negative photoperiod (T4). Complete blood cell count (CBC), levels of T, OB and CAT were determined. All data were analysed using one-way ANOVA (Minitab_18©). Age categories were established following previous research [1]. Selected parameters were analysed for correlation by using Pearson correlation test (r). Significance was set for P-value<0.05.Horses appeared healthy throughout the trial. OB decreased significantly (P-value=0.022) from the beginning of stud season as well as until the end of positive photoperiod (follow-up). OB and T were negatively-correlated with sampling time (r=-0.650, P-value=0.000 and (r=-0.338, P-value=0.033, respectively). In agreement previous observations [1], horses aged >13 ys. confirmed a progressive decline of circulating T (P-value=0.013). OB and T are positively correlated in horses aged >13 ys (r=0.412, P-value=0.046). In conclusion, the metabolic pattern of selected hormonal profile along with nutritional status and activity during the stud season turned out to be significantly affected in stallions older than 13 years, thus pointing at age as an animal factor, beyond management.
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77° CONVEGNO SISVET

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EVALUATION OF THE ERYTHROCYTE MEMBRANE LIPIDOME PROFILE IN GROWING LAMB BEFORE AND AFTER SUPPLEMENTATION WITH HEMPSEED CAKE

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Recently, there has been a growing interest on hemp (Cannabis Sativa L.) due to the plant's pharmaceuticals properties that offer a wide variety of applications, and research studies are currently focusing on the investigation of its properties on human and animal nutrition. Hemp can be a source of amino acids as well as of essential fatty acids and it can be used as a supplement in feed. In dairy ruminant nutrition, hempseed and its derivatives (oil, cake and meal) has been given as a dietary supplement to sheep and ewes in the past [1], however a systematic study to evaluate the possible changes on the erythrocytes' membranes of the animal after such supplementation has not yet been conducted. The aim of this study was to compare the lipidomic profile of the erythrocytes' membranes of growing lambs with and without the supplementation of hempseed cake – HSC, using methods of lipidomic analysis.
This study was conducted at a farm located in the Abruzzo region, Italy. For the present study, 46 Bergamasca lambs (20 males, 26 females) with an age of 45 days, initial body weight (BW) of 18k (min=15,2, max=20.8) and a typical Body Condition Score (BCS) of 3/5 were selected and equally divided in 2 groups: control (CTR) and experimental group (HSC group). All animals received a total mixed ration diet (TMR), formulated to support their growing needs. CTR lambs were given TMR with soybean and by-products of soybean meal as the main protein source, while a 12% DM of these feed was replaced by hempseed cake in the HSC group. Hay and water were offered to lambs ad libitum. Whole blood samples in EDTA were collected from each animal after 60 days of receiving the two different diets. The erythrocytes' membranes was then isolated and by Gas-Chromatography a group of 10 fatty acids (FA), specifically: saturated (SFA: stearic, palmitic), mono-unsaturated (MUFA: palmitoleic, oleic, vaccenic), polyunsaturated FA ω-6 (PUFA-ω6: linoleic, dihomo-gamma-linolenic [DGLA], arachidonic [ARA]) and PUFA ω-3 (eicosapentaenoic [EPA], docosahexaenoic [DHA]) was determined, along with additional lipid parameters (SFA/MUFA, SFA/PUFA, ω6/ω3, PUFA balance, unsaturation [UI] and peroxidation [PI] indexes).
Between the two groups no differences were observed in the SFA concentration, however there was a significant decrease in the levels of oleic acid (p=0.01) in HSC group, that lead to the decreased levels of the total MUFA (p=0.0046). On the other hand, at the HSC group there was an increase of the concentration of the omega-3 (p=0.0046) as well as the omega-6 (p=0.0021) resulting in the increase of the total PUFA (p=0.0021). Specifically, the levels of the omega-6 fatty acid ARA were significantly higher (p=0.0001) and both of the omega-3 EPA and DHA were in increased levels as well (p=0.0028 equally). Equally significant (p = 0.0003) were the decrease of the triene/tetraene ratio (ω6/ω3) and the increase of PUFA balance ω3/(ω3+ω6) for the HSC enriched diet. The UI and PI were found in significantly higher levels for the HSC lambs (p=0.0001 and p<0.0001 respectively).
In conclusion, an evaluation of the lipidic profile of ovine erythrocytes has been occurred for the first time and our data indicate that a diet rich in HC can result in increased levels of PUFA, with a specific importance on the increase of the essential fatty acids EPA and DHA that are generally known to play a beneficial role in cell functions.
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77° CONVEGNO SISVET

Stato: INVIATO - ID: 13833

Comprehensive Evaluation of Mango and Avocado Fruit Byproducts: Nutritional Potential, In Vitro Digestibility, rumen fermentation, including Encapsulated Phenolic Extracts as Feed Supplements for Ruminants

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The intensification of food-feed competitions and concerns over environmental footprints have driven research efforts towards identifying unconventional feed sources for optimizing ruminant feeding strategies. Fruit by-products, such as peels and seeds of mangoes and avocados, emerge as potential feed ingredients. Alongside serving as a feed source, these by-products also contain a significant amount of bioactive substances that help in the mitigation of greenhouse gas emissions [1]. In this study, we explored the potential use of mango peel (MP), mango seed (MS), mango seed coat (MSC), avocado peel (AP), and avocado seed (AS) in two experiments. Experiment 1 evaluated the feed potential of these fruits by-products by assessing their chemical composition, in vitro true digestibility, gas production, and volatile fatty acid production. In vitro true digestibility was determined over 48 hours of incubation using the Ankom DaisyII incubator, while in vitro fermentation parameters were analysed following 24-hour fermentation with the Hohenheim Gas syringe system. Among the various by-products, AP and MP exhibited higher total phenolic content ranging from 121.50 to 243.69 (mg GAE/g) and antioxidant capacity from 342.92 to 366.63 (mg TE/g), indicating their potential to positively influence the rumen ecosystem. MP, MS, and AS showed higher digestibility (86.4–89.5%), elevated metabolizable energy (8.41-9.59 MJ/kg DM), while MSC and AP exhibited lower values. MS, AS and MP had the highest cumulative gas production (50.33 ml/0.2g DM, 47.92 ml/0.2g DM and 43.83 ml/0.2g DM respectively), while AP and MSC showed lower gas production (20.75 ml/0.2g DM and 14.33 ml/0.2g DM). The ruminal pH varied, being higher in MSC (6.92) and AP (6.83) and lower in MS, AS, and MP (6.6, 6.67, and 6.74, respectively). The acetate-to-propionate ratio, a crucial indicator of methane, is found to be higher in AS (3.96), MSC (3.71), MP (3.53) and lower in MP and AP (3.06 and 3.07, respectively). Experiment 2 was conducted to assess the effects of supplementing dairy cow diets with microencapsulated extracts derived from mango and avocado by-products, specifically mango peel extract (MPE), avocado peel extract (APE), mango seed extract (MSE), avocado seed extract (ASE), and mango seed coat extract (MSCE), with alfalfa hay serving as the control group. The encapsulation of these by-product extracts was conducted utilizing β-cyclodextrin as the encapsulating agent. These microencapsulated by-products, at 20 mg (fresh matter), were added to alfalfa hay (200 mg DM) as a basal substrate during a 24-hour incubation. Gas production at 24 hours varied, with the highest observed in the MSE group (42.33ml/0.2g DM) and control group (42.00ml/0.2g DM), followed by ASE (41ml/0.2g DM), MSCE (39.83/0.2gDM) APE (37.67/0.2gDM), and MPE (36.5ml/0.2g DM). The ruminal Ph was found lowest in APE as compared to control and other groups. APE significantly reduced acetate to propionate ratio compared to control and other groups ($P < 0.001$). Additionally, the ammonia nitrogen concentration was lower in the encapsulated groups compared to the control. In conclusion, all evaluated by-products, particularly MP, MS, and AS, demonstrated promising potential as feed ingredients, with APE and MPE showing potential as a feed supplement to mitigate methanogenesis in ruminant diets.
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77° CONVEGNO SISVET

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Fluctuation in Body Weight, Body Condition and Muscle Condition of Breeding Stallions during and after the stud season

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Stallions entering the stud season are required to face the energy demand to serve for semen donation used in artificial insemination or for natural mating. Despite those are established practices in studs, knowledge on variation in body weight (BW), body condition score (BCS), and muscle condition (MCS) of stallions in relation to reproduction performance is limited. We aimed to investigate if variations related to the nutritional status of breeding stallions during and after the stud season could occur. To achieve this goal, ten stallions were enrolled in the study and monthly evaluated during and after the stud season 2022/2023. All animals were fed a diet to cover individual requirements [1]. Each stallion was weighed and assessed for BCS (1-9 points [2]), and MCS (1-5 points). The effect of time on the variables was analyzed using a one-way ANOVA, with a significance level of $\alpha = 0.05$.Stallions showed normal BW throughout the period of observation. A significant decrease of BCS during the stud season was observed ($p = 0.046$). MCS increased significantly ($p < 0.0001$) during the stud season. Stallions exhibited a higher BCS and lower MCS after the stud season. There is evidence of the different proportion between lean and fat masses despite constant BW throughout the period of observation. In conclusion, our study showed that the BW, BCS and MCS of breeding stallions varies during the stud and non stud season. Significant fluctuation as to lean and fat mass proportion could be assessed.
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77° CONVEGNO SISVET

Stato: INVIATO - ID: 13874

Comparative aspects of feeding habits of mammal predators of Sardinia

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Feeding preference and peculiarities of gastrointestinal tracts through a comparative approach of Sardinian fox (Vulpes vulpes ichnusae Miller, 1907), Sardinian wildcat (Felix silvestris lybica Forster, 1907) and Sardinian marten (Martes martes latinorum Barrett-Hamilton, 1904) living on Sardinia island were comparatively studied. To achieve this goal, a total of n. 10 individuals of Sardinian fox, n. 5 Sardinian wildcat and n. 16 Sardinian martens found dead for traumatic causes (car bumping) and collected from different areas of Sardinia were dissected and studied. Somatometric measurements, linear measurements and the capacity of the complete digestive system were assessed [1]. For each subject, a sex- and age-based classification was conducted, followed by weight determination. Lengths, capacities and relative indices were determined. Stomach stuff-ratio and relative capacity were also explored. On stereomicroscope, stomach contents were analyzed for qualitative data and taxonomic composition [2]. The stomach stuff ratio (%) (mean±SD) was: 23.3%±10.42 for the Sardinian fox, 17.5%±8.8 for Sardinian wildcat and 12%±9.1 for Sardinian marten, respectively. The digestive tract of Sardinian marten shows the absence of the cecum, differently from the other two species. The analysis of stomach content allowed identify the presence of small rodents (mice) in all the three species, and seasonal fruits ingested both by the Sardinian fox and Sardinian marten. This suggests different feeding behaviour than expected from morphology, in particular for the marten: in fact, Sardinian fox confirmed as omnivore, while the Sardinian marten exhibited to behave as facultative carnivore, although the anatomy of the digestive system is more similar to that of an obligate carnivore, like the wildcat [3]. In conclusion, the differences observed shed a light on the understanding of how the composition of the diet in the natural habitat is dictated by the capacity and functionality of each tract of the digestive system, with a more plastic feed preference in the marten, as unexpected from the anatomy of the digestive tract. Indeed, plant material seems to require limited degradation and thus very limited fermentation and short retention time of digesta. The common pray selected in all the three species alike was rodents.
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RNIV

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Two sardinian ASFV isolates with a sustained genomic deletion presented an attenuated phenotype *in vitro*

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African swine fever virus (ASFV) causes a devastating disease affecting domestic and wild pigs. ASF was first introduced in Sardinia in 1978 and until 2019 only genotype I isolates were identified. A remarkable genetic stability of Sardinian ASFV isolates was described, nevertheless in 2019 two wild boar strains with a sustained genomic deletion (4342 base pairs) were identified (7303WB/19, 7212WB/19) [1]. In this study, we performed *in vitro* experiments with monocyte-derived macrophages to unravel the phenotypic characteristics of these deleted strains. Macrophages are the main target of ASFV and virulent isolates developed several strategies to efficiently replicate in these cells [2].

Five healthy pigs were used as blood donors to generate macrophages (authorization n° 1232/2020-PR). Porcine macrophages were infected with virulent 26544/OG10 or deleted 7303WB/19, 7212WB/19, alongiside mock-infected controls. The ability of these isolates to replicate in macrophages was first assessed, using a high (1) or a low (0.01) multiplicity of infection (MOI). Cells supernatants were collected at 0, 24, 48, 72 hour post infection (hpi) and then titrated. Subsequently, interaction of ASFV with these cells was investigated using flow cytometry and multiplex ELISA. Macrophages were infected using a MOI of 1; 24 h later flow cytometry was employed to assess the intracellular levels of viral proteins. In parallel, culture supernatants were collected by centrifugation, and stored at -80°C until analysed. Levels of IL-1α, IL-1β, IL-6, IL-10, IL-12, IL-18, TNF were monitored using Porcine Cytokine/Chemokine Magnetic Bead Panel Multiplex assay. We observed that both 7303WB/19 and 7212WB/19 presented a lower growth kinetic in porcine macrophages compared to virulent Sardinian 26544/OG10, using a high (1) or a low (0.01) MOI. In addition, flow cytometric analysis showed that both 7303WB/19 and 7212WB/19 presented lower intracellular levels of both early (p30) and late ASFV (p72) proteins. Infection with either deleted or non-deleted ASFV strains did not trigger release of pro-inflammatory or anti-inflammatory cytokines from macrophages.

Overall, we observed the deleted virus isolates in Sardinia only in 2019, at the end of a strong eradication campaign, and our data suggested that it might possess an attenuated phenotype. *In vivo* studies should be performed to analyse the phenotype of these deleted isolates, to better their role in ASFV persistence in Sardinia.

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ID: 13199

Targeting Toll-like Receptor 2: synthetic diacylated lipopeptides polarize equine macrophages towards a pro-inflammatory phenotype

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Toll-like receptors (TLRs) are a group of pattern recognition receptors (PRRs), which play critical roles in initiating host immune defenses. TLR-ligands are attracting increasing attention as prophylactic and/or therapeutic agents against infectious diseases or in cancer immunotherapy [1, 2]. In particular, TLR-2 agonists have shown promise against both pathogens and tumors [2]. We previously observed that three synthetic diacylated lipopeptides based on a surface protein of Mycoplasma agalactiae (Mag-Pam2Cys_P48, MagPam2Cys_P80, Mag-Pam2Cys_MAG1000) strongly activated innate immune cells, including porcine monocyte-derived macrophages (moMΦ) [3]. In this study, we investigated the immunomodulatory effects of these molecules on equine moMΦ.

Heparinized blood was collected from four healthy horses and monocytes were differentiated into moMΦ though incubation in media supplemented with autologous plasma (20%) and human M-CSF (50 ng/mL). After seven days, equine moMΦ were harvested, counted, seeded in 12 well plates and stimulated with these TLR-2 agonists (at 100 ng/mL), alongside untreated controls. After 24 hours, the immunomodulatory effect was measured through RT-qRT (expression of key immune genes) and ELISA multiplex (release of cytokines). The cytokine profile of moMΦ stimulated with a TLR-2 ligand (Mag-Pam2Cys_P80) was also compared to that of classically activated macrophages (IFN-γ + LPS, moM1). Finally, the impact of Mag-Pam2Cys_P80 on the phenotype of macrophages stimulated with IL-4 or IL-10 ('M2-related' cytokines) was assessed with ELISA multiplex and RT-qPCR.

Stimulation with the synthetic diacylated lipopeptides polarize macrophages towards a pro-inflammatory phenotype, with enhanced induction/release of pro-inflammatory cytokines. In particular, increased expression of *IL1B*, *IL8*, *IL12B* was observed, as well as release of TNF, IL-1β, IL-8, with no differences between compounds. We selected one of the these molecules (Mag-Pam2Cys_P80) and we compare it with moM1. Our data revealed that stimulation with IFN-γ + LPS resulted in higher release of several pro-inflammatory cytokines compared to Mag-Pam2Cys_P80: IL-6, IL-18, TNF. Finally, we investigated how MagPam2Cys_P80 affected the functionality of equine macrophages stimulated with two 'M2- related' polarizing factors: IL-4 and IL-10. Our data revealed that stimulation with MagPam2Cys_P80 did not significanly affect the induction/release of pro-inflammatory cytokines from moM(IL-4) or (IL-10), with increased expression and release of IL-8 from both moM(IL-4) and moM(IL-10) 24 h after MagPam2Cys_P80 stimulation. Anyway, few differences between subsets were observed: stimulation with that TLR-2 ligand resulted in higher levels of TNF from moM(IL-4), but not from moM(IL-10). In addition, MagPam2Cys_P80 did not trigger release of IL-1β from both moM(IL-4) and moM(IL-10), nevertheless increased expression of *IL1B* was observed in all subsets.

Overall, these preliminary data suggest that these synthetic diacylated lipopeptides polarize macrophages toward a pro-inflammatory phenotype, but weaker than classical activation (IFN-γ + LPS). Our results suggest also that the inflammatory activity evoked by these compounds could be mitigated in vivo by release of anti-inflammatory molecules (e.g. IL-10), avoiding potentially harmful consequences.

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“Analysis of the Correlation between Welfare, Biosafety, Drug Consumption, and the Number of Heads in Buffalo Farming".

Author:

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The World Health Organization (WHO) promoted the Global Action Plan on Antimicrobial Resistance (AMR) in 2015, emphasizing the need for close cross-sector collaboration between public and animal health, as well as in food safety (1). This approach is known as "One Health" and is based on the idea that human health is closely linked to the health of animals and the environment. Increasingly aware consumers are also demanding respect for farmed species and an increase in the level of safety and quality of the food they consume. This is made possible by ClassyFarm, an integrated system for categorizing farms based on risk assessment (RA) methodology. This system can be applied to different animal species, including buffalo, to ensure high standards of animal welfare and food safety. Risk assessment (RA) as part of systems such as ClassyFarm becomes essential to identify farm management practices that could promote antimicrobial resistance and to take appropriate preventive measures. In the present study, a total of 70 buffalo farms. For each, the average herd size was surveyed and assessments were performed using the ClassyFarm checklist. The checklist assesses animal welfare, biosecurity and medication use, expressed in Defined Daily Dose for Italy (DDDAit). Analyses were conducted and found that there were correlations between these by Statistical analysis was performed by Spearman’s rank correlation using GraphPad Prism 8.0.1, for measurements welfare and biosecurity, welfare and DDDAit, and biosecurity and DDDAit. During the observation period, 20% of the barns were excluded from the assessment due to temporary closures for health reasons or incomplete data. Consequently, for the remaining 80% buffalo barns considered in the analysis, bulk milk samples were taken for further evaluation. The results show that the average herd size was 349, while the average value of DDDAit was found to be 0.54. The mean value for animal welfare was 80%, while the mean score for biosecurity was 69.5%. Welfare and biosecurity were positively correlated (Rho=0.712; $p< 0.001$) as welfare and DDDAit (Rho=0.568; $p< 0.001$) and Biosecurity and DDDAit (Rho=0.549; $p< 0.001$) The analyses of mastitogen found that 26% of the stables were positive. The farms were then divided into two groups: mastitogen-positive and mastitogen-negative. It was observed that, biosecurity, number of animals, welfare, and DDDAit values for the two groups were not statistically significant. In conclusion, the statistical analysis revealed important correlations between the variables considered, but also highlighted the need for further investigation to fully understand the impact of these factors on the health and welfare of the animals in question.

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Proinflammatory and anti-inflammatory cytokines in calves infected with alphaherpesvirus: comparison of Bovine aphaherpesvirus 1 (BoAHV-1) and Bubaline alphaherpesvirus 1 (BuAHV-1)

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Cytokines are small proteins with an essential role in controlling the homoeostasis of the immune system; their levels are often monitored as indicators of infection or vaccine-induced immune responses [1, 2]. Bovine alphaherpesvirus 1 (BoAHV-1) is one of the major respiratory pathogens in cattle worldwide [2]. Bubaline alphaherpesvirus 1 (BuAHV-1) is an important pathogen of water buffalo and it is able to infect cattle, causing significant economic losses to the dairy industry worldwide [3]. To date, little is known about the impact of both alphaverphesvirus on the bovine immune system, therefore we investigated the serum levels of key cytokines in calves infected with BoAHV-1 or BuAHV-1.

Ten healthy calves 3-6 months of age were used in the study. Six animals were infected with a virulent BoAHV-1, whereas four were infected with a virulent BuAHV-1; intranasal administration was used in both experiments. Clinical signs and immunological parameters were monitored over-time. In particular, serum samples were collected at 0, 2, 4, 7, 10, 14 days post-infection and the levels of ten cytokines were investigated by multiplex ELISA tests (IFN-γ, IL-1α, IL-1β, IL-4, IL-6, IL-10, CXCL10, MIP1β, IL-36Ra, TNF). Levels post-infection (day 2, 4, 7, 10, 14) were compared to those pre-infection (day 0).

After infection with both viruses, calves presented respiratory symptoms up to day 10 typical of herpesvirus infections. BoAHV-1 triggered early raise of IFN-γ and IL-10, but not IL-1β and IL-4, in agreement with previous studies [2]. For the first time, the impact on this alphaherpesvirus infection on serum values of two chemokines (CXCL8, MIP1β) was investigated and we observed that BoAHV-1 infection resulted in raise of MIP1β values, but not CXCL8. Infection with BuAHV-1 resulted in a similar transient rise of IFN-γ early postinfection (day 2), but also a second wave was observed (day 10). In addition, a transient rise of serum levels of four cytokines was observed: IL-1α (day 4, 7), IL-4 (day 4, 7), IL-10 (day 4, 7), and MIP1β (day 10). For both viruses, no major alteration were observed in circulating levels of pro-inflammatory IL-1β, IL-6, and TNF, whereas a transient decrease of circulating levels of IL-36Ra (a receptor antagonist) was observed at day 14.

Overall, our results evidenced that for both viruses the raise of IFN-γ, IL-10 and MIP1β circulating values were transitory and associated to animals' ability to overcome the infection. The serum levels of these cytokines should be monitored in studies with vaccine candidates as indicators of vaccine-induced immune responses.

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Novel Insights into Feline Immune Response Following Squamous Cell Carcinoma Electrochemotherapy

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Within the One Health approach, comparative oncology finds in spontaneous animal tumors a crucial study model. Dogs and cats, due to their similarity to humans and shorter lifespan, offer fertile ground for research (1). Although feline tumors have not been explored as extensively as canine ones, studies highlight a higher frequency of common tumors such as lymphoma, sarcoma, and squamous cell carcinoma (SCC) (2). The latter represents approximately 70-80% of all oral tumors in domestic cats. In humans, head and neck tumors account for about 10-12% of all malignant tumors in men and 4-5% in women. In Italy, it is estimated that there are about 6,500 new cases of oral cavity and pharyngeal tumors each year, and slightly fewer, about 5,500, of laryngeal tumors (2). Most of these are SCC affecting the oral cavity, oropharynx, ear, and nose. Considering this evidence, it becomes evident how the cat can serve as an excellent model for understanding and developing effective therapies for such tumors. Recent studies suggest electrochemotherapy (ECT) based on bleomycin (BLM) as a possible therapy both for humans and animals (2). Some studies show regression following ECT even of untreated lesions. This highlights a possible involvement of the immune system; hence the importance of characterizing the immune response related to the tumor and therapy as well as the need to identify immunomodulatory therapies which, in combination with ECT, can improve the outcome. In this context, the present project proposes to use the spontaneous SCC present in cats as a model in comparative oncology. To this end, this study focuses on the analysis of clinical biochemistry and hematocytometry data obtained in cats subjected to ECT (n=21) and cats not treated due to too severe lesions (n=16). The results demonstrated an absence of significance difference before ECT treatment (T0) for each parameter analysed following the comparison between the group of cats selected for ECT treatment and cats with lesions judged too severe to be treated. A subsequent comparison of the values obtained in untreated cats (T0) belonging to the group selected to receive treatment with ECT, and cats treated with ECT (T1) highlighted an increase for albumin ($p<0.05$), the width of the platelet distribution ($p<0.05$), and neutrophils ($p\approx0.05$) in the treated group. Differently, lymphocyte and monocyte population underwent a reduction (respectively, $p<0.05$ and $p\approx0.05$) in the cats subjected to treatments. The subsequent comparison between the data measured at T0 and T1 with the data obtained following samples taken 15 days after the treatment (T2) highlighted a significant reduction ($p<0.05$) in monocytes. Nonetheless, the comparison of the hematocytometric data obtained by performing the T2 sampling with the data obtained previously highlighted a further significant reduction in platelets ($p<0.05$), platelet hematocrit ($p<0.01$) and basophil population ($p<0.01$).
However, future studies involving a larger number of subjects, should be carried on validating our observations.

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The Swine nephroblastoma as a model for human Wilms' tumor.

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Nephroblastoma is a relatively common renal neoplasm of swine, is typical of youngest subjects, affecting animals under 1 year of age, and represents the animal counterpart of Wilms' tumour (WT) of children [1]. WT is the most common paediatric renal tumour, with an annual incidence of 1 in 10.000 children occurring in both sporadic and congenital forms [2]. Standard therapy consists of chemotherapy before or after surgical intervention. Unfortunately, some patients do not respond to treatment or undergo disease relapse. The importance of inflammatory microenvironment is a key component of many tumours, where it may promote both the epithelial-mesenchymal and the mesenchymal-epithelial transitions (EMT and MET). It has been documented that WT is characterized by an immunosuppressive microenvironment. The cell infiltrate, with a low number of lymphocytes, is usually detected in the stromal component of the tumour, and the most abundant cells are tumour-associated macrophages (TAMs): WT is classified as a cold tumour due to a lower presence of activated CD8+ cytotoxic T cells [3]. This project focuses on the histological and molecular characterization of nephroblastoma and its inflammatory microenvironment in pigs, aiming to propose the spontaneously occurring porcine tumour as a novel disease model for WT. Thirty FFPE archival samples of porcine nephroblastoma were examined histologically (H&E staining) and immunohistochemically with antibodies against CD3 or CD20 by using an automatic stainer (Leica bond bax). In the same samples, we analysed the expression of genes involved in tumour progression and MET (IL10, IL17a. CDH1, CDH2, CXCL8, RANKL, ß-catenin, IL1B2, TP53, TGF-ß and INΦß) by RT-qPCR (CFX Bio-Rad). Samples were classified as epithelial-mesenchymal-stromal subtypes showing the same characteristics as their human counterpart. CD3 and CD20 staining was revealed in 11 cases (31%) and 22 cases (73%) respectively. Within these, 11 cases were "double-expresser" (CD3+CD20+) while 11 cases were positive only for CD20. These results indicate that also swine nephroblastoma can be divided into two tumour types: cold (CD3-CD20-) or inflamed (CD3+CD20+, CD3-CD20+).We found different gene expression profiles between CD3-CD20- and CD3+CD20+ cases: in particular, CD3+CD20+ cases showed downregulation of ß-catenin, CDH2, IL1B2, TGF-ß, TP53, INΦß, and up-regulation of CXCL8 (IL8) expression and CDH1. These findings align with the observations in human WT where there is an upregulation of TGF-ß and a downregulation in epithelial WT associated with a poor prognosis.
Our preliminary data recalls what occurs in children's tumour, indicating that swine nephroblastoma is a suitable model for WT.

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SICLIMVET

77° CONVEGNO SISVET

SOCIETA´ SCIENTIFICA DI RIFERIMENTO **SICLIMVET**

TITOLO **FLOW CYTOMETRIC CD44 AND CD45 EXPRESSION TO CHARACTERIZE NON-HEMOPOIETIC NEOPLASTIC, REACTIVE, EPITHELIAL AND MESOTHELIAL CELLS IN CANINE EFFUSIONS**

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Testo e Riferimenti bibliografici

The classification of non-hematopoietic (NH) cells in cavitary effusions is challenging and flow cytometry (FC) can help to characterize them [1]. CD44 is a versatile molecule with different functions, ranging from basic cellular processes to inflammation, cancer, and tissue regeneration and its expression was investigated as a potential biomarker in various human and canine tumors [2]. This preliminary study aims to investigate CD44 expression and autofluorescence in CD45-negative NH cells in canine effusions. Dogs were privately owned and underwent sampling for diagnostic purposes with signed informed consent from the owners. Thus, specific formal approval by the authors' Institution Committee for Animal Care was not required (protocol 1965–2017, Ethical Committee, University of Turin). Thirty-one effusions with CD44 vs CD45 analyzed by FC were retrospectively included. They were grouped in 20 neoplastic (N) and 11 reactive (R) cases based on clinical and clinicopathological data and in 11 epithelial (E) and 18 mesothelial (M) cases according to cytokeratin (CK), vimentin (VIM), and desmin (DES) expression [3]. M group was subclassified in 7 MN and 11 MR. Only sample with >1% of CD45-negative cells were included. The proportion of CD45-negative cells (%CD45neg), and the Median Fluorescence Intensity (MFI) of CD45 (autofluorescence) and CD44 were recorded. The CD44/CD45 ratio was also calculated. %CD45neg, CD44MFI, CD45MFI and CD44/CD45 ratio were compared between groups (N vs R; E vs M; MN vs MR) (Mann-Whitney test). ROC curves were prepared for statistically different parameters and cut-off values to distinguish N and R favoring specificity over sensitivity were defined. N had higher %CD45neg cells compared to R (21,35 vs 3,38; p=0,007), and R had higher CD44MFI (p=0,026) and CD44/45 ratio (p=0,023) compared to N. No difference in CD44/45 ratio was detected between E and M both in general and restricted to the N group. On the contrary, CD44/CD45 ratio was significantly higher in MR compared to MN (p=0,0268). A CD44/CD45 ratio value of 141,6 was the best cut-off to detect MN (Sp=91%; Se=86%) and N (Sp=91%; Se=65%).
In conclusion, a combined FC CD44/CD45 labelling can contribute to distinguish neoplastic and non-neoplastic NH cells in effusions by measuring the %CD45neg cells, autofluorescence and CD44MFI. High %CD45neg are suggestive of neoplastic effusion and a CD44/CD45 ratio lower than 141,6 is suggested to detect neoplastic mesothelial cells. Further perspective studies on larger case series are needed to confirm these results.

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77° CONVEGNO SISVET

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Exploratory evaluation of homotaurine as wellbeing enhancer in cognitive impaired dogs

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Thanks to the progresses in veterinary medicine, the life expectancy of dogs has increased and, consequently, the incidence of degenerative modifications associated with aging. As in humans, different life stages are characterized by different cognitive features, and some elderly dogs tend to develop an age-related disease called cognitive dysfunction syndrome (CDS), that affects the brain and causes deterioration similar to Alzheimer's disease in humans. Affected dogs may display one or more of the following symptoms as disorientation, decrease in social interactions, changes in sleep-wake cycles, loss of prior housetraining, increased anxiety, and changes in their level of activity.[1] Currently, only few pharmacological options are available to counteract the deleterious effects of CDS in dogs (selegiline and propentofylline). Nutraceutical supplementation has been extensively studied for CDS. Scientific research has shown that, beside environmental enrichment, dietary supplements containing cellular antioxidants, mitochondrial cofactors, omega-3 fatty acids and other neuroprotective agents may play a role in diminishing the risk factors associated with brain damage caused by CDS.[2]Homotaurine (HT), also known as Tramiprosate is a small, orally administered compound that binds to soluble amyloid beta substance, reduces amyloid aggregation and subsequent deposition, would seem to have a promising protective role against oxidative stress on DNA caused by free radicals produced by the oxidation of catecholamines. In a previous study, HT has been shown likely to improve cognitive ability in aged dog affected by CDs when administered over a 8-month period.[3]A possible short effect of HT on wellbeing, attitude, and emotional state during CDS has not been ascertained yet. Six dogs affected by CDS received HT supplementation (500mg once daily) over a period of 4 weeks and their owners, who were kept blind about the possible effect of HT, were asked to fill a short questionnaire regarding their dog's behavior and wellbeing on a weekly basis for the length of observational period.Data obtained were analyzed using ANOVA for repeated measure and a value of $p<0.05$ was considered significant. Over the 4-week supplementation period, all dogs revealed a decrease in anxiety and confusion accompanied by a restoration of sleep-wake cycle, enhancements in learning and memory tasks, and reestablishment of previous social behavior. This preliminary evaluation indicates HT may deserve to be better investigated for its possible role in amelioration of wellbeing and mental state of elderly CDS affected dogs. Notably, HT supplementation has improved the quality of life for both dogs and owners and a larger, randomized, and placebo-controlled study should be performed to confirm these preliminary findings. References [1] Landsberg, G. M., & Malamed, R. (2017). Clinical picture of canine and feline cognitive impairment. Canine and Feline Dementia: Molecular Basis, Diagnostics and Therapy, 1-12.[2] Neilson, J. C., Hart, B. L., Cliff, K. D., & Ruehl, W. W. (2001). Prevalence of behavioral changes associated with age-related cognitive impairment in dogs. Journal of the American Veterinary Medical Association, 218(11), 1787-1791.[3] Benedetti, R., Marchegiani, A., Tambella, A. M., Fruganti, A., Serri, E., Malfatti, A., & Spaterna, A. (2019). Effects of chronic supplementation of homotaurine on cognitive processes and spatial cognition in aged dogs: preliminary results. Journal of Veterinary Behavior, 33, 90-95.

77° CONVEGNO SISVET

SOCIETA´ SCIENTIFICA DI RIFERIMENTO **SICLIM-VET**

TITOLO **EVALUATION OF GALECTIN-3 IN DOGS WITH ATRIAL FIBRILLATION**

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Testo e Riferimenti bibliografici

Galectin-3 (Gal-3) is a novel biomarker associated with fibrosis and inflammation. In humans, Gal-3 plays a role in cardiac remodelling and elevated levels are associated with an impaired outcome in patients with heart failure. Furthermore, an increase in Gal-3 concentration was found to be a risk factor for the development of atrial fibrillation (AF) [1]. Few recent studies have investigated the role of Gal-3 in dogs with cardiac disease, but the results were quite controversial [2,3]. As in humans, AF is the most prevalent canine supraventricular arrhythmia, particularly in those with cardiac disease associated with left atrial enlargement. The purpose of this study was to evaluate the serum concentration of Gal-3 in dogs with cardiac disease with and without AF.
In this retrospective study, clinical data of dogs visited from July 2017 to October 2023 were reviewed. Dogs with congenital heart diseases (CHD), myxomatous mitral valve disease (MMVD) and dilated cardiomyopathy (DCM) associated with AF were selected. A control group of dogs was then created including clinically healthy dogs and dogs with CHD, MMVD, and DCM but without AF. All dogs included in the study underwent blood sampling and the serum concentration of Gal-3 was assessed using a commercial canine specific ELISA kit. Statistical analysis was performed to assess normality of data using the Shapiro-Wilk test. Not normally distributed data were compared using nonparametric tests, whereas normally data were compared using the t-Student test and ONE-WAY ANOVA for comparisons between two groups or among more than two groups, respectively. Associations between Gal-3 and clinical and echocardiographic variables were evaluated using Spearman's rank correlation coefficient.
Eighty dogs were included, of which 17 (21.2%) were clinically healthy dogs and 63 (78.8%) had heart disease. Among these latter, 30 (47.6%) dogs had AF and 33 (52.4%) dogs maintained a sinus rhythm. Statistical analysis showed no significant difference in Gal-3 concentration between healthy dogs (3.90±0.38 ng/mL) and dogs with heart disease, either with or without AF (3.45±0.28 ng/mL, P=0.226 and 4.46±0.27 ng/mL, P=0.286, respectively). Among dogs with heart disease, dogs with MMVD had higher serum concentration of Gal-3 (4.61±0.22 ng/mL) compared to that of dogs with DCM (2.75±0.34 ng/mL, P<0.001) or CHD (3.17±0.52 ng/mL, P=0.007), but no significant difference was found regarding MMVD stages (P=0.29). In dogs with MMVD, increased Gal-3 concentration was found in those without AF (5.35±0.27 ng/mL, P<0.001), whereas in dogs with DCM no difference was found according to presence or absence of AF (P=0.716). Finally, Gal-3 showed a significant positive correlation with age (r=0.46) and fractional shortening (r=0.41), and a significant negative correlation with body weight (r=-0.40) and aortic root diameter (r=-0.44). Based on the results of this study, Gal-3 neither has a role in distinguishing healthy dogs from dogs with heart disease nor has a predictive value for the development of AF in dogs with heart disease.

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77° CONVEGNO SISVET

SOCIETA´ SCIENTIFICA DI RIFERIMENTO **SICliMVet**

TITOLO **OLALIAMID PROTECTS OBESE DOGS. DOUBLE-BLIND, RANDOMIZED, PLACEBO-CONTROLLED STUDY**

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Testo e Riferimenti bibliografici

Canine obesity is a common nutritional disease, progressively increasing in western countries over the years. In overweight dogs, quality of life is poor and lifespan is shortened; furthermore, canine obesity can predispose to the development of specific pathologies. The present study aimed to investigate whether an olive oil derivative enriched in N-acyl-ethanolamines (Olaliamid, OLA) [1] may protect dogs against obesity-induced metabolic and cardiovascular changes. After obtaining the owner's written consent, 24 dogs aged 2-10 years, weighting ≥ 4.5 kg and with body condition score (BCS) ≥ 7/9 were included, provided they were otherwise healthy. The dogs received a commercial maintenance diet no later than two weeks before enrollment and their lifestyle remained unchanged throughout the study. According to a computer-generated randomization list, dogs were divided in two groups: one supplemented with the study product (OLA) and the other with placebo (OLA vehicle), packaged in indistinguishable bottles. The product was administered once a day orally for 3 months at 0.7 ml/5kg body weight (BW). At baseline (V0) and 3 months later (V1), dogs underwent physical examination and echocardiography (Mindray DC-90, Cina), while owners were administered an 8-item questionnaire on a 10-cm visual analog scale (VAS) about their dog's general condition. The thickness of the interventricular septum (IVSdN) and the posterior wall of the left ventricle (LV) in end-diastole (LVPWdN), as well as the internal dimensions of the LV (LVIDdN and LVIDdsN), were measured in M-mode and normalized for BW. At each time point, blood samples were collected and processed immediately for biochemical analyses, or stored in aliquots at −80 °C until batch assay for leptin (Millipore, USA), IL-6 (Genorise, USA), Reactive Oxygen Metabolites (d-ROMs) and biological antioxidant potential (BAP) (Diacron, Italy). The study was approved by the Ethical Committee of the University of Naples Federico II (PG/2021/0119942). Generalized linear mixed model with Tukey-Kramer post-hoc test for multiple comparisons was used to compare changes between groups, while t-test to compare changes within group at different times. The signed rank test was preferred for robustness in the presence of outliers. P was set at <0.05. The mean age of dogs was 7.5 years, while mean BW was 26.3 ± 13 SD kg. Groups were homogenous at baseline. According to dog owners, difficulty rising from lying down significantly increased in the placebo (P=0.035) but not in the OLA group. No differences were observed in BW and BCS between or within groups. A significant difference was observed in serum ALT (P=0.005), whose level decreased by 20% in the OLA group, while increased by 16% in the placebo group. Similar results were observed for azotaemia (P=0.051); furthermore, bilirubin decreased in the OLA (P=0.030) but not in the placebo group. OLA exerted an anti-inflammatory and antioxidant effect, since it counteracted the increase in leptin observed in the placebo group (P=0.011), decreased IL-6 (P=0.042) and d-ROMs (P = 0.008), and increased BAP compared to the placebo group (P=0.032). Finally, OLA showed a cardioprotective effect, with a significant decrease of IVSdN (P=0.028), LVPWdN (P=0.047), IVSd/LVIDd (P=0.015) and LVPWd/LVIDd (P=0.034) *vs* placebo. Overall, our data showed that OLA was effectiveness against obesity-induced meta-inflammation and oxidative stress, as well as metabolic and cardiovascular dysfunctions.

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77° CONVEGNO SISVET

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Ketosis in dairy cows: assessment of preventive protocols efficacy through biochemical analysis

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Fatty acid metabolism, gluconeogenesis, and Krebs cycle functioning through their precursors (especially propionate, gluconeogenic amino acids (AA), vitamins and co-factors) are essential to reduce the risk of hyperketonemia or subclinical ketosis during post-partum period of dairy cows. The aim of this study was to assess the efficacy of preventive protocols for hyperketonemia through biochemical analysis.
594 Holstein-Friesian dairy cows were selected from the same farm at the begging of the dry period and received the same diet. Blood sampling was performed on each animal from the coccygeal vein at -21 and -7d pre-partum (pre-P), at calving, and at +7, +14, +28, and +55d post-partum (PP). Animals were randomically and equally divided in four groups following a time-series design (ethical approvals n. 204359/2023):
1. CTR-control without preventive treatment;
2. CPP-complete preventive protocol with two treatments of AA, inositol, and cyanocobalamin at 15 and 12d during the pre-P, 2 mL/10 kg of BW (IM), Bograss and seven treatment with acetyl-methionine, α-Lipoic acid and cyanocobalamin, 20 mL/animal, Erbacolina Plus each other day from calving to 12d PP;
3. SPP-simplified preventive protocol group received two treatments of Bograss with previously dosage and Erbacolina Plus (70 mL/animal per treatment) at 12d pre-P and 6d PP);
4. MON-monensin group received one monensin bolus (35.2 g/animal, Kexxtone) at 21d pre-P.
Serum NEFA and BHB concentrations were assessed in all animals. Biochemical analyses were performed on 45 animals per group (30 multiparous and 15 primiparous). Liver functionality index (LFI) was calculated based on albumin, total bilirubin, and cholesterol values at +7 and +28d PP. Differences in biochemical parameters were assessed with a linear mixed-effects model. A post hoc pairwise comparison was performed using Bonferroni correction. A p-value<0.05 was considered significant, whereas a 0.05≤p-value≤0.10 was considered a trend.
Lower NEFA and BHB levels were found in CPP and SPP compared to CTR and MON during PP, with an hyperketonemic state in primiparous cows on CTR at 28d PP and pluriparous cows at 14d PP. Furthermore, the CTR incurred in hypoglycemia at 7d PP. Serum albumin concentrations decreased from 7d pre-P in CTR, 7d PP in CPP and from 14d PP in MON, while increased in SPP with the greatest level at 14, 28, and 55d PP. Serum urea was lower in CPP and SPP at 7 and 14d PP compared to CTR. Serum AST levels were within the physiological range, but lower concentrations were noted in CPP and SPP from calving to CTR and MON. Serum GGT was greater in the MON from calving to 55d PP with the lowest level in CPP at 28 and 55d PP. Serum ALP concentration was greater in CPP and SPP from 7d AP to 55d PP compared to CTR and MON with greater level of Ca from calving to 14d PP. Furthermore, serum Cl and Na were greater in the same groups around calving. Lastly, SPP cows had the greatest LFI with no differences found between CTR and CPP cows.
The preventive protocols CPP and SPP reduced lipomobilization, metabolic stress, and protein catabolism, and improved liver health status and calcium mobilization. In addition, SPP resulted in a better liver functionality and support to albumin production. In conclusion, providing precursors for gluconeogenesis and Krebs cycle, especially in both pre-P and PP, had a better effect on the animal biochemical profiles.

77° CONVEGNO SISVET

Stato: INVIATO - ID: 13107

Ultrasound evaluation of mammary gland cistern in Holstein-Friesian cows affected by clinical mastitis

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The objective of this study is to assess ultrasound mammary gland evaluation as a reliable on field diagnostic tool for mastitis in dairy cows.This study was carried out within Agritech National Research Center. During a period of 10 months, 356 mammary quarters of 89 primiparous and multiparous Holstein Friesian dairy cows from a single farm were evaluated. Animals treated in the same lactation with antibiotic, or presenting other diseases differing from mastitis were excluded. Animal care and procedures were in accordance with the European directive 2010/63/EU and the national law D.L.2014/26. During this study, sterile milk pool samples were examined for antimicrobial analysis and somatic cell count(SCC); clinical examination of all animals was performed. Based on the results, animals were divided into 3 groups: “healthy”(11), having negative microbial analysis and SCC< 100.000 cells/ml; “subclinical”(55), having at least either positive microbial culture or SCC>100.000 cells/ml; “clinical”(23), having positive microbiological culture and clinical signs of mastitis. After 6 hours from milking, B-mode mammary ultrasound evaluation was performed. Based on the echogenic aspect of the mammary cistern, a possible grading has been proposed. 0 anechoic, 1 little echogenic spots, 2 massive echogenic spots, 3 widely echogenic. Statistical analysis was performed through R software. Shapiro Wilk test showed non-normal distribution of data. Chi-squared test, Kruskal-Wallis test and Spearman’s correlation test have been performed. P-value<0,05 was considered significant.From microbiological culture, the majority of animals were positive to coagulase-negative staphylococci, Streptococcus uberis and Escherichia coli. From ultrasound images, 144 quarters were classified as 0, 143 as 1, 51 as 2 and 16 as 3. Considering the distribution of gradings within the 3 groups, Chi-squared test was performed, highlighting significant difference within groups. Subsequently, a media per animal of the 4 quarters’ grading has been calculated. Kruskal-Wallis test confirmed a significant difference of the media between groups, with the highest value in clinical animals and the lowest in healthy subjects. Finally, Spearman’s correlation between SCC and grading media has been applied. The test showed a Spearman correlation of 0,59 and significant p-value.Ultrasonography of the normal udder parenchyma shows homogenous hypoechogenic parenchyma with interspersed anechoic blood vessel, milk alveoli and lactiferous duct. The gland cisterns appear instead as a large homogenous anechoic area. Several studies previously assessed ultrasonographic changes in parenchyma structure and possible gradings based on them has already been proposed[1,2]. Our study focused on the gland cistern instead. The statistical analysis highlighted a significantly higher echogenicity of gland cistern affected by clinical mastitis, in accordance with previous articles[3]. Moreover, a moderate direct correlation between echogenicity and SCC has been proved. The possibility to predict and evaluate the degree of damage during mastitis through ultrasound mammary gland evaluation, particularly of the gland cistern, could represents an important on-farm tool. Moreover, the establishment of an objective mammary ultrasound score, might provide useful directions not only in terms of animal health and welfare, but also for the evaluation of the most proper therapeutic protocol.
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77° CONVEGNO SISVET

Stato: INVIATO - ID: 13108

Bovine respiratory disease: healing process of lung lesions after florfenicol and meloxicam treatment in veal calves

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Clinical examination (CE) of respiratory signs during bovine respiratory disease (BRD) is not indicative of the healing process in consolidated lungs following treatment. In contrast, lung ultrasonography (LUS) has gained recognition as performant tool to detect BRD that can be used to document the healing process. The aim of this study was to use the LUS as investigation tool to evaluate the lung healing process following florfenicol and meloxicam treatment.
Animal care and procedures were in accordance with the European Directive 2010/63/EU and the national law D.L. 2014/26. A single stock of 84 veal calves were enrolled with an average age of 30.6±9.6d at arrival. CE and LUS examinations were performed on each animal. Nasal and ocular discharges, rectal temperature, cough, ear position, and abnormal breathing were assessed to calculated two clinical scores: Wisconsin (BRD≥4) and California (BRD≥5) Scores. The LUSs were used to establish ultrasonography score (US; 0-5 points score) and modified lung lesion score (LLS; BRD≥10.5). Lung consolidations were measured to provide thickness (cm) and area (cm2) of each lesion. The sum of all consolidated areas provided the total lung consolidation area (TLC; cm2). Animals with the US≥3 or consolidation thickness ≥3cm on cranial region were treated with one-shot of florfenicol and meloxicam (40mg/Kg+0.5mg/Kg; Zeleris®, Ceva Santé Animale). Treated group (TRT; n=36) was monitored at +1, +3, +5, +7, +9, +11, and +14d post-treatment. The non-treated animals during the production cycle were classified as control group (CTR; n=48).
Differences over production cycle, CE and LUS follow-ups were assessed by PROC GLIMMIX procedure of S.A.S.-software. Groups comparisons were performed at arrival, treatment days, and at the day before slaughter of each animal. A post-hoc pairwise comparison was performed using Bonferroni correction. A p-value<0.05 was accepted.
At their arrival, no differences were found between groups. Clinical scores, US, LLS, and TLC were significantly higher in TRT vs. CTR at the treatment day. At the end of the study, clinical scores and LUS examinations were similar or marginally different between groups. In addition, growth performances and beef quality were similar in both groups. A vast majority of BRD cases (88.9%) occurred within first 30d after arrival with another 11.1% occurring until 60d. Overall BRD treatment success rate was 94.3%. BRD chronicity rate was 2.9%; and fatality rate was 2.9%. Interestingly in TRT calves, overall clinical scores were indicative of disease at +5d after treatment (Wisconsin 4.5±0.6; California 5.7±0.8) but not at treatment day. US, LLS and TLC were high at the day of BRD diagnosis (US=4.7±0.3; LLS=15.6±1.9; TLC=30.06±1.98cm2) with cranial regions showing the largest lesions as determined by consolidation thicknesses and areas. Following treatment, a swift lung healing process was observed with significative decrease in US at +3d, +5d, +11d; in LLS at +1d, +5d, +7d; and in TLC at +1d, at +5d, and at+9d.
In this field study in veal calves, BRD mainly occurred within the first month after arrival. Systematic LUS examinations, both US and LLS, allowed to detect BRD five days before clinical scores which ensured prompt treatment. The evaluation of the lung healing process also revealed the fast and beneficial effects of florfenicol and meloxicam in affected calves.

77° CONVEGNO SISVET

SOCIETA´ SCIENTIFICA DI RIFERIMENTO **SICLIMVET**

TITOLO **OCCURRENCE OF BACTEREMIA AND/OR BACTERIURIA IN DOGS AND CATS WITH CHRONIC KIDNEY DISEASE. A PROSPECTIVE STUDY**

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Testo e Riferimenti bibliografici

Chronic kidney disease (CKD) is the most recognised form of kidney disease as well as an important cause of morbidity and mortality in dogs and cats [1,2]. In human medicine, major infections are the most significant and critical non-cardiovascular complications in patients affected by CKD, with bacteriuria being the primary source of bloodstream infections and its evolution toward sepsis [3]. Unlike in humans, there is a lack of data on the prevalence of bloodstream infections, in dogs and cats with CKD, with the only available data concerning the elevated prevalence of bacteriuria. Data on the potential association between bacteraemia and bacteriuria in companion animal with CKD are also lacking. The aim of this observational prospective study was to determine the occurrence of bacteremia, bacteriuria and bacteriuria-related bacteremia in dogs and cats affected by CKD. Client-owned dogs and cats with a documented history of CKD undergoing disease follow-up were enrolled. Each included animal underwent a comprehensive physical examination, clinico-pathological and microbiological analyses of blood and urine, along with molecular detection of the 16S rRNA bacterial gene in blood. Aseptically collected blood and urine were obtained through jugular venipuncture and cystocentesis, respectively. After collection, blood and urine samples underwent bacteriological culture within one hour. In the population enrolled, 2/47 dogs and 1/41 cats presented bacteriemia. Moreover, 8/47 dogs and 6/41 cats presented a positive urine culture. Additionally, in one out of the 47 dogs the same pathogen (i.e. *Serratia marcescens*) was identified from blood and urine samples, with a final diagnosis of urosepsis. No instances of bacteriuria-related bacteriemia were observed in the cat population. This study provides preliminary results on the occurrence of bacteremia in dogs and cats affected by CKD, demonstrating a low prevalence in both species examined. This is the first-time data was prospectively obtained from concomitant blood and urine cultures executed in the entire population of enrolled dogs and cats. Furthermore, this study confirms a high prevalence of bacteriuria in companion animals with CKD, being evaluated for the first time in a prospective observational study in dogs. This study was approved by the Ethics Committee of the Department of Veterinary Medicine of Bari, Italy (Approval number,
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77° CONVEGNO SISVET

Stato: INVIATO - ID: 13114

Evaluation of the efficacy of a nucleotide and lactoferrin product in maintaining stable/improving the clinical picture and laboratory findings in leishmaniotic dogs: a randomized controlled study.

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Canine leishmaniosis (CanL) by Leishmania infantum is a potentially life-threatening sand fly-borne disease in dogs. The outcome of the disease is closely linked to the immune response of the dog, which is the definitive host and the main peridomestic reservoir of the parasite. Several molecules with immunostimulant activity have proven effective in enhancing the protective cell-mediated immune response to the protozoan in dogs [1]. In this regard, nucleotides have been previously studied in association with the Active Hexose Correlated Compound for the management of CanL, showing encouraging results. To date, lactoferrin has not been used for this purpose, although its ability to stimulate the cell-mediated immune response in dogs has already been demonstrated. This six-month-long, prospective, randomized, controlled, therapeutic study aimed to evaluate the efficacy of a product containing nucleotide and lactoferrin in maintaining or improving the clinical picture and laboratory findings of dogs affected by leishmaniosis. Forty dogs that tested seropositive for L. infantum and did not require leishmanicidal and/or leishmaniostatic treatment according to the available guidelines [2] were enrolled in the study and randomized into two groups: treatment group (TG) and placebo group (CG). Both products were blindly administered by both veterinarians and dog owners as palatable tablets at a rate of 1 tablet per 10 kg of weight once every 24 hours for the entire 6-month duration of the study. Following inclusion (T0), dogs were followed up after 3 (T90) and 6 (T180) months. At each time point, for all animals enrolled physical examination and laboratory tests (complete blood count, biochemical panel including acute phase protein [APP], and serum protein electrophoresis) were performed. The immunofluorescence antibody test to detect antibodies for L. infantum, Ehrlichia canis, and Anaplasma phagocytophilum was also executed. Furthermore, a CanL-dedicated clinical score following a previously validated assessment scale ranging from 0 (i.e., absence of clinical signs) to 19 [3] was assigned. During the clinical trial, no statistically significant differences were found between CG and TG at both T90 and T180 in terms of CanL clinical score. However, at the end of the study, more than 40% of the dogs enrolled in the CG developed an active form of leishmaniosis. This was evidenced by increased positive APP (i.e., C-reactive protein and ferritin) and total protein values, accompanied by hypergammaglobulinemia and hypoalbuminemia. In contrast, none of the animals in the TG developed an active form of leishmaniosis at T180. Furthermore, the number of dogs with total protein, albumin, and globulin values in the normal range significantly increased. Additionally, positive APP remained stable throughout the clinical trial in the TG. In conclusion, the administration of a supplement containing nucleotides and lactoferrin for six months was found to be effective in maintaining a stable clinical score and improving laboratory parameters in L. infatum seropositive dogs. The study was approved by the Ethics Committee of the Department of Veterinary Medicine of the University of Bari (Prot. 26/2021).

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77° CONVEGNO SISVET

SOCIETA´ SCIENTIFICA DI RIFERIMENTO **SICLIM-VET**

TITOLO **SMARTPHONE-BASED 6-LEAD ECG: A NEW DEVICE FOR ELECTROCARDIOGRAPHIC RECORDING IN CATS**

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Testo e Riferimenti bibliografici

Growing research on smartphone-based technology for electrocardiographic recording has been developed and has become part of the new concept of mobile health both in human and veterinary medicine. The clinical reliability of smartphone-based ECG for electrocardiographic recording has been shown both in humans and in animals [1,2], with only two recent studies demonstrating the reliability of smartphone-based 6-lead ECG in dogs [3]. The aim of this study was to assess the feasibility and the diagnostic reliability of the eKuore 6-leads ECG, a new smartphone-based 6-lead electrocardiograph (smECG), in comparison to standard 6-lead ECG (stECG) in cats.

This was a prospective, observational study. The study protocol was reviewed and approved by the Institutional Welfare and Ethics Committee of the University of Pisa [20/2024]. All included subjects underwent physical examination, echocardiography and simultaneous electrocardiographic recording with both methods (stECG and smECG) in right lateral recumbency for at least 30 seconds. All ECG traces were reviewed blindly by an experienced operator, who judged whether the traces were acceptable for interpretation, performed electrocardiographic measurements, and assigned a diagnosis. Agreement in electrocardiographic interpretation and diagnosis between smECG and stECG was assessed using the Bland-Altman test and the Cohen's k test.

The study included 43 client-owned cats, 20 females and 23 males, with a median age of 5.5 years [interquartile range (IQR), 3-10 years] and a median body weight of 4.4 kg (IQR, 3.6-5.4 kg). Twenty-one cats were affects by different cardiac disease, and the remaining 22 cats had normal echocardiographic findings. Forty-one cats were in sinus rhythm, one cat had atrial fibrillation and one had third-degree atrioventricular block. Four cats had left anterior fascicular block, 3 right bundle branch block, 1 left bundle branch block, and 1 bifascicular block.

Electrocardiographic tracings obtained with the smECG were interpretable in all cases (100%). Perfect agreement between smECG and stECG was found in the detection of heart rhythm (k=1). No clinically relevant differences were found in the assessment of heart rate (bias, 0 bpm), P wave duration (bias, -0.24 ms), PQ interval duration (bias, 0 ms), QRS complex duration (bias, -2.2 ms) and QT interval duration (bias, 0 ms), P wave amplitude (bias, 0.02 mV) and R wave amplitude (bias, 0.1 mV).

Our study suggests that the tested smECG is a clinically reliable device for assessing heart rate, heart rhythm and electrocardiographic measurements in cats. The device could be a new electrocardiographic tool in cats, particularly useful for telemedicine and mobile health thanks to the easy-to-use smartphone-based system.

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77° CONVEGNO SISVET

Stato: INVIATO - ID: 13141

Analysis of candidate genes for cryptorchidism congenital disorder in pigs

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Cryptorchidism represents a considerable problem in swine breeding since it causes direct economic losses, as it determines a decrease in survival and litter size. Moreover, the testes retained in the abdomen are proven to develop testicular tumours at a higher rate than scrotal testes, and to produce pheromones that alter the organoleptic characteristics of meats, producing the undesirable boar smell [1]. For these reasons, piglets are usually castrated short after birth by adequately trained staff according to Italian laws 122/2011 and 146/2001. In case of cryptorchid piglets, however, a veterinarian must intervene since the operation involves exploration of the abdominal cavity.Cryptorchidism is a disease with complex aetiology, in which hormonal, genetic, anatomical, and environmental elements are involved [2]. To better understand whether hormonal disturbances in the uterus or other mechanisms that regulate the expression of genes are involved in testis descent, ten genes, reported to have a role in this disease in other species, were selected as candidate gene for the identification of causative mutations of cryptorchidism in swine [3]. The DNA of eighteen male pigs divided into two groups of nine wealthy and nine affected animals was extracted and analysed by next generation sequencing of the aforementioned regions to compare the two groups of animals in search of the potential causative mutations. The piglets included in the study were managed according to standard protocols for treating cryptorchidism in compliance with veterinarians' professional ethics and regulations for protecting pigs. Publication of data from the reuse of material derived from the activity of University Departments was approved by the Ethics Committee of the University of Milan (approval number 2/16, Feb. 15/ 2016). We identified 516 single nucleotide polymorphisms (SNPs) and, based on the distribution of the alleles in the two groups, 13 SNPs were identified as putatively related to cryptorchidism. The significance of these SNPs was checked using the Wilcoxon-Mann-Whitney test. This work is a starting point for further studies on a wider population to validate them as potential causative mutations of cryptorchidism. If the causative mutations of the disease will be confirmed, measures could be taken to avoid the breeding of genetically predisposed pigs. This would reduce the expenses incurred by farmers for this disease and increase the quality of life of pigs because surgery and related procedures are a source of reduced animal welfare and economic costs.

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77° CONVEGNO SISVET

SOCIETA´ SCIENTIFICA DI RIFERIMENTO **SICliMVet**

TITOLO **Salivary diagnostic markers of stress in horses affected by stereotypies**

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Testo e Riferimenti bibliografici

Stereotypies are defined as compulsive behavior patterns performed repetitively without a defined target largely described in animals and people. In equine medicine, stereotypies can be easily exhibited by domesticated horses living in human controlled environments especially when natural needs cannot be satisfied. In this perspective, as happens in human beings, stress is considered a major event that disrupts the stabilization of homeostasis in an animal's physiology, psychology, and cognition increasing disease predisposition and occurrence. The most frequent type of stereotypes includes cribbing and weaving. Several studies pointed out the positive correlation between cortisol concentration and stereotypical behavior occurrence since cortisol can produce detrimental effects on health status. In this study we hypothesized that cortisol, salivary alpha-amylase (sAA), and butyrylcholinesterase (Bchol) levels in saliva could correlate with behavioral stress responses in horses. Twenty-six thoroughbreds were included in the study and divided into 3 groups: Group A: 10 clinically healthy sport horses (4 males, 6 females, mean age 2.7±1.1 years), Group S: 11 sport horses (5 males, 6 females, mean age 3.4±1.3 years) showing stereotypic behaviors (cribbing n. 7, weaving n.2, box walking n.2), group L included 5 healthy horses (3 females, 2 geldings, mean age 7.2±3.5 years) used for leisure purpose. Saliva samples were collected in the morning (07:00 a.m.-08:00 a.m.), using cotton swabs (Salivette® Sarstedt AG & Co., Nümbrecht, Germany) inserted into a customized hollow mouthpiece that horses chewed for 5 minutes. All experimental procedures were approved by the Institutional Animal Care and Use Committee of Camerino University (Registration number: 10/2023).

Cortisol, butyrylcholinesterase (Bchol) and salivary alpha-amylase (sAA) levels were measured by using dedicated horse ELISA Kit (Bioassay Technology Laboratory). One-way ANOVA ($p<0.05$) was performed to assess statistical differences between leisure, athletic, and behavioural-impaired horses. sAA, Bchol and cortisol concentrations from different groups resulted as follow: sAA (U/L): group A 38.48 ± 7.82, group S 38.96 ± 10.71, group L 52.22 ± 12.44. Bchol (mU/mL): group A 8.54 ± 2.35, group S 6.08 ± 1.65, group L 6.52 ± 2.25. Cortisol (ng/mL): group A 30.10 ± 9.26, group S 19.29 ± 11.39, group L 9.30 ± 8.40.

sAA in leisure horses was significantly different with respect to athletic and behavioural-impaired ones ($p=0.03$ and $p=0.03$, respectively). Group S had lower level than L but not than A. No differences for Bchol were found.

Cortisol was significantly lower in leisure horses than athletic and behavioural-impaired ones ($p=0.003$), with athletic horses showing a significant increase of cortisol when compared to leisure horses ($p=0.002$).

sAA is considered a reliable marker of stress or anxiety in humans and of acute stress in horses. Further studies could clarify the effect of chronic stress on sAA salivary values. Cortisol resulted to be much higher in athletic than leisure horses and this could represent a consequence of the stress resulting from different management. Cortisol level in horses presenting stereotypies had value in between group A and L. Group A and S had the same management and, although not statistically different, it could be speculated that stereotypies have an anti-stress function as demonstrated in people.

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77° CONVEGNO SISVET

SOCIETA´ SCIENTIFICA DI RIFERIMENTO **SICliMVet**

TITOLO **Prevalence and risk factors associated with aberrant geometric airway smooth muscle arrangement in severe equine asthma**

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Testo e Riferimenti bibliografici

Equine asthma is a chronic inflammatory respiratory disease affecting about 15% adult horses living in temperate climates. In its severe form, the disease manifests with recurrent episodes of increased respiratory effort at rest, reversible following pharmacologic treatment or prolonged antigen avoidance [1]. The main determinant of the clinical signs observed in severe equine asthma is an exaggerated airway smooth muscle (ASM) contraction. The reasons underlining this abnormal activation of ASM in asthma are ill-defined, mostly due to technical difficulties preventing direct study of ASM function in vivo [2-3]. The work presented is based on the recurrent observation of a previously undescribed, aberrant, geometric arrangement of ASM cells (ASMC) in endobronchial biopsies (EBB) of asthmatic horses. In the geometric arrangement, elongated cells are tidily aligned; ASM nuclei are arranged in series at their center and form unusual nuclear lines within the tissue. As the first step in the study of this newly recognized disease trait, the present work aimed at estimating its prevalence in asthmatic and non-asthmatic horses, and evaluating its relationship with clinically relevant risk factors. We retrospectively evaluated EBB samples available in our archive from asthmatic and non-asthmatic horses. For each EBB, sex, age and breed of the horse, clinical group (disease vs. non-disease), and disease status (exacerbation vs. remission) were recorded. Overall, 52/1173 EBB studied presented the aberrant geometric ASM arrangement (apparent 4% prevalence, 95%CI 3-6%). We observed the aberrant geometric arrangement of ASM in 37/276 (13.9%) EBB from asthmatic horses in exacerbation, in 11/834 (1.3%) EBB from asthmatic horses in remission of the disease, and in 4/72 (5.5%) EBB in control horses exposed to antigen challenge in a similar way to asthmatic horses in exacerbations. Based on our data, asthmatic horses in exacerbation of the disease are at increased risk of presenting the aberrant geometric ASM arrangement compared to asthmatic horses in clinical remission of the disease, either pharmacological or obtained by antigen avoidance strategies (risk ratio [RR] 10.5, 95%CI 5.4-20.3), and compared to control horses living in the same unhealthy environment (RR 2.4, 95% CI 0.92-6.77). Our data support an increased prevalence of the newly described aberrant geometric arrangement in asthmatic horses in exacerbation compared to those in remission of the disease, supporting a possible role in asthma pathophysiology.

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77° CONVEGNO SISVET

Stato: INVIATO - ID: 13191

Endogenous symmetric dimethylarginine (SDMA) and asymmetrical dimethylarginine (ADMA) levels in healthy cows and cows affected by subclinical and clinical mastitis.

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Symmetric dimethylarginine (SDMA) and asymmetrical dimethylarginine (ADMA) are indirect and direct inhibitors, respectively, of nitric oxide's (NO) synthesis. NO is a regulator of vascular tension, inhibits the adhesion of inflammatory cells to the vascular wall and the aggregation of platelets [1]. ADMA and SDMA increase in plasma during a disease to modify NO synthesis [2]. The study evaluates plasma SDMA and ADMA levels in healthy cows (H), cows with subclinical mastitis (SCM) and cows with clinical mastitis (CM) at different sampling times (Institutional Animal Care and Use Committee, University of Pisa, N: 18/2023 of 19.04.2023). Cows were included in H group based on clinical examination, California Mastitis Test (CMT) < 1 and somatic cell count (SCC) < 250.00 cells/ml, while mastitic cows had CMT >1 and SCC ≥ 250.000 cells/ml. Group SCM had no clinical signs and no milk alterations, while CM group had clinical alteration both in udder and in milk. The project included 196 samples, of which 96 from H cows and 100 pathological cows (58 SCM and 42 CM quarters). Blood samples were collected in lithium heparin tubes and the harvested plasma was frozen at #80 °C. ADMA and SDMA was assessed according to the method of Teerlink [1]. Data distribution was assessed with the Shapiro-wilk test and resulted not normally distributed. A Kruskal-Wallis test with Bonferroni post-hoc correction was performed to evaluate differences among the groups (H vs CM vs SCM). If the difference between healthy and diseased animals was significant ($p < 0,05$), the cut-off was calculated by Receiver Operating Characteristic (ROC) curve along with the sensitivity and specificity. For ADMA, statistically significant differences were highlighted between H (0.11 micromol/L; 2.22 microg/dl) and both mastitis type (SCM 0.26 micromol/L, 5.14 microg/dl; CM 0.26 micromol/L, 5.22 microg/dl), while SCM and CM showed no differences. The cut off value found was >3.27 microg/dl with a Se of 88.64% and a Sp of 82.95 %. No differences were highlighted for SDMA concentrations between groups (H - 0.11 micromol/L; 2.25 microg/dl, SCM - 0.10 micromol/L; 2.09 microg/dl and CM - 0.08 micromol/ L; 1.68 microg/dl). Correlation between mastitis, oxidative stress and NO levels have been found [3] which can explain our results. In conclusion, ADMA levels are higher in mastitic cows compared to H ones, thus it may serve as a diagnostic marker for mastitis. Further study will be focused on the evaluation of ADMA concentration in relation to specific bacteria.

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77° CONVEGNO SISVET

Stato: INVIATO - ID: 13194

Evaluation of different anatomic regions as potential sites of microneedle implantation in Dairy Cattle

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Continuous monitoring of biomarkers allows early detection of diseases, even in their subclinical forms [1]. Biomarker monitoring in cattle necessitates repetitive blood sampling or other stressful procedures. Micro-needle technology represents a minimally invasive approach for continuous monitoring (maximum length of needles: 1.5 mm) [1,2]. Studies in cattle are limited, but it holds significant potential for dairy industry [2].The first step in determining the feasibility of this technology in cows was to investigate the anatomical characteristics of potential implantation sites.
This study explores the feasibility of 4 different anatomic regions: regio auricularis (pinna), inguinalis (groin), radicis caudae (tail ventral side), fossa retromandibularis (parotid region) as potential sites of microneedle implantation in cattle throughout ultrasonographic and histological skin thickness evaluation (Institutional Animal Care and Use Committee, University of Pisa, N: 20/2023 of 19.04.2023). Fifteen Holstein Friesian healthy cattle, selected for culling, were included. Ultrasonographic evaluation of the mentioned anatomic regions were performed at the slaughterhouse, in standing, not sedated animals. Each anatomic site was scanned with a portable device (Blu, Draminsky SA, Poland), using two different probes (transrectal probe, linear hokey stick probe), with or without a gel pad. Skin samples for histological evaluation were collected after slaughter (post-mortem). The anatomical samples were fixed, embedded in paraffin, and stained with hematoxylin and eosin. Skin thickness measurements were performed on ultrasonographic (ImageJ software) and histological images (NDP.view2). Ultrasound images were assessed for quality (resolution, image uniformity and transducer related artifacts). Results of skin thickness were reported as median and percentile (25%P - 75%P).A total of 240 ultrasound images and 60 skin samples were analyzed. Best ultrasound images were obtained using the linear hokey stick probe and gel pad. The median ultrasonographic measures were 2.46 mm (2.2 - 2.8 mm) for pinna, 4.84 mm (3.6 - 6.06 mm) for parotid region, 3.77 mm (3.28 - 5.38 mm) for groin region and 3.33 mm (2.67 - 4.27 mm) for tail's ventral side. The median histological measures were 3.4 mm (1.7 - 4.8 mm) for pinna, 9.25 mm (6.79 - 10.17 mm) for parotid region, 7.31 mm (6.13 - 8.22 mm) for groin region and 6.79 mm (4.66 - 8.31 mm) for tail's ventral side. The pinna was the area with the lower skin thickness, but all the investigated anatomic sites showed a ultrasonographic and histologic skin thickness greater than 1.5 mm.Results from this study were essential to provide foundational insights for application of mini-invasive monitoring systems in cows. In conclusion, micro-needle technology seems not applicable in dairy cattle due to the high skin thickness. Further studies will investigation alternative technologies for continuous monitoring with a greater penetration potential.
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77° CONVEGNO SISVET

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Evaluation of PCT, PCC, ADMA and SDMA plasma concentrations in healthy lactation dairy cows at different time points.

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Procalcitonin (PCT), protein carbonylated content (PCC), asymmetric dimethylarginine (ADMA) and symmetric dimethylarginine (SDMA) have been investigated as biomarkers of bacterial infection and inflammation [1-3]. Lactation stage may influence biomarkers concentrations due to underline paraphysiologic inflammatory and oxidative stress conditions.The aim of the study was to evaluate plasma concentrations and potential variations of PCT, PCC, ADMA and SDMA in healthy lactating dairy cows at different days in milk (DIM): 15 DIM (T0), 60 DIM (T1) and 150 DIM (T2) (Institutional Animal Care and Use Committee of the University of Pisa, prot. N: 2825/2014). Italian Friesian cows were included based on physiological dry periods, normal general examination, specific udder examination with CMT <1 and somatic cell count <150,000 cells/ml (primiparous) and <250,000 cells/ml (multiparous) at each sampling time (T0, T1, T2). Subjects that developed mastitis or other pathologies during the study were excluded. Blood samples in lithium heparin tubes were collected at T0, T1, T2. The harvested plasma was frozen at -80 °C. PCT, PCC, ADMA and SDMA were assessed using methodologies described previously [1-3]. Data distribution was assessed with Shapiro-Wilk test and the results were reported as median and percentile (25%P - 75%P). One way ANOVA was used to assess potential physiological changes in biomarkers concentrations and difference were significant with p value < 0.05. A total of 17, 19, 21 and 21 cows were included for PCT, PCC, ADMA and SDMA analysis, respectively. Median concentrations of PCT were 64.29 pg/ml (0,00-143,23), 75.36 pg/ml (16,90-161,47) and 77.5 pg/ml (32,49-120,18) at T1, T2 and T3, respectively. Median concentrations of PCC were 0,17 nmol/ml/mg (0,10-0,27), 0,14 nmol/ml/mg (0,08-0,23) and 0,20 nmol/ml/mg (0,08-0,22) at T1, T2 and T3, respectively. Median concentrations of ADMA were 0,11 micromol/l (0,09-0,15) and 2,21 microg/dl (1,74-3,10), 0,11 micromol/l (0,09-0,13) and 2,08 microg/dl (1,83-2,67), 0,10 micromol/l (0,09-0,14) and 2,08 microg/dl (1,89-2,83) at T1, T2 and T3, respectively. Median concentrations of SDMA were 0,11 micromol/l (0,09-0,14) and 2,28 microg/dl (1,71-2,79), 0,12 micromol/l (0,09-0,15) and 2,56 microg/dl (1,88-3,18), 0,10 micromol/l (0,09-0,16) and 2,10 microg/dl (1,72-3,31) at T1, T2 and T3, respectively. No statistically significant differences were found for any biomarker among the three sampling times. Therefore, specific reference intervals for different lactation stages may not be necessary for plasma PCT, PCC, ADMA and SDMA evaluation.

Acknowledgment: Agritech National Research Center funding.

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77° CONVEGNO SISVET

Stato: INVIATO - ID: 13208

Antioxidant activity of a nutraceutical supplement in endurance horses undergoing exercise field test

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Endurance is an equestrian discipline that relies primarily on aerobic metabolism. Intense aerobic exercise produces reactive oxygen species (ROS), that can induce an imbalance between oxidant and antioxidant substances, called oxidative stress, and may be associated with a reduction of athletic performance. In the present study, the effects of the administration of a feed supplement, containing natural antioxidants and omega-3 fatty acids, on the blood antioxidant activity and the athletic condition of endurance horses undergoing an exercise test were evaluated. The study was approved by the Animal Welfare Organization of the University of Milan (Protocol Number OPBA_16_2022) and funded by Tecnozoo s.r.l. Eleven Arabian endurance horses (6.5 ± 3.6 years old, 5 mares, 6 geldings) coming from the same stable and subjected to the same training and dietary regimen, were randomly assigned to treatment or control groups. At T0, a blood sample was collected at rest to assess blood lactate concentration and antioxidant capacity. Then, the horses performed an exercise test on a track with heart rate and GPS monitoring, including a 15-minute warm-up at trot, and 60-minute gallop at 20 km/h. Mean and maximum heart rate (HR) reached during exercise were recorded. After the end of exercise, horses were cooled down and showered, and the HRs at 5-, 10-, 15-, and 30-minutes post-exercise were recorded. At 30 minutes, a second blood sample was collected for blood lactate, antioxidant capacity and serum creatin-kinase (CK) evaluation. Treatment group (6 horses) received 100 gr/daily of the dietary supplement (Algaphyt; Equiplanet by Tecnozoo, Padua, Italy) for 21 days, while controls (5 horses) maintained their diet. After 21 days, the protocol was repeated (T1). The antioxidant capacity of whole blood and red blood cells (RBC) was assessed by the Kit Radicaux Libres (KRL) test (Laboratoires Spiral, France) [1]. Variables (resting and post-exercise blood lactate, resting and post-exercise whole blood and RBC KRL values, post-exercise serum CK, and mean, maximum and post-exercise HRs) were statistically compared within and between groups through two-way ANOVA and post-hoc Fisher's LSD tests. Statistical significance was set at $p<0.05$. Significant time*group effects were observed for serum CK ($p=0.026$), RBC antioxidant capacity at rest ($p=0.034$) and post-exercise ($p=0.019$). At T1, in treatment group, CK decreased ($p=0.006$), while RBC antioxidant capacity increased at rest ($p=0.037$) and after exercise ($p=0.006$). The results showed that the administration of the nutraceutical supplement enhanced RBC antioxidant capacity, probably due to the content of several plant extracts rich in polyphenols and vitamin C, with antioxidants properties [2]. Moreover, the reduced CK concentration in treated horses suggests a mitigated exercise-induced muscular damage, that can be associated to the presence of natural anti-inflammatory substances such as omega-3 fatty acids [3]. The inclusion of this nutraceutical supplement in the diet may be helpful for horses engaged in intense aerobic exercise that involves a significant level of stress on the skeletal muscle system.

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77° CONVEGNO SISVET

Stato: INVIATO - ID: 13227

Computed tomography and clinical findings in 44 foals diagnosed with osteomyelitis

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Bacterial sepsis in foals is a common cause of morbidity and mortality in foals and can produce local septic foci such as osteomyelitis.[1] Diagnosis of osteomyelitis by CT examination has been suggested to be superior but no comprehensive studies are available.[2] The aim of this study was to investigate clinical cases of osteomyelitis in foals and to establish an association between CT findings and survival. The hypothesis was that variables acquired by CT examination would predict survival significantly better than other clinical variables. Foals that presented to a single equine referral hospital between July 2019 and December 2022 were included in this retrospective clinical study. Inclusion criteria were 1. < 9 months of age. 2. Clinical diagnosis of suspected osteomyelitis. 3. CT examination confirming osteomyelitis. Statistical analyses were performed using SPSS 29. Significance was set at p= <0.05. Analysis was carried out for two outcomes, survival to discharge and long-term survival. Fourty-four (44) foals were included in the study. Twenty nine (66%) foals survived to discharge and 23 (55%) long term. Variables significantly associated with survival to discharge and long term survival on multivariable analysis included joint collapse (p=0.011, OR 0.054, 95%CI 0.006-0.506) and bone sclerosis (p=0.014, OR 0.16, 95%CI 0.037-0.693) respectively. Contrarily to prior studies, survival was not significantly influenced by clinical variables, only variables identifiable through CT examination had significant associations with survival. The results of this study advocate for the adoption of early CT evaluation in cases suspected of osteomyelitis for refined treatments and prognostic assessments.

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77° CONVEGNO SISVET

SOCIETA´ SCIENTIFICA DI RIFERIMENTO **SICLIMVET**

TITOLO **PRELIMINARY INVESTIGATIONS ON INHALED MINERAL PARTICLE EXPOSURE AND RELATED EFFECTS IN HEALTHY RACEHORSES**

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Testo e Riferimenti bibliografici

Air quality is a crucial determinant of respiratory health [1]. Mineral particles are commonly inhaled, and some of them can induce lung inflammation [2]. No data is currently available on the relationship between inhaled mineral particles and equine respiratory health. This first investigation was undertaken: i) to assess air quality at a racetrack, ii) to assess mineral particle load in bronchoalveolar lavage fluid (BALF) and its relationship with BALF inflammation and, iii) to investigate potential determinants of increased BALF mineral particle load.
Air quality assessment was performed at a racetrack on two different days (training vs. racing) in September 2023.
To this aim, three particle counters were employed as well as three filter integrated air samplers positioned at 30 (n=1) and 100 cm (n=2) height, at the edge of a dirt (sand) racetrack.
Clinical and BALF data from a cohort of 26 Thoroughbred racehorses (TBR) trained at the same racetrack between June 2021 and October 2022, and for which BALF cytospin samples were still available for re-assessment, were obtained retrospectively. Horses' available data included: age, sex, endoscopic findings, BALF cytology, and bloodwork results. Intracellular mineral particle counts have been performed on BALF slides using polarized light microscopy. Number of races performed and racetrack surface (dirt vs. turf) was obtained by consulting electronic databases. Air quality data were compared using T-test with Welch correction. Correlations were assessed by the Spearman correlation test for not-normally distributed data.
Air quality assessment revealed higher values of PM1, PM2.5, and PM10 during racing vs. training days ($p<0.001$), but climate conditions differed. PM values did not specifically increase during or immediately after horses passing close to the particle counters. Respirable particles exceeded the current allowed daily threshold of 15 µm for PM2.5 when assessed by integrated filter analysis.
Twelve male and 14 female horses were studied; aged 3.6±1.3 (mean±S.D.) years, free of clinically relevant respiratory problems, and with no complaints of poor performance. Fifteen out of 26 horses (58%) had mild lung inflammation based on lung cytology, using the more restrictive thresholds [3]. Intracellular mineral particles were noticed exclusively within macrophages and absolute counts revealed 0.144±0.09/µl and 1.1±0.97 particles*103 macrophages. The number of particles*103 macrophages increased with age ($rs=0.458$, $p= 0.019$), with the total number of races ($rs=0.650$; $p<0.001$), and with the number of races on dirt ($rs=0.517$, $p= 0.008$). The number of intracellular particles was not associated with BALF differential or absolute cell counts.
Intracellular mineral particles are commonly found in the BALF of clinically healthy TBR, and increase with aging and with the number of races performed. Racing is associated with increased exposure to respirable mineral dusts. The presence of inhaled mineral particles within BALF macrophages was not associated with local or subclinical inflammation, and should not be considered as a pathological finding per se. More data are needed to understand the potential implication of such exposure on long term equine respiratory health.

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77° CONVEGNO SISVET

SOCIETA´ SCIENTIFICA DI RIFERIMENTO **SICLIMVET**

TITOLO **APPLICATION OF AN ARTIFICIAL INTELLIGENCE-BASED ALGORITHM TO PREDICT THE SEVERITY OF DEGENERATIVE MITRAL VALVE DISEASE FROM LATERAL THORACIC RADIOGRAPHS USING TWO GRADING SYSTEMS**

Autori

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Testo e Riferimenti bibliografici

Myxomatous mitral valve disease (MMVD) is the most common acquired heart disease in dogs and is classified by the American College of Veterinary Internal Medicine (ACVIM) guidelines into four different stages based on clinical, radiographic and echocardiographic criteria [1]. Recently, an echocardiographic classification of MMVD severity, namely the Mitral INsufficiency Echocardiographic (MINE) score has been proposed [2]. Even if echocardiography is the reference method for the definitive diagnosis of MMVD through the direct visualization of valvular lesions, an automatic evaluation of the cardiac silhouette on thoracic radiographs could aid in the early diagnosis and staging of MMVD [3]. The aim of our study was to develop an artificial intelligence (AI)-based algorithm able to predict the stage of MMVD based on both ACVIM and MINE scores from canine lateral radiographic images of the thorax.
In this retrospective multicentric study, lateral radiographs of dogs with concomitant radiographic and echocardiographic examination were selected from the internal database of the Veterinary Teaching Hospital of the University of Padua and from the AniCura Arcella Veterinary Clinic (Padua) in the period between 2012 and 2023. Animals were classified as healthy, B1, B2, C and D and as healthy, mild, moderate, severe and late stage, according to ACVIM and MINE score, respectively [1,2]. The AI-based algorithm was equally trained on both right and left lateral radiographic views.
A total of 795 lateral radiographs were collected. According to the ACVIM classification system, 81 (10.2%), 236 (29.7%), 159 (20%), 294 (37%) and 25 (3.1%) were labelled as healthy, B1, B2, C and D, respectively. According to the MINE score, 81 (10.2%), 227 (28.6%), 113 (14.2%), 316 (39.7%) and 58 (7.3%) radiographs were labelled as healthy, mild, moderate, severe and late stage, respectively. The area under the curve (AUC) showed a good performance of the developed algorithm in classifying MMVD stages using both classification system. An AUC of 0.88, 0.88, 0.79, 0.89 and 0.84 was achieved for healthy and ACVIM stage B1, B2, C and D, respectively. Based on the MINE score, the AUC was of 0.90, 0.86, 0.71, 0.82, and 0.82 for healthy, mild, moderate, severe, and late stage, respectively. The overall precision was 67% and 60% for ACVIM and MINE score, respectively.
Results of this study showed that the developed AI-based algorithm is a useful tool to predict the severity of MMVD from lateral radiographs in dogs. Moreover, AI-algorithm achieved high performance in the classification of radiographs based both on ACVIM and MINE scores and can be used in the early screening of dogs with MMVD.

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77° CONVEGNO SISVET

SOCIETA´ SCIENTIFICA DI RIFERIMENTO **SICLIM-VET**

TITOLO **Feasibility of electrocardiographic recording in foals using a new smartphone-based digital stethoscope**

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Testo e Riferimenti bibliografici

Smartphone-based technology is becoming useful in human and veterinary medicine for ECG recording as a complementary tool for screening and monitoring in both ambulatory practice and educational settings [1-3]. This prospective observational study was approved by the Ethical committee (University of Pisa, 3/22) and aimed to evaluate the feasibility and reliability of electrocardiographic recording with a new smartphone-based device in foals. The study included 29 foals who underwent auscultation and recording of an ECG tracing using the "Eko DUO ECG + Digital Stethoscope" device. All recorded data were at least 30 seconds in duration. ECG waves and intervals were assessed for data distribution using the D'Agostino & Pearson test and results were expressed as mean±standard deviation or median, minimum, and maximum values. ECG artifacts were classified as present/absent and categorized in 3 groups: 1) < 2 sec, 2-4 sec or >4 sec. ECG tracings were analyzed blindly by an expert operator to assess quality. The study sample was composed by 21/29 (72.5%) trotter, 4/29 (13.8%) saddlehorses, 3/29 (10.3%) quarter horses and 1/29 (3.4%) thoroughbred. Of the 29 foals, 13/29 (44.8%) were fillies and 16/29 (55.2%) colts, with a median age of 18 days (4-59 days). All the ECG tracings recorded were found to be legible and interpretable for the assessment of HR, heart rhythm and ECG measurements, leading to a 100% feasibility. All foals showed sinus rhythm, positive P waves and negative QRS complexes. HR was 114±24.2 bpm; P, PR, QRS and QT intervals were 0.08 (0.05-0.09) sec, 0.14±0.03 sec, 0.10±0.02 sec and 0.25 (0.18-0.47) sec, respectively. Thirteen out of 29 (45%) ECG did not show artefacts, while 16/29 (55%) showed artefacts with a median duration of 1 sec (0-5.2 sec). Artefacts were < 2 sec in 8/16 (50%), between 2-4 sec in 6/16 (37.5%), and >4 sec in 2/16 (12.5%) ECG tracings. No ECG tracings showed artifacts lasting >5 sec in the 30 seconds recording. The "Eko DUO ECG + Digital Stethoscope" device proved effective in assessing PR and QT intervals, as well as the polarity and duration of the QRS complex and it is reliable for assessing HR and measuring ECG waves and intervals in foals. The P wave polarity was consistent with what reported in literature for standard [1-3] and eKuore smartphone-based ECG for adult horses and foals [3], while partially agree with Kardia ECG recording in foals [2]. Further research is needed to evaluate the device's sensitivity in detecting deflections and cardiac arrhythmias.

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77° CONVEGNO SISVET

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Effect of treatment of gastric ulcers on selected fitness parameters in saddlebred horses used for show-jumping competitions: preliminary results

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Equine Gastric Ulcer Syndrome (EGUS) has been associated with poor performance in racehorses, but the effect of the disease on the performance of show-jumping horses is not completely elucidated. Often, in saddlebreds and animals performing at low intensities, the only recorded clinical sign is girthiness during saddling.Aim of this study was to evaluate some fitness parameters in jumping horses before and after treatment for EGUS. The research was approved by the Bioethical Committee of the University of Perugia (protocol number. 21/2022). Nine horses used for jumping competitions at various levels, but with at least 1 year of experience, and with Equine Squamous Gastric Disease (ESGD) at least grade 3 at gastroscopy were included in the study. The horses performed a ridden test with a fitness tracker validated for the use in equids (Equimetre, Arioneo, France), and then were treated with omeprazole (4mg/kg orally once daily for 30days, Gaster, Acme, Italy). After healing (confirmed by endoscopy), a new ridden test was performed. Data of maximal and mean heart rate during saddling, and information on maximal heart rate, maximal speed, stride length and frequency at maximal speed during the ridden test, were compared before and after healing using Wilcoxon rank test for paired samples, with significance set at $p<0.05$. During saddling, maximal heart rate ($p=0.02$) showed a significant decrease, while the mean one was not different, despite a slight tendency to decrease after healing. Maximal speed ($p=0.01$) and stride length ($p=0.03$) showed an increase after treatment, while no changes were seen for maximal heart rate and stride frequency. These preliminary results show that saddling appears to cause discomfort in horses with EGUS. The decrease in maximal heart rate during preparation after healing can be related to a reduction in gastric pain and consequently a decrease in sensitivity of the muscles and anatomical structures beneath the girth and saddle. Performance seems to improve with healing: rise in maximal speed appears to be caused by an increase in stride length, with no change in stride frequency and in maximal heart rate. These preliminary findings indicate that performance and welfare of horses used for show-jumping are negatively impacted by the presence of gastric ulcers: healing of the disease reduces the discomfort during preparation and allows the animals to perform better. Further studies, including a larger number of subjects, are needed to better define the effect of gastric ulcers on fitness parameters in jumping horses.

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77° CONVEGNO SISVET

SOCIETA´ SCIENTIFICA DI RIFERIMENTO **SICLIMVET**

TITOLO **COMPUTED TOMOGRAPHY FEATURES OF PRESUMED ILIAC LATERAL LYMPH NODES IN DOGS WITH CUTANEOUS OR SUBCUTANEOUS MALIGNANT TUMORS**

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Testo e Riferimenti bibliografici

In dogs, the iliosacral lymph center (*lymphocentrum iliosacrale*) consist of the medial iliac, internal iliac and sacral lymph nodes. The lateral iliac (*iliaci laterales*) lymph nodes (LILNs) are well described in bovine, equine, swine and ovine species. However, to our knowledge, these lymph nodes have not been yet described in dogs [1,2]. In daily veterinary practice, indirect Ct lymphangiography is commonly performed to map the sentinel lymph nodes of cutaneous or subcutaneous neoplastic lesions (e.g. mast cell tumor, MCT) in dogs [3]. The aim of this descriptive anatomical study is to illustrate the CT features of presumed lateral iliac lymph nodes in dogs with cutaneous/subcutaneous tumors that underwent indirect CT lymphangiography for neoplastic staging. Three dogs (two English setters and one Golden Retriever) were included; among them, 2 had a cutaneous low-grade mast cell tumor, located on the right flank and on the left thigh, respectively, and one had an apocrine carcinoma of the left thigh. On CT examination, in all dogs a well-defined, oval-shaped structure with soft tissue attenuation was observed in the area corresponding to the presumed lateral iliac lymph node projection. On indirect CT lymphangiography, a high contrast enhancement of the presumed LILN ipsilateral to the primary lesion was observed in all cases, heterogeneous in two cases and homogeneous in one, respectively. In one case, the contralateral LILN was also observed. Only one dog underwent surgery for resection of the cutaneous MCT on the left thigh and of the sentinel lymph node. The LILN was found intraoperatively in the subcutaneous region of the flank following the proximal portion of the ventral branch of the deep circumflex artery. The intraoperative methylene blue dye injection around the tumor helped to visualized the LILN. On histopathologic examination, the presumed LILN was consistent with an early metastatic (HN2) lymph node. These findings suggest that an ovoidal structure consistent with the previously reported anatomical features of lateral iliac lymph node in pigs, horses, sheep and cows may also be detected in canine CT examinations as a sentinel lymph node in dogs with cutaneous/subcutaneous neoplasia. Further studies are needed to confirm the presence of presumed normal LILNs in canine CT examinations.

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77° CONVEGNO SISVET

SOCIETA´ SCIENTIFICA DI RIFERIMENTO **SICLIM-VET**

TITOLO **Plasma mitochondria-containing extracellular vesicles as biomarker of oxidative stress in dogs with preclinical myxomatous mitral valve disease**

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Testo e Riferimenti bibliografici

Myxomatous mitral valve disease (MMVD) is the most common canine heart disease, often causing heart failure. In MMVD, oxidative stress can harm mitochondria causing cell senescence and disease progression. However, this relationship in pre-clinical MMVD remains unclear [1]. In Human medicine, circulating extracellular vesicles (EVs) are attracting for their potential role as biomarkers of oxidative stress in cardiovascular disease. In particular, circulating mitochondria-containing extracellular vesicles (mt-EVs) are large cell-derived lipid-bound vesicles that mediate intercellular communication in ischemic myocardium in humans [2]. Recently, veterinary studies evaluated changes in EVs storage in various diseases, such as leishmaniasis, neoplasms or doxorubicin cardiotoxicity in dogs [3]. However, studies on circulating EVs in small animal cardiovascular diseases are lacking. We evaluated plasma levels of mt-EVs and their content in lipofuscin, a leading marker of oxidative stress-induced cell senescence, in dogs with preclinical MMVD. Client-owned healthy dogs (stage ACVIM A - control group, n=5), stage ACVIM B1 dogs (n=5) and stage ACVIM B2 dogs (n=5) underwent blood sampling. The study protocol was reviewed and approved by the Institutional Welfare and Ethics Committee of the University of Pisa (authorization number: 41/20203). Extracellular vesicles were isolated from plasma via serial centrifugations and the presence of intact mitochondria inside the EVs was evaluated using electron and confocal microscopy. We determined the percentage of plasma mt-EVs using MitoBrilliant™fluorescent probe and we measured lipofuscin content of mt-EVs by assessing red autofluorescence (PC5.5A) with flow cytometry.
The median number of plasma EVs was not different among the groups (P=0.19). The median percentage of plasma mt-EVs was lower in preclinical dogs [B1, 55.8%, interquartile range (IQR) 36.6-59.5%); B2, 44.7%, IQR 13.4-59.0%] in comparison to stage A (79.3%, IQR 77.2-90.6%) (P=0.035 and P=0.041, respectively). Moreover, dogs in stage B2 showed a higher lipofuscin content (median signal intensity 6528, IQR 6293-7350 signal intensity) in comparison to stage A (median signal intensity 2101, IQR 155-2884 signal intensity) (P=0.014).
In conclusions, lower plasma mt-EV levels with increased lipofuscin content might reflect oxidative stress and cellular senescence in stage B2 of canine MMVD. Plasma mt-EVs could potentially serve as a diagnostic tool to assess oxidative stress in preclinical MMVD in dogs.

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77° CONVEGNO SISVET

SOCIETA´ SCIENTIFICA DI RIFERIMENTO **SICliMVet**

TITOLO **Use of a low-fat diet for controlling hypertriglyceridemia in epileptic dogs receiving phenobarbital: a preliminary study**

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Testo e Riferimenti bibliografici

Phenobarbital is commonly used for treating seizure disorders in dogs and has been linked to hypertriglyceridemia (HT) in dogs. [1,2] Hyperlipidemia (HL) can cause pancreatitis, insulin resistance, liver enzyme elevation, gallbladder issues, behavioral changes, seizures, and other neurologic signs. Managing triglyceride and cholesterol concentrations is vital to reduce these risks.[3] Nonetheless, evidence regarding the effectiveness of dietary therapy in patients treated with phenobarbital which develop HL is scarce.
The study aims 1) to evaluate the prevalence of HL in epileptic dogs treated with phenobarbital and 2) to evaluate the efficacy of a low-fat diet in dogs with HT who also receive phenobarbital.
The study is divided into two phases. Phase one was an observational longitudinal prospective study. Client-owned dogs presented to the Veterinary Teaching Hospital who required or were already on phenobarbital therapy were enrolled. At the time of inclusion, all dogs underwent a thorough physical examination, complete blood count, serum chemistry panel (including triglycerides and cholesterol) after a minimum 12-hour fast, complete urinalysis with proteinuria, and abdominal ultrasound. Exclusion criteria were a) pre-existing endocrine disorders like hypothyroidism, hyperadrenocorticism, or diabetes mellitus; b) ongoing corticosteroids or progestin therapy; c) cholestatic disorders (e.g., gallbladder mucocele) when initiating antiepileptic treatment; d) HL before starting antiepileptic therapy. Triglycerides and cholesterol were measured three weeks after inclusion, then every three months.
Phase two was an uncontrolled clinical trial. Dogs that develop HT (triglycerides >150 mg/dL) during treatment with phenobarbital were included and treated with a low-fat commercial diet (fat content <10% metabolizable energy). Triglycerides and cholesterol were monitored every four weeks following the HT diagnosis. Triglycerides and cholesterol concentrations before and after treatment were compared using the Wilcoxon matched pairs signed rank test. Statistical significance was set at $P < .05$.
Twenty-four dogs were included in phase 1. Nine out of 24 (37.50%) dogs developed HT with median value of 280 mg/dL (range: 153-753 mg/dL). The median time of onset of HL was 280 days (range: 30-2052 days) after the beginning of phenobarbital treatment. Instead, 2/24 (8.33%) had hypercholesterolemia (HC) with median value of 310 mg/dL (range: 204-628 mg/dl).
Hypertriglyceridemia resolved after treatment with a low-fat diet in 7/9 (77.78%) dogs, with a median value of 79 mg/dL (range: 35-228 mg/dL). Serum triglycerides concentrations after dietary therapy were statistically lower compared with pre-treatment values [difference of medians (DM) = -204 mg/dL, 95% confidence interval (CI) -420 to -67.5; P=0.008].
Hypercholesterolemia was observed in 3/9 dogs (33.33%) following dietary treatment, with a median value of 301 mg/dL (range: 151-415 mg/dL). Serum cholesterol concentrations after dietary treatment did not differ compared with pre-treatment values (DM = -20 mg/dL, 95% CI -90.3 to 20.1; P=0.097).
The preliminary results of this study confirm the high frequency of hypertriglyceridemia in dogs treated with phenobarbital. A low-fat commercial diet appears to effectively normalize serum triglycerides in these patients, while no effect was detected on serum cholesterol.

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Serum Bicarbonate Deficiency in Cats with Acute and Chronic Kidney Disease

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Bicarbonate deficiency is a frequent condition in both human and canine patients with acute (AKI) and chronic kidney disease (CKD), as kidneys play a fundamental role in generation, and reabsorption of bicarbonate [1]. In uremic patients, serum bicarbonate deficiency is responsible for the enhancement of bone demineralization, and calcium-phosphate disorders. This nexus has been recently reported in dogs, as abnormalities of CaxP product were associated with higher frequency and severity of bicarbonate deficiency [2].

The aim of the present study was to assess the frequency and the severity of serum bicarbonate deficiency in cats with AKI, CKD and acute on chronic kidney disease (ACKD), and its possible association with the degree of azotaemia, and disorders of calcium-phosphate metabolism.
A retrospective evaluation of serum biochemical panels (serum creatinine, urea, ionized calcium, total calcium, phosphate, calcium-phosphate product, and bicarbonate) of cats referred to the nephrology and urology service of the VTH of University of Pisa Veterinary between August 2013 and November 2023, with a diagnosis of AKI, ACKD and CKD was performed. Cats were not included in the study in case they missed one or more biochemical parameters, and/or in case they were already on oral or IV supplementation of sodium bicarbonate. Bicarbonate deficiency was defined for serum bicarbonate < 16 mmol/L, and classified as moderate (12-16 mmol/L) or severe (< 12 mmol/L), according to the IRIS guidelines [3]. Data were analysed through GraphPad Prism™ ($p<0.005$).

Serum bicarbonate deficiency was found in 276/618 cats (45%), of which 173/276 (63%) showed moderate deficiency and 103/276 (37%) showed severe deficiency. Cats with AKI and ACKD showed significantly higher frequency of bicarbonate deficiency (58% and 60%) compared to CKD (38%) cats ($p = 0.002$).
In AKI and CKD cats, a negative linear correlation was found between serum bicarbonate, and serum creatinine, phosphate and CaxP product, while in ACKD cats this correlation was only present between serum bicarbonate, and creatinine and phosphate.
The frequency of bicarbonate deficiency was higher in later stages/grades of the disease in both AKI ($p=0.0145$), and CKD ($p<0.0001$) cats. Cats with serum CaxP $\geq 70 mg^2/dL^2$ showed a higher frequency of bicarbonate deficiency ($p<0.0001$) compared to cats with CaxP $<70 mg^2/dL^2$, but no association was found with the severity of bicarbonate deficiency ($p = 0.2147$).
Serum bicarbonate deficiency seems to be a very frequent disorder in both AKI and ACKD cats, with an increasing frequency and severity in more advanced stages/grades of renal disease. The higher frequency and severity of bicarbonate deficiency in AKI, and ACKD may be caused by a more severe and sudden loss of renal function, whereas the lower frequency of the disorder in the CKD group might be related to the use of a renal prescription diet, or a slower progression rate of CKD in cats.
The association between frequency and severity of bicarbonate deficiency, and abnormal CaxP may suggest a potential connection between metabolic acidosis, and bone-mineral disorders in cats, similarly to what previously reported in dogs [2]. However, the finding of a 21% of cats with normal serum bicarbonate in the group with elevated CaxP product, suggests that bone-mineral disorders may also occur independently by serum bicarbonate abnormalities.

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SICLIMVET

Application of contrast-enhanced ultrasonography in traumatic renal injury: a case series.

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Traumatic injuries resulting from blunt or penetrating abdominal trauma can lead to acute fatalities in both companion animals and humans, with a 10% mortality rate observed in animals. Contrast-enhanced ultrasonography (CEUS) is a safe, radiation-free, and cost-effective method with the potential to detect injuries of abdominal organs such as ruptures, hematomas, lacerations, and active bleeding. In pediatric blunt trauma cases, kidney ranks as the third most commonly affected organ, following spleen and liver. While various studies and reviews describe the application of CEUS in renal traumatic injuries in humans[1], only a limited number of reports has been published in veterinary medicine regarding CEUS features during traumatic kidney injuries[2,3].

Therefore, the aim of this study is to describe CEUS features in traumatic renal injuries of small animals. We reviewed imaging records of dogs and cats with abdominal trauma that underwent to CEUS examination. Among a total of 6 cases, there were 3 dogs and 2 cats with suspected renal laceration or rupture resulting in renal hematoma, confirmed through cytology, clinical pathology, ultrasonographic follow-up, surgery, or CT, and 1 cat displaying subcapsular renal hemorrhage.

In all cases of renal hematoma (n=5), an extended area of enhancement defect within the renal parenchyma was observed, associated with an irregular mass of varying dimensions, deforming the renal profile, and exhibiting avascular behavior after CEUS examination. In the single case of suspected subcapsular renal hemorrhage, severe distension of the renal capsule with evidence of minimally enhanced spots moving centrifugally from the renal cortex and floating in the subcapsular space was noted, representing bubbles of ultrasonographic contrast medium leaking from damaged vessels.

Based on our findings, CEUS demonstrated a high potential to identify traumatic renal injuries and could serve as a reliable tool in the initial diagnosis of cases with suspected or confirmed blunt abdominal trauma in dogs and cats.

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77° CONVEGNO SISVET

SOCIETA´ SCIENTIFICA DI RIFERIMENTO **SICLIMVET**

TITOLO **FIRST AUTOCHTHONOUS BABESIA VULPES INFECTION IN A DOG FROM ITALY**

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Testo e Riferimenti bibliografici

Babesiosis is a global emerging tick-born disease. Large and small babesias are protozoa that infect red blood cells of several wild and domestic animals, including humans, and are potentially responsible for severe forms of hemolytic anemia [1]. Babesia vulpes is a small babesia that mainly causes infections in red foxes. Cases of canine infection have been reported in some European and non-European countries, but never in Italy [2]. In this report, we describe the first documented case of canine babesiosis caused by B. vulpes in Italy.
A 10 months old, intact female, Cane Corso dog was referred to the Veterinary Teaching Hospital of the University of Perugia (PG-VTH) for severe anemia. A month earlier, the dog was presented to the referring veterinarian for lethargy and, after hemolytic anemia was detected, treatment with prednisone (2 mg/kg q24h), mycophenolate mofetil (10 mg/kg q12h) and doxycycline (10 mg/kg q24h) was started. Despite treatment and a blood transfusion administration, the anemia did not improve. When referred to the PG-VTH, physical examination revealed lethargy, weight loss and pale mucous membranes. On serum biochemical analysis, an increased concentration of aspartate aminotransferase, alanine aminotransferase, alkaline phosphatase, gamma-glutamyl transferase and total bilirubin was present. The complete blood count (CBC) showed moderate macrocytic hypochromic anemia and thrombocytopenia; a Coombs-positive test result was assessed. The blood smear evaluation revealed a marked regenerative response and the intraerythrocytic presence of small babesia merozoites. The serological test (IFAT) for Babesia spp. was positive, and B. vulpes was identified by PCR analysis and gene sequencing performed at the Experimental Zooprophylactic Institute of Venice. PCR analysis for Ehrlichia spp., Anaplasma spp., Rickettsia spp. and Leishmania infantum were negative. Another blood transfusion was performed. Treatment with imidocarb dipropionate at 4,25 mg/kg was administered on days 0 and 14, while prednisone and mycophenolate mofetil doses were gradually decreased. The dog showed progressive yet complete improvement of the anemia until a few months later when, coinciding with the first heat, a new decrease in hematocrit was found. On blood smear evaluation, intraerythrocytic merozoites were not observed, but PCR analysis for B. vulpes was still positive. Based on these findings, therapy with azithromycin (10 mg/kg q24h) and compounded atovaquone (20 mg/kg q12h) was administered for 10 days. After treatment, the dog had resolution of anemia and the subsequent PCR analysis yielded a negative result.
To the author's knowledge, this is the first confirmed case of B. vulpes infection in a dog in Italy, where the protozoan had rarely been identified only in red foxes and wild boars [3]. The dog of this report had never traveled outside Italy and, although owners report regular application of ectoparasite anti-feeding products, the transmission by an infected tick remains the most probable hypothesis. In conclusion, B. vulpes should be considered in dogs with severe hemolytic anemia in Italy. Additionally, in our report, treatment with Imidocarb dipropionate was not completely effective, in contrast to therapy with azithromycin and compounded atovaquone which allowed the successful resolution of the infection.

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77° CONVEGNO SISVET

SOCIETA´ SCIENTIFICA DI RIFERIMENTO **SICLIMVET**

TITOLO **Circulating Endocannabinoids in canine cutaneous mast cell tumor**

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Testo e Riferimenti bibliografici

Cutaneous Mast Cell Tumor (cMCT) is the most common cutaneous neoplasia in dogs, accounting for approximatively 7-21% of all skin tumors. Endogenous lipids such as N-arachidonoylethanolamine (anandamide, AEA) and 2-arachidonoylglycerol (2-AG) are integral to the endocannabinoid system (ECS). Besides these lipids, there are structurally related compounds known as N-acylethanolamines (NAEs). The N-acylethanolamines most extensively studied, such as N-palmitoylethanolamine (PEA) and N-oleoylethanolamine (OEA), are involved in the regulation of a wide range of biological pathways. Dysregulation of these compounds has been associated with inflammatory conditions and cancers in both humans and dogs. In a recent study, the receptor component of the ECS was assessed demonstrating CB1-CB2 expression in low-grade cMCT.The present study aimed to determine the plasmatic levels of AEA, 2-AG, OEA, and PEA in dogs affected by cMCT and compare them with healthy control dogs.The study has been approved by CEISA (UNICHD12 N. 1168).Dogs with diagnosis of cMCT presented to the Veterinary Teaching Hospital (VTH) of the Department of Veterinary of University in Teramo, Italy, between February 2023 and June 2023, were enrolled in the study. For inclusion, all dogs without evidence of concurrent disease, must underwent complete clinical staging and histological diagnosis of cMCT and in the same period healthy dogs were enrolled as control. All blood samples were collected and centrifugated and plasma was stored at #80#C until analysis. The measurements of the eCBs were performed by CNR of Pozzuoli, Napoli, Italy. The eCBs were analyzed and quantified after the extraction and purification procedures. All data was evaluated using a standard descriptive statistic. Normality was checked using the D'Agostino Pearson test and the comparisons between the two groups were performed using the unpaired t test or the Mann-Whitney test.Seventeen dogs with cMCT and 11 healthy dogs were included. Dogs affected by cMCT, regardless of the WHO stage and histologic grading, had higher plasma levels of 2-AG (p =0.0001) and lower levels of and AEA (p =0.0012) and PEA (p =0.0075) compared to the control group. No differences were observed at the OEA level between healthy and cMCT dogs (p =0.9264). The ability of eCBs to discriminate between healthy and cMCT was interrogated through the area under the ROC curve (AUC). An accuracy of 0.98 (95% confidence interval [CI], 0.94-1.02) was found for 2-AG, of 0.85 (95% CI, 0.71-0.99) for AEA and of 0.81% for PEA (95% CI, 0.64 – 0.69). Values > 52.75 pmol/ml for 2- AG showed 94% sensitivity and 90% specificity in distinguish cMCT from healthy dogs.The present study provides insights into specific lipid metabolism profiles in cMCT but show some limitations. Firstly, the sample size was relatively small, patients in IV clinical stage were not enrolled and a limited number of dogs affected by high grade cMCT was included. Secondly, the study was conducted using a cross-sectional design, which may not fully capture the dynamic changes in eCBs levels during different stages of cMCT development and treatment. Future prospective studies exploring the expression pattern of endocannabinoids in the cMCT tumor tissue may further enhance our understanding of the ECS involvement in tumor pathogenesis and progression.

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77° CONVEGNO SISVET

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Hyperlipemic dogs with biliary tree disease: lipidogram analysis pre and post fenofibrate supplementation

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Hyperlipemia is common in dogs with biliary tree disease; the cholestasis may be a cause or a consequence of hyperlipidemia because it can induce gall bladder dysmotility and dysfunction [1]. The fibrate therapy has been shown to significantly reduce lipemia in dogs with both primary and secondary hyperlipemia [2]. Our hypothesis is that fenofibrate therapy may change the lipoprotein pattern in hyperlipemic cholestatic dogs. In fact, our aim is to study lipoprotein electrophoretic patterns and changes pre and post fibrate therapy in dogs with biliary tree disease and hyperlipemia.Retrospective cohort study on left-over frozen-stored (-80°C) serum samples of client-owned dogs with chronic biliary disease with hyperlipidemia (hypercholesterolemia and/or hypertriglyceridemia) that underwent fenofibrate therapy at 4-10 mg/kg once daily (T0) and had a re-check after 4-6 weeks of therapy (T1). Medical records were cross searched for dogs with hypercholesterolemia (>280 mg/dL) and/or hypertriglyceridemia (>90 mg/dL) that had a concurrent increase of two or more between alkaline phosphatase (ALP) >250 U/L, gamma-glutamyl transferase (GGT) >11 U/L and total bilirubin >0.3 mg/dL. Afterwards, abdominal ultrasound were reviewed for a chronic biliary tree disease (immobile biliary sludge, cholelithiasis, increased gallbladder wall thickness, intrahepatic biliary tree dilatation, mineralization of the intrahepatic biliary tree, and common biliary duct dilatation). Dogs withmodification of diet between T0 and T1 were excluded. Frozen-stored (-80°C) serum samples were collected and used to perform lipoprotein electrophoresis [3]. Comparison between T0 and T1 chylomicrons, VLDL, LDL and HDL percentages values were analyzed using Friedman test (repeated-measures non-parametric ANOVA) with Durbin-Conover pairwise comparisons.A total of 25 dogs were included in the present study. Most dogs were mixed breed (9 dogs; 36%) and the remaining 16 dogs belonged to other breeds. Median age was 9.8 years with a range of 3.2 and 16.6 years, respectively. T1 serum cholesterol and triglycerides were significantly lower than T0 [cholesterol: median 293 (range: 177-513) vs 368 (235-873) mg/dL, p<0.001]; triglycerides [70 (43-404) vs 181 (39-790 mg/dL, p=0.003)]. In total, 15/25 dogs (60%) had a significant reduction in both cholesterol and triglycerides, whereas 6/25 (24%) and 2/25 (8%) had a significant reduction only in cholesterol and only in triglycerides respectively. No differences (p 0.19 were found in serum total protein at T0 [7 (4.9-8.3 gr/dL)] and T1 [6.8 (3.9-8.7)]. Based on lipoprotein electrophoresis at T1, there was a significant increase of HDL% (from 51% to 62.9%; p=0.006) and reduction of VLDL% (from 33.2% to 18%; p=0.02) and chylomicrons% (from 3.2% to 1.7%; p=0.02). Our result showed a good effectiveness of fenofibrate in reducing lipemia in cholestatic dogs. Lipidogram pattern significantly improved at T1, especially with VLDL and chylomicrons reduction. The rapid control of lipemia can be an important goal to limit hepatic injury in primary and secondary cholestasis.[1] Xenoulis and Steiner. Canine hyperlipidaemia. J Small Anim Pract. 2015.[2] Miceli et al. Fenofibrate treatment for severe hypertriglyceridemia in dogs. Domest Anim Endocrinol. 2021.[3] Bunn et al. Lipoprotein profiles in Miniature Schnauzer dogs with idiopathic hypertriglyceridemia and hypercortisolism. J Vet Diagn Invest. 2024.

77° CONVEGNO SISVET

Stato: INVIATO - ID: 13476

LEFT AND RIGHT-SIDE ECHOCARDIOGRAPHIC MEASUREMENT OF PULMONARY ARTERY STIFFNESS (PAS) IN HORSES

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The Pulmonary Artery Stiffness (PAS) is a non-invasive echocardiographic index of pulmonary artery elasticity that allows the assessment of structural features and function of the pulmonary vascular bed [1]. In equine medicine, it has been demonstrated that PAS can be easily measured non-invasively by pulsed-wave Doppler echocardiography across the pulmonary valve from the right parasternal short axis view [2]. To the best of the author's knowledge, there are no studies in literature that measured PAS by pulsed-wave Doppler echocardiography from the left side in horses. Therefore, the present study aimed to measure PAS by pulsed-wave Doppler echocardiography from both right and left side and to evaluate whether there was a difference between the two measurements in healthy horses. This study was approved by the Institutional Animal Care Committee of the University of Milan (OPBA_14_2023). Fifteen horses of different sex, age and breed were included in this prospective study. Based on history and physical examination, all horses were deemed healthy and underwent echocardiographic examination to measure PAS. Pulsed-wave Doppler of the pulmonary outflow was acquired from both sides of the thorax: from the right parasternal short-axis view at the level of the pulmonary artery with the sample volume on the arterial side of the pulmonary valve [2]; from the left parasternal angled view of the right ventricular inlet/outlet [3], with the sample volume positioned just above the bifurcation of the pulmonary artery. Maximal frequency shift (MFS) and acceleration time (AT) were measured from at least three nonconsecutive Doppler flow traces, and PAS was calculated as the ratio of MFS to AT. From the right side, the median PAS value was 12.03 kHz/s; from the left side, it was 14.93 kHz/s. A Wilcoxon signed-rank test was used to compare the ranks between the two sides. No statistically significant difference was found in PAS measurements when right and left sides were compared in this study. The absence of differences could be an advantage in equine clinical practice because, as already reported in literature [3], echocardiographic images sometimes can be of poorer quality when obtained from the left side compared to the right side. Moreover, when the images were taken from the left side, the more cranial probe placement was less tolerated by some horses in this study. Hence, if the side does not affect PAS measurement, the possibility of choosing the one best tolerated by the horse can facilitate the echocardiographic examination and may reduce discomfort related to the procedure itself. In conclusion, this study showed that PAS values were not influenced by measurement taken from the right or left side of the thorax in horses. Furthermore, we found that pulsed-wave Doppler measurement of the pulmonary artery was better tolerated by most of the horses when taken from the right side compared to the left.

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77° CONVEGNO SISVET

Stato: INVIATO - ID: 13478

A path towards the biological age estimate in dogs using circulating blood biomarkers.

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Chronological age (CA) is a suboptimal descriptor of the aging process [1]. In order to better predict life expectancy, a concept of “biological age” (BA) was introduced. BA is usually calculated using a variety of aging biomarkers. Aging biomarkers have been intensively studied and validated in humans, whereas in dogs such studies are limited and the concept of biological age has only seldom been applied. The aim of this study was to propose a novel method to calculate canine BA, based on routine blood laboratory tests.
208 privately owned, clinically healthy, dogs above 5 years of age were enrolled for this study. A questionnaire addressing lifestyle, health status, and cognitive function, was administered to the owners [2]. A physical capability test (time on a 10-meters-long on-leash trail) was performed in each dog. Furthermore, a complete clinical examination and routine blood tests were performed in each dog. To calculate the biological age of each dog, a four steps procedure was used: 1) the laboratory parameters showing a linear variation with age were identified. 2) a linear regression model correlating the previously selected biomarkers and age was developed. 3) the normalized difference between the actual and the expected value, based on the regression model, was calculated for each parameter for each dog 4) the biological age was calculated as the sum of all the normalized differences for each dog. The BA distribution was normal, with dogs categorized as "biologically younger", "equivalent to CA," or "biologically older" based on their BA’s deviation from the mean.
Five biochemical (azotemia, cholesterol, Cl, C-reactive protein, and K) and nine hematological parameters (red blood cells count, hematocrit, hemoglobin, mean cell hemoglobin, mean corpuscular hemoglobin concentration, red blood cells distribution width, platelet count, plateletcrit, and basophil count) showed a robust linear variation with age. Dogs deemed "biologically older" exhibited lower cognitive scores and poorer physical capability test performances than their younger or age-equivalent counterparts. Finally, "biologically older" and "biologically younger" dogs had an equivalent mean and median CA.
We hypothesize that the age category calculated using the proposed model based on laboratory parameters could be used as a proxy to the biological age calculation in dogs. Finally, we discuss the effects of breed, weight and the body condition score to the biological age calculated this way. The biomarkers included in this project will be assessed every six months for an 18-month period, thus allowing a more accurate estimation of BA.

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77° CONVEGNO SISVET

SOCIETA´ SCIENTIFICA DI RIFERIMENTO **SICLIMVET**

TITOLO **ASSESSMENT OF THE QUALITY OF LIFE OF CATS AFFECTED BY PARAPARESIS/PARAPLEGIA AND URINARY RETENTION AND THEIR IMPACT ON CAREGIVERS**

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Testo e Riferimenti bibliografici

Severe spinal cord injury (SCI) can result in irreversible and significant neurological impairments, including loss of mobility and micturition disorders. These concerns impose physical and emotional strains on their caregiver. Certain studies mention the time and owner effort needed to assist dogs with chronic SCI and pet quality of life (QoL) [1,2]. Nevertheless, comparable data for client-owned and shelter cats are currently unavailable.
This study aimed to analyze the QoL of cats affected by paraparesis/paraplegia and urinary retention, in shelter and household settings. The secondary aim was to assess how these conditions affect the owners' QoL.
One veterinary technician evaluated the QoL of 45 cats in shelter and private settings by using an assessment framework priorly developed and adapted for the purpose of this study. Observations focused on pain, hunger, hydration, hygiene, happiness, mobility (paraplegia or paraparesis), and stress using standardized scoring systems. The second part of the study investigated time commitments, care techniques, and social impacts on caregivers, through an analysis of two questionnaires distributed to cat owners and shelter volunteers.
The median age of cats was 4 years (range 1-13 years). All cats experienced a traumatic SCI; 18/45 were paraplegic, and 15/45 developed neurological deficits within their first year of life, while the remaining 30/45 after surpassing their first year. Observed cats maintained a good QoL (median score 64/70, range 50-70). No significant differences were observed in the QoL score of 8 private-owned cats (median 65, range 62-68) and 37 shelter cats (median 64, range 50-70, p=0.6130). Cats with ambulatory paraparesis showed a higher score (median 67/70, range 58-70) compared to non-ambulatory cats (median 64/70, range 50-67, p=0.0026). However, both ambulatory and non-ambulatory cats showed satisfactory scores.
40 owner-directed and 38 shelter volunteer-directed questionnaires were analyzed. Most caregivers seem to perceive the temporal dedication required for caring for cats, in both domestic environment and shelter, as not imposing a substantial burden. The anticipated and perceived challenges seem to be smaller than initially imagined. Although, as expected, caring for a cat with a severe SCI revealed a decrease in the owner's QoL, most owners still believed that the effort invested in caring for their pets was worthwhile.
One limitation of the study is the population bias. It is evident that owners or volunteers who opt to care for these cats instead of choosing euthanasia must possess a significant level of dedication to their pets. Another limitation of the study is the QoL assessment made by a single qualified person. Although it could be a subjective bias, the framework is created as a simple-to-use and straightforward QoL scale, with objective scoring.
This study aids prospective owners of paraplegic cats with urinary retention and veterinarians in making informed decisions regarding whether to maintain or euthanize the afflicted animal. Although much debate remains on the QoL of cats with severe SCI and further studies are needed to address this topic, these preliminary results suggest that these cats can have a good life and be managed adequately, even in a shelter environment.

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77° CONVEGNO SISVET

SOCIETA´ SCIENTIFICA DI RIFERIMENTO **SICLIMVET**

TITOLO **PROTEOMIC INVESTIGATION ON SERUM OF HORSES AFFECTED BY OBSTRUCTIVE COLIC**

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Testo e Riferimenti bibliografici

The evaluation of new biomarkers during obstructive colic can improve early diagnosis, help to define their severity, or evaluate the effectiveness of any therapeutic procedures. Given the premises, the objective of the present study was to identify and characterize the functional proteins expressed in horses suffering from colic due to large intestinal obstruction using a proteomic technique to characterize the ongoing pathological process. With the owners' prior written consent, all horses underwent a complete routine clinical examination and venous blood samplings. A total of 17 samples were analyzed using a technique based on Liquid Chromatography with tandem mass spectrometry (LC-MS/MS), to identify and characterize functional proteins expressed[1]. Of these, (i) 14, belonging to 7 sick horses sampled before and after conventional conservative therapy leading to clinical recovery, were used for an intra-group comparison (recovery vs. acute phase); while (ii) 3 belonging to healthy horses were used for an inter-group comparison (acute phase vs. healthy group). Intra-group differences were evaluated using paired-sample Student's t-test while inter-group differences by Student's t-test for independent samples. Changes in protein expression were calculated as abundance ratios (Fold Changes) expressed in terms of label-free quantification intensity. A total of 537 proteins were identified in the intra-group comparison while 556 proteins were instead recorded in the inter-group one. In the first case, proteins belonging to the processes of the immune response (e.g., Complement C2 – log2FC 1.27; Hemopexin – log2FC 2.88, etc.), blood coagulation (e.g., Von Willebrand factor – log2FC -0.89; Coagulation Factor XIII B Chain – log2FC -0.82, etc.), antioxidant activity (e.g., Apolipoprotein E – log2FC -0.61; Apolipoprotein E – log2FC -0.83, etc.), biosynthesis and lipid homeostasis (e.g., Apolipoprotein E – log2FC -2.94; Lecithin-cholesterol acyltransferase – log2FC -0.79) were significantly up or down-regulated. While in the inter-group comparison, the functional analysis did not allow significant enrichment groupings, although differences related to the individual proteins involved in the innate immune response (e.g, Serum amyloid A protein - log2FC 6,28; lactotransferrin - Log2FC 1.90, etc) and coagulation (e.g., Fibrinogen alpha chain -log2FC 2,42, etc.) were observed. Proteomic analysis proved to be an interesting tool for the assessment of biomarkers for evaluating the effectiveness of the employed therapy, while the results evaluating the possibility of making an early diagnosis of this type of colic were less encouraging. However, overall, the method has allowed to formulate in horses affected by obstructive colic interesting hypotheses regarding the simultaneous presence of intestinal dysbiosis associated with an inflammatory (explained by the up-regulation of proteins able to destroy bacteria and limit their spread), tissue damage (justify by up-regulation of proteins involved in vascular and tissue damage) and lipid mobilization (confirmed by up-regulation of proteins related to lipid homeostasis and transport). Further studies are desirable to confirm the hypotheses on a larger sample population.

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77° CONVEGNO SISVET

Stato: INVIATO - ID: 13507

Retrospective evaluation of therapeutic response in 148 dogs diagnosed with immunosuppressant-responsive enteropathy

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Immunosuppressant-responsive enteropathy (IRE) is a subtype of chronic enteropathy that, if severe enough, can lead to protein loss (protein losing enteropathy, IRE-PLE) [1-2]. Currently, there is no ideal protocol for IRE, nor it is possible to predict the clinical response to different dietary and pharmacological approaches [3]. Therefore, the aims of this study were to describe the clinical response to different dietary and pharmacological approaches, and to identify factors associated with clinical response in a population of dogs with IRE.
Clinical records of dogs with IRE were retrospectively evaluated. Inclusion criteria were: presence of chronic gastrointestinal signs, failure to respond to dietary and microbiota manipulations only, presence of inflammatory gastrointestinal infiltrate on histological examination, response to immunosuppressive therapeutic trial and appropriate follow-up. Dogs with serum albumin ≤ 2.0 g/dL were classified as IRE-PLE. Data studied at diagnosis (T0) and follow-up (T1= 1 month; T6= 6 months) were: signalment, chronic canine enteropathy clinical activity index (CCECAI), serum albumin concentration, type of diet, type of immunosuppressive therapy (monotherapy or multitherapy), cobalamin supplementation. Dogs with reduction in >25% CCECAI at T1 were classified as responders (nonresponders = reduction ≤ 25% or death). ΔCCECAI was defined as the difference between the CCECAI at T6 and T1. Dogs classified as responders at T1, alive at T6 and having a ΔCCECAI <3 were classified as medium-term responders. Dogs classified as nonresponders at T1, with a ΔCCECAI >2, that died or were euthanized for IRE related causes were classified as medium-term nonresponders.
One hundred forty-eight dogs with IRE (53 IRE-PLE) were included. At T0, median (IQR) age, CCECAI and albumin were 5.25 years (IQR 5), 8 (IQR 4) and 2.39 g/dL (IQR 1.44), respectively. At T0, 113 dogs received prednisolone, 21 prednisolone and cyclosporine, 14 prednisolone and chlorambucil, and 95 cobalamin supplementation. Fifty-six, 45, 29 and 18 dogs received hydrolyzed protein, restricted antigen, home-cooked (low-fat and ultra-low fat formulations) and highly digestible (low-fat and non low-fat formulations) diets, respectively. At T1, 109 dogs were considered responders (27 IRE-PLE), 29 nonresponders (23 IRE-PLE). Median CCECAI was 2 (IQR 4). From T1 to T6, the type of immunosuppressant was not modified, modified from monotherapy to multitherapy (and viceversa), or suspended in 34, 5 (and 4), and 58 dogs, respectively. At T6, 88 dogs were considered medium-term responders (14 IRE-PLE), 40 medium-term nonresponders (29 IRE-PLE). The median CCECAI was 0 (IQR 2). Twenty-one dogs (19 IRE-PLE) died or were euthanized during the study period. No statistically significant difference in clinical response was found regarding the type of immunosuppressive therapy (monotherapy vs multitherapy). Presence of PLE, cobalamin supplementation and home-cooked diet resulted significantly associated with non-response both at T1 and T6 in the multivariate analysis.
In conclusion, factors associated with clinical response were not related to the type of immunosuppression, but to the presence of hypoalbuminemia and hypocobalaminemia, and the need to adopt low-fat and ultra-low fat formulations (home-cooked diets) to reduce protein and lipid loss through the intestinal mucosa.
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77° CONVEGNO SISVET

SOCIETA´ SCIENTIFICA DI RIFERIMENTO **SICLIMVET**

TITOLO **Systemic inflammatory response syndrome in horses affected with acute gastro-intestinal diseases associated with colic syndrome.**

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The systemic inflammatory response syndrome (SIRS) is a complex pathophysiologic response often associated in equine medicine to acute gastrointestinal diseases as colic. The main objective in the management of equine patient affected by SIRS is early treatment. Thus, an early diagnosis may lead to a prompt and adequate treatment and a better prognosis. The study was aimed to investigate the prognostic variables related to SIRS in a population of adult horses affected with acute gastrointestinal diseases.
Forty-one horses referred for acute gastrointestinal disease associated with colic syndrome were included. Clinical data including the signalment, physical examination findings, final diagnosis, and outcome were recorded. Also, at the time of admission all horses were submitted to hematological and biochemical tests including blood lactate concentration. The identification of SIRS was performed and scored in the presence of at least two of the four following parameters, recorded during the course of the clinical examination at the time of admission in emergency: hypothermia/hypothermia (lower than 37 or higher than 38.5 °C), tachycardia (>52 bpm), tachypnea (>20 bpm) and abnormalities in white blood cell count (WBC) >12,500 cells/µL or <5000 cells/µL and 10% band neutrophils). Horses, who were euthanized for poor prognosis or dead spontaneously, were considered in the same group as non-survivors. Based on the presence of almost two SIRS criteria horses were grouped as positive (p-SIRS group, n= 24), while horses with one 0 or 1 abnormal SIRS criterion on admission were considered negative (n-SIRS group, n= 17). Mann–Whitney test was used to compare categorical variables. Fisher's exact test compared the survival proportions for p-SIRS and n-SIRS groups and reported as odds ratio (OR). Also, the case fatality rate has been calculated. The median age was 6 years old (mean 7.9 years old; range 2–20 years old; IQR 4–12.2 years old). The overall case fatality rate in the population study was 34.1% (n = 14/41). The fatality rate for the p-SIRS group was of 50% (n=12/24), while 11.7% for n-SIRS group. Seven horses (29%) in the p-SIRS group were euthanized. Horses in the n-SIRS group died spontaneously. Thirty-two horses (n.17 p-SIRS and n.15 n-SIRS, respectively) were affected by non-strangulating colic, while nine were affected by strangulating colic (n=7 p-SIRS and n=2 n-SIRS, respectively). A significant presence of eosinopenia (P=0.04) was detected in the p-SIRS *vs* n-SIRS. Horses in the p-SIRS group showed values significantly higher in heart rate (P=0.01) and in respiratory rate (P<0.001) than n-SIRS. The OR showed a significant high risk in SIRS horses (P=0.07). A significant level of blood lactate concentration (P= 0.04) was recorded comparing survivors (1.18 ±0.35 mmol/l) and non-survivors (5.92 ±2.96 mmol/l) belonging the p-SIRS group. Although the enrolled population is limited, data reported emphasize the clinical relevance of SIRS in colic horses, and the role of several factors such as heart rate, respiratory rate, blood lactate and eosinopenia in the assessment of colic cases.

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77° CONVEGNO SISVET

Stato: INVIATO - ID: 13517

Diagnosis and treatment of cystitis in dogs: an Italian survey

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Cystitis is a common reason for veterinary visits and antimicrobial administration in dogs; therefore, it is an important target for antimicrobial stewardship activities [1-2].
An observational cross-sectional study was designed to gather information about diagnosis and treatment of cystitis in dogs, by surveying veterinarians from different regions in Italy. Veterinarians were recruited through a link to an online survey that was shared among forums as well as Provincial Professional Association mailing lists.
The survey consisted of 11 mandatory answers, it was accessible from 12 February through 10 March 2024. All responses were anonymous. Descriptive analysis was reported and post-hoc analysis was performed to compare different workplaces using a Chi-squared test. Significance was set at $P<0.05$.
Three-hundred fifty-nine veterinarians answered. The most represented regions were Emilia Romagna (18.9%), Lombardia (17%), Veneto (14.5%), and Toscana (9.5%). Two-hundred twenty-seven (63.2%) veterinarians worked in primary care, 92 (25.6%) in clinic, and 40 (11.1%) in 24h hospital.
Regarding diagnosis of cystitis, 162 (45.1%) were guided by symptoms, bloodwork, urinalysis and abdominal ultrasound, 157 (43.7%) by symptoms and urinalysis, 28 (7.8%) by symptoms, bloodwork and urinalysis, 12 (3.3 %) by symptoms alone.
Urine culture and sensitivity (UCS) was performed by 113 (31.5%) respondents in less than 25% of patients, by 88 (24.5%) in 25-50%, by 77 (21.4%) in 50-75%, and by 81 (22.6%) in more than 75%. The percentage of respondents that performed UCS in more than 50% of dogs differed significantly between primary care (34.4%), clinic (55.4%), and 24h hospital (72.5%) ($P<0.00001$). The main reasons not to perform UCS for respondents were 'owners financial limits' (268, 74.7%), 'delayed results' (73, 20.3%), 'difficulties in urine collection' (72, 20.1%), 'not necessary' (39, 10.9%), and 'difficulties with shipping to external laboratory' (16, 4.5%). Concerning treatment, 142 (39.6%) used antibiotics, anti-inflammatories and supplements (e.g., d-mannose, probiotics), 137 (38.2%) anti-inflammatories and supplements, 28 (7.8%) antibiotics and anti-inflammatories, 22 (6.1%) antibiotics and supplements, 10 (2.8%) anti-inflammatories, 10 (2.8%) supplements, 10 (2.8%) antibiotics. Overall, antibiotics were included in first-line treatment protocol by 202 (56.3%) of respondents.
The most empirically prescribed antibiotic classes were enhanced penicillins (211, 58.8%), chinolones (79, 22%), penicillins (28, 7.8%). Prescription of enhanced penicillins, chinolones and penicillins did not differ significantly between primary care (54%, 24.7%, and 8.8%, respectively), clinic (61.9%, 22.8%, and 5.4%, respectively), and 24h hospital (77.5%, 5%, and 7.5%, respectively) ($P=0.09$). When analyzed separately, prescription of chinolones differed significantly between different workplaces ($P=0.02$). Regarding duration of antibiotic therapy, 157 (43.7%) answered 7 days, 155 (43.2%) 10-14 days, 24 (6.7%) ≤5 days, 23 (6.4%) >14 days. When grouped as short-term antibiotic treatment (7 days or less) and log-term antibiotic treatment (10 days or more), there was no statistical difference between workplaces ($P=0.08$).
Clinicians were asked to answer about management of subclinical bacteriuria, defined as presence of bacteria in urine in absence of clinical signs [1]: 166 (46.2%) prescribed probiotics, 149 (41.5%) d-mannose, 71 (19.8%) no therapy, 70 (19.5%) antibiotics, 37 (10.3%) anti-inflammatories.
In this study, slightly over 50% of prescriptions for cystitis in dogs were consistent with (International Society for Companion Animal Infectious Diseases) ISCAID treatment guidelines; nevertheless, chinolones were prescribed empirically in 22% of patients. Despite ISCAID recommendation to use short-term therapies to reduce risk of adverse effects and antimicrobial resistance, 49.6% of clinicians still prescribe antibiotics for more than 7 days [1-2].

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77° CONVEGNO SISVET

SOCIETA´ SCIENTIFICA DI RIFERIMENTO | **SICLIMVET**

TITOLO | **DIAGNOSIS AND TREATMENT OF CYSTITIS IN CATS: AN ITALIAN SURVEY**

Autori | I. Tirelli[1], F. Fidanzio[1], S. Bertini[1], S. Crosara[1], L. Intorre[2], I. Lippi[2], V. Marchetti[2], A. Corsini[1]

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Testo e Riferimenti bibliografici

Cystitis is a common diagnosis in companion animals and is responsible for a significant prescription of antimicrobials in veterinary medicine[1].
An observational cross-sectional study was designed to gather information about diagnosis and treatment of cystitis in cats, by surveying veterinarians from different regions in Italy. Veterinarians were recruited through a link to an online survey that was shared among forums as well as Provincial Professional Association mailing lists.
The survey consisted of 11 mandatory answers, it was accessible from 12th February through 10th March 2024. All responses were anonymous. Descriptive analysis was reported and post-hoc analysis was performed to compare different workplaces using a Chi-squared test. Significance was set at $P<0.05$.
Three-hundred seventeen veterinarians answered. The most represented regions were Emilia Romagna (18.3%), Lombardia (18%), Veneto (16.4%), Toscana (13.9%). Two-hundred (63.1%) veterinarians worked in primary care, 86 (27.1%) in clinic, and 31 (9.8%) in 24h hospital.
Regarding diagnosis of cystitis, 151 (47.6%) were guided by signs and urinalysis, 121 (38.2%) by signs, bloodwork, urinalysis and abdominal ultrasound, 29 (9.1%) by signs, bloodwork and urinalysis, 15 (4.7 %) by signs alone, 1 (0.3%) by signs and bloodwork.
Urine culture and sensitivity (UCS) was performed by 147 (46.4%) respondents in less than 25% of patients, by 74 (23.3%) in 25-50%, by 46 (14.5%) in 50-75% and by 50 (15.8%) in more than 75%. The percentage of respondents that performed UCS in more than 50% of cats differed significantly between primary care (22%), clinic (38%), and 24h hospital (61%) ($P<0.0001$). The main reasons not to perform UCS for respondents were 'owners financial limits' (211, 66.6%), 'difficulties in urine collection' (87, 27.4%), 'not necessary' (60, 18.9%), 'delayed results' (57, 18%), and 'difficulties with shipping to external laboratory' (15, 4.7%).
Concerning treatment, 185 (58.4%) used anti-inflammatories and supplements (e.g., d-mannose, probiotics), 66 (20.8%) antibiotics, anti-inflammatories and supplements, 31 (9.8%) antibiotics and anti-inflammatories, 15 (4.7%) anti-inflammatories, 10 (3.2%) antibiotics and supplements, 8 (2.5%) supplements, 2 (0.6%) antibiotics. Overall, antibiotics were included in first-line treatment protocol by 109 (34%) of respondents.
The most empirically prescribed antibiotic classes were enhanced penicillins (160, 50.5%), chinolones (77, 24.3%), penicillins (21, 6.6%). Prescription of enhanced penicillins, chinolones and penicillins differed significantly between primary care (44.5%, 29%, and 6%, respectively), clinic (61.6%, 20.9%, and 5.8%, respectively), and 24h hospital (61.3%, 3.2%, and 12.9%, respectively) ($P=0.009$). When analyzed separately, prescription of chinolones differed significantly between different workplaces ($P=0.005$).
Regarding duration of antibiotic therapy, 138 (43.5%) answered 7 days, 133 (42%) 10-14 days, 32 (10.1%) ≤5 days, 14 (4.4%) >14 days. When grouped as short-term antibiotic treatment (7 days or less) and long-term antibiotic treatment (10 days or more), there was no statistical difference between workplaces ($P=0.44$).
Antibiotics were prescribed for management of cats with urinary tract obstruction by 98 (30.9%) respondents in less than 25% of patients, by 90 (28.4%) in more than 75%, by 71 (22.4%) in 25-50%, and by 58 (18.3%) in 50-75%.
Considering the low rate of bacterial cystitis in cats (<2%)[2], overuse of antimicrobials in these patients is likely common. Following the International Society for Companion Animal Infectious Disease (ISCAID) guidelines[1], limiting treatment to cats with positive urine culture test and using first line treatments (e.g., penicillins) for a shorter period is recommended and is a key component of antimicrobial stewardship.

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77° CONVEGNO SISVET

SOCIETA´ SCIENTIFICA DI RIFERIMENTO **SICLIMVET**

TITOLO **Preanalytical effect of acidification on urinary calcium in dairy cows.**

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Testo e Riferimenti bibliografici

Urinary calcium (uCa) concentration is considered a reliable indicator of the success of acidogenic diets. It is commonly asserted that the uCa concentration may be underestimated by chemical analysis because of the precipitation of uCa in urine's alkaline pH. According to this, acidification of urine samples before analysis is suggested to ensures the solubilization of calcium [1]. However, to date, the effectiveness of this process has not been validated and remains a subject of debate among researchers [2]. The aims of this trial were to define if preanalytical acidification affects uCa measurement and stability of uCa after 21 days of storage. To these aims, at least 15 ml of free catch urine samples were obtained from 20 dairy Holstein, multiparous cows, balanced for body condition score, and immediately delivered to the Clinical Pathology Laboratory of the University of Parma. From each sample, 15 mL of urine were equally divided in three separated plastic tubes (5 mL each) and mixed with 80 µL of a 50% sulfuric acid solution (Group 1) (as described previously [3]), 80 µL of distilled water (Group 2), or left unaltered (Group 3). After pH determination (Hanna Instruments Inc., USA), all tubes were centrifuged. Urinary calcium (cresolphthalein method) and urinary creatinine (uCrea, picric acid methods) concentration were determined on urine surnatants immediately after centrifugation (T0) using an automated chemistry analyser (BT3500 Biotecnica Instruments). The same analysis was repeated after 21 days on the same samples stored at -20°C (T21). The uCa to uCrea ratio (uCa/uCrea) was also calculated.
Differences over groups (Group 1 vs Group 2 vs Group 3) were compared by Friedman test followed by post-hoc analysis in case of significant results. Differences between the two time points (T0 vs T21) were compared by means of Wilcoxon test.
In Group 1, prior to acidification, the pH (mean±SD) was 8.75±0.19 and dropped to 1.89±0.14 following acidification. The values of uCa did not differ between Group 1 (0.75; 0.10 – 4.90 mg/dL) and Group 3 (0.70; 0.10 – 4.80 mg/dL), while Group 2 (0.65; 0.10 – 4.70 mg/dL) showed significant lower results compared to the other groups ($p < 0.003$ vs Group 1; $p < 0.007$ vs Group 3; overall $p < 0.004$). The uCa/uCrea values were not different between Group 1 (0.016; 0.002 – 0.08 mg/dL), Group 2 (0.04; 0.003 – 0.08 mg/dL), and Group 3 (0.014, 0.002 – 0.008 mg/dL) (overall $p = 0.06$). No significant differences were observed between the groups for both parameters after storage for 21 days at -20°C and results did not varied according to the different storage time.
Even if our results differed between Group 1 and Group 2, when analysis is performed immediately after sampling, it is unlikely that the observed variation may impact the monitoring of acidogenic diet in dairy cows in practice. This is also supported by the absence of differences between acidified urine and unaltered urine on fresh samples and between all groups after storage for 21 days. In conclusion our study suggests that free catch urine sample from cows do not need pretreatment with acidification to properly determine calcium and creatinine concentration for monitoring purposes both on fresh and frozen samples. The present work was carried out in accordance with Italian law on animal ethics (DL 4/3/2014 n26).

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77° CONVEGNO SISVET

Stato: INVIATO - ID: 13525

Long-term follow up of 20 dogs with chronic hepatitis

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In humans, chronic hepatitis (CH) may progress to liver cirrhosis and consequent liver failure or to hepatocellular carcinoma [1,2]. In veterinary medicine, scarce literature is available on long-term follow-up and outcome of CH dogs, also considering the wide variability in clinical presentation, disease stage and therapeutic approach.
This study aimed to evaluate outcome (survival time, death CH-related, neoplastic progression), clinical signs, biochemical findings and signs of liver disease progression and liver failure in CH dogs. CH was histologically diagnosed according to WSAVA criteria [3]. Clinical and biochemical findings along with abdominal ultrasonography features were collected at various timepoints: diagnosis (T0), 6 (T6), 12 (T12), 18 (T18) and more than 18 months from diagnosis (T>18). Dogs with follow-up shorter than 6 months were excluded.
Twenty dogs with CH were retrospectively included. Median age was 8 years (2-12 years). One dog died between T0-T6, 1 dog died between T12-18 and 1 dog between T18-T>18 for CH-related causes. Two dogs died for non CH-related causes.
At T0, 14/20 (70%) dogs presented overrange ALT, 4 dogs (20%) presented ultrasonographic secondary signs of portal hypertension (ascites in absence of hypoalbuminemia, acquired portosystemic shunts), 7/20 (35%) presented at least one biochemical signs consistent with liver failure (hypoproteinemia, hypoglycemia, hypocholesterolemia, hyperbilirubinemia, increased blood ammonia or bile acids). At T12, 13/17 dogs (76%) presented increased ALT and 1 dog (6%) presented signs of portal hypertension. At T>18, 9/15 (60%) dogs presented overrange ALT, 1 dog (6%) presented signs of portal hypertension and 1 dog (6%) biochemical findings consistent with liver failure. One dog (6%) developed hepatocellular carcinoma at T>18. Between T0-T6 7/20 (35%) required immunomodulant therapy (ciclosporine and/or prednisolone), between T6-T12, 1/17 (6%) required immunomodulant therapy while at T>18, 3/15 (20%) dogs were currently treated. All dogs received mixed support therapy (antioxidants, hepatoprotectants, symbiotics) and, when needed, vitamin supplementations, ursodeoxycholic acid or d-penicillamin (2/20; 10%).
CH dogs showed to have overall good outcome with long life-expectancy and improvement of liver function despite frequent persisting increased ALT activity. Interestingly, one patient developed hepatocellular carcinoma, as reported for human CH patients. To better identify variables with prognostic value, further studies are needed with larger cohort of dogs evaluating possible correlation between CH dogs' outcome and clinical, hematobiochemical, therapeutic, ultrasonographic and histological features.References: [1] Ogunwobi OO, et al. Mechanisms of hepatocellular carcinoma progression. World J Gastroenterol; 2019[2] Ginès P, et al. Liver cirrhosis. Lancet; 2021. [3] VandenIngh, et al. Morphological classification of parenchymal disorders of the canine and feline liver: Hepatocellular death, hepatitis, and cirrhosis-2 (updated version). In WSAVA Standards for Clinical and Histological Diagnosis of Canine and Feline Liver Diseases; Society of Comparative Hepatology; Saunders Elsevier: St. Louis, MO, USA, 2016.

77° CONVEGNO SISVET

Stato: INVIATO - ID: 13591

Treatment and monitoring of feline hyperthyroidism: preliminary results of an Italian survey

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Feline hyperthyroidism (FH) is the most common endocrine disease in cats, affecting about 10% of senior cats [1-2].
The aim of this observational study was to describe the approach of Italian veterinarians to the different treatment options for FH (i.e., anti-thyroid medications (ATM), iodine-restricted diet, thyroidectomy, and radioactive iodine [RAI]). A survey consisting of 38 questions was published on social networks and sent through the mailing list of an endocrine consultation service in Italy from January 10th to 20th, 2024. The relevance of different reasons to not perform or suggest a specific treatment option was defined using a Likert scale ranging from 1 (not relevant at all) to 5 (extremely relevant).
One hundred and fifty-two veterinarians were included; 120 (73%) had more than 10 years of clinical experience, 100 (66%) worked in primary care, 106 (70%) were based in northern Italy. Eighty-nine (59%) respondents diagnosed between 1 and 5 cases/year, 43 (28%) 6 to 10, and 20 (13%) more than 10 cases/year. At the time of diagnosis 46 (30%) respondents proposed exclusively ATM, while 25 (16%) proposed all treatment options; ATM was the treatment of choice for 127 (84%) respondents, 9 (6%) indicated diet, 5 (3%) RAI and 2 (1%) thyroidectomy.When asked what treatment option would they prefer in an ideal setting (e.g., no cost limitation, easy access to all treatments) 79 (52%) veterinarians answered RAI. Concerning ATM, 110 (72%) respondents prescribed methimazole oral solution as first choice drug. The first follow up was performed after 3-4 weeks by 118 (78%) respondents. One-hundred and thirty-nine (91%) respondents measured total thyroxine, either alone or included in larger thyroid profile, to monitor treatment with ATM; only 15 (10%) included TSH in the monitoring. Fifty-two (34%) respondents had not experienced any side effect of ATM in the previous year; when asked to report side effects experienced at least once, most frequent were vomiting (66, 43%), facial dermatitis or itching (45, 30%), and diarrhea (40, 26%). One-hundred and two (67%) veterinarians stated they never had to discontinue ATM in their practice; the most common reasons leading to ATM discontinuation were ‘impossibility of administering the drug’ (66, 43%), ‘side effects’ (40, 26%) and ‘inadequate control of the disease’ (36, 24%). Only 19 (12%) respondents treated at least one cat with RAI in the previous year: the main reason why this procedure was not performed was ‘difficulty in accessing the treatment’ (median 5, range 1-5). Twenty-one (14%) respondents worked in a practice where thyroidectomy is performed; only 17 (11%) treated at least 1 cat with surgery in the previous year: the main reasons not to perform thyroidectomy were ‘old age’ (4, range 1-5), ‘comorbidities’ (4, range 1-5), ‘inability to perform pre-surgery scintigraphy’ (4, range 1-5), ‘few centers available’ (4, range 1-5). Seventy-six (50%) respondents proposed diet when ATM was not possible, 28 (18%) as an additional treatment to ATM, and 27 (18%) never proposed it.
This study highlights that ATM is by far the most common therapeutic approach in Italy for FH. Only a small percentage of Italian veterinarians discuss all available options, mainly due to the difficulty in accessing definitive treatments. Comprehensive communication at the time of diagnosis would ensure greater choice and awareness for the owner, which would likely benefit hyperthyroid cats [2].

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77° CONVEGNO SISVET

SOCIETA´ SCIENTIFICA DI RIFERIMENTO **SICLIMVET**

TITOLO **Complications and outcome in 208 dogs undergoing echo-guided interventional cardiac procedures**

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Testo e Riferimenti bibliografici

Minimally invasive interventional procedures are routinely performed in veterinary cardiology. Fluoroscopy is used for procedure guidance aside ultrasound guidance. Common therapeutic indications for interventional procedures in veterinary cardiology include transcatheter closure of patent ductus arteriosus (PDA), pulmonary balloon valvuloplasty (PBV) and transvenous permanent artificial pacemaker implantation (PMI). This retrospective study aims to describe procedural complications and outcome in dogs that underwent PDA embolization, PBV and PMI, performed at the Veterinary Teaching Hospitals of Perugia and Camerino University between 2009 and 2023. Short-term post procedural complications have been taken in account if were observed during the hospitalization time, considered 24 hours for PDA occlusion and PBV and 7 days for PMI. All the procedures were performed in echocardiographic guidance, transthoracic (TTE) or transoesophageal (TEE) for PDA occlusion, TTE for pulmonic PBV and PMI. The number of dogs presented for PDA occlusion, PBV and PMI were 103 (72 females, 31 males; median age 7 months, range 2-122), 99 (36 females, 63 males; median age 9 months, range 2-71) and 29 (13 males, 16 female, median age 120 months, range 26-174) respectively. In the PDA and pulmonary stenosis (PS) groups, 11 and 12 dogs respectively were excluded from the interventional procedure because the morphology/dimension of the ductus was incompatible with the Amplatz Canine Ductal Occluder (ACDO) or the valvular dilation had anatomical, clinical or procedural counter-indications. In the PDA group 6 dogs (6,5%) had intra-procedure complications, divided into major complications (2,16%), where the embolization was unsuccessful or created potential hemodynamic imbalance or cardiovascular failure, and minor complications (4,3%) where the procedure duration was longer than expected or required more than one attempt. Sixteen (17,39%) dogs had short-term post-procedural complication, where 1 dog (1,16%) had device embolization in right pulmonary artery and 15 dogs (16,23%) had hematoma formation at the vascular access point. In the PS group, 64 dogs (74%) were presented for type A stenosis, 10 (11%) for type B stenosis and 13 (15%) for mixed type stenosis. Twenty-two dogs (25,3%) had intra-operatory complications, divided into major complication (9,20%), where the procedure had negative results or created potential hemodynamic imbalance or cardiovascular failure, and minor complication (16,1%), where the procedure created or worsened pulmonary or tricuspid insufficiency or had technical or procedural complications that prolonged the procedure expected duration or required more than one attempt. No dog had short-term post-procedure complications. In the PMI group, 20 dogs (69%) were presented for third-degree atrio-ventricular block (AVB), 5 (17,2%) for sick sinus syndrome, 3 (10,3%) for advanced second-degree AVB and 1 (3,4%) for atrial standstill. Two dogs (6,9%) had complications during the procedure whereas 4 dogs (13,8%) had post-implantation complications. The short-term survival rate in PDA embolization, PBV and PMI groups was 100%, 99% and 93,1%, respectively. Our study demonstrates that ultrasound guidance in interventional cardiology procedures is safe and effective in PDA occlusion, PBV and PMI. The complication outcome-rate is similar to what reported for the same procedures performed under fluoroscopic guidance in other veterinary institutions.

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77° CONVEGNO SISVET

Stato: INVIATO - ID: 13650

Effect of oral supplementation of Hazelnut Skin Extract on health, morbidity and mortality in hospitalized calves with neonatal diarrhoea

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Using by-products with nutraceutical qualities for animal feed is a win-win strategy to reduce wastes from the food chain and to provide functional feed to animals. Italy is the second largest producer of hazelnuts in the world, and, contrary to other by-products, hazelnut skin (HS) is still managed as waste. HS is rich in phenolic compounds that have shown benefits for animal health, such as reducing oxidative stress and inflammation[1]. This study aims to evaluate the potential impact of a HS bioactive green extract containing 872 mg of gallic acid equivalents (GAE)/g, in the treatment of neonatal calf diarrhea.The study involved 36 Italian Friesian breed calves, aged between 1 and 21 days, suffering from neonatal diarrhea, admitted to the Veterinary Teaching Hospital of Lodi without concomitant diseases. Upon admission, calves received clinical scores, blood and fecal sampling. Drug and fluid therapy followed a described standard protocol[2]. The calves were randomly divided into: Control Group (CG n=19) with a standard milk diet, and Treated Group (TG n=17) received the same meal added with 10 g of HS polyphenolic extract for five days. During hospitalization, parameters as weight, milk intake, clinical status, fecal score, sepsis score, fluid therapy, and mortality were monitored. The data were analyzed using SPSS v.29.0. Non-normally distributed data were identified with the Shapiro-Wilk test. Data were examined using the Chi-square test for categorical variables and the Mann-Whitney U test for continuous variables. Upon admission, the median age was 9 days. There was no difference in blood gas values between the groups. Aetiological investigations showed prevalences of Escherichia coli (100%), Cryptosporidium parvum (48%), Rotavirus (39%), and Coronavirus (27%). The mortality rates at the end of hospitalization resulted similar in both groups (36.8% for the CG and 35.3% for the TG). The faecal score in the TG was significantly lower on day 5 (p=0.034). This improvement is probably due to the antioxidant effect of HS and its ability to regulate the microbiota of the digestive tract, resulting in better stool consistency and shorter duration of diarrhea. In addition, HS extract showed astringent properties similar to other phytotherapeutic substances explored in literature[3]. No difference in mortality was observed, considering that this trial was carried out in a hospital setting where admission concerns more severe cases. Therefore, further research is warranted to evaluate the therapeutic efficacy of HS extract under field conditions, the optimal levels of inclusion in the diet of diarrheic calves, and its efficacy in preventing neonatal diarrhoea.

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77° CONVEGNO SISVET

SOCIETA´ SCIENTIFICA DI RIFERIMENTO **SIClimVet**

TITOLO **Evaluation of Eko DUO digital stethoscope with 1-lead electrocardiogram in healthy sheep**

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Testo e Riferimenti bibliografici

Examination of cardiovascular system in veterinary medicine consists of physical examination and additional diagnostic tests as electrocardiography (ECG), radiology and echocardiography. However, diagnostic tests commonly used in companion animals are not readily available in small ruminant clinical practice. Recently, a new smartphone-based digital stethoscope featuring simultaneous one-lead ECG has been assessed in dogs and cats, showing a good diagnostic accuracy in the detection of heart sound abnormalities and cardiac arrhythmias. The aim of our study was to assess the reliability of this new digital stethoscope to evaluate heart sounds and ECG findings in healthy sheep. Additionally, we compared effect of the breed (dairy vs meat breed) on the ECG tracings.
Forty healthy sheep (20 “Bergamasca” and 20 “Comisana” sheep) were recruited from two different teaching flocks of Perugia University. The study was approved by the Ethical Committee of the University of Perugia (19/2022). Only healthy sheep, based on history and physical examination, and aged >1 year were included. All sheep were restrained using a self-catching feeder while feeding. Each sheep was subjected to cardiac auscultation with a conventional stethoscope and Eko DUO ECG digital stethoscope by a clinician. Then, a standard base-apex ECG and digital stethoscope ECG were recorded simultaneously. At the end of the recording, the trace (audio, phonocardiography and ECG) was automatically stored online for each animal. Blinded analysis of each recording was performed by two different authors. Conventional and Eko DUO ECG tracings were analyzed by a single author assessing heart rate, P wave polarity/duration/amplitude, PQ interval duration, QRS complex polarity/duration/amplitude, QT interval duration, T wave polarity and electrocardiographic diagnosis. Cohen’s k was used to calculate the agreement between blinded re-auscultation of the audio recordings and the agreement for T wave morphology between the two devices. Bland-Altman analysis was used to compare the conventional and Eko DUO ECG tracings. Mann-Whitney test was used to compare ECG data between the breeds.
Conventional and digital stethoscopes allowed assessment of physiological heart sounds without abnormalities in all sheep. The blinded re-auscultation of the audio recordings allowed to consider these interpretable in all sheep. For a first observer the audio tracings were of high quality in 15 (37.5%) sheep, acceptable in 20 (50%) and low quality in 5 (12.5%). The re-auscultation of the audio tracings by a second blinded observer showed good agreement (k = 0.755) in the audio recording quality. Conventional and digital stethoscope ECG tracings showed sinus rhythm in all sheep. Bland-Altman analysis showed a perfect agreement for heart rate and a good agreement for P wave duration/amplitude (bias -5, 95%CI -6.64 to -3.36; bias 0.02, 95%CI 0.01 to 0.04) and PQ interval (bias 13, 95%CI 9.79 to 16.21) between the two methods. P wave duration showed a proportional bias. QRS duration and QT interval showed a good agreement between the methods (bias -4.25, 95%CI -6.44 to -2.06; bias -11.25, 95%CI -15.28 to -7.22), although a proportional bias was demonstrated for QRS duration and the digital stethoscope slightly overestimated the QT interval. S wave amplitude showed substantial difference (bias 0.28, 95%CI 0.21 to 0.35). A moderate agreement (k=0.569) for the T wave morphology between the two devices was observed. Comparison of ECG variables between “Bergamasca” and “Comisana” breed showed differences ($p<0.05$) for heart rate, QRS duration and QT duration.
Our study demonstrated that the Eko DUO ECG digital stethoscope can assess the cardiac rhythm and simultaneously record a single EGC trace in healthy sheep. The measurements of ECG variables are similar between the two devices but some of these were not interchangeable. The ease of accessibility of the digital stethoscope could increase the use of ECG “in the field”, considering that the ECG recording can be obtained during the cardiac auscultation. In clinical practice, early detection of arrhythmias could provide useful information for treatment and prognosis in sheep with metabolic or electrolytic disturbances.

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77° CONVEGNO SISVET

SICLIMVET

ADVERSE EVENTS AND EFFICACY OF MEGLUMINE ANTIMONIATE IN DOGS WITH LEISHMANIASIS: “what doesn’t kill you, makes you stronger!"

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Antimoniate therapy, in association with allopurinol, remains among the first-choice protocols for the treatment of canine leishmaniasis (CanL) [1]. This study evaluates potential adverse effects associated with the meglumine antimoniate (aNm) in the treatment of CanL through a retrospective analysis of the medical records of treated dogs, as well as through a long-term prospective study also aimed to investigate its efficacy. In the retrospective study, the records of 87 dogs with CanL at different Leishvet stages [2], having at least one follow-up available during (and/or at the end of) therapy with aNm (Glucantime®)- at a dose of 100mg/kg SC BID for 30 days in association with allopurinol, were reviewed. During treatment, 30 (34%) animals showed adverse effects of various types, such as local reactions at the injection site (n= 6), systemic reactions related to the pain from the injection site (n = 4), systemic disease due to renal function worsening (n = 4), acute pancreatitis (n = 1), diarrhea (n = 5), vomiting (n = 3) and severe idiosyncratic skin reactions (n = 3). Of these dogs, 13 (14.9%) required treatment suspension. The prospective study included 16 dogs, selected among the Leishvet stages II and III CKD IRIS 1 and treated with the same protocol as in the retrospective study and observed for 360 days with follow-up at 30, 60, 90, 180 and 360 days. All possible adverse effects reported by owners during aNm therapy were reported and documented. Two dogs had severe reactions at the injection site and were excluded from the study. For the other 14 dogs the following adverse events were reported during treatment: mild and transient subcutaneous reactions appeared few days after starting protocol (n=2), recurrent episodic diarrhea throughout treatment (n=1), laboratory self-limiting changes indicative of liver disease at D30 (n=1). No cardiac adverse effects were registered using conventional and strain echocardiography by using Esaote Mylab X75. The criteria used to evaluate the efficacy of treatment with aNm were: reduction of the clinical score and improvement and/or normalization of laboratory parameters, negativization of PCR on the bone marrow material and disease-free interval time. PCR results showed complete negativity between D0 and D60 in 78.5% of animals. The percentage of reduction of the clinical score reached 91.9% at D180. No animals showed clinical laboratory relapse during the whole study duration. Veterinarians must be vigilant regarding the potentially serious adverse effects associated with aNm and be aware to immediately stop the drug administration if unexpected clinical manifestations occur. On the other hand, they should not discard its use for CanL treatment since it is confirmed that aNm in association with allopurinol is highly effective in controlling CanL. A higher percentage of PCR negativization after treatment is documented if compared with miltefosine [3]. Thus, this protocol, when tolerated, has to be suggested as the gold standard for the treatment of CanL, at least in dogs with preserved renal function.

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77° CONVEGNO SISVET

SOCIETA´ SCIENTIFICA DI RIFERIMENTO **SICLIMVET**

TITOLO **Validation of a new technological device for monitoring vital functions in newborn foals**

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Testo e Riferimenti bibliografici

Accurate clinical evaluation of newborn foals is essential for the identification of early neonatal pathologies. The use of technological devices capable of constantly monitoring vital parameters could prove extremely useful [1], although no technology is currently available for these animals. The study aimed to validate the performance of using a multiparametric wearable device developed for the telemonitoring of large dog breeds (Dinbeat UNO ®, Dindog Tech, Spain) in nine newborn foals. The study was approved by the Ethics Committee for the Care and Use of Animals of the University of Naples Federico II (PG/2023/0120380). The following eligibility criteria were applied for the enrollment of the animals: (i) eutocic birth; (ii) Apgar scoring system > 9; (iv) to be healthy at clinical examination performed within two hours after birth. The device employed included electrodes for heart rate (HR), electrocardiogram and respiratory rate (RR), an automatic thermometer for body temperature (T°C) recording, accelerometers defining the positions (standing bouts/lying bouts) and the activities (resting time/moving time) of the animals; it was applied approximately 8 hours after birth on all the animals, and a continuous recording period of 6 hours was performed. The gold standard was represented direct clinical procedures including: (I) measurement of body temperature using a digital thermometer (SC 1091 Veterinary thermometer Flex), (ii) assessment of HR through direct auscultation, (iii) definition of arrhythmias by portable electrocardiograph (BTL-08 SDecg); (iv) recording of RR, position, and activity through direct observation. Each parameter was assessed every 10 minutes along the entire recording time. Differences between continuous variables (T°C, HR, RR) were analyzed using the Mann-Whitney U test, those between the expected and observed frequencies of the categorical data (standing bouts/lying bouts, resting time/moving time, and presence/absence of arrhythmias) were evaluated using the $\chi 2$ test contingency tables. Correlation between continuous variables was assessed by Pearson's test (r), while for categorical variables the Spearman's test (r2) was used. During the recording process, 344 pairwise determinations were compared for the continuous variable RR, 326 for HR, 448 for T°C. For the categorical ones, the measurements were 448 for the parameter "standing bouts/lying bouts", 438 for that one "resting time/moving time", 300 for "presence/absence" of arrhythmias. Regarding the correlation between continuous variables, the RR showed a moderate value ($r= 0.546$; $P<0.0001$); the HR showed a strong value ($r=0.700$; $P<0.0001$). The T°C instead showed a weak correlation ($r=0.371$; $P <0.0001$). For all the categorical variables a complete agreement between the device and gold standard was instead detected ($r2=1.000$ - very strong, $P=0.01$). In the absence of specific technologies, the use of the current device seems to be encouraging. However, the position of sensors within the harness might have influenced the results observed. Indeed, for those positioned along body areas showing obvious differences between the two species (cranial part of the chest) results less reliable were found (T°C and RR). For those well-fixed within the harness or placed in anatomic areas with similar characteristics (lateral part of the chest), the data were more precise ("standing bouts/lying bouts", "resting time/moving time", HR and "presence/absence of arrhythmias"). Overall, the device seems to give interesting indications regarding the vital parameters of the subjects, however, the development of specific devices to monitor the health status of newborn foals remains a priority.

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77° CONVEGNO SISVET

SOCIETA´ SCIENTIFICA DI RIFERIMENTO **SICLIMVET**

TITOLO **ETIOPATHOGENETIC, CLINICAL AND DIAGNOSTIC INVESTIGATIONS OF BACTERIAL CHONDRONECROSIS WITH OSTEOMYELITIS OF THE FEMORAL HEAD IN BROILERS: PRELIMINARY NOTES.**

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Testo e Riferimenti bibliografici

The bacterical chondronecrosiswith osteomyelitis of the femoral head (BCO) is considered a significant cause of lameness in broilers, affecting 1-2% of poultry from 5 weeks of age onwards, leading to substantial economic losses [1]]. Actually, BCO is indicated as a bacterially etiological pathology, with the most frequently isolated strains including Staphylococcus aureus, Staphylococcus spp., Escherichia coli, Enterococcus cecorum, Streptococcus spp., Enterococcus spp., and Salmonella spp. [1-2]. A recent study has also demonstrated that the presence of deoxynivalenol and fumonisins, in the feed, increases the predisposition to BCO development [3].
This work aims to contribute to the definition of the etiopathogenesis of BCO using diagnostic imaging and investigating the microbial populations found in the synovial fluid and other target matrices of animals affected by femoral head necrosis confirming and/or adding predisposing and triggering factors about this pathology, with a particular focus on the bacterial etiology of lameness.
This study was carried out in an intensive poultry farm in southern Italy that breed ROSS 308 broilers. A total of 17.500 chicks of mixed sex were housed in an industrial building during a production cycle lasting 48 days. Throughout the cycle, the percentage of discarded animals was 2.38% (417 specimens); of these, 28.78% (120 specimens) presented lameness, with 56 subjects showing suspected femoral head necrosis (unilateral or bilateral).
These subjects, after being euthanized by cervical dislocation, as prescribed by Reg. (CE) 1099/2009, underwent the following protocol: macroscopic description of lesions and classification based on laterality; aseptic sampling from selected sites (coxofemoral joints, synovial fluid, spleen) [3] for microbiological analysis to identify bacterial populations associated with the pathology; performance of imaging diagnostic procedures to detect and stage the pathology from a morphological standpoint. The diagnostic protocol was carried out on broiler carcasses; it was therefore not necessary to request an ethical opinion.
The expected outcomes will suggest that the development of pathology in broilers is directly linked to specific isolated bacterial populations.
From this initial study on the evaluation of the etiopathogenesis of BCO, conducted in a broiler farm located in southern Italy, the accurate identification of pathogens involved in major pathologies compromising ambulation could facilitate the development of targeted preventive and therapeutic strategies to breast this significant health and economic issue in the poultry industry.

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77° CONVEGNO SISVET

SOCIETA´ SCIENTIFICA DI RIFERIMENTO **SICLIMVET**

TITOLO **EFFECT OF METRONOMIC CHEMOTHERAPY ON THE ABSOLUTE NUMBER OF CIRCULATING REGULATORY T CELLS IN DOGS WITH CANCER**

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Testo e Riferimenti bibliografici

Immunosuppressive regulatory T cells (Tregs) are part of the tumor microenvironment and contribute to the establishment of neoplastic tolerance, promoting tumor development and progression by dampening anti-tumor immune responses. Metronomic chemotherapy (MC) is defined as the oral administration of low doses of chemotherapy on a continuous schedule of treatment, without extended drug-free breaks. MC is a multitarget therapy, acting not only on neovascularization, cancer stem cells and tumor quiescence, but also interacting with the patient's immune response to cancer, reversing the state of immune tolerance by selectively depleting the number of Tregs and impairing their function.[1-2]
This study aimed to evaluate the effects of MC, consisting of daily administration of cyclophosphamide, meloxicam and thalidomide, on the absolute number of circulating Tregs in a population of patients with different cancer histotypes, over the medium/long term. It was hypothesized that during MC, the absolute number of circulating Tregs would progressively decrease. Through an analysis of variance (ANOVA), we evaluated which factors were significant ($p \leq 0.05$). For significant factors, Least Square Means were estimated. The study protocol was approved by the Ethics Committee for Animal Experimentation (PROT. N.21/CESA/2020).
This prospective study included 29 canine cancer patients: 13 epithelial, 8 mesenchymal, and 8 round cell tumors. Sixteen patients (55.2%) received MC as adjuvant to surgery. To determine circulating Tregs, we performed a complete blood count and flow cytometry analysis on peripheral blood mononuclear cells (PBMC). A blood sample was collected before and at 15, 30, 90 and 180 days after the start of the metronomic treatment. The percentage of circulating CD4+CD25+FoxP3+ Tregs obtained by flow cytometry was then related to the absolute number of circulating lymphocytes to obtain the absolute number of circulating Tregs of each sample. This study showed that the duration of MC administration has a statistically significant influence on the reduction of circulating Tregs absolute number ($p = 0.019$), but not on the circulating Tregs percentage. Furthermore, in this cohort of patients, tumor type or previous surgery did not seem to have any effect on the circulating Tregs absolute number. This is the first study that evaluated the effect of the metronomic combination of cyclophosphamide, meloxicam, and thalidomide on circulating Tregs in a canine population with cancer, showing a statistically significant reduction in their absolute number over time. Future studies, more homogeneous in terms of tumor type and patients' clinical stage, aiming at correlating trends in circulating absolute Tregs number during MC with an objective response to the same therapy could enable the use of the absolute Tregs value as a marker of disease progression during MC in clinical practice.

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77° CONVEGNO SISVET

SOCIETA´ SCIENTIFICA DI RIFERIMENTO **SICLIMVET**

TITOLO **Disseminated *Mycobacterium abscessus* infection in a Rottweiler dog**

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Testo e Riferimenti bibliografici

A 1.5-year-old female Rottweiler was referred to the VTH of Teramo for a 4-5 cm mass on the left ventral side of the neck, non-responsive to antimicrobials and anti-inflammatory corticosteroids. No other concerns were reported and the dog appeared otherwise healthy. The mass was hard, non-tender, not warm, and well-adhered to the underlying planes. Peripheral lymph nodes, including the corresponding mandibular lymph node, were normal. Physical examination did not reveal any further abnormalities. Laboratory workups were unremarkable and vector-borne pathogens serologies were negative. An incisional biopsy of the mass was performed and histological examination was consistent with pyogranulomatous panniculitis. Given the diagnostic suspect of sterile panniculitis, an immunosuppressive therapy with prednisolone 2 mg/Kg q24h was started. After an initial response, one week later the dog showed severe enlargement of the retropharyngeal lymph nodes and facial edema. To rule out a foreign body, the dog underwent head and neck CT, yet the sole finding was the lymphadenopathy. The lymph node cytology was consistent with a severe pyogranulomatous reaction, according to the previous histological diagnosis. Serology and PCR for *Bartonella* spp., bacterial cultures and Ziehl-Neelsen staining on cytological samples were requested. Pending the results, ampicillin-sulbactam 20 mg/Kg q8h and enrofloxacin 10 mg/Kg q24h were initiated resulting in a reduction of the lymph nodes size and the resolution of edema. However, in the next days, the dog become jaundiced and an increase in abdominal volume was observed. Thrombocytopenia, increased liver enzymes activity and hyperbilirubinemia were noted. Abdominal ultrasound revealed hepatomegaly with hypoechoic liver nodules and abdominal effusion, classified as modified transudate probably due to portal hypertension. Test for *Bartonella* spp., aerobic and anaerobic cultures were negative, while Ziehl-Neelsen staining evidenced the presence of alcohol acid-resistant bacilli, identified by sequencing as *Mycobacterium abscessus*. The patient died after seven days from and the necroscopy results are pending at this writing. To the author knowledge this is the first report of *M. abscessus* infection in a dog. *M. abscessus* belong to a group of rapidly growing mycobacteria (RGM), multidrug-resistant nontuberculous mycobacteria (NTM) species that are ubiquitous in soil and water. The dog had access to a farm and may have brought it into contact with multiple potential sources of infection. RGM are involved in nontuberculous skin infections in immunocompetent companion animals and panniculitis caused by RGM have been previously reported in dogs [1,2]. In this case, the histological diagnosis of pyogranulomatous panniculitis allowed to hypothesize a localized disease in the early stage of the infection. Indeed, RGM are generally constrained by the immunological response that prevent hematogenous or lymphatic spread. In this case, a rapid progression despite antibiotic therapy was observed and the immunosuppression due to corticosteroids may have contributed to the spread of the infection, underscoring the importance of an early and accurate diagnosis of NTM. Despite the thoughtful diagnostic work-up, the diagnosis was initially missed according to available literature, in which mycobacterial infection was not initially suspected in any reported case. These features highlight the challenges of diagnosing mycobacterial infections, emphasizing the importance of considering rare or re-emerging pathogens, including NTM, in the differential diagnosis of solitary masses in dogs, even in absence of systemic signs.

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77° CONVEGNO SISVET

SOCIETA´ SCIENTIFICA DI RIFERIMENTO **SICLIM-VET**

TITOLO **Cyclophosphamide overdose in a patient with undifferentiated soft tissue sarcoma**

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Testo e Riferimenti bibliografici

Cyclophosphamide is an alkylating cytotoxic chemotherapeutic drug widely used in human and veterinary medicine. It is usually well tolerated. The most prevalent adverse effects include reversible dose-limiting myelosuppression, gastrointestinal symptoms such as nausea and vomiting and sterile hemorrhagic cystitis caused by the metabolites acrolein and 4-hydroxymetabolites in the urine, which cause submucosal edema, hemorrhage, necrosis, and fibrosis of the urinary bladder mucosal epithelium.[1-2-3]
An adult spayed female 5-years old mixed-breed dog of 11 kg was referred to the Veterinary Teaching Hospital of the University of Parma because of a glandular epithelial subcutaneous poorly differentiated tumor, with atypic cytological characteristics, located on the left pectoral area. Three weeks later, computed tomography (CT) and indirect lymphangiography revealed a mass in the left axillary cavity not bounded by the pectoral muscles, whereas the sentinel lymph nodes (SLNs) did not detect aqueous contrast medium. After four months from the diagnosis, she underwent surgical excision, and the histological report revealed an undifferentiated sarcoma of muscular origin with marked anisocariosis and anisocytosis removed with adequate margins, while the axillary lymph node was free from metastasis.
Therefore, 6 cycles of dose-intense intravenous chemotherapy based on doxorubicin were started (30 mg/m^2 every three weeks); after a negative end-staging performed at the end of the intravenous chemotherapy, metronomic chemotherapy based on cyclophosphamide (12.5 mg/m^2) and thalidomide (4 mg/kg) was begun. One week from the start of this therapy, the patient was visited at the Veterinary Teaching Hospital after having received 11 times the normal daily dose of metronomic cyclophosphamide for 7 consecutive days because of owner's mistake, leading to grade IV symptomatic neutropenia and thrombocytopenia. Two days after receiving her last dose of cyclophosphamide, the patient developed hyperthermia, anorexia and rear tremors. Despite the low overall dosage (385 mg, 740,40 mg/m^2), hematology indicated severe nonregenerative symptomatic neutropenia and thrombocytopenia. The patient never developed hemorrhagic cystitis.
Therefore, chemotherapy was immediately stopped and a combination of broad-spectrum antibiotics (ampicillin-sulbactam, enrofloxacin and piperacillin), intravenous supportive therapy, gastroprotectants, and granulocyte colony stimulating factors were established, while being kept in a sterile environment, being manipulated wearing disposable gloves, face masks, shoe covers and continuous cleaning.
The peripheral neutrophil count improved within 7 days, and the total blood count was almost normal 10 days later. The dog was discharged from the hospital on day 10 from the presentation, without further chemotherapy.
At the time of writing, the dog had entirely recovered and had no long-term complications, including a normal blood count. A local recurrence developed 610 days from surgery and 397 days after the chemotherapy overdosage, but the dog is still alive, without treatment at the time of writing.
This is the first publication to describe how dogs can be treated and survive after receiving improperly high doses of metronomic cyclophosphamide. Even dogs with significant clinical symptoms and bone marrow suppression can recover from chronic cyclophosphamide toxic exposure by receiving appropriate supportive care.

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77° CONVEGNO SISVET

Stato: INVIATO - ID: 12946

The combined use of triamcinolone and platelet-rich plasma improves pain in equine fetlock osteoarthritis and promotes the chondrocyte viability

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The use of corticosteroids to treat osteoarthritis (OA) has established benefits, including relief of pain and increased range of motion but they, including triamcinolone (TA), may alter the cartilage metabolism. [1] Platelet-rich plasma (PRP) may limit triamcinolone cytotoxic effects and give a clinically better outcome by reducing clinical signs of OA and maintaining an acceptable safety profile. [2] This study hypothesized that the sequential use of PRP after a single dose of TA would reduce the negative effects of corticosteroids; the aim of this study was to determine how the combination of TA and PRP might improve the clinical signs of fetlock OA in racehorses. An “in vitro” study was performed using chondrocytes isolated post-mortem from metacarpophalangeal/metatarsophalangeal joints of 6 Thoroughbreds/standardbred horses between 3-6 years, death for reasons unrelated to the study. Briefly, cells were exposed to different concentrations (0.25, 0.50, 1.0, 2.0 and 4.0 mg/ml) of TA (Kenacort-Bristol Mayers Squibb) for 24 hours in presence or in absence of PRP. Cell viability was assessed using the 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT) assay based on the conversion of MTT into a purple-coloured formazan product by viable cells. For the “in vivo study”, 33 Thoroughbred racehorses in race training, aged between 2 and 5 years were recruited. The inclusion in the study involved one or more of the following criteria: unilateral or bilateral forelimb fetlock joint effusion and/or synovitis, pain at passive flexion of the forelimb fetlock and /or lameness, and radiographic findings of OA. They were divided in 2 groups, of which “TA group” received a single intraarticular injection of TA and “TA+PRP group” received an additional dose of PRP after 1 week from the TA injection. Flexion, effusion and lameness scores were evaluated at 1 and 2 weeks after treatment and compared statistically using Friedman’ ANOVA with Tukey’s post hoc correction to determine the difference for multiple comparisons. The “in vitro” study showed that exposure of condrocytes to TA significantly decreased cell viability; this effect was prevented by the simultaneous culture of PRP. The presence of PRP significantly limited the negative effect on chondrocyte viability at tested dosages of TA. PRP in addition to TA significantly improved chondrocyte proliferation. The “in vivo” study showed that TA+PRP group maintained effusion scores low after 2 weeks, while TA group returned quicker towards the initial score. The comparison of flexion test scores before and after one week showed a significant decrease in both groups. However, after two weeks, the TA+PRP group maintained its flexion scores unchanged, while the TA group returned to the baseline score. The TA+PRP group showed a longer effect in maintaining the flexion score unchanged for a longer time. In conclusion, our findings suggest a promising strategy to alleviate any adverse effect on chondrocyte viability after the corticosteroid administration, highlighting the potential for mid-term pain relief through a separate platelet-rich plasma (PRP) injection. Further clinical trials are crucial for a comprehensive evaluation of the therapeutic potential and safety profile associated with the integration of PRP with triamcinolone in the treatment of osteoarthritis in equine athletes.

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77° CONVEGNO SISVET

DIAGNOSTIC IMAGING AND SUCCESSFULL TREATMENT OF LATERAL COLLATERAL LIGAMENT DESMITIS OF THE ELBOW JOINT IN A JUMPING MARE

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Injury of collateral ligaments of elbow joint is an uncommon condition, more frequently associated with traumatic events [1]. This paper describes a case of lateral collateral ligament desmitis of the elbow joint in a 13-year-old Belgian jumping mare. The horse was presented with a history of acute onset of severe left forelimb lameness after a jumping competition. Radiographic images showed the presence of periosteal new bone formation at the proximal and distal insertion of the lateral collateral ligament of the humeroradial joint and ultrasonography images indicated a severe injury of the lateral collateral ligament associated with severe proliferative synovitis. A therapy with ultrasound and PEMF (pulsed electromagnetic field) was set up. Radiography and ultrasonography images after 3 month of follow up were performed. In contrast with poor prognosis of traumatic injuries of the elbow joint, the outcome for horses with lateral collateral ligament enthesopathy and desmitis due to indirect trauma during sports activity seems promising for return in athletic state with appropriate treatment and rest.

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77° CONVEGNO SISVET

Stato: INVIATO - ID: 13070

Unilateral nephrectomy in three horses with positive athletic outcome

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Unilateral nephrectomy is a rare surgical procedure in horses, indicated for neoplasia, hydronephrosis, abscessation, pyelonephritis, nephrolithiasis, ureterolithiasis, nematodiasis, idiopathic renal hematuria and ectopic ureter(1).
Different surgical techniques are described: transcostal and transthoracic approaches in lateral recumbency, standing laparoscopic nephrectomy, standing hand-assisted laparoscopic transperitoneal (LTP) nephrectomy, ventral midline celiotomy(1,2).
Nephrectomy is considered a safe surgery if the controlateral kidney is compensating, although few reports underline the importance of serial monitoring of renal function and the outcome on athletic activity(2,3).
Three horses were presented for nephrectomy: case-1 (11-year-old) had a history of inappetence and increased serum creatinine, correlated to the presence of a right ureterolith with secondary degenerative changes in the ipsilateral kidney; case-2 (16-year-old) and -3 (12-year-old) had a neoformation at the right and left kidney, respectively, with a previous long-term history of weight loss and polyuria without hematological alterations.
Horse-1 underwent a standing LTP right nephrectomy.
In horse-2, a right flank standing laparoscopy was planned to collect biopsy samples, followed by a nephrectomy under general anesthesia in lateral recumbency by a trans-costal approach.
Horse-3 underwent a standing LTP left nephrectomy; after complete isolation of the kidney, due to its dimensions and a notable catecholaminergic response, general anesthesia was required for removal through a ventral laparotomy.
No significant complications occurred in the postoperative period.
A severe, chronic hydronephrosis was diagnosed in case-1, while a papillary renal carcinoma and a papillary renal adenocarcinoma resulted in case-2 and case-3, respectively.
A positive outcome was recorded in all horses. Long-term follow up is present for horse-1 and -2 (27 months and 20 months, respectively): case-1 persisted with mild increase in serum creatinine (mean 2.8 mg/dl, reference ranges 0.9-2) without other hematologic alterations nor clinical signs and it is competing at high-level show jumping; case-2 has not shown hematologic alterations and returned to full show jumping activity. Case-3 has a shorter 8-month follow-up but it is back to jumping activity without hematologic alterations.
In this case series, standing LTP was performed in two horses, entirely in case-1 and partially in case-3, where isolation of the kidney by laparoscopy was followed by its removal by ventral midline laparotomy under general anesthesia. In case-2, renal biopsies by standing laparoscopy allowed the surgeon to plan nephrectomy in lateral recumbency through a trans-costal approach, given the large dimension of the neoplasia. Therefore, three different cases requiring nephrectomy have been approached with different surgical techniques, highlighting the importance of considering each case alone to identify the best approach.
Even if monolateral nephrectomy has been performed with a good prognosis for normal life(1,2,3), there is poor evidence in literature about the return to athletic activity of horses(2).
In this case series, 3 horses affected by unilateral renal disease underwent nephrectomy with complete resolution of the initial complaint and a good prognosis on athletic activity after short- and long-term follow-up. No clinical signs of renal insufficiency have been reported; the elevation in serum creatinine in case-1 could be explained by the complete loss of activity of the right kidney, which probably overloaded the controlateral before surgery, while the neoplasia of case-2 and -3 coexisted within a partially functioning kidney with an adequate compensatory response, explaining the absence of blood alteration before and after surgery.

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77° CONVEGNO SISVET

Stato: INVIATO - ID: 13075

Patient-Specific 3D-Printed Osteotomy Guides and Titanium Plates for Distal Femoral Deformities in Dogs with Lateral Patellar Luxation

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Bone deformities are complex musculoskeletal pathologies that lead to varying degrees of patellar luxation (PL) during growth. Lateral patellar luxation (LPL) is less common than medial patellar luxation (MPL) and is more commonly diagnosed in large or giant breeds. Distal femoral osteotomy (DFO) is one of the most performed surgical techniques to correct grade IV PL secondary to femoral deformity. Virtual surgical planning (VSP) and three-dimensional (3D) printed patient-specific guides (PSGs) are widely used in human medicine and have recently been described in veterinary medicine to correct limb deformities. The aim of this study was to describe the restoration of alignment in the sagittal, frontal and transverse planes in two adult large breed dogs with complex femoral deformities and grade IV LPL treated with DFO using VSP and patient-specific 3D-printed osteotomy guides and titanium plates. Both patients were sedated and underwent pelvic limb computed tomography (CT) to identify and characterize deformities. DICOM images obtained from CT scans were exported to computer-aided design (CAD) software for 3D reconstruction and manipulation of the femoral deformities, allowing to perform both VSP and 3D printing of PSGs. Of the two patients, one was affected bilaterally and the other unilaterally, but both dogs were from the same litter. Therefore, the healthy femur of the unilaterally affected patient was used as a template. Three DFO followed by reduction and internal fixation were performed using patient-specific 3D-printed osteotomy guides and plates. Reduction was performed directly by applying the plate without using a reduction guide. This limited the size of the surgical approach, making it smaller and less invasive and avoiding overly aggressive soft tissue manipulation. Despite the limited surgical approach, no difficulties or errors were encountered with either the osteotomy guide or the plate placement. No major complications requiring revision surgery were observed. Preoperative, expected, and postoperative femoral angles were compared to evaluate the efficacy of VSP and surgical correction. The postoperative angles were consistent with expected ones in sagittal and transverse plane, but the frontal plane showed a mild overcorrection of the valgus deformity which resulted in a varus deformity of less than 5 degrees, probably due to a loss of primary reduction during plate application. Despite this, clinical improvement until full functional recovery was observed in both patients. The use of PSG eliminated the need for intraoperative diagnostics and ensured a more accurate osteotomy, while the patient-specific titanium plates reduced surgical complications and postoperative recovery time. Overall, the synergy between the patient-specific 3D-printed osteotomy guides and plates significantly reduced intraoperative times, thereby reducing the associated risks. This study suggests that the treatment used is a viable surgical alternative to restore limb alignment in patients with complex femoral deformities, improving the accuracy of corrective osteotomies, reducing the complication rate and consequently improving the animal's quality of life.

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A quilting subcutaneous suture pattern in cats undergoing caudal median laparotomy: incidence of postoperative complications and influence of a different postoperative recovery regimen.

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Median ventral celiotomy is among the most used approach in cats undergoing surgery. However, this procedure isn't free from incisional complications, and the most common are seroma formation and surgical site dehiscence. Although seroma is frequently categorized as minor complication, this fluid collection can cause pain for the patient as well as a state of worry and anxiety for the owner, leading to additional outpatient visits, increasing costs and treatment. In this study (Ethical Committee n. 2019/02) two different suture patterns of the subcutaneous tissue were compared: a simple continuous suture (CTR) and an anchoring continuous suture (ANC) to the underlying abdominal fascia in a direction parallel to the linea alba, aiming to reduce the dead space between the two tissue planes therefore preventing the accumulation of fluid and subsequent seroma formation. In addition, two different post-operative resting regimens were compared: home resting (HOME) and hospital confinement in a cage (HOSP), aiming to assess how the different environment may influence the healing of the surgical wound and the degree of comfort and perceived pain, accordingly. All cats received the same anesthetic protocol and were operated by the same surgeons. A five-point scale [0=no alteration, 1=mild hyperemia without swelling, 2=mild wound thickening (<0.5cm), 3=severe swelling (>0.5cm)/seroma, 4=wound dehiscence] was designed to assess the surgical wound and was applied right after surgery and on the tenth day post-surgery. Cats scored as 3 were checked by ultrasound to confirm the seroma diagnosis. To evaluate animal's comfort and pain perception, the UNESP-Botucatu scale was applied from 1 hour after surgery and once a day for 5 post-operatively. Furthermore, any intra- and post-operative complication was recorded. Results showed a significant difference ($p=0.035$) between the CTR and ANC groups in the occurrence of seroma as 7 (21%) and 0 cats were scored as 3 in group CTR and ANC, respectively. Difference were recorded also comparing the resting regimens for the UNESP-Botucatu score, as the mean score of the group HOSP (2.4; range 0-15) was significantly higher than group HOME (0.2; range 0-4); this result is probably derived from the class of subjects included in HOSP group as, being non-owned cats, they basically have a more adverse and less compliant temperament. The anchoring suture pattern of the subcutaneous tissue does not affect the total surgery ($p=0.09$) and anesthesia ($p=0.486$) time, furthermore it does not cause additional pain to the animal compared to a non-anchoring suture pattern ($p=0.401$), thus guaranteeing greater safety without posing additional risks to surgery. Similarly, a resting regimen in the home environment, carried out ignoring the veterinary surgeon's instructions (HOME group), combined with the presence of a non-anchoring suture pattern (CTR) is an additional risk factor ($p=0.002$ and OR 0.15, confidence interval 0.045-0.50) in the occurrence of post-operative complications of the surgical wound. In conclusion, an anchoring suture could positively influence the healing of the laparotomic wound without affecting the duration of surgery but should always be coupled by a correct post-operative resting regimen.

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77° CONVEGNO SISVET

Stato: INVIATO - ID: 13086

AUTOLOGOUS PLATELET-RICH PLASMA TREATMENT IN LAPAROTOMIC WOUNDS OF HORSES UNDERGOING SURGERY FOR COLIC SYNDROME: PERSPECTIVE ON BENEFICIAL HEALING SUPPORT.

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Platelets (PLT) play a significant role in wound healing, being crucial for hemostasis and tissue regeneration given their high content in growth factors, cytokines and extracellular matrix modulators. For these reasons Platelets Rich Plasma (PRP) therapy has been largely used both in animals and humans[1], however, in horses PRP has been essentially used in dermatology and orthopedics[2]. Considering that the incidence of wound complications after laparotomy for colic (40-57%), including exudation (76-88%), dehiscence (3-5%) and hernia (6-17%)[3] is a critical issue in equine medicine, we believe that the application of a PRP-enriched patch within the laparotomic wound could ameliorate the healing process, limiting complications and providing analgesia. For this purpose, we used a bioabsorbable patch based on autologous PRP combined with a biopolymeric mixture (Ematik® Kit dermatologico).

Ethical approval was obtained for this study, and 15 horses undergoing laparotomy for colic were randomly allocated into two groups receiving (EMA: n=8) or not receiving (CTRL: n=7) the patch within the wound layers. The patch is composed by a polymeric sponge overlapped to a 3D support and obtained adding autologous PRP (6 mL), extracted from 60 mL of blood collected from the jugular vein upon arrival. After jellification, the patch was aseptically cut and applied with the sponge over the linea alba between the two layers of the incision. A covering stent was maintained for 5 days, and the wound received visual inspection and ultrasound evaluation (USe) on day 5, 7 and 10. The USe included 6 specific levels: cranial, medial, and caudal at both sides. Scores (from 0-2=absent-moderate) were assigned to the presence of subcutaneous edema, signs of infection (hyperechoic spots, heterogeneous material, fistulas, and suppuration) and pain at palpation and registered in a dedicated score sheet. A Pain Scale (PS; maximum score=64, cut-off=17) was applied at 11 specific time-points for 3 days post-surgery.

The obtained PRP had a mean PLT concentration of 1.1x106 PLT/μL and a mean PLT concentration factor of 7x. 4/8 and 4/7 horses in EMA and CTRL group, respectively, showed wound drainage during hospitalization. No significant difference between the groups was observed for USe and wound assessment. However, EMA showed a tendency for lower subcutaneous edema and a lower reaction at palpation from day 5 to 10 ($p<0.001$).According to PS, CTRL group showed a significant reduction from T0 (22±7) to T7 (11±8, $p<0.004$) and T11 (9±5, $p<0.001$) (day 2-3, respectively). In EMA, no significant difference was detected, but a lower variability of the PS was observed (T0= 13±9; T7= 11±3; T11=7±3).

Given the low number of enrolled animals, these are considered as preliminary results, however, we believe that the use of PRP applied within a patch could contribute to a better wound healing, possibly providing antimicrobial activity and positive aesthetic and functional outcomes. If so, its use might find wide consensus in the scientific and clinical fields. Meanwhile, a different anchoring of the 3D-scaffold to the tissue could have limited its movement within the SC layer, providing a greater outcome. Nevertheless, this patch deserves further consideration given the positive reduction of local inflammation, edema and overall pain sensation in the treated horses.

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77° CONVEGNO SISVET

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INDIRECT INTRACRANIAL PRESSURE (ICP) EVALUATION IN DOGS WITH BRAIN TUMORS: A RETROSPECTIVE MRI ANALYSIS IN 54 CASES

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Brain tumors constitute just under 1% of all canine tumors with an incidence of 20 per 100.000 dogs per year [1], and generally occur in older subjects, with a median age of 9 years. Intracranial hypertension (ICH) is a common sequela of several brain diseases that can cause impairment of neurological status until death. The most accurate practice to measure ICP is by direct measurement, but in clinical practice it is very difficult to perform. Clinical evaluation of ICH in dogs showed a sensitivity of 70% and specificity of 46% so we cannot presumptively recommend treating dogs with mild to moderate neurological dysfunction for ICH based on clinical assessments, while it has been evaluated that sensitivity of MRI for predicting ICH was 90% and the specificity 69% [2]. The aim of this study was to investigate the incidence of suspected ICH at the time of diagnosis taking into consideration qualitative features as midline shift (MS), sub-falcine herniation (SFH), caudal trans-tentorial herniation (CTH), foraminal herniation (FMH), lateral ventriculus compression, displacement of the quadrigeminal lamina and presence of perilesional edema, considering that increased ICP is considered possible with ≥ 3 of these signs [2]. The correlation between tumor volume and indirect signs of ICH in MRI was evaluated. This observational retrospective study was performed by reevaluating MRI exams of fifty-four dogs with suspected and histologically confirmed intracranial tumors from January 2022 to December 2023 at San Francesco Veterinary Hospital (TV). The median age of dogs was 108 (84-144) months. Twenty-seven dogs were spayed females and twenty-seven intact males. Masses were intra-axial in 31 dogs and extra-axial in 23. 94% of cases had 3 or more signs in MRI referable to ICH. Tumors were in the temporal-piriform lobes (26%), in parietal lobes (24%), in frontal lobes (22%), in olfactory lobes (14%), into the lateral ventriculus (8%), intro III ventriculus (2%), in diencephalon (2%) and in occipital lobe (2%). 81% of the cases showed MS, 81% SFH, 46% CTH, 33% FMH while 48% showed MS-SFH, 20% MS-SFH-CTH-FMH, 13% MS-SFH-CTH, 7% CTH-FMH, 6% CTH alone and 6% FMH alone. 83% of tumors presented perilesional edema while in 17% was not present. The compression of lateral ventricle was evident in 75% and the displacement of the quadrigeminal lamina in 59%. Kendall's Tau test was performed to find correlations between indirect signs of ICH and relative volume of the tumor, which was positively related with the frequency of lamina quadrigemina dislocation (p=0,0122). Relative volume of the tumors was not correlated with perilesional edema, which was positively related with compression of the lateral ventricle (p=0,0243) or extra-axial location of the masses (p=0,0022). In conclusion, at the time of diagnosis, most cases have indirect signs of ICH that may need treatment for increased ICP.

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77° CONVEGNO SISVET

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Clinical comparison between carprofen and meloxicam for post-surgical pain management in pet rabbits undergoing orchiectomy

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Pain in rabbits is associated with prolonged recovery times, heightened stress, and diminished gastrointestinal motility, leading to reduced appetite and fecal output [1]. Composite pain assessment tools like CANCRS (Centro Animali Non Convenzionali Rabbit Scale) are valuable for evaluating pain in rabbits [1,2]. NSAIDs such as meloxicam and carprofen possess analgesic properties and are frequently employed following elective surgical procedures. While meloxicam is extensively studied as an NSAID in rabbits, the evaluation of carprofen in this regard is comparatively limited [3]. This study aims to compare the post-operative effects of meloxicam and carprofen following orchiectomy in rabbits from a clinical point of view.
Twenty-two mixed-breed domestic rabbits undergoing elective orchiectomy were enrolled in this study and allocated into 2 groups, each comprising 11 subjects. Signed informed consent was obtained. An intranasal administration of a ketamine, medetomidine, and butorphanol combination (20, 0.4, and 0.2 mg/kg) was performed using a Mucosal Atomization Device to induce anesthesia, followed by orchiectomy. Post-operatively, NSAIDs were administered for three days subcutaneously once daily: Group M received 1 mg/kg meloxicam, while Group C received 2 mg/kg carprofen. Additionally, all rabbits received 5 mg/kg enrofloxacin subcutaneously once daily and 1 mg/kg metoclopramide subcutaneously twice daily for three days.
Rabbits were assessed using the CANCRS at 5 time points (T0: baseline, T1-T4: 6 hours post-surgery and at 9 am, 1:30 pm, and 6 pm the following day). Additionally, the time of spontaneous feeding and fecal output after recovery was recorded. Statistical analysis was conducted using R (v. 4.3.0), with a significance level set at $p<0.05$. Friedman's test and subsequent Wilcoxon signed rank test were employed to analyze CANCRS scores over time, while the Wilcoxon rank sum test was utilized for comparisons of CANCRS scores and times of spontaneous feeding and fecal output between groups.
No significant differences were observed between groups in CANCRS scores at any time point or in times of spontaneous feeding and fecal output. Statistically significant differences in CANCRS scores within each group were identified (C: $p = 0.033$; M: $p = 0.009$).
No significant differences were found between treatment groups, suggesting equivalent analgesic effects of post-operative meloxicam and carprofen in rabbits undergoing orchiectomy. No clinically detectable adverse effects were noted with either NSAID, supporting their safety in rabbits at the studied doses. Considerations for drug choice may include familiarity, volume, cost, and ease of administration, with meloxicam's oral suspension availability potentially advantageous for home use. Limitations of the study include lack of remote assessment tools and absence of a placebo group due to ethical considerations. In conclusion, both carprofen and meloxicam are viable options for post-operative pain management in rabbits, with additional research warranted to enhance understanding and reliability of outcomes.

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Surgical technique and evaluation of the incidence of intraoperative and postoperative complications following surgical management of Porto Azygos Shunt attenuation in Thoracoscopy

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Congenital extrahepatic portosystemic shunts (CEPSS) frequently occur in small breed dogs, approximately 25% of CEPSS cases terminate on the azygos vein within the thoracic cavity, known as portoazygos (PA) shunts. They are conventionally attenuated intra - abdominally. The aim of this study is to evaluate the PA shunt attenuation by cellophane band using thoracoscopic approach in twelve dogs.

Twelve patients were positioned in sternal position, and three incisions were made, with 11 out of 12 cases having incisions at the levels T9, T10, and T11 on the right side, while in 1 case, incisions were made at T7, T8, and T9 on the left side. In all instances, the shunts were secured using a 4mm cellophane band. Thoracoscopic exploration allowed to identify the PA shunt close to the azygos vein. The aberrant vessel was dissected and isolated for at least 2 cm. A 4-mm-wide triple-layer cellophane band previously prepared was placed around the vessel using a right-angled forceps. Two clips were applied in an alternating manner from both sides of the cellophane aiming to achieve the shunt correct attenuation. After thoracoscopic procedure in 3 of 12 cases were done laparoscopic liver biopsy. The patients were changed position, were in dorsal recumbency, optica of 5 mm 30° was positioned in preumbelical site and forceps for biopsy in right position.

No major intraoperative or postoperative complications were observed. One minor complications was observed in 1/12 patient, a intercostal artery was accidentally sacrificed, mild self-limiting bleeding was observed. The average surgical duration was 45 minutes (range: 26-90 minutes) for 9/12 cases, while the surgical duration time was 45, 50, 60 minutes for the 3/12 cases were the laparoscopic liver biopsy was associated. In one case, a follow-up total body CT scan revealed shunt patency, prompting subsequent surgical ligation of the shunt. The mean follow-up period was 389 days (range: 14-820 days). At the conclusion of the follow-up, all patients were alive and exhibited no sign associated with the PA shunt. Thoracoscopy offers a direct path to the terminal PA shunt with minimal soft tissue dissection. Cellophane bands application to the PA shunt using thoracoscopic approach appears to be feasible.

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Evaluation of the incidence of intraoperative and postoperative complications following laparoscopic extirpation of enlarged and no enlarged sentinel medial iliac lymph node in four dogs

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The evaluation of sentinel lymph node (SLN) known as lymph node mapping, is a critical process in assessing the stage of various solid tumors, such as mast cell tumors (MCTs). Several sentinel lymph node (SLN) mapping techniques, have been investigated in the last 10 years in veterinary oncology. If SLN is a Medial Iliac Lymph Node (MILN) the methods to evaluate it, include diagnostic imaging, fine needle aspiration (FNA) or excisional biopsy. MILN extirpation can be a challenge for localization between the deep circumflex iliac and the external iliac arteries.
The aim of this study is to evaluate incidence of intraoperative and postoperative complications following laparoscopic extirpation of enlarged and no enlarged sentinel medial iliac lymph node in four dogs (SMILN), after SLN mapping.

A Golden retriever male, 8 yo, 30 kg, a mixed breed neutered male, 25 kg, 7 yo, a English Bouledogue, male, 6 yo, 30kg, and a mixed breed male, 13 yo, 18 kg presented for extirpation of SMILN secondary to neoplasia. The first case a Golden retriever had an enlarged SMILN. CT scan described maximum size of approximately 3-4 cm with irregular and undefined margins, resulting in moderate osteolysis of the underlying bone tissue and clear destruction of the bone cortex. The SMILN's mixed breed were mild enlargement. The SMILN's English Bouledogue was no enlargement. For the first one the enlarged SMILN no use of blue di metilene was used intra operatory for identify the SMILN, in other cases the blue of metilene was used.

The dogs were positioned in dorsal recumbency. The first portal and the creation of pneumoperitoneum was done by open modified Hasson technique in midline 2 cm under umbelica. After exploring the entire abdomen with a 5 mm × 29 cm 30° to evaluate the organs for iatrogenic damage, the second cannula 6 mm × 6.5 cm was inserted at a point equidistant from the midline and the camera portal on the contralateral side of the MILN under laparoscopic guidance. The third portal was established in the caudal abdomen at a location approximately one third of the distance between the pubic brim and the ipsilateral instrumental portal. The retroperitoneum was incised between the right external iliac artery and the testicular vessels. The tissue surrounding the retroperitoneum and the small vessels attached to the lymph nodes were dissected using blunt dissection and sealing/transection by the vessel-sealing device, respectively. During the procedure, the lymph node was retracted away with laparoscopic Babcock forceps from the large vessels.

SMILNs were successfully identified and excised by using the ventral approach in all dogs. There were no major complications defined as a treatment-related adverse event requiring further therapy with increase in the level of care or prolonged hospitalization, or minor complications defined as a treatment-related adverse event requiring nominal therapy or no treatment with or without overnight hospitalization for observation were. No animal required conversion from laparoscopic procedure to open laparotomy.

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77° CONVEGNO SISVET

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Comparison of butorphanol, lidocaine or butorphanol/lidocaine for local anesthesia in intraoperative pain management in sedated calves undergoing umbilical hernia repair.

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Abstract
The aim of the study was to compare the analgesic effects of butorphanol and lidocaine administered locally, alone or in combination, during umbilical hernia repair in sedated calves [1]. Thirty calves were enrolled and divided into three groups: L group (n=10), B group (n=10) and LB group (n=10). All subjects were sedated with xylazine 0,3 mg/kg administered intramuscularly (IM). For the local analgesic protocol the L group received lidocaine 4,5 mg/kg, both by infiltration of the surgical planes and intraperitoneally, the B group received butorphanol 0,02 mg/kg by infiltration of the surgical planes and intraperitoneally and the BL group received lidocaine by infiltration of the surgical planes 4,5 mg/kg and butorphanol 0,02 mg/kg intraperitoneally. The drug dose was divided into two syringes, with the total dose shared in the B and L groups and the syringes differentiated by drug in the BL group. The individual syringes were then used for infiltration into the surgical plane and for intraperitoneal administration. To achieve greater diffusion, the volume of each syringe was increased to 40 mL with the addition of saline solution (0.9% sodium chloride). Infiltration in the umbilical region involved both skin and muscle planes, while intraperitoneal injection was performed in the hernia sac. Heart rate (HR), respiratory rate (RR), arterial pressure (SAP, MAP, DAP) and hemoglobin oxygen saturation (SpO2) were recorded. These parameters were recorded before sedation (T0), 15 min after xylazine administration (T1), 10 min after local drug administration (T2), then at 10 (T3), 15 (T4), 20 (T5), 25 (T6), 30 (T7), 35 (T8), 40 (T9) min and on awakening (T10). To assess the response to intraoperative noxious stimulation, we used a cumulative numerical scale that considered percentage changes in HR, RR and SAP compared to baseline (T0) according to the following procedure: (time point value – basal value)/ basal value x 100 = % change. Scores were assessed as follows: Score 0 = variation ≤ 0%; 1 = variation ≤ 10%; 2 = variation > 10% but ≤20%; 3 = variation > 20% but ≤30%; 4 = variation > 30%. Scores were assigned by the evaluators who were blinded to treatment. A final score ranging from a minimum of 0 to a maximum of 12 was obtained by summing the scores of the selected variables; if the score was 6 or higher (HR, RR and SAP increased by more than 20%), rescue analgesia was administered and the surgical area was infiltrated and sprayed intraperitoneally with 2 mg/kg lidocaine 2%. The intraoperative noxious stimulation response scale showed a significant reduction from T3 to T10 compared to baseline in the B and L groups ($p<0.001$). The Bl group showed a significant reduction from T3 to T7 ($p<0.001$). When comparing between groups, the B group showed significant differences with the L group at many time points (T1, T2, T4, T5, T6, T9, $p<0.001$), while the BL group only showed a difference only at T1 ($p<0.05$). The comparison between the L group and the BL group showed significant differences at T1 and T6 ($p<0.001$). The results obtained showed a change in the monitored parameters (HR, RR, SAP) that did not require rescue analgesia in any case. The time from the start of the surgery to the animals' return to the standing position was significantly different between the B, BL group and the L group ($p = 0.000$) and was as follows: 180 min (160/210; 185± 15,5) B group, 128 min (95/180; 131± 25,6) L group and 192 min (160/240; 196± 23,1) BL group. The assessment of the postoperative pain score using the UNESP-Botucatu Unidimensional Composite Pain Scale showed a significant variation in the B and BL groups at 4 h and 5 h ($p < 0.001$), remaining ≤4 throughout the observation period. In the L group, the UNESP-Botucatu showed changes from 2 h to 5 h ($p < 0.001$), with 4 subjects at 4 h and 5 subjects at 5 h requiring rescue analgesia by administration of 3.3 mg/kg intravenous flunixin meglumine (Finadyne, Schering-Plough Animal Health, Oss, The Netherlands).

Comparison of the groups with regard to UNESP scores showed a significant difference between B and L ($p < 0.001$) and between BL and L ($p < 0.001$), as the scores of B and BL were lower than those of L throughout the postoperative period. The number of legally authorized anesthetics and analgesics for livestock, including calves, is limited. Therefore, it is necessary to propose therapeutic alternatives to veterinarians designed to ensure patient welfare, surgeon comfort and staff safety. The protocol used in this study consisted of intramuscular administration of xylazine followed by butorphanol or lidocaine, alone or in combination, along the incision lines and intraperitoneally [2]. This protocol provided adequate sedation and analgesia in calves undergoing umbilical hernia repair, with no clinically demonstrable adverse effects [3].. The combination of butorphanol and lidocaine administered locally gave a better effect on the monitored parameters than the same drugs used alone. Therefore, this combination could be proposed as a viable alternative in calves undergoing umbilical hernia repair.

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77° CONVEGNO SISVET

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Clinical findings in the treatment of canine degenerative lumbosacral stenosis. A pilot study on the analgesic activity of amantadine through the analysis of the ground reaction forces.

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Degenerative lumbosacral stenosis represents a multifactorial pathology and is a frequent cause of cauda equina syndrome in canine patients. The therapeutic approach encompasses both conservative and, ultimately, surgical interventions. The resulting pain from degenerative lumbosacral stenosis is maladaptive. Arising from abnormal activation of neuronal pathways underlying pain perception, traditional therapies involving the use of steroidal and non-steroidal anti-inflammatory drugs may, therefore, prove insufficient for effective pain management in affected patients. The objective of this study was to assess the efficacy of amantadine within a therapeutic protocol for managing chronic neuropathic pain in dogs affected by degenerative lumbosacral stenosis through the analysis of ground reaction forces (GRFs), including Peak of Vertical Force (PVF), Vertical Impulse (VI), and stance time (ST). Client-owned dogs, over 12 months old and 20 kg of body-weight, with a confirmed diagnosis of DLSS, were included in this randomized study approved by the Ethics Committee (prot. No. PG/2023/0059191 of 22/05/2023). The dogs were randomly assigned to two groups: Group A, administered a combination of meloxicam for 7 days (0.2 mg/kg PO SID as a loading dose, followed by 0.1 mg/kg PO SID for 6 days) and amantadine (3 mg/kg PO SID) for 21 days, and Group B, receiving only amantadine (3 mg/kg PO SID) for 21 days. To evaluate the treatment efficacy, each subject underwent gait analysis using a force platform at the time of enrollment (T0), after 7 days (T1), and after 21 days (T2). The recorded ground reaction forces included peak vertical force (PVF), vertical impulse (VI), and stance time (ST). The Mann-Whitney U-test was used to examine the differences between groups A and B at time T0. The two-tailed Wilcoxon matched-pairs signed rank test was used to examine differences between pre-and post-treatment values (at baseline T0 and at T2). Friedman's ANOVA test for related samples was used to measure the significance of the GRF increase from T0 to T2 for both groups. The Fisher LSD test was used as a post-hoc test. The p-value was set to <0.05.Fourteen dogs were enrolled. At T0, the groups were equal in terms of GRFs, body weight, and age. In both groups, there was a statistically significant increase in VI%BW and PVF%BW over time points. In group A, there was a statistically significant difference between T0-T2 (p-value= 0.0037), and between T1-T2 (p-value=0.0187) for VI%BW; similarly, for group B, there was a statistically significant difference between T0-T2 (p-value=0.0021) and between T1-T2 (p-value=0.0151). For PVF%BW, in group A, there was a statistically significant difference between T0-T2 (p-value=0.0078) and T1-T2 (p-value=0.0194); while in group B, there was a statistically significant difference between T0-T1 (p-value=0.0046), T1-T2 (p-value=0.0007) and T0-T2 (p-value<0.0001).Results indicated that amantadine can be considered a valid alternative to the administration of anti-inflammatory drugs for managing neuropathic pain associated with degenerative lumbosacral stenosis in dogs.

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77° CONVEGNO SISVET

Stato: INVIATO - ID: 13361

Assessment and validation of the sterilization process of surgical equipment

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Postoperative surgical site infections (SSIs) have been described as a complication of 0.8% to 18.1% of small animal surgical procedures, with significant variation associated with surgery type [1]. Preventive strategies represent the most economical and effective means of reducing SSIs impact [2]. The sterilisation cycle of surgical equipment represents an important factor in the infection control during the surgical procedures, to guarantee the patient safety [3]. Currently, many cleaning and sterilisation protocols are used for sterilising surgical instruments, but not precisely defined is the sterility duration time of the equipment as well as the best packaging method. The aim of our study is to verify the validity of our sterilisation process, assess the shelf-life of our surgical equipment and compare two different packaging methods, single pouch, or double pouch.Two hundred and fifty non-sterile surgical screws of different dimensions were used for the study and divided into two groups; Group 1, composed of 125 screws packaged individually in a single sterilisation pouch, and Group 2, composed of 125 screws packaged in a double sterilisation pouch.In double pouch packaging, the pouches were placed in the same fashion: plastic faces plastic, and paper faces paper. All screws were autoclaved by an experienced operator using the same cycle (210kPa, 134°C, 50 min) and adequately stored in a clean steel cabinet. Furthermore, each pouch was handled every day to simulate the routine activities in the surgical room. The bacteriological examination of 25 screws from each group was performed after 24 hours, 168 hours, 360 hours, 720 hours, 1440 hours (60 days), 2160 hours (90 days), 4320 hours (180 days) from sterilisation at the Bacteriology Laboratory of the Department of Veterinary Medicine and Animal Production (University of Naples Federico II). Once opened, the collected screws were inoculated in Brain Heart Infusion (BHI) broth and incubated aerobically at 37°C for 24 hrs. After the overnight incubation, broth turbidity was assessed, and, when necessary, broth sub-cultivation was performed on Columbia blood agar base plates, a medium for the isolation of both Gram-positive and Gram-negative bacteria. The investigation currently performed up to 2160 hours (90days) has not detected any bacterial growth on the surgical equipment packaged in both single and double pouches.The obtained results are promising, and the use of a single pouch seems to be sufficient, on account of the considered parameters such as temperature, humidity, and light exposure present in our storage location, suggesting the possibility of maintaining the sterility of the screws in the pouches even beyond 4320 hours (180 days).

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EVALUATION OF AN INTRA-ARTICULAR CARBOXYMETHYLCELLULOSE HYDROGEL IN HORSES WITH OSTEOARTHRITIS

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Introduction

Osteoarthritis (OA) is one of the most prevalent and debilitating diseases affecting horses, with a notable economic impact on the equine industry [1]. Numerous treatment methods (physical, biological, and pharmaceutical) have been advocated either to prevent OA or to minimize clinical signs of pain (lameness), reduce joint deterioration, and prolong the competitive career of athletes [2]. Carboxymethylcellulose (CMC), is one of the derivatives of cellulose belongs to the class of cellulose ethers. is used in veterinary medicine for various purposes [3]. Aim of this study is to test the effficacy of a commercial CMC hydrogel in reducing lameness due to osteoarthritis

Materials and Methods

To be included, horses needed to be 2 or more years of age and less than 650 kg. Horses with lameness from a single site of OA in forelimb feetlock joint. The lameness had to have a minimum duration of 4 weeks and be positive to flexion test of the affected joint. The diagnosis was confirmed with diagnostic intra-articular anesthesia and radiographic examination of the joint. Exclusion criteria were intra-articular therapy of the affected joint within 4 weeks before study enrollment or during the study period, systemic application of corticosteroids, NSAIDs or other pain medication, shock wave therapy, acupuncture, or any homeopathic or oral supplements within 4 weeks before study enrollment or during the study period. The horse owner had to agree to adherence to the study protocol and sign an informed consent agreement. The veterinarian had to agree to the study protocol and provide all follow-up data. The study was approved by applicable institutional animal care and use committees. Horses that met enrollment criteria were assigned a lameness score based on the AAEP scoring system. After njections horses were asked to handwalk for days 1–5, up to 20 minutes on the walker for days 6–15, then from16 to 20 if serviceably soundwere allowed to jog up to a mile a day before returning to full work. The horses were re-evaluated after 15, 30 and 90 days. Treatment group included horses treated with CMC (intra articular injection), control group included horses treated with 10 mg of triamcinolone acetonide and sodium hyaluronate (intra articular injection). Improvement was defined as reduction of at least one lameness grade.

Results

Eleven horses were included in the treated group and five in the control group, in the treated group four horses were thoroughbreds and six were show jumpers. In the control group all the horses were thoroughbreds.
The overall success rate for the study was 20% at 15 days for treated group an 100% for control group. At 30 days the overall success rate was 90 % for treated group and 70 % for control group.
At 90 days the overall success rate was 90 % for treated group and 40% for control group.

Conclusions

In conclusion carboxymethilcellulose hydrogel injected IA significantly improved lameness in affected joints. Carboxymethilcellulose could be considered a safe option for the treatment of horses suffering from OA.

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77° CONVEGNO SISVET

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STRAIN ELASTOGRAPHY EVALUATION OF PATELLAR LIGAMENTS IN HEALTHY DOGS AND AFTER TPLO/TTA, FOCUS ON SEMIQUANTITATIVE EVALUATION

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Patellar ligament desmopathy in dogs has been reported after TPLO and TTA, resulting in tendon thickening and disruption of normal fiber orientation (1).Strain elastography (SE) is a sonographic technique able to determine tissue stiffness, applying repeated manual compression with the transducer, obtaining the amount of lesion deformation relative to the surrounding normal tissue measured and displayed as a color map called elastogram (2). SE allows semi-quantitative evaluation of the tissue comparing the relative grade of compression, expressed as elasticity index (EI), with a reference tissue. A strain ratio (SR) >1 indicates that the target lesion compresses less than the normal reference tissue, indicating greater stiffness.Patellar ligament has been qualitatively described as soft healthy dogs and hard in dogs affected by cranial cruciate ligament rupture (2), however the use of strain ratio with reference tissue as semiquantitative indicator of tendon stiffness has not been assessed, especially in dogs after TPLO or TTA surgery.This work mainly focused on semiquantitative evaluation of patellar ligament elasticity in healthy and post TPLO/TTA dogs comparing the tendon elasticity with infrapatellar fat pad and cutis/subcutis dorsal to the tendon.Dogs were divided in two groups, healthy (G1) and after at least 1 month after surgery (G2). B-mode and SE examinations were performed with Logiq S8 imaging device (GE Healthcare; Milwaukee, WI, USA) with a probe L11, 8.5–10 MHz in association with strain elastography software. Tendon mean thickness was recorded and a B-mode score from 0 to 3 was determined based on the severity of tendon abnormalities. SE was qualitatively performed with a score 1-4 based on the stiffness, then EI was recorded on the whole tendon area and in proximal, intermediate and distal area excluding the patellar and tibial crest insertions, then SR was respectively collected with both fat pad and cutis/subcutis; value of SR > 1 represented increased tissue stiffness relative to the reference healthy tissue selected.Statistical analysis was performed with Past4 software (significance set with $p < 0.05$).24 healthy (G1) and 11 after surgery (G2) tendons were included, G1 was significantly thinner and obtained a lower score than group 2 (median 0.18 cm and score 0 for group 1, 0.35 cm and score 2 group 2 $p< 0.05$). In 7 of 11 dogs of G2 infrapatellar fat pad was inhomogeneous, hyperechoic and with variable amount of free fluid.EI for G1 was significantly lower than G2 for both whole tendon and the single portions, EI for the distal portion was significantly lower than proximal and intermediate in G2.SR was < 1 in G1 and >1 in G2 ($p < 0.05$) for both fat pad and cutis/subcutis.SR in G1 was significantly different ($p < 0.05$) in the three-tendon portion only with fat pad as reference.These data suggest that after TPLO o TTA surgery the tendon may become stiffer and that the increase of stiffness may not be uniform.Cutis/subcutis seems to be a more reliable reference tissue than infrapatellar fat pad due to lower variability in healthy tendons and lower prevalence of B-mode abnormalities.

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77° CONVEGNO SISVET

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PROCEDURES FOR SCAFFOLD RECONSTRUCTION OF TENDON: AN EXPERIMENTAL STUDY

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The occurrence of tendon injuries/defects caused by trauma, age-related degeneration, or excessive loading of the musculoskeletal system is a common clinical problem in Human and Veterinary patients. Currently, the conventional clinical treatments includeimmobilization, physiochemical therapy, and surgical suturing.Although suture surgery provides temporary restoration of tendoncontinuity, it fails to retain the structural integrity and mechanical strength. Biomaterials have been demonstrated to promote the regeneration and the treatment of tendon injuries with some advantages such as morphological restoration, unrestricted material source, excellent biocompatibility and absence of immune reactions. Various fabrication methods have been developed such as 3D bioprinting, wet-spinning, and electrospinning. Our group had the possibility to validate an electrospinning bioabsorbable scaffold obtained from a new technology that uses an electrically-driven method to produce fibers of nanometric or micrometric diameter that can reproduce the hierarchical structure and the mechanical propriety of tendinous and ligamentous tissues in the Achilles tendon of anovine model. The aim of the stud , authorized by the Italian Ministry of Health (n° 733/2023-PR) , was to find a surgical system that could guarantee the tightness of the tendon itself, even in the presence of a large loss of substance, which had to be left free to carry out its normal functions during the tendon regeneration. In this study we report the surgical employed techniques and the first results. 12 sheep were trichotomized and underwent premedication and subarachnoid anesthesia in preparation for surgery. A longitudinal skin incision was made corresponding to the Achilles tendon of approximately 7 cm and then the tendon was isolated. The common tendon sheath that envelops the two terminal heads of the tendons was incisedallowing the two ends to be highlighted and isolated with a mosquito forceps placed between them. At the two ends of the sheath incision, two full-thickness n.2 nylon sutures were applied to join the two tendon heads together. At this point two technique of implant of scaffold were applied:1) a medial portion of 4 cm x 5 mm of Gastrocnemius tendon was cut and removed and replaced with a scaffold of the same measure that was sutured proximally and distally tothe excised tendon with a Bunnell suture in nylon 3.0 monofilament and laterally with a monofilament of PDS 3.0. Then the tendon sheath was sutured with a reabsorbable monofilament 3.0.2) after the incision of the common tendon sheath and the application at the two ends of the sheath incision of the two full-thickness nylon n.2 sutures, the Gastrocnemiustendon was isolated and a partial longitudinal incision was made to get a pocket to receive the scaffold. The tendon incision and the common tendon sheath wassutured with a 3.0 monofilament reabsorbable suture. In the two techniques the subcutaneous tissue was sutured with a reabsorbable monofilament 3.0 suture and the skin was closed with staples. Clinical and ultrasound checks were performed in the post operative period to check the mechanical tightness of the systems and evaluate the regenerative and mechanical capacity of the scaffold . The first results demonstrated the absence of contamination, mechanical resistance at full load and partial integration of the scaffold.
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2D Shear wave elastography of the equine suspensory ligament branches: preliminary data.

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The suspensory ligament branches disorders (SLBs) are an important source of lameness or poor performance in the equine athlete (1). Although many cases of these injuries are straightforward to diagnose, detecting subclinical abnormalities and correlating them with the future development of clinically relevant SLBs desmopathy is challenging (1-2). Although MRI offers higher sensitivity than ultrasound (US) in detecting most types of soft tissue injury, US has remained an important and more cost-effective imaging modality for diagnosing and monitoring such injuries (2). 2D-Shear Wave Elastography (2D-SWE) is an innovative ultrasound-based technique that quantifies tissue stiffness, providing information about its mechanical properties through measurement of the velocity of propagation of the shear waves within the examined structure (3). Aim of the study was to evaluate the feasibility and repeatability of 2D-SWE in assessing SLBs in sound horses. Quarter Horses in full training were prospectively recruited (Prot. 11/2019). Each horse underwent a comprehensive orthopedic evaluation, followed by radiographic and US examination of both fore and hind limbs. 2D-SWE was performed by a single experienced operator in both longitudinal and transverse scans, with the horse in a weight-bearing position and without a stand-off pad. Elastographic images were then analyzed independently and randomly by 2 experienced observers to assess interobserver agreement. Quantitative analysis of elastographic images involved manually drawing a region of interest (ROI) in the most rigid area of the SLBs, with measurements taken for Shear Wave velocity (m/s) and Young's Modulus (kPa). Descriptive statistic and T-test were performed for studying the interobserver agreement and to compare branches between the same limb or between different limbs, with a significance level set at $p<0.05$. A total of nine horses were examined. Elastograms could be acquired both in transverse and longitudinal plans, in 59 out of 72 branches. Qualitatively, they revealed essentially non-deformable SLBs depicted in light blue to marked blue colorations. Inter-observer agreement was excellent in both from transverse and longitudinal scans. Some differences were found when comparing medial and lateral branches of all right forelimbs with all right hindlimbs, and all left forelimbs with all left hindlimbs only in longitudinal images, and when comparing all right forelimbs with all left forelimbs, and all right hindlimbs with all left hindlimbs only in transverse images. T-Test comparing medial with lateral branches within the same limb highlights some differences both in transverse and longitudinal scans. The results of our study demonstrated excellent repeatability between observer, with no statistical difference highlighted, proving the feasibility of the 2D-SWE technique for the examination of the SLB in sound horses. Further research involving a larger number of cases is necessary to establish a standardized protocol and determine a range of stiffness in sound and pathologic horses, to extend its usage as diagnostic modality on field for evaluation of the SLBs desmopathies.

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77° CONVEGNO SISVET

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Computer-Aided Design Planning and 3D-printed Patient-specific Guide to Address an Oblique Plane Antebrachial Deformity in a Dog

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Abstract
Computer-aided design (CAD) and patient-specific 3D-printed guides have been demonstrated to improve the accuracy of antebrachial deformity correction in dogs (1,2). This report describes a successful application of CAD and a patient-specific 3D-printed guide to correct a complex antebrachial deformity in a 6-month-old Maltese dog presented with left forelimb lameness. Radiographs of the affected limb showed elbow incongruity, a short ulna, and a long curve radius. A multiplanar antebrachial deformity was diagnosed based on CT scans. Employing CAD (Grasshopper algorithmic modeling tool integrated into Rhinoceros software), the center of rotation of angulation was identified in the distal radius. An oblique plane deformity was diagnosed, characterized by 21° valgus, 30°excessive procurvatum, and 42° external torsion. Using CAD, the surgery was stimulated virtually, and an osteotomy guide with high-profile features was custom-designed for the radius. Intra-operative models and surgical guides were 3D-printed in polylactic acid. The surgery involved a bi-oblique ulnar osteotomy and an oblique-plane closing-wedge ostectomy of the radius. The guide was perfectly positioned and stabilized with K-wires to the bone. After the ostectomy, a mini-series Fixin plate was placed dorsally on the radius. Postoperative radiographs showed resolution of deformity and elbow incongruity. Radiographical union of the osteotomy site was observed 60 days post-op. After seven months, radiographs showed a decrease in radial bone density underneath the plate, indicative of stress protection ascribed to the plate's rigidity. Staged plate dynamization was performed at 7 and 9 months, followed by complete plate removal at 11 months. This case report highlights the benefits of using CAD planning and patient-specific 3D guides in an antebrachial corrective osteotomy; as well as the importance of long-term follow-ups for early diagnosis and management of stress protection. Future research should focus on objective assessments of CAD and 3D technologies in corrective procedures, comparing them to free-hand surgeries (2,3).

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77° CONVEGNO SISVET

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2D-Shear Wave elastography for evaluation of the intermediate patellar ligament in horses: preliminary data

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Diagnostic imaging of the stifle in horses relies exclusively upon radiography and ultrasound examination. Of all the three patellar ligaments, intermediate patellar ligament (IPL) desmopathy is the most common [1]. 2D-Shear Wave Elastosonography (SWE) is an emerging technique in veterinary medicine [2], which assesses tissue elasticity measuring the propagation of transverse shear waves through the tissue itself. Aim of the study was to evaluate the feasibility and repeatability of 2D-SWE for equine intermediate patellar ligament in horses. Horses included in the study underwent a complete orthopedic examination, radiography and ultrasound of both stifle joints. They were allocated to Group S if no lesions were found at the IPL and Group D if signs suggesting desmopathy of the IPL were detected. 2D-SWE (in longitudinal and transverse scans in full weight-bearing stance) and elastograms evaluation were performed by 2 experienced operators (OP1-OP2). Cranio-caudal thickness and maximum diameter of the IPL were measured on B mode images, and their ratio calculated. The region of interest (ROI) was outlined on the elastogram over the margins of the IPL in transverse scan and at its middle third in longitudinal scan. Statistical analysis evaluated repeatability of measures between OP1 and OP2 (paired T test) for thickness and diameter of IPL, velocity (m/s) and Young's modulus (kpa), and compared the same variables between Group S and D, and between left and right limb. Level of significance was set at $p<0.05$. Twenty horses mixed for age and breed were included in the study. Fifteen were assigned to Group S, 5 to Group D. No differences were found either between the measures from OP1 and OP2, or between left and right limb. In Group S mean values of velocity in transverse scan were 4.7±0.32, in longitudinal scan 19.2±20.15; whereas Young's modulus was 73.8±10.15 and 59.5±21.38 respectively in transverse and longitudinal scan. In Group D mean values of velocity in transverse scan were 4.9±0.04, in longitudinal scan 4,8±0.01; whereas Young's modulus was 76.8±0.73 and 91.2±24.88 respectively in transverse and longitudinal scan ($p>0.05$).A mild significative correlation was found between the diameter/thickness ratio and velocity in transverse scan and between the diameter/thickness ratio and Young's modulus in longitudinal scan. As previously demonstrated [2,3], the technique was feasible and repeatable for evaluation of IPL in horses. In the present study it didn't highlight differences between sound horses and horses affected by IPL desmopathy. IPL dimension (diameter and thickness) was only partially correlated to its elasticity. The small sample size and the small representation of horses with IPL desmopathy are the main limitation of the study. The potential utility of the technique as an adjunctive diagnostic tool for stifle evaluation in horses should lead to more extensive research on the topic to reach stronger conclusions.

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77° CONVEGNO SISVET

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Predicting lymph node metastatic status in canine mast cell tumor with pre-operative planar lymphoscintigraphy: an explorative study

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The aim of this study was to assess the potential pre-operative predictive value of planar lymphoscintigraphy regarding the sentinel lymph node (SLN) metastatic status in dogs with mast cell tumor (MCT). Sentinel lymphadenectomy is fundamental for staging, prognosis and therapy of MCT [1]. Lymphoscintigraphy has been successfully used in veterinary medicine for SLN mapping [2], especially in MCT, demonstrating high sensibility in SLN detection.In humans, lymphoscintigraphy, a gold standard for breast carcinoma and melanoma, has a pre-operative prognostic value regarding the presence of metastases in SLN [3]. In this cross-sectional retrospective study, dogs with MCT referred to the Veterinary teaching Hospital of Lodi, University of Milan, between May 2017 and June 2023, that underwent pre- and intraoperative SLN mapping with lymphoscintigraphy for therapeutical purpose, were selected. Other inclusion criteria were presence of non-palpable/normal sized lymph nodes, absence of loco-regional or distant metastasis; surgical excision of the primary tumor and sentinel lymphadenectomy guided by pre-operative planar lymphoscintigraphy (PPL) and intraoperative gamma probe, and Weishaar histological classification of the excised SLNs. Images of PPL were evaluated quantitatively, considering the minimum, maximum and mean count of the SLN. A qualitative evaluation was also performed: lymph nodes uptakes were divided using a scale from 0 to 5 (0: no uptake; 5 marked nodal uptake). Statistical analysis was performed in order to evaluate a correlation between the PPL nodal uptake and the nodal histological status after surgical extirpation (4-point scale: HN0-HN3 classification; binary outcomes: HN0-HN1 vs HN2-HN3). To assess the potential ability of the counts to distinguish between metastatic and non-metastatic nodes, the Receiver Operating Characteristic (ROC) curve was examined and the area under the curve (AUC) and the Youden index J were extrapolated to identify the best cut-off value. Additionally, positive and negative predictive values were calculated along with sensitivity and specificity values.In the study, 52 dogs were included for the quantitative evaluation and 48 for the qualitative evaluation. A total of 68 SLN were excised: histophatologically, 16 SLNs were classified as HN0, 11 as HN1, 31 as HN2 and 10 as HN3.Quantitatively, the value of nodal uptake of radiopharmaceutical tracer in preoperative phase was significantly lower in metastatic SLN compared to that observed in non-metastatic lymph nodes. Threshold uptake was also identified to predict the absence of metastases with a sensitivity of 100%: a maximum uptake value higher than 219 counts and a minum uptake value higher than 76 counts, identified a definitely non-metastatic SNL. Regarding qualitative aspects, in this study, a significant association was found between the presence of nodal metastases and reduced/absent visualization of the node itself and between the absence of metastases and nodes with maximum uptake. The findings of this study showed the predictive value of SLN radiotracer uptake evaluated on PPL regarding sentinel nodal status in canine patients with mast cell tumors using both quantitative and qualitative approaches.

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77° CONVEGNO SISVET

Stato: INVIATO - ID: 13403

Evaluation of the effect of autologous Leukocyte and Platelet Rich Fibrin membranes in the treatment of dairy calves after disbudding: preliminary results.

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In dairy industry, disbudding is a common practice that induces pain and discomfort to the treated calves and requires a long healing period [1]. The treated areas remain severely damaged for at least 3-4 weeks and complete healing takes 6-13 weeks. In addition, local disinfectants and antibiotics may be required to prevent further complications. Considering all the welfare aspects associated with hot-iron disbudding, a technique to enhance the healing process and shortens the re-epithelialization time must be considered as a clinical treatment option. Therefore, the use of the Leukocyte-Platelets Rich Fibrin (L-PRF) could fulfill this need. The L-PRF is a platelet-based autologous, hemostatic biological scaffold that has been used in other animal species for wound healing [2] and whose production protocol has recently been validated in dairy cows [3]. The aim of this randomized, controlled clinical trial is to investigate the efficacy of L-PRF on wound healing after disbudding in dairy calves. Seventeen Holstein-Friesian calves were randomly enrolled in the study and disbudded with a hot-iron within three weeks of birth. A whole blood sample of 20 ml was collected from each calf by jugular venipuncture and used for L-PRF production according to the procedures described [3]. All patients received bilateral procaine-based cornual nerve block (5 ml/side Procamidor duo®, IZO S.r.l.), followed by disbudding 15 minutes later. After disbudding, each calf received two L-PRF membranes on the right wound (treated side_TS) while the left one was left untreated (control side_CS). During follow-up, the healing process was assessed weekly by digital photographs and monitored until complete re-epithelialization of the wounds. The clinical procedure proved to be well tolerated by the animals and easy to perform. No side effects were observed. On average, the TS healed within 6 weeks, while the CS healed within 8 weeks ($p<0.05$). The digital analysis of revealed that wound on the TS, which healed faster, required 5 weeks and showed a percentage decrease in wound area of 80%, while the wound that took longer to heal required 9 weeks with a percentage decrease in wound area of 80%. In contrast, for the CS, the time interval for the same parameter ranged from 6 weeks to 10 weeks with a percentage decrease of 80%. Preliminary data show that L-PRF has an overall beneficial effect on the regeneration of wounds caused by hot-iron disbudding in dairy calves. Its use in dams might be hypothesized as support for a complete clinical calf management program to reduce the use of disinfectants and antibiotics while maintaining a high level of animal welfare. Nevertheless, further studies are needed to confirm the encouraging results observed.
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77° CONVEGNO SISVET

Stato: INVIATO - ID: 13448

Effects of tecar therapy on subclinical post-anaesthetic myopathy in horses: preliminary data.

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Post-anaesthetic myopathy (PAM) is one of the most common peri-anaesthetic complication in horses and may affect single groups of muscles. It may occur in a generalized or a subclinical form, with an increase in serum muscular enzymes like creatine-kinase (CK), aspartate-transferase (AST), and lactate-dehydrogenase (LDH) [1]. PAM has been associated with decreased muscle perfusion related to hypotension (mean arterial blood pressure <65-70 mmHg), prolonged duration of general anaesthesia (>3 hours), lateral recumbency, and larger body mass [1]. Muscle enzymes increase in horses undergoing general anaesthesia but are significantly elevated in horses suffering from hypotensive episodes. Tecar (energy transfer capacitive and resistive) is an endogenous thermotherapy that uses electrical current to generate energy by moving electrical charges within tissues [2]. It is commonly used in rehabilitation due to its diathermic effect secondary to blood circulation increase that could reduce muscular spasms and contractions and help muscle oxygenation [3]. Aim of the study was to determine the effects of tecar therapy on muscles after general anaesthesia in horses undergoing elective and emergency surgeries. Horses included in the study receiving tecar therapy were assigned to Group A-T after elective surgery, and to Group B-T after emergency surgery. A non-treated control group was provided for A-T and B-T (respectively Group A-C and Group B-C). Treatment consisted of 3 tecar applications on the muscles involved in the recumbency at regular intervals in the two days following the anaesthesia. A blood sample was collected before the induction of the anaesthesia (T0) and 6h (T1), 24h (T2) and 48h (T3) after induction in all horses, and the concentration of serum muscle enzymes (AST, CK, LDH) and electrolytes (Na, K, Cl, Ca, P) were assessed for each sample. Descriptive statistic and T-test were performed to compare the trend of muscular enzymes between all treated and all non-treated horses and to compare the same variables individually between Group A-T and Group A-C and between Group B-T and Group B-C, with a significance level set at $p<0.05$. Fifteen horses were included in the study. Five were allocated to Group A-T, three to Group B-T, two to Group A-C, and five to Group B-C. No adverse reactions were observed during and after treatment. Some notable differences for AST and LDH concentrations were found when comparing all treated horses (Group A-T and B-T together) with all control horses (Group A-C and B-C together). T-test comparing Group A-T and Group A-C highlighted a difference only for AST concentration, while the comparison between Group B-T and Group B-C found a significance only for LDH concentration.
The results of our study demonstrate that tecar therapy is a safe and well-tolerated technique, which can help to limit the muscular damage in horses undergoing general anaesthesia for elective or emergency surgeries. Further studies involving a larger number of horses are required to establish the optimal treatment protocol and parameters to better assess the effects of tecar therapy on subclinical PAM in horses.

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77° CONVEGNO SISVET

Stato: INVIATO - ID: 13464

Retrospective preliminary evaluation of dexmedetomidine-ketamine-midazolam chemical immobilization in pumas (Puma concolor)

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Due to their wild nature, chemical immobilization of pumas is necessary for safe clinical management. However, literature on the chemical immobilization of pumas remains scarce, and few pharmacological combinations have been used, including tiletamine-zolazepam, ketamine and xylazine, or detomidine and ketamine, or medetomidine and ketamine [1,2]. The combination of ketamine and an alpha2-agonist with midazolam has not been described in pumas. However, anesthetic mixture including midazolam have been shown to provide cardiovascular stability and uneventful recovery in Panthera tigris [3].
The aim of this retrospective study is to evaluate the clinical effects following the intramuscular (IM) administration of dexmedetomidine, ketamine, and midazolam in eight pumas (Puma concolor) undergoing clinical or surgical procedures performed under field conditions or at the veterinary teaching hospital of the University of Padova.
Animals included 4 males and 4 females, with a median age of 7 years (range: 0.3-17) and body weight of 32.5 kg (range: 8-55). Dexmedetomidine (7.8 ± 2.7 mcg/kg), ketamine (2.4 ± 0.2 mg/kg), and midazolam (0.16 ± 0.04 mg/kg) were administered IM via blowpipe darting. Three animals were recumbent but still responsive to stimuli 25 minutes after the injection, and received butorphanol 0.1 mg/kg IM to facilitate a safe approach. An intravenous catheter was inserted within 17.5 min (range: 11-35) after injection. Anesthesia was maintained with intravenous propofol in all animals, except for one, which was intubated after propofol administration and maintained under volatile anesthesia with isoflurane. Throughout anesthesia, clinical parameters including pulse rate, oxygen saturation, respiratory rate, arterial blood pressure, ECG, end-tidal carbon dioxide, rectal temperature, muscle tone, and palpebral reflex were continuously monitored and recorded every 5 minutes in all animals. Cardiorespiratory parameters remained stable during immobilization and within ranges reported in this animal species. Two pumas received atipamezole 40 mcg/kg IM after the end of the procedures. All animals recovered within 25 ± 5 minutes after the conclusion of clinical or surgical procedures. No peri-anesthetic complications were observed.
The combination of dexmedetomidine, ketamine, and midazolam may provide effective and safe immobilization in pumas, ensuring cardiorespiratory stability. However, the addition of butorphanol may be considered to safely approach the animal.

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77° CONVEGNO SISVET

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Plasma serotonin concentration biomarker of stress responses in chronic joint disorders undergoing analgesic therapy with tapentadol in horse: preliminary results

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Plasma serotonin concentrations increase or decrease in stress responses[1]. The aim of the study was to evaluate serotonin concentrations in different degrees of lameness due to chronic joint disorders in horses undergoing analgesic therapy with tapentadol. PROT. No. 20/CESA /2023. Thirty horses were selected in this study. The orthopedic examination included flexion tests, radiological and ultrasound examinations. The degree of lameness due to chronic osteoarthritis (OA) has been estimated from 0 to 5 according to the American Association of Equine Practitioners (AAEP)[2]. The horses were divided into group C (control group): healthy horses (n = 10), group A, horses with chronic OA, grade 3-4 lameness (n = 10); Group B, horses with chronic OA, grade 5 (n10) lameness. Heart rate (HR), respiratory rate (RR), systolic (PS), diastolic (PD) and mean (PM) blood pressure were recorded and the serotonin concentration was determined, at baseline and at the beginning of every week for three weeks. Subjects suffering from OA were treated with Tapentadol at a dose of 0.5 mg/kg. The response to painful stimulus on flexion tests was assessed using the NRS (modified Numeric Pain Rating Scale 0-7) from baseline and the CPS (Cumulative Pain score 0-4) after the first week of treatment with tapentadol. Scores were assigned to the percentage changes, compared to baseline, in heart rate, respiratory rate and systolic blood pressure according to the following scheme: 0 ≤ 0%; 1 ≥ 0% but ≤ 10%; 2 ≥ 10% but ≤ 20%; 3 ≥ 20% but ≤ 30%; 4 ≥ 30%. The sum of the three scores gave the total CPS and the score of 10 was the cut-off point for satisfactory analgesic therapy. The degree of lameness decreased along the time line in both groups (score from 3-4 to 1 in group A and score from 5 to 1in group B) $p<0.05$. The NRS score decreased along the time sequence in both groups $p<0.05$, respectively from mild pain to no pain in group A (score 1-3 to 0) and from moderate pain to no pain in group B (score from 4 to 0). Physiological variables did not change along the time line. CPS scores ranged from 0.5 to 4 in group A and 1.5 to 7 respectively in group B $p=0.008$. Serotonin concentrations remained unchanged across the time line in all groups $p=1.000$ but OA groups were lower than control $p=0.000$. Tapentadol has been shown to be effective in the management of chronic OA pain in horses and serotonin may be a biomarker in the stress response.

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77° CONVEGNO SISVET

Stato: INVIATO - ID: 13505

INTERNAL FIXATION FOR PALATAL FRACTURE AND MANDIBULAR SYMPHYSEAL SEPARATION REPAIR AND COMPUTED TOMOGRAPHY ASSESSMENT OF BONE HEALING IN FELINE HEAD TRAUMA CASES.

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The aim of this retrospective study was to evaluate the efficacy of submucosal internal fixation using titanium miniplates for palatal fracture and mandibular symphyseal separation (MSS) stabilisation with Kirschner wires (KW) and to assess bone healing with computed tomography (CT) in 12 cats.
The hospital records of cats with palatine bone fractures repaired by miniplates presented at the Veterinary Teaching Hospital of the University of Milan between September 2019 and January 2023 were reviewed. Signs related to orofacial injuries were recorded. Preoperative diagnostic imaging included skull radiographs, obtained with patients under general anesthesia, and whole-body multi-detector CT (MDCT) with high resolution head scan (GE Brightspeed, 16 slice).The surgical procedure was carried out with reduction of palatal fractures and stabilization with one or two 2-holes, 11 mm bridge, 0,55 mm thickness titanium miniplate, and 4 mm thread length, cross drive, 1,6 mm diameter screw. MSS was stabilized by one or two threaded or unthreaded KW, 1 mm or 0.8 mm diameter. Radiographic examination and MDCT-scan were performed postoperatively in each cat. The clinical and imaging (radiography and MDCT scan) follow-up were scheduled concurrently with implant removal.Twelve cats were included in this study: 11 domestic shorthair, 1 domestic longhair; 2 female, 4 male, 6 neutered male; mean age at first presentation 44,8 months (median 40,5 months; range 12-120 months); mean body weight 3.7 kg (median 3.5 kg; range 3.1-5.3 kg); 4 owned cats and 8 stray cats living in feline colonies. Obvious signs of craniofacial trauma included: epistaxis (n=12); facial soft tissue wounds (n=12); ocular changes (n=7; 2 bilateral); neurological disfunction (n=11); upper airway related sounds (n=12); intraoral soft tissue wounds (n=12); incisive and palatine bone fracture (n=12; 3 with intact palatal mucosa); MSS (n=11); parasymphyseal mandibular fracture (n=1). Fractures identified on diagnostic imaging included: incisive/palatine (n=12); temporomandibular joint (TMJ) (n=9; 3 bilateral); mandibular symphyseal (n=11); mandibular parashymphyseal (n=1); presphenoid (n=11); zygomatic (n=12); frontal/temporal (n=12); TMJ luxation (n=1).
Reduction and stabilization of palatine bone fracture was achieved with one miniplate in 8 cats and two miniplates in 4 cats. MSS was stabilized with 2 KW in 7 cats, 1 KW in 3 cats; 2 cats were treated conservatively. Hard palatal mucosa and soft palate healed within 7 days in all cases. MDCT exam showed direct palatine bone healing within 4 months in all cases, and partial incisive bone healing in four cats. MSS healed within 8 weeks in all patients. Post-operative complications included screw loosening (n=1) and incisive bone screw-related osteolysis (n=1).
Mini titanium plates used in the current study provided anatomic reduction and direct bone healing in all cases. MSS internal fixation enabled proper oral cavity occlusion. Skull radiographs were useful for the initial screening by having the whole head in a single image but MDCT was superior both to identify the fracture sites and to assess the progression of bone healing.

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77° CONVEGNO SISVET

Stato: INVIATO - ID: 13511

ANALGESIC EFFECT OF ULTRASOUND-GUIDED TAP (TRASVERSUS ABDOMINIS PLANE) BLOCK IN RABBIT UNDERGOING OVARIOHYSTERECTOMY: PRELIMINARY DATA

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In the current literature, several studies demonstrated the effectiveness of the TAP block (Transversus Abdominis Plane Block) technique in different species in the control of peri-operative pain related to abdominal surgery [1]. In rabbits, there are only cadaveric studies showing the applicability of the TAP block performed via ultrasound guidance but the clinical efficacy of this technique has not yet been evaluated in this species [2]. Based on this background, the aim of this study was to evaluate the analgesic efficacy of the TAP block in rabbits undergoing ovariohysterectomy. In this prospective, randomized, clinical study, 12 female rabbits were selected and subjected to ovariohysterectomy. All animals were premedicated with dexmedetomidine (80 mcg/kg), ketamine (5 mg/kg), and methadone (0.2 mg/kg) IM. After 10 minutes, the sedation score was assessed (1 = poor; 11 = excellent) and the cephalic vein was cannulated. Then, patients were randomly divided into two groups. In the TAP group (6 rabbits), the TAP block was performed with an ultrasound-guided preiliac approach with 0.15 ml/kg of 2% lidocaine diluted with NaCl to obtain a total volume of 1 ml; group C (6 rabbits) did not receive any locoregional analgesia. During all described procedures the subjects received oxygen via face mask. Then, all patients were induced with alfaxalone (1 mg/kg) IV and underwent endoscopic intubation. General anesthesia was maintained with isoflurane in pure oxygen. Subsequently, the auricular artery was catheterized for invasive pressure detection. During the intraoperative period, the main cardiovascular and respiratory parameters were monitored at different study times: 10 minutes before the start of the surgery (BASE), during the skin incision (SKIN), the traction of the first (TRAC 1) and of the second ovarian ligament (TRAC 2), the suture of the muscular plane (MUSCLE) and the application of the skin suture (SUTURE). If heart rate (HR) increased 20% above BASE, we would administer 5 μg/kg of fentanyl IV. In the post-operative period, the quality of recovery were recorded and HR, respiratory rate (RR) and temperature (T) were monitored every 10 minutes from extubation for 1 hour (POST10, POST20, POST30, POST40, POST50, POST60). Furthermore, the time elapsed from extubation to first food intake and defecation was recorded. All values of $p<0.05$ were considered statistically significant. The results obtained show that 2/6 rabbits in group C required rescue analgesia during the abdominal wall incision, while in the TAP group none required additional analgesia. Furthermore, in the postoperative period, at POST10 and POST20, the RR was significantly higher in the C (POST10 = 89,6 ± 10,01 breaths/min; POST20 = 82,4 ± 12,5 breaths/min) than in the TAP group (POST10 = 48 ± 7,32 breaths/min; POST20 = 59,6 ± 9,56 breaths/min). There were no differences in the times of resumption of feeding and defecation. In conclusion, the preliminary data obtained from this study demonstrate that the TAP block technique could guarantee better pain management both in the intra- and post-operative period in rabbits undergoing ovariohysterectomy.

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77° CONVEGNO SISVET

Stato: INVIATO - ID: 13515

REAC NEUROBIOLOGICAL TREATMENTS IN DOG'S CRANIAL CRUCIATE LIGAMENT (CCL) RUPTURE. FIRST RESULTS.

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Radioelectric asymmetric conveyor (REAC) technology is a platform designed to optimize cell polarity. Cell polarity is a universal biological phenomenon that is implicated in cell differentiation, proliferation, morphogenesis, aging, and rejuvenation. Rupture of the cranial cruciate ligament in dogs is a pathology of surgical interest in which several factors such as age, physical condition and activity level are very important in making the decision on the best therapeutic option. In the field of biomodulation, REAC technology are effective not only in post traumatic outcomes, regenerative medicine, and in neurodegenerative diseases, but also in direct cellular reprogramming. REAC protocols have been used for years, especially in human medicine, in the treatment of post-traumatic lesions in various types of tissues, as muscle, ligament and tendon. On these premises, in this study we have used REAC technology to treat CCL dog's rupture with the aim to verify short- and long-term rates of successful clinical outcomes.The study was conducted by subdividing subjects classified by age, sex and race into two groups with respect to body weight: Group A (<15Kg) and Group B (> 15 Kg). All owners have refused surgery for their animals and opted for non-surgical management. The disease was chronic since it had been present for more than three weeks. All patients were subjected to REAC. The first session initially provided the VNPO, aimed at initiating the proper neuro-psychic biological feedback mechanisms to environmental conditions and improving motor and postural strategies. As a second protocol, we employed the VNPPO which provided for the application of a special laminar probe positioned in the cervical back area with the aim of promoting an optimization of the nervous system control over bodily regions modified by traumatic events or localized pathologies. All dogs were also subjected to regenerative treatment (RGN) and the laminar probe was applied directly to the knee region. Conservative management from 3 to 8 weeks provided controlled movement through leash walks and, when indicated, restoring ideal weight conditions. The 7 dogs of group A were examined within 15 days of the end of treatment, and two months after the end of the test, showing progressive and gradual improvement over the initial clinical examination. 7 out of 7 dogs (100%) were classified as "normal". Of the 9 cases treated (group B), 5 were classified as clinically "normal", 3 dogs classified as "improved", 1 did not have any improvement over the 2 months (non-responder). After 12 months all animals confirmed improvement or disappearance of clinical symptoms without worsening.In dogs, as humans, traumatic events such as knee ligament injuries may represent a stressful event affecting not only the joint stability, but also the physical condition of the subject. All that may affect the rehabilitation outcome and the complete functional recovery. When the integrity of a tissue is compromised by a lesion or trauma, there is an alteration of the delicate mechanism of regulation of the electrochemical properties of the cells, inhibiting the production of ionic flows. REAC biomodulation treatments, activate the recovery of endogenous bioelectric fields, reduce oedema and pain, limit the chronicization of the processes, and accelerate their reparative operations.
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77° CONVEGNO SISVET

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COMPARISON OF SURGICAL COMPLICATION BETWEEN ELECTIVE NECK DISSECTION AND GUIDED SENTINEL LYMPH NODE BIOPSY IN HEAD AND NECK TUMORS IN DOGS

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The histological evaluation of the draining lymph node (LN) has become a standard procedure in managing head and neck tumors (HNT) in dogs. Two possible surgical approaches for lymphadenectomy have been reported: 1 - elective neck dissection (END) consisting in the removal of mandibular and retropharyngeal +/- parotid nodes; 1 2- sentinel lymph node biopsy (SLNB) consisting of a targeted selection of draining LN.2,3 This study aims to compare the surgical complications after END and SLNB in dogs with HNT. This retrospective cohort study included dogs that underwent END or SLBN for HNT. Dogs with previous head and neck regional lymphadenectomy have been excluded. Data collected included signalment, tumor types, primary tumor surgical data, node surgical data (type of surgical approach, number of LN stations, and LN removed), and surgical complication data on primary tumor and lymphadenectomy sites. Given the presence of competing risks, Fine and Gray's regression model was used to compare the incidence of complications among groups in uni- and multivariate models. Eighty-one dogs were included. Regional nodes were enlarged in 21 dogs. Fluorescence- and/or lymphoscintigraphy-guided SLNB was performed in 38 dogs, and END in 43 dogs. No intraoperative complication was observed. Postoperative complications at the lymphadenectomy site occurred in 41 dogs. The cumulative incidence of postoperative complication is significantly greater for END than SLNB ($p<0.001$; 30 days Relative Risk=3.56). Additionally, in univariate analysis, clinically enlarged RLN, an increasing number of resected lymphocenters, and LN increased the cumulative incidence of postoperative complications. In multivariate analysis, only END is significantly associated with a higher risk of postoperative complications ($p=0.0036$). Considering that both END and SLBN seem reliable for nodal staging in HNT,1-3 guided SLBN should be preferred to avoid a higher risk of postoperative complication.

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77° CONVEGNO SISVET

Stato: INVIATO - ID: 13541

COMPARISON BETWEEN TOTAL INTRAVENOUS ANESTHESIA WITH PROPOFOL AND INHALATION ANESTHESIA WITH ISOFLUORANE IN DOGS UNDERGOING BALLON PULMONARY VALVULOPLASTY

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Pulmonary stenosis is among the most frequent congenital heart defects in dogs. The main therapeutic treatment is represented by balloon valvuloplasty. This procedure entails various anesthetic risks represented by the interruption of arterial flow during the opening of the balloon, arrhythmias, hypoxemia and hypotension [1]. The aim of this study was to compare anesthesia with propofol versus isoflurane and evaluate which was more suitable in dogs undergoing valvuloplasty. For this purpose, 10 dogs were randomly divided into two groups: Group P (5 dogs) in which general anesthesia was maintained with a constant rate infusion (CRI) of propofol at 0.2-0.5 mg/kg/min IV and group I (5 dogs) in which inhaled isoflurane was administered. All dogs were premedicated with 0.3 mg/kg of methadone (IM) and induced with midazolam (0,3 mg/kg) and propofol (3 mg/kg) IV. Following orotracheal intubation, pure oxygen (FiO2= 1) was administered. All animals received 5 ml/kg of balanced crystalloid fluids and lidocaine (50-100 μg/kg/min) IV. In group P, propofol CRI started immediately after the induction bolus. In group I, administration of isoflurane began after intubation. In both groups, the delivery of anesthetic was stopped at the end of the procedure. Main cardiovascular and respiratory parameters were recorded at baseline, introduction of the guidewires, introduction of valvuloplasty catheter, opening of balloon and at the end of the procedure. The depth of the anesthetic plane was assessed via the eyelid reflex and the position of the eyeball. The rate of administration of propofol and the percentage of isoflurane were modified based on the depth of anesthesia and were recorded at the aforementioned study times. If mean arterial pressure (MAP) <was less than 60 mmHg, a CRI of dopamine (5-10 μg/kg/min) was administered. Moreover, 30, 60, 120 and 180 minutes after extubation, the quality of recovery (QR = 1 - 6), HR, RR, T° and MAP were monitored. A p value < 0.05 was considered statistically significant. Group P received a lower quantity of dopamine compared group I (P = 2,1 ± 0,5 μg/kg/min; I = 6,77 ± 1,2 μg/kg/min), showed a more stable cardiovascular condition. Moreover, P group showed a slower and gentler awakening with a significantly lower QR compared I group [P = 2 (1-2); I = 3 (2-4)]. In conclusion, general anesthesia maintained with propofol infusion could guarantees a more stable cardiovascular condition during valvuloplasty procedures in dogs, reducing the risk of hypotensive episodes. Furthermore, awakening appears to be slower and more peaceful, reducing episodes of post-operative dysphoria and delirium, and allowing operators to better monitor the patient at the end of the procedure. These results are in agreement with a previous study in which the use of propofol is recommended over isoflurane to obtain a smooth and quiet anesthetic recovery [2].

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77° CONVEGNO SISVET

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Optimizing Ovariectomy in Cats: Comparative Analysis of Laparoscopic vs. Open Surgical Approaches and Postoperative Pain Management

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To evaluate the surgical variables and postoperative pain in cat ovariectomy associated with three techniques: the open surgery and the classic suture, the open surgery and the bipolar vessel-sealing device compared with two-port laparoscopic ovariectomy.A total of 30 cats were selected based on the inclusion criteria to eliminate variability. The subjects were randomly divided into three groups (open-suture group: open laparotomy and classic suture for ovarian pedicle resection; Open-BSVD group: open ovariectomy and bipolar vessel sealing device; LOVE group: two-port laparoscopic ovariectomy and bipolar vessel sealing device) of ten each. All cats in the current study were anesthetized using the same protocol. In the present study, the Glasgow scale was used to assess postoperative pain. Pain was assessed before anesthesia and at one, two, three, and four hours after extubation. In addition, the present study evaluated operative time and intraoperative bleeding score.The present study observed that bleeding was easily controlled in all groups. The mean operative time was significantly shorter ($p < 0.05$) in the bipolar vessel sealing device group (27 ± 9.59 min) and LOVE (30.2 ± 5.19 min) compared with the open-suture group (43.89 ± 14.37 min). In addition, it has been observed that suturing the pedicle with classic sutures increases the risk of intraoperative bleeding. In the current study, a significant increase in pain scores one hour after extubation was observed in both open techniques compared to LOVE ($p<0.05$). However, rescue analgesia was administered in 70% of animals in both open surgical techniques compared to 10% in the LOVE group.The results of this study showed that all techniques used were appropriate, feasible and safe in the feline species. However, the use of a bipolar vessel sealing device was associated with a faster procedure and lower bleeding rates compared to the suture group. LOVE showed similar time and safety outcomes to the open BSVD group, although the need for rescue analgesia was significantly lower than in the open surgery groups, showing less surgical impact on immediate postoperative pain. In addition, the use of the laparoscopic technique reduced the level of postoperative pain up to four hours after extubation without the need for further analgesia compared to open surgery. Therefore, it can be assumed that the use of a bipolar vessel sealing device reduces the operative time and the risk of intraoperative bleeding, even if controlled in all cases, compared to open surgery and the use of hand sutures. Although, LOVE need technological investment and specifically trained surgical skills, showed that the technique is not time-consuming and provides less surgical trauma, which impact on providing no requirements to postoperative analgesia in 90% of patients. [1]Case JB, Boscan PL, Monnet EL, et al. Comparison of surgical variables and pain in cats undergoing ovariohysterectomy, laparoscopic-assisted ovariohysterectomy, and laparoscopic ovariectomy. J Am Anim Hosp Assoc 51: 1-7;2015. [2]Pereira MAA, Gonçalves LA, Evangelista MC, et al. Postoperative pain and short-term complications after two elective sterilization techniques: ovariohysterectomy or ovariectomy in cats. BMC Vet Res 14: 335;2018. [3]Gauthier O, Holopherne-Doran D, Gendarme T, et al. Assessment of postoperative pain in cats after ovariectomy by laparoscopy, median celiotomy, or flank laparotomy. Vet Surg 44 Suppl 1: 23-30;2015.

Assessment of movement asymmetries on circles on a soft surface in adult racehorses using an objective smartphone camera markerless system

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Lameness examination can be aided by various objective motion measurement devices (OMDs). OMDs allows detection of smaller asymmetries difficult to detect by the human" eye", also overcoming the limited agreement between veterinarians, and the expected bias. The most used OMD require inertial measurement units (IMUs) with marker mounted on specific anatomical area of the horse's body. OMDs are very expensive and difficult to use in practice. A new OMD (Sleip AI) that use a smartphone application using a markerless video recorded tracking has been develop [1]. The system has been proved to have a high sensibility and sensitivity compared to other IMUs [2]. Lameness assessment commonly involves evaluation of a horse trotting on a circle. At trot, specific forces and 3D joint movements are present compared to the straight line, possibly providing key information to aid clinical decision making [3]. Only asymmetry threshold for circle on hard surface are available, even if surfaces not significantly affect the movement symmetry [2] . The aim of this study is to evaluate the prevalence of movement asymmetries on circles on a soft surface in adult racehorses with the use of an objective smartphone video computed method.24 racehorses (20 Thoroughbred, 4 AngloArabian) (mean age = 7) involved in the "Palio of Faenza" were included in the study. All the horses were perceived to be sound by their trainers. The horses underwent clinical examination, followed by dynamic inspection. All of them were trotted in-hand on both reins on a soft surface in a 12-15 m circle for at least 45 s in each direction to perform both the subjective and the objective lameness evaluation. Movement asymmetries were assessed using a marker-less smartphone computer vision method. The system divides asymmetries with different thresholds into very mild, mild, moderate, and severe. Only horses with mild and above asymmetries were counted. For each horse and or each rein, number of stride, differences between the two vertical displacement minima and maxima of head (HDmin, HDmax) and pelvis (PDmin, PDmax) per stride were collected. Descriptive statistics were calculated. The analysis assessed both the most frequently affected leg, both the type of asymmetry (push-off vs impact). All 24 horses displayed some degree of asymmetry (from very mild to severe). The main findings indicate that on the right rein, 41% of horses displayed some degree of asymmetry (from mild (12,5%) to moderate (8%)), with the right forelimb (inside leg) as the most affected (50%), and with total prevalence of push-off asymmetry (70%). On the left rein 29% of horses displayed asymmetry (only mild (100%)) with the left forelimb (inside leg) as the most affected (71%), with total prevalence of push-off asymmetry (57%). Change of asymmetry between the two reins were recorded. Racehorses display asymmetries and some of them may be consistent with lameness. Due to the type of circuits in which these horses race, with hyperbolic curves, high biomechanical stress affects the joints, for which an assessment on lungeing can be more meaningful than the straight one. The use of this OMD has the potential to early identify asymmetries and treatable underlying conditions. Further application may require the comparison of these data with this threshold to distinguish if these asymmetries are physiological or pathological.

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ULTRASOUND-GUIDED PERCUTANEOUS BALLOON PERICARDIOTOMY IN DOGS: AN EXPLORATIVE CADAVERIC STUDY

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Pericardiocentesis represents the life-saving therapy during cardiac tamponade. However, this is a temporary solution that gives relief to the animal but does not solve the problem properly.
The creation of a pericardial window using a percutaneous balloon catheter technique has been used in human medicine as a safe and effective non-surgical method to relieve clinical signs in patients with cardiac tamponade which cannot undergo surgery, due to the high risks (Jackson et al., 1992).
The objective of our study was to evaluate the feasibility of performing a balloon dilatation pericardiotomy in cadaver dogs using an ultrasound guided Seldinger technique.
Five canine cadavers of various breeds, ages, weights, and sexes were recruited. Pericardial effusion was created in 4 cadavers, while in one dog a significant pericardial effusion was already present. To produce pericardial effusion an intercostal thoracotomy was performed to access the pericardium and inject 2 mL/kg of NaCl. For the balloon dilatation pericardiotomy we used a high pressure balloon catheter that was percutaneously inserted into the pericardial space with a Seldinger technique, on the opposite site of the thoracotomy. Skin and tissue were dilated using 10–12 F dilators to allow the subsequent passage of the balloon catheter. The diameter of the balloon was between 20 and 25 mm, with a length of 60 mm to decrease the tendency of the balloon to slip. The entire procedure was performed under ultrasound guidance, with an in-plane technique.
The time required to complete the technique was recorded, and measurements of the size of the pericardial window were measured post procedure. Percutaneous ultrasound-guided balloon catheter placement was successful in 4 out of 5 cadavers. The time required to complete the technique was 240 $\pm$ 35 seconds. The mean diameter of the pericardial window created by the balloon was 1.2 $\pm$ 0.3 cm.
Percutaneous ultrasound-guided pericardiotomy by balloon dilation is a procedure that can be performed in about 5 minutes, although success was not 100%. This technique can be cheaper than thoracotomy or thoracoscopic pericardiotomy surgery, less invasive, and is easy to perform. Further research is required to determine if the technique is applicable to live animals.

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77° CONVEGNO SISVET

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EVALUATION OF DIFFERENT INSTRUCTIONAL MODALITIES FOR ANAESTHETIC PROCEDURES LEARNING

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Higher education attempts to ameliorate learning experience through match between learning subjects and instructional modalities. Recent studies showed interest for advancements in and development of veterinary anaesthesia teaching (1), however no standardized, worldwide-accepted pedagogical method has been identified. To date, simulators and multimedia supports (i.e. photos and videos) are demonstrated to be beneficial to the provision of clinical scenarios to the students (2). The hypothesis of our study was to compare five different teaching methods for an anaesthesia procedure simulation and to verify the learning capacity of the last year veterinary students.A cohort of 72 fourth-year students of a master's degree course in Veterinary Medicine were enrolled. All students provided informed consent (Declaration of Helsinki), and the study was approved by the Bioethical Committee of the University of Pisa (Review No. 18/2021). Each student was exposed the following anaesthesia practice skills: how to insert a peripheral vein catheter and how to perform an endotracheal intubation. The class was randomly split into five groups, which were provided with five different learning materials format: scheme for Group Sc (GSc) (15/72 students), muted video for Group Vi (GVi) (15/72 students), audio recording for Group Au (GAu) (13/72 students), text for Group Tx (GTx) (16/72 students) and oral explanation with practical demonstration (a method usually used at our University) for Group Cl (GCl) (13/72). Both procedures were carried out on phantoms, and all the students used the same anaesthesia equipment. Each student was evaluated by an experienced operator (A.B.), unaware of the learning materials administered. Data were analyzed for distribution and Kruskal-Wallis test and Dunn's post hoc were applied for comparison between different teaching methods for both procedures. A Friedman test was used to compare the scores for each teaching method. All the students (72/72 students) completed the anaesthesia procedure simulation. For the intravenous catheterization, the GSc obtained significant lower scores for wash hand procedure in comparison to GTx (p-value = 0.002), and for catheter handling procedure in comparison to GAu ($p<0.001$). In the GCl a significant lower score was detected concerning the last procedure (positioning of the tape) in comparison to the previous (catheter positioning) ($p=0.006$). Regarding the endotracheal intubation, GSc showed significantly lower scores than GAu, GVi and GTx groups ($p<0.0001$). GCl last procedures (assessment of the correct position of the tube and fixation) resulted in a significantly lower score than intubation ($p= 0.003$).The present findings showed that the use of scheme as instructional modality had the worst performance in our student population for both the evaluated procedures. Video, audio recording and text showed the best performances. The present study provided feedback on modern teaching modalities in which cognitive aids play a key role (1,2).
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77° CONVEGNO SISVET

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EVALUATION OF EQUINE IN-VIVO ULTRASOUND TECHNIQUE FOR THE MEDIAL BRANCH OF THE DORSAL RAMUS OF THE CERVICAL SPINAL NERVES ASSESSMENT

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In the equine species, pain in the cervical area is cause of neck stiffness, lameness referred to the forelimbs, and ataxia (1,2). Ultrasound-guided local anaesthesia of nerves is a diagnostic method routinely used in veterinary orthopaedics in horses. To date, only one ex-vivo study described the ultrasound (US) technique and local infiltration of the medial branch of the dorsal branch of the cervical spinal nerves (MB-DBCSNs) in horses (1). The study aimed to assess the US technique for the MB-DBCSNs in-vivo horses and evaluate performances between clinicians with different experiences. The study was approved by the Institutional Animal Care and Use Committee of the University of Pisa (Prot. N. 45/23). The cervical area of 10 skeletally adult horses was included. All the animals were sound on orthopaedic and neurological examinations. Horses underwent radiographic and US examinations of the cervical area. During examinations, the horses were manually restrained in the weight-bearing position. A radiographic examination was executed for C3-C7 to categorise healthy and pathological articular facet joints (3). US examination of the MB-DBCSNs of C3-C7 articular facet joints was performed on both left and right cervical areas, using the US technique previously described (1), with a portable machine (MyLabSigma, Esaote, Italy) and a 10MHz linear array transducer. Four operators with different experiences performed the US, and the number of cervical nerves successfully visualized was recorded. Statistical analyses were conducted using SPSS 29.0 statistical software for Mac (IBM, Armonk, USA). The chi-square test was performed to assess whether training practice and anatomical location could influence the percentage of identified facet joints. US agreement among operators was evaluated using Cohen's K test, both on the total number of analysed facet joints and divided by anatomical location. Each operator assessed a total of 80 MB-DBCSNs. According to radiographic examination, 70/80 articular facet joints were classified as healthy and 10/80 as pathological. The training practice significantly influenced the number of cervical nerves successfully visualized for all the operators, which reached 90% in the latest training session. Moreover, the cervical nerves of cranial facet joints (C3-C5) were displayed significantly more times (up to 81%) compared to the caudal ones (C5-C7) (59%). Agreement between operators resulted in slight to fair for all cervical nerves. The US practice gradually improved the successful visualization of the cervical nerves in in-vivo horses. Moreover, the cranial cervical nerves were easier to identify compared to the caudal ones. Nevertheless, agreement between operators of different experiences was low, which might be influenced by the long learning curve associated with the described ultrasound technique (1,2).

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77° CONVEGNO SISVET

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Effectiveness of Pectin-Honey-Hydrogel to Reduce the Incidence of Surgical Site Infection following Laparotomy in Horses

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Surgical site infection (SSI) is a common complication after celiotomy in horses, leading to increased morbidity and costs [1-3]. The contribution of perioperative antibiotics to antibiotic resistance underscores the necessity to evaluate alternative preventive approaches. The effectiveness of Manuka-honey in promoting wound healing has been recently demonstrated as well as the ability to reduce the risk of wound infections after colic surgery [4]. While the use of liquid honey has some limitations, the use of Pectin-Honey-Hydrogels (PHHs) may be warranted. The aim of the study was to evaluate the effectiveness of PHHs to reduce the incidence of surgical site infections (SSI) after laparotomy. The study received approval from the Ethical Committee of the Department (n.1514 21st July 2020) and owner consent was signed for each horse. Horses undergoing laparotomy were randomly assigned to two groups. In Group 1, PHH was applied on the sutured linea alba before skin closure, in Group 2 no treatment was applied. Pre-, intra- and postoperative medications were the same for both groups. Horses that survived less than 5 days postoperatively were excluded. The incidence of SSI prior to discharge was compared between groups. Data were analyzed with the Fisher's exact test or chi-square test. Eighteen horses per group were included in the study. One out of eighteen horses in group 1 and 6 out of eighteen horses in group 2 developed infection (p value = 0.035). Horses not treated with PHH have 8.5 times greater risk of incisional infection. Treatment with PHHs reduces the incidence of SSI in horses undergoing colic surgery. The use of alternative treatments may be beneficial towards the reduction of the use of antimicrobials and increase of antimicrobial resistance. [1] Isgren CM, Salem SE, Archer DC, Worsman FCF, Townsend NB. Risk factors for surgical site infection following laparotomy: Effect of season and perioperative variables and reporting of bacterial isolates in 287 horses. Equine Vet J, 49:39-44, 2017.[2] Bischofberger AS, Brauer T, Gugelchuk G, Klohnen A. Difference in incisional complications following exploratory celiotomies using antibacterial coated suture material for subcutaneous closure: Prospective randomized study in 100 horses. Equine Vet J,42:304-309, 2010.[3] Gandini M, Cerullo A, Giusto G. Scoping review: Occurrence and definitions of postoperative complications in equine colic surgery. Equine Vet J,55(4):563-572, 2023.[4] Gustafsson K, Tatz AJ, Slavin RA, et al. Intraincisional medical grade honey decreases the prevalence of incisional infection in horses undergoing colic surgery: A prospective randomised controlled study. Equine Vet J, 53(6):1112-1118, 2021.

77° CONVEGNO SISVET

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POST-MORTEM RADIOGRAPHIC AND COMPUTED TOMOGRAPHIC EVALUATION OF GUNSHOT LESIONS IN DOGS. PRELIMINARY RESULTS

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Gunshot wounds are frequently encountered in both human and veterinary forensic investigations. These injuries can result in varying degrees of damage to soft tissues and skeleton and are influenced by multiple factors such as type, calibre, proximity, and velocity of the projectile used [1]. Survey radiographs, and more recently, computed tomography (CT), are used as complementary exams and are usually performed before necropsy [2]. Both modalities are particularly useful in detecting bullets and cartridge elements, but they can also determine their spatial location and help track the bullet's trajectory in the victim's body. Unfortunately, despite the considerable increase in practical applications of forensic radiology and imaging in Veterinary Medicine, published data are still scarce.
This prospective study aimed to compare and assess the usefulness of radiography and CT in the detection and characterization of skull gunshot wounds in dog cadavers.

To this aim, nine mesaticephalic skulls of medium and large-size dogs euthanized for reasons unrelated to this study were prospectively included in the sample. All the gunshot lesions were provoked by the same operator, firing the skulls from different distances and using different guns and munitions. More in detail, an air pistol (Pardini K58) with a specific lead cartridge (Diablo Flat Head .177) was used to fire 1 skull at point-blank range. A semi-automatic pistol (Benelli cal. 9) equipped with two different types of 9mm bullets (full metal jacket and copper coated) was used to fire at 6 skulls at point-blank range and then from one and six meters. Finally, 2 skulls were fired using a semi-automatic rifle (Benelli cal.12) equipped with a shotgun shell (slug) from six and twelve meters. On the same day, both the radiographic and CT images of the skulls were acquired. For the radiographic examination, LL and DV projections were acquired using a direct digital radiography system. Computed tomographic studies were obtained with a multi-detector unit using a standardized institutional protocol. The CT images were evaluated using transverse images, multiplanar reconstructions and 3D volume renderings. The performance of both modalities in detecting the bullets inside the skull, their trajectory and the lesions caused to soft tissues and skeletal structures were consequently compared.

The advantages of CT over radiographs, already reported by previous studies, were confirmed in the present study. Specifically, CT was superior to the radiographic exam in the characterization of skeletal lesions and the detection of the bullet trajectory. However, radiography still plays a pivotal role as a screening method thanks to its quick execution, cost-effectiveness, relative ease of use, and ability to easily detect metallic bullets and fragments without artefacts.

This study is part of the Research Project "Validation of diagnostic algorithms for the identification and characterization of traumatic injuries, penetrating or non-penetrating, in Veterinary Forensic Medicine" (IZS ME 09-22 RC),

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Effectiveness of different respiratory strategies for the management of postoperative hypoxemia in dogs

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Dogs undergoing general anesthesia may have transitory hypoxemia during the recovery period for changes caused by anesthesia and/or surgery. If its duration is prolonged, it may increase the risk of postoperative pulmonary complications.(1) For these reasons, the AAHA guidelines emphasize the importance of monitoring SpO_2 during the recovery period from general anesthesia.(2) The purpose of this study is to evaluate the effectiveness of using a face mask (FM), a Continuous Positive Airway Pressure (CPAP) helmet, and High Flow Nasal Cannula (HFNC) in treating transient hypoxemia in dogs after general anesthesia and to compare the three treatments in terms of time to restore normoxemia (TRN). The study received approval from the Ethical Committee of the University of Bari, Italy (Approval number: 03-2017). It was conducted at the Section of Veterinary Clinics and Animal Production of the DiMePRe-J. This prospective, randomized clinical study included 600 dogs classified as ASA I-III, with a body weight > 5 kg, without cardiovascular or respiratory disease, undergoing general anesthesia for various procedures not involving the thorax and lasting no more than 3 hours. In the postoperative period oxygenation was continuously assessed with determination of SpO_2 at room air (SpAT), and hypoxemia was defined as an SpO_2 < 95%. Dogs that resulted to be hypoxemic at 5 minutes after extubation (TPOST) were randomized to receive face mask (FM-group), CPAP helmet (CPAP-group), or HFNC treatment (HFNC-group). Oxygen therapy lasted one hour, during which four therapeutic sessions were performed, each consisting of 10 minutes of treatment followed by the SpAT determination at T15, T30, T45, T60. If SpO_2 values < 85% were detected at any time after extubation, dogs were reintubated and supported with invasive ventilation. Dogs in these cases were excluded from the study. The TRN was defined as the time (min) elapsed from starting treatment to achieving SpO2 $\geq$ 95% during SpAT. Tolerance of the interface (face mask, nasal cannula and helmet) was scored with a validated scale (TS) during recovery. Data were tested for normal distribution with the Shapiro-Wilk test. Two-way ANOVA was used to compare the SpO_2 and the TS at different times during the study between groups, with time and treatment as variables. $P < 0.05$ was considered statistically significant.

At TPOST 168 dogs (28%) were hypoxemic. Of these, 34 dogs were excluded for impossibility to perform the study (uncompliant behavior, poor SpO_2 signal, surgical related postoperative complications), and 104 were randomly assigned to a treatment group. Seventy-six of them completed the study (CPAP-group *n*=31,FMgroup *n=24* or HFNC-group n=21). All treatments were able to restore normoxemia. TRN was faster in the CPAP-group compared to HFNC-group and FM-group (16.93±15.69min; 27.86±20.83min and 40.42±12.85, respectively). No differences in the TS were observed among groups.

The results of this study showed that postoperative hypoxemia in dogs can be effectively treated with FM, HFNC, or CPAP, but the latter treatment provided a more rapid restoration of normoxemia.

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77° CONVEGNO SISVET

Stato: INVIATO - ID: 13659

Pneumocephalus and pneumorrachis after transoral transphenoidal hypophysectomy in a dog

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Pneumocephalus and pneumorrhachis consist of intracranial and intraspinal trapped air and are recognized after neurosurgery in humans. In veterinary literature, pneumocephalus and pneumorrhachis are rarely described and their association has been reported in a single case report after transfrontal craniotomy. The aim of this report is to describe a case of pneumocephalus and pneumorrachis after transoral hyphophysectomy in a dog.An 8-year-old male Belgian Malinois was referred for pituitary-dependent hyperadrenocorticism. The pituitary macroadenoma (pituitary-brain ratio 0.5) was treated by transsphenoidal hypophysectomy, without any intraoperative complications. Seven days after surgery and after discharge, the dog presented with acute progressive painful ambulatory tetraparesis, consistent with C1-C5 neurolocalization. Differential diagnoses were infective/inflammatory (i.e., meningitis) or degenerative/traumatic (i.e., intervertebral disc disease causing compressive myelopathy).Inflammatory markers were unremarkable at blood works. The Magnetic Resonance Imaging (MRI) detected free air in the third and lateral ventricles and in the epidural space at C1-C2 and C5-C6, causing severe compressive myelopathy. The diagnosis was of pneumocephalus and pneumorrhachis. Rest and pain management was set. Neurological signs improved within 72 hours. Four weeks later, pneumocephalus and pneumorrhachis disappeared on control MRI, analgesia was progressively tapered, and the dog completely recovered. Pneumocephalus represents an expected finding following intracranial surgery, especially in human medicine: it is generally asymptomatic and spontaneously resolves within one month from surgery. The air is stored inside the cranial cavity during surgery due to a valve effect, assuming an anti-declining position. Less commonly, tension pneumocephalus occurs, i.e. when the air inside the cranial cavity causes an increase in intracranial pressure leading to neurological deterioration. A decompressive surgery should be considered as an option in these cases. Pneumorrhachis rarely occurs after intracranial surgery, despite an existing communication between cerebral and spinal subarachnoid spaces, possibly causing spinal compression. In the case herein described a progressive air accumulation in the cervical subarachnoidal space might have been caused by a possible communication between the rhinopharynx and the ventricular system due to failure of sphenoid bone closure by bone wax, or air migration from the cerebral ventricles, entered during surgery. In this case pneumocephalus and pneumorrachis completely resolved by conservative treatment and pain management within 4 weeks from surgery, as already reported in human medicine. Pneumocephalus should be considered as postoperative consequence of transsphenoidal hypophysectomy; pneumorrhachis may occur as a rare complication.

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77° CONVEGNO SISVET

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COMPARISON OF THREE DIFFERENT SEDATIVE-ANAESTHETIC PROTOCOLS IN CAPTIVE BABOONS (Papio hamadryas)

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Baboons handling typically requires chemical restraint to reduce animal stress and risks of injury/exposure to humans [1].
This study aimed to compare the sedative and cardiopulmonary effects of three balanced sedative-anaesthetic protocols in captive baboons (Papio hamadryas) undergoing clinical examination, microchip application and sample collection for routine screening. Vasectomy was also performed in males, to prevent overpopulation and inbreeding.
Based on estimated body-weights, group TZD (G_TZD) were darted intramuscularly (IM) with tiletamine/zolazepam combination (3mg/kg) and dexmedetomidine (20μg/kg); group KDM (G_KDM) received IM ketamine (6mg/kg), dexmedetomidine (30μg/kg), methadone (0.2mg/kg), whereas group MDM (G_MDM) received IM midazolam (2mg/kg), dexmedetomidine (60μg/kg) and methadone (0.2mg/kg). Once recumbent, a cephalic venous access was placed, and arytenoids were irrigated with lidocaine. If necessary, intravenous propofol was titrated to effect (PPF-induction-dose) to achieve orotracheal intubation (T0) and anaesthesia maintenance (PPF-maintenance). Sedation time and quality [2], cardiopulmonary parameters and rectal temperature were recorded every 10 minutes (T0 to T30). All baboons received IM injection of atipamezole post-procedure (G_TZD 0.2mg/kg, G_KDM 0.3mg/kg, G_MDM 0.6mg/kg). Flumazenil (0.02mg/kg) was also administered in G_MDM. Anaesthesia time, recovery time and quality [2] were recorded.
Preliminary data resulting from 18 individuals (n=6; three males and three females per group) of the baboon colony at the Ravenna Safari Park Zoo, were evaluated. Estimated ages, mean estimated weights (G_TZD 11.2±7.8 kg; G_KDM 8.3±2.6 kg; G_MDM 6.8±4.8 kg) and mean actual weights (G_TZD 9.3±5.6 kg; G_KDM 8.1±2.2 kg; G_MDM 5.6±3.5 kg) did not differ between groups. In G_MDM, the sedation quality was significantly lower (4.5; 4-5) compared to G_KDM and G_TZD (5; 5-5 both; p=0.02), but without differences in sedation times between groups. Both PPF-induction-dose (0-2.2mg/kg) and PPF-maintenance (0-1.2mg/kg as boluses if necessary) did not differ between groups. In G_MDM mean heart rate (77±9 bpm) was significantly higher compared to both G_TZD (57±10 bpm; p=0.01) and G_KDM (62±11 bpm p=0.02). Similarly, in G_MDM mean respiratory rate were significantly higher than other groups (G_MDM 33±6 rpm; G_TZD 22±6 rpm, p=0.01; G_KDM 21±3 rpm, p=0.003). Non-invasive systolic, diastolic and mean (G_MDM 79±17 mmHg; G_TZD 73±14 mmHg; G_KDM 74±13 mmHg) blood pressures, rectal temperature (G_MDM 34.4±2.1°C; G_TZD 33.3±0.8°C; G_KDM 34.6±1.5°C), end-tidal carbon dioxide concentration (G_MDM 40±4 mmHg; G_TZD 42±5 mmHg; G_KDM 35±8 mmHg) and peripheral oxygen saturation (G_MDM 92±9%; G_TZD 94±6%; G_KDM 92±7%) were similar between groups.
Baboons in G_TZD experienced significantly longer (25±10 min; p=0.02) and poorer quality recoveries (8.5; 5-11; p=0.001) compared to other groups, while G_MDM had significantly shorter recoveries (G_MDM 4±2 min; G_KDM 14±8 min) with good quality similar to G_KDM (G_MDM 3; 1-7; G_KDM 3.5; 2-7).
The three protocols maintained clinically acceptable cardiopulmonary parameters, therefore they appear to be suitable and safe for baboon anaesthesia. TZD and KDM protocols provided deeper sedation compared to MDM. MDM protocol could be advised for minor and brief procedures.

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77° CONVEGNO SISVET

Stato: INVIATO - ID: 13669

Clinical effects of perineurial dexmedetomidine or magnesium sulphate as adjuvants to ropivacaine for sciatic and saphenous nerve blocks in dogs undergoing tibial plateau leveling osteotomy

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In dogs undergoing tibial plateau leveling osteotomy (TPLO), peripheral nerve blocks (PNBs) provide better perioperative pain relief and comfort than systemic analgesics [1]; unfortunately, most of their advantages are short-lived. Combinations between local anaesthetics and adjuvant drugs have gained popularity in human medicine because they prolong sensory nerve block, enhance patients' satisfaction, and reduce postoperative opioid requirements [2]. However, a limited number of studies have investigated the effects of adjuvant drugs combined with local anaesthetics for PNBs in dogs. This study aimed to compare the quality of perioperative analgesia, the motor block duration, and the effects on main cardiovascular parameters of dexmedetomidine (1 μg/kg per nerve block) or magnesium sulphate (2 mg/kg per nerve block) combined with 0.3% ropivacaine for sciatic and saphenous nerve blocks in dogs undergoing TPLO. It was approved by the Institutional Ethical Committee for Animal Care at the University of Milan (OPBA_52_2023) and all dogs were enrolled after obtaining the owner's written informed consent. Dogs were administered intramuscular acepromazine (0,02 mg/kg) and methadone (0,2 mg/kg). Anaesthesia was induced with intravenous propofol titrated to effect and maintained with isoflurane in oxygen. Dogs randomly received perineural dexmedetomidine-ropivacaine (D group, n = 10), magnesium sulphate-ropivacaine (M group, n = 10), or ropivacaine (C group, n = 10) for ultrasound-guided sciatic and saphenous PNBs [3]. Fentanyl was administered in case of intraoperative nociception. Postoperative pain was assessed using the Short Form-Glasgow Composite Measure Pain Scale (SF-GCMPS) and VAS scale. The duration of motor blockade and intra- and postoperative cardiovascular parameters were also recorded. Group M required significantly more fentanyl than D group ($p = 0.04$). Group M had a significantly higher SF-GCMPS score than group C at 4 ($p = 0.002$) and 5 hours after extubation ($p = 0.01$), and a significantly higher VAS score than group D at 3 hours after extubation ($p = 0.03$), and at 4 hours if compared to group C ($p = 0.009$). No significant differences regarding the duration of motor blockade were detected between groups ($p = 0.07$). The heart rate was significantly lower in group D than in M and C groups intraoperatively and during the first 1.5 hours post extubation ($p < 0.05$). The addition of dexmedetomidine or magnesium sulphate as adjuvants to perineural ropivacaine did not improve the quality of perioperative analgesia and did not prolong the motor blockade in dogs undergoing sciatic and saphenous nerve blocks for TPLO surgery. Perineural dexmedetomidine, at the dose proposed in this study, promotes the appearance of not clinically relevant systemic effects. Further studies are needed to determine whether higher doses of perineural dexmedetomidine and magnesium sulphate would increase the analgesic effect and prolong motor blockade in dogs undergoing PNBs of the sciatic and saphenous nerves.

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77° CONVEGNO SISVET

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BIOSURGEX TITANIUM VETERINARY LOCKED MINI PLATE SYSTEM FOR STABILISATION OF RADIUS AND ULNA FRACTURES IN TOY BREED DOGS

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The Biosurgex system represents one of the latest innovation in internal fixation devices in Veterinary medicine. This work aims to offer a detailed description of the Biosurgex titanium veterinary locked mini plate system, of the instruments necessary for its use, focusing on the biomechanical aspects of implants and to evaluate the clinical aspect of this system for stabilization of radius and ulna fractures in toy breed dogs . The Biosurgex mini series , designed for use in cats and small dogs (up to 10-15 kg of body weight), consists of supports of various shapes with a thickness of 1.2 or 1.5 mm, capable of hosting screws of 1.5 or 2.0 mm diameter.The essential instrumentation for inserting the mini locked plate system consists of titanium alloy plates ,self-tapping screws ,bend plates ,drill bit with a diameter of 1.5 or 2.0 mm for the mini series , hexagonal screwdriver, drill center with conical coupling on the bush meter.The main advantage of the Biosurgexsystem is the number, the shape and type of implants that can be used as well as their ductility also linked to the possibility of being able to use multi-compound and cuttable plates. Furthermore, unlike what happens with traditional systems, it is not necessary to perfectly model the plate on the bone. Small adjustments (bends) of the support to the skeletal base can be useful, not only to avoid protrusions of the device, but also to bring it as close as possible to the bone so as to increase the stability and rigidity of the implant. We present in this retrospective study the results obtained with the use of Biosurgex blocked mini implant in the treatment of 22 radio-ulna fractures in 21 toy breed dogs of ± 3.5 kg of body weight in the period between 2020 and 2024. The criteria for inclusion were: fracture of the radius and ulna with open reduction and internal fixation utilizing Biosurgex veterinary locked bone plate of 2.0 mm. Data pertaining to breed, gender, age, body weight, clinical history, time from injury to surgery, fracture description, previous repair attempts, duration of surgical repair, plate size and configuration, postoperative fracture alignment, postoperative management, postoperative complications, lameness outcome, and time from fracture fixation until last follow-up radiographs were recorded. Complications were classified into major and minor based on criteria proposed by Cook et al. We operated on 12 diaphyseal fractures, 8 distal diaphyseal fractures, one proximal ulnar fracture and one post-surgical radioulnar pseudarthrosis. For the fixation of the fractures, 13linear plates of 2 mm., (9 cuttable) and 9,2mm T-plates (4 cuttable). In 12 cases, 1.5 mm screws were used. In 10 cases 2 mm screws. In all cases treated we achieved good alignment of the stumps, good reduction and good fixation of the treated fracturesconfirmed by post operative x rays.In the post operative period we do not observed minor complication in all animals treated but we observed two major complication (9%) represented by a break of T plate and in another case an exposition of a T plate used in distal fractures. In the other cases we obtained bone healing without complications (91%).The use of Biosurgex implant is a suitable choice for stabilization of radio-ulnar fractures on toy dogs.

77° CONVEGNO SISVET

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Evaluation of liver parenchyma in dogs with hyperlipidaemia using ultrasound attenuation imaging (ATI)

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Pathological hyperlipidemia is a significant emerging condition in dogs, presenting as either primary or secondary pathology. In both humans and dogs, hyperlipidemia is associated with liver disease, such as the hepatocellular accumulation of triglycerides, referred to as hepatic lipidosis or steatosis [1]. Ultrasound attenuation imaging (ATI) is a feasible method for assessing hepatic parenchyma in dogs and is used in humans as a non-invasive technique to quantitatively evaluate hepatic steatosis with ultrasound imaging [2,3]. The aims of our prospective study (OPBA permission number: 43/2020) were to verify the applicability of the ATI on hyperlipemic dogs, to compare the attenuation coefficient (AC) (dB/cm/MHz) between hyperlipemic and non-hyperlipemic dogs, and to determine whether AC correlates with visual ultrasound evaluation of the liver. Fifty-three dogs underwent clinical examination, blood tests (cholesterol, triglycerides, alkaline phosphatase, alanine aminotransferase, gamma-glutamyltransferase, total protein, albumin), abdominal ultrasound and ATI between January 2021 and January 2024. They were categorized into 2 groups: 21 healthy dogs (A), and 32 hyperlipemic dogs (B). Within Group B, dogs were further divided based on blood values into mild hyperlipemia (B1; n=15) and moderate/severe hyperlipemia (B2; n=17). Based on the qualitative ultrasound evaluation, the grade of severity of hepatic steatosis was classified into: G0 (normal), G1 (mild), G2 (moderate) and G3 (severe) in 2, 16,14 and 2 dogs, respectively. During ATI investigation, AC was measured 5 times for each dog and the median value was used. The mean AC value was significantly higher in group B than in group A (respectively 0,81 ± 0,10 vs 0,95 ± 0,23 dB/cm/MHZ) using an unpaired t test. No statistically significant differences were found between the mean AC value of group B1 and group B2 using an unpaired t test. Although no statistical difference was identified between mean AC values and different grades of ultrasound severity using Fisher's exact test; a progressive increase in the AC value was observed between G0 and G3 (G0: 0,80 ± 0,08; G1: 0,95 ± 0,27; G2: 0,93 ± 0,26; G3: 1,10 ± 0,3 dB/cm/MHZ). In conclusion, ATI has proven a useful method for the non-invasive identification of hepatic steatosis in dogs, although it does not allow the evaluation of the degree of severity.

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77° CONVEGNO SISVET

Stato: INVIATO - ID: 13701

Tomographic findings of feline ischiatic limph nodes

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In recent years, interest in diagnostic imaging of lymph nodes has surged, particularly with the advent of specialized techniques like computed tomography (CT). However, interest in feline species remains limited. Nevertheless, certain feline patients undergoing abdominopelvic CT have revealed structures resembling lymph nodes in specific anatomical locations. Notably, the ischial lymph center has been identified in feline species, a feature not observed in dogs [1]. This retrospective, multicenter, anatomical study aimed to determine the prevalence and characteristics of ischial lymph nodes in cats undergoing CT. A group of 250 cats was included in the study, and pre- and post-contrast total body CT scans were reviewed by three expert readers to identify the presence or absence of these lymph nodes. Dimensions (width, height, and length) were measured in multiplanar reformation, with descriptions based on homogeneity and enhancement compared to surrounding muscles. Additionally, the study considered the presence of regional or systemic lymphadenomegaly and any concurrent pathologies. Ischiatic lymph nodes were visualized in 161 patients, with 77 showing bilateral presence, 35 only on the right side, and 46 only on the left side. All cats included were adults, except for six cats under 12 months of age. These lymph nodes were typically located dorsal to the ischial tuberosity, lateral to the IV coccygeal vertebra, deep to the gluteofemoralis muscle and medial to the caudal gluteal vein. Variability in position was noted, with nodes sometimes being ventral to the muscle, slightly caudal, or even cranial in rare cases. Additionally, smaller nodular formations were observed in seven cases caudal to the main lymph node, likely representing additional lymph node structures within the lymph center. The highlighted lymph nodes, totaling 242, typically exhibited elongation in a craniocaudal direction and displayed various shapes in transverse scanning, including oval (n=118), roundish (n=89), bean-shaped (n=9), or irregular (n=26). The dimensions of the right and the left ischiatic lymph nodes were expressed as median values along with their respective ranges. For the right lymph node, the width was 2 mm (range: 1-8 mm), height 2 mm (range: 1-6 mm), and length 3.6 mm (range: 1-12 mm). For the left lymph node, the width was 2.2 mm (range: 1-7 mm), height 2 mm (range: 1-5.3 mm), and length 3.6 mm (range: 1-15 mm).Most lymph nodes typically exhibit homogeneous parenchyma; however, defining homogeneity and enhancement for some was challenging due to their small size. Regarding contrast enhancement, it was categorized as mild (n=62), moderate (n=57), and marked (n=27). Among the 161 cats, 14 presented with neoplastic (n=11) or inflammatory (n=3) pathologies affecting the drained area, all showing at least one altered regional lymph node (iliac, popliteal, or sacral). Interestingly, the size of ischial lymph nodes in these subjects did not differ from the population average. The evaluation of ishiatic lymph nodes in cats is feasible using CT. Factors such as the amount of adipose tissue and contrast administration subjectively enhanced lymph node visualization. The measurements and morphological characteristics are proposed. Recognition of ischiatic lymph nodes in feline patients may significantly aid disease staging, facilitating identification of disease spread and therapeutic planning.

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77° CONVEGNO SISVET

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Medetomidine and vatinoxan premedication in isoflurane anesthetized dogs: occurrence of complications in a clinical setting

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Medetomidine is an alpha2-adrenoceptor agonist commonly used in veterinary anaesthesia to obtain sedation, analgesia and muscle relaxation, causing dose-dependent cardiovascular side effects characterized by increased systemic vascular resistance and reflex bradycardia [1]. When medetomidine (M) is administered to isoflurane anesthetized dogs, the vasodilatory effect of isoflurane might only partially attenuate its vasoconstrictive effect, thereby reversing the bradycardia. Vatinoxan (V) is a peripheral alpha2-adrenoceptor antagonist and, when administered with medetomidine, in dogs undergoing inhalation anesthesia, no changes in peripheral vascular resistance are observed but the heart rate (HR) is better maintained compared with medetomidine alone [1]. Drawbacks of the MV combination are the risk of incurring in a superficial aesthetic plane and in poor recoveries [2]. The aim of the study was to retrospectively evaluate the stability of the anesthetic plane, the incidence of cardiovascular side effects and the recovery quality in dogs premedicated with MV combination and undergoing isoflurane anesthesia. We also evaluated the effects of the concurrent administration of acepromazine.The anaesthetic records of dogs submitted to isoflurane anaesthesia and receiving MV as part of the premedication protocol between January 2023 and March 2024 were retrospectively evaluated. The anesthetic protocol, the mean fraction of expired isoflurane (FeISO) and the occurrence of the following events were considered: hypotension (mean arterial pressure, MAP < 60 mmHg), bradycardia (HR < 60 bpm), measures for correction of hypotension and recovery quality. Data were elaborated with a descriptive statistic. A Wilcoxon rank sum test was used for comparison between the two treatments. A Fisher exact test was used to test the association between the evaluated events and the addition of acepromazine. A $p < 0.05$ was considered statistically significant.In the study 334 anesthetic records were included. Opioids (methadone n=265/334 – 79.3% or butorphanol n=69/334 - 20.7%) or acepromazine (n= 75/334 - 22.5%) were administered in combination with MV. Overall, 19/334 (5.7%) episodes of bradycardia occurred, with 7/334 (2.0 %) requiring atropine. Hypotension occurred in 37/334 – 11% anesthetic procedures. A fluid bolus was administered in 33/334 -9.9% occasions, while dobutamine was administered during 22/334- 6.6% procedures (6.6%). The occurrence of hypotension was significantly correlated with the administration of acepromazine (14/75 - 18.6% versus 23/257- 9% when acepromazine was not administered; $p < 0.035$); also, the occurrence of administration of a fluid bolus for treatment of hypotension was significantly correlated with the administration of acepromazine ($p < 0.001$). The median FeISO in dogs receiving acepromazine [1.20 (0.7-1.5) %] did not differ significantly from the dogs in which it was not administered [1.20 (0.6-1.8) %]. The addition of acepromazine was not significantly associated with the quality of recovery ($p=0.950$). In conclusion, MV premedication in isoflurane anesthetized dogs results in a lower incidence of bradycardia than previously reported for medetomidine alone [2]. Furthermore, although episodes of hypotension cannot be excluded only a small percentage have required inotropic support. The addition of acepromazine to MV premedication was associated with an increased incidence of hypotension without providing a more significant isoflurane sparing effect or improving the recovery quality.

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77° CONVEGNO SISVET

Stato: INVIATO - ID: 13727

DOPPLER PARAMETERS OF THE LATERAL PALMAR DIGITAL ARTERY IN LAME SPORT HORSES

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During the weight-bearing and stance phase of the stride, lame horses usually try to “unload” the affected limb. In the literature, increases in blood flow velocities evaluated by eco-Doppler examination of the lateral palmar digital artery (LPDA) were documented when the limb is maintained in a non-weight-bearing position. The aims of the study were to evaluate the ultrasonographic alterations of the blood flow of the LPDA of the front limb (FL) of horses affected by unilateral lameness and to verify whether these parameters are correlated with the degree of lameness. In this study, 25 lame horses (LH) and 12 sound horses (SH) were enrolled: LH presented unilateral forelimb lameness previously diagnosed by equine practitioners. All the animals underwent eco-Doppler examination, during which peak systolic velocity (PSV), end-diastolic velocity (EDV), and resistivity index (RI) of the LPDA of both FLs were recorded. The ultrasonographic examination was carried out on non-sedated horses, standing squarely, and by the same equine practitioner, who was blinded to the lameness examination results. A portable ultrasound machine, equipped with a linear transducer, was used for the exam. The transducer, with a silicone pad, was placed on the lateral aspect of the fetlock region, over the neurovascular bundle. The LPDA was identified using longitudinal scans. Pulsed wave Doppler (PW) was then applied to the LPDA in triplex mode, and PSV, EDV, and RI were obtained. The examination was repeated for the contralateral limb, and for both limbs, three measurements of each blood flow parameter were recorded. Normality was tested with Shapiro-Wilk’s W test, and data were reported as mean ± standard deviation or median (range). Differences between PW parameters of the right and left FL (delta) for both LH and SH were calculated by subtracting the values of the left limb from those of the right limb. Student’s t-test or a Mann-Whitney U test was used to compare delta absolute values of LH and SH. Spearman’s rho was applied to test the correlation between blood flow parameters and the degree of lameness. Three horses were excluded from the study, as their arteries were non-resistive. Median PSV was higher in LH (32.9-123.5) compared to SH (14.7-130). No differences were recorded between EDV and RI values of LH and SH. The degree of lameness was not correlated with Doppler parameters. The ultrasound examination was well tolerated by all the animals; however, different sources of variability, mainly relating to the inspection technique, may have interfered in the examination of both groups. During lameness, horses tend not to load the affected limb, resulting in an increase in PSV values, as reported by Hoffman et al. (2001) and Pietra et al. (2004). However, by evaluating individual data and not medians, some SH horses presented higher PSV values than LH, suggesting that other anatomical, pathological (type, severity, and chronicity of the lameness), and hemodynamic features may be responsible for variations in Doppler parameters. No correlation between PSV and the degree of lameness was found, suggesting that further studies involving a larger number of animals are needed to investigate additional potential causes of variation in LPDA eco-Doppler parameters.

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77° CONVEGNO SISVET

Stato: INVIATO - ID: 13740

Analysis of the surgical outcomes in the tibial plateau leveling osteotomy procedure using the Vitcone® system and analysis of the case studies collected over 5 years and in 859 cases.

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Cranial cruciate ligament disease currently appears to be the most widespread orthopedic pathology in the canine species, affecting a percentage of between 3% and 5% of the total population (1), causing joint instability, lameness and pain. Among the techniques identified as gold standard for the surgical treatment of this pathology is the TPLO, i.e. the leveling osteotomy of the tibial plateau (TPLO), which consists in neutralizing the functionality of the cranial cruciate ligament by eliminating the cranial tibial thrust , bringing the tibial plateau back to an angle of 5°. Introduced by Dr. Slocum in 1993, is currently recognized as one of the most widespread techniques with the highest success rate and the best surgical outcomes, thanks to extreme standardization. This procedure requires a tibial osteotomy which determines the creation of stumps, the small proximal one requiring stable fixation, which over the years has been perfected thanks to the introduction of stable angle implants with dedicated plates with specific profiles. This study examined the use of the Vitcone® system, angular stable implants, over the course of 5 years of practice, all procedures were carried out by the same surgeon, for a total of 859 operations, carried out on 653 subjects . 504 single TPLO, 149 bilateral TPLO in two sessions, 28 TPLO in the same session were carried out. The average weight of the treated subjects was 22 kg, with 218 subjects under 10 kg, 196 subjects weighing between 10 and 20 kg, 196 subjects weighing between 20 and 30 kg, 155 subjects between 30 and 40 kg, 59 subjects weighing between 40 and 50 kg, 28 subjects weighing between 50 and 60 kg, and 7 subjects over 60 kg. In this collection of cases, the sample of subjects based on sex shows 40% males of which 12% were neutered, and 60% females of which 43% were sterilised. The number of screws used per implant was also evaluated. The statistics collected in the case studies do not present any discrepancies based on the sex of the subjects and their weight. The Vitcone® system shows a reduction in the number of screws required for stabilization of the implant, in fact 4 screws were used for 91% of the procedures, 5 screws in 2%, 6 screws in 7%. In the sample group, 5 valgus knees, 10 osteomyelitis, 12 serious intraoperative hemorrhages, 6 roll backs, 42 fistulas, 8 screw uncouplings, 10 fibula fractures, 10 slow healing and 3 dissatisfied customers were observed as complications.

77° CONVEGNO SISVET

Stato: INVIATO - ID: 13804

Refining the TNM staging system for canine oral Malignant Melanoma: A novel approach for primary tumour description and the role of lymphadenectomy

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Malignant melanoma (MM) represents the most common tumour of the canine oral cavity. It has an aggressive biological behaviour characterized by a high local invasiveness and a elevate metastatic rate, mainly to regional lymph nodes and lungs.The prognosis of MM is determined by several factors, including clinical staging, histological and immunohistochemical factors, and the therapy employed (1).The choice of the proper treatments depends on the TNM clinical staging system, that describes the anatomical extent of tumour on the dogs. However, the current TNM classification presents several limitations, in particular, T (maximum diameter of the primary tumour) does not consider the anatomical differences among breeds and the different tumour histotypes, and N (status of regional lymph nodes) does not specify which and how many lymph nodes should be considered and removed (2, 3). Therefore, the study aims to develop a novel parameter “I” that more effectively identifies the patient-specific anatomical extent of the primary tumour. By incorporating the histological prognostic factors, this project aims to more accurately evaluate the parameter’s ability to predict dogs’ prognosis and its potential association with lymph nodes and distant metastases. Moreover, the study assesses the status of regional lymph nodes along with the importance of lymphadenectomy.Dogs affected by MM and treated at the Veterinary Teaching Hospital of Grugliasco (Turin, Italy) since December 1st, 2014, were retrospectively considered for this study.The inclusion criteria were surgical excision of primary tumour and regional lymphadenectomy, histological diagnosis of MM, immunotherapy treatment (Aut. Min. 0015537-28/06/2017-DGSAF-MDS-P), absence of distant metastases at presentation, at least one year of follow-up and no concurrent life-threatening disease.For each patient were collected all the clinical and histological data and were calculated the different oncological endpoints: median survival time (MST), disease-free interval (DFI), time to recurrence (TTR).The “I” parameter was assessed by analysing the skull CT images and represents a ratio between the tumour size, measured as tumour implant surface and the oral cavity dimension, measured by the combined mandibular and maxillary surfaces.A total of 72 dogs with MM met the inclusion criteria and were enrolled in the study. Of these, 42 were staged with total-body CT scan. In this group, the "I" parameter was calculated and showed a statistically significant correlation with oncologic endpoints, unlike the "T" parameter.A mathematical model, built using linear and logistic regression, was subsequently created to predict patient prognosis based on “I” and its histological characteristics. Using ROC curves, classes were created for 'I', which revealed a significant difference in MST between them, as opposed to the classes based on 'T'.In all the dogs, the status of lymph nodes and the importance of regional lymphadenectomy were evaluated. The type of lymphadenectomy, whether ipsilateral or bilateral, was not found to be associated with disease progression or the early onset of distant metastases. Furthermore, the presence of lymph node metastases at presentation (33% of cases; 24/72) did not affect MST or DFI. 1) Bergman PJ. Canine oral melanoma. Clin Tech Small Anim Pract, 22:55-60, 2007. 2) Owen LN. TNM classification of tumors in domestic animals, I ed. Geneva, WHO, 1980. 3) Grimes JA et al. Histologic evaluation of mandibular and medial retropharyngeal lymph nodes during staging of oral malignant melanoma and squamous cell carcinoma in dogs. J Am Vet Med Assoc,254:938-943, 2019.

SIFTVET

IN VITRO EFFICACY OF GREEN TEA EXTRACT AGAINST FELINE HERPESVIRUS TYPE 1 (FHV-1)

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Feline Herpesvirus type 1 (FHV-1), the main etiological agent for rhinotracheitis and ocular symptoms in cats, may affect felines of any age and health condition [1]. Young cats are more susceptible to this infection, due to their immature immune system, as well as elderly cats or those with chronic diseases, as they frequently present immune system impairments [2]. In severe cases, a systemic antiviral therapy based on famciclovir administration can be used [3]. Furthermore, cats with recurrent conjunctivitis requires topical ocular therapy, as antiviral ophthalmic drops. In these cases, it is important to treat corneal ulcers promptly to prevent permanent eye damage [3].

To date, phytotherapeutic treatments represent a relatively underexplored option for addressing FHV-1 infections and reactivations. In this scenario, natural compounds could provide several advantages, such as reduced side effects, less resistance to the commonly used antivirals, and low toxicity.

The current study aimed to evaluate the *in vitro* efficacy of a Green tea extract (GTE) from *Camellia sinensis* with 50% of polyphenols (Italfeed) for the treatment of FHV-1 infection. For this purpose, Crandell-Reese feline kidney (CRFK) cells were treated with different doses of GTE (10-400 µg/mL) during the viral adsorption phase and throughout the following 24 hours. The MTT and $TCID_{50}$ assays were performed to determine the citotoxicity and the EC_{50} of the extract, which was found to be approximately 90 µg/mL. Therefore, the subsequent experiments have been carried out using GTE amounts that exceeded the estimated EC_{50}.

The western blot assay showed a reduction in the expression of viral glycoproteins (i.e., gB and gI) after GTE treatment when a monoclonal anti-FHV-1 was used. GTE induced not only an almost complete suppression in viral proliferation, but also in the phosphorylation of Akt protein, generally involved in viral entry [4]. Moreover, the increase in cell proliferation observed in infected cells upon GTE addition was supported by enhanced expression of BAX and BCL-xL anti-apoptotic proteins.

Since GTE presents a high content of polyphenols with antioxidant activity, the level of reactive oxygen species (ROS) has been quantified by DCFH-DA (2',7'-Dichloro-dihydro-fluorescein diacetate) staining. The ROS burst observed during FHV-1 infection was mitigated after GTE treatment, leading to a reduction in the oxidative imbalance.

In conclusion, the results of this study demonstrated the effectiveness of GTE *in vitro* against FHV-1 infection. Its easy availability suggests that GTE may offer reliable therapeutic support for managing FHV-1 infection in feline patients, warranting further investigation in clinical settings.

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Polychlorinated biphenyls cause metabolic alterations in mature 3T3-L1 adipocytes via modulation of aquaglyceroporin levels

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Polychlorinated biphenyls (PCBs) are ubiquitous persistent organochlorine pollutants that still exist in different environments. Due to their high chemical stability and lipophilicity, PCBs accumulate in fat-containing tissues and undergo strong biomagnification processes, thus achieving higher concentrations in predatory species [1] and posing a serious risk to animal and human health. Long-term exposure to PCBs leads to several metabolic diseases, such as type 2 diabetes and obesity [2]. Aquaglyceroporins (AQPs), including AQP3, AQP7, and AQP9, are a subclass of aquaporin water channels involved in adipose tissue metabolism through the regulation of glycerol uptake and release. Hence, these channels are key factors in the maintenance of lipid balance and energy homeostasis, and their dysregulation is involved in several disorders, including insulin resistance and obesity [3]. We aimed to investigate if PCBs could contribute to the onset of metabolic dysfunctions through the alteration of AQP expression in adipocytes. 3T3-L1 cells were differentiated into mature adipocytes by a standard procedure of adipogenic differentiation and then treated for 48 hours with 1 µM PCB 101, 153, or 180. A modulation of AQP protein expression was revealed in treated cells. Specifically, AQP3 and AQP7, which are involved in glycerol release, were reduced by all PCBs. On the contrary, AQP9, which is involved in glycerol uptake, was increased by PCB 153 and 180. This modulation suggests a greater glycerol accumulation in treated cells confirmed by the reduced amount of released glycerol in the culture media of cells exposed to PCBs, measured by using an enzymatic colorimetric method. Moreover, the increased mRNA levels of glycerol kinase in PCB 153-treated cells, indicate the conversion of accumulated glycerol into glycerol 3-phosphate. An increased gene expression of key targets involved in lipid uptake and storage, such as *Fabp4* and *Pparg*, was also evidenced in adipocytes exposed to PCB 153. Finally, PCB 153 also increased mRNA levels of *Dgat1* and *Agpat9* genes, codifying enzymes involved in the synthesis of triglycerides from glycerol 3-phosphate and free fatty acids. All these results suggest an increase of lipid storage in adipocytes treated with PCB 153, confirmed by Oil Red O staining. The role of AQPs in the above effect was also evaluated by pre-treating cells with phloretin, a well-known AQP9 inhibitor, which partially restored intracellular lipid levels. Overall, our results showed the involvement of AQPs in PCB-induced adipocyte dysfunction, contributing to better define the toxicity mechanisms by which these pollutants cause obesity and metabolic disorders.

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Ecotoxicity assessment of fluoroquinolones and sulfonamides, in two saltwater organisms

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Abstract

Fluoroquinolones (FQs) and Sulfonamides (SAs) are extensively utilized in human and veterinary medicine due to their broad-spectrum antimicrobial properties. Consequently, they have become prevalent in aquatic ecosystems through various sources such as pharmaceutical discharges, urban sewage, aquaculture, and agricultural runoff. Whilst the impact of these antimicrobials on freshwater environments is well-documented, their effects on marine ecosystems remain largely unexplored. This study assessed the ecotoxicity of eight antibiotics - four FQs (ciprofloxacin, enrofloxacin, flumequine, and levofloxacin) and four SAs (sulfadiazine, sulfadimethoxine, sulfamethazine, and sulfamethoxazole) - on two saltwater organisms: the alga *Phaeodactylum tricornutum* and the crustacean *Artemia salina*. Evaluating effects on these primary trophic levels is crucial for understanding potential bottom-up repercussions on marine ecosystems. Algal growth inhibition tests (72h) were conducted on *P. tricornutum* in accordance with the ISO 10253 standard protocol [1], whilst acute immobilization tests (48h) were performed on *A. salina*, based on the Standard Operational Procedure provided by Microbiotest™ [2]. Results indicated significant toxic effects of SAs on *P. tricornutum*, with EC_{50} values of 0.4, 1.3, 57.8, and 1.1 mg L^{-1} for sulfadiazine, sulfamethoxazole, sulfadimethoxine, and sulfamethazine, respectively. Moreover, the three most active compounds (sulfamethazine, sulfamethoxazole, and sulfadiazine) displayed algicidal effects, and their toxicity was unaffected by the addition of folic acid (100 ng L^{-1}) to the culture medium. In contrast, FQs exhibited effects only at relatively high concentrations, with EC_{50} values for enrofloxacin, flumequine, and levofloxacin calculated as 120.4, 670.0, and 429.8 mg L^{-1}, respectively. Notably, at the highest concentration tested, instead of inhibiting, ciprofloxacin stimulated algal growth, a phenomenon that deserves further investigation. Comparison with existing literature data on freshwater algae revealed a lower sensitivity of *P. tricornutum* to FQs but similar sensitivity to SAs [3]. Overall, *A. salina* displayed very low sensitivity to the assayed antibiotics, with adverse effects observed only with 100 mg L^{-1} of ciprofloxacin. Preliminary Risk Quotient (RQ) assessment based on EC_{50} values obtained in *P. tricornutum*, highlighted sulfadiazine and sulfamethoxazole as antibiotics of possible concern, with RQs of 0.145 and 0.237, respectively.

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Therapeutic monitoring of ampicillin in newborn hospitalised foals

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Ampicillin (molecular formula $C_{16}H_{18}N_3NaO_4S$) is a broad-spectrum antibiotic that belongs to the penicillin class [1]. This drug acts by inhibiting the synthesis of bacterial cell walls, ultimately resulting in the destruction of the pathogen. It is commonly used in veterinary medicine to treat a variety of infections in cats, dogs, and horses. As a time-dependent beta-lactam antibiotic, monitoring the concentration of administered ampicillin in animals over time is essential [1].
To the best of our knowledge, to date, no pharmacokinetic (PK) studies on ampicillin in critically ill foals have been published. Additionally, no liquid chromatography-tandem mass spectrometry (LC-MS/MS) approaches have been proposed for its quantification in equine plasma. Therefore, the objective of this work was to develop a LC-MS/MS technique for the quantification of ampicillin in equine plasma and apply it to samples collected from hospitalised newborn foals enrolled in a Therapeutic Drug Monitoring (TDM) study. This research was approved by the Committee for Animal Welfare of the University of Bologna (Protocol Number 358467 and conducted at the Veterinary Teaching Hospital of the same university.
The proposed method uses a simple and fast sample preparation, followed by analysis in LC-MS/MS with chromatographic runs of just 4.0 min. Plasma samples (100 µL) were precipitated with acetonitrile and injected in the analytical system. Ampicillin separation was obtained with a Waters Acquity Ultra Performance Liquid Chromatography pump equipped with an Ethylene Bridged Hybrid C18 column, pumping a mixture of 0.1% formic acid in water and acetonitrile under programmed conditions. The quantification of the analyte was performed on a Waters XEVO TQ-S Micro, operating in positive electrospray ionization mode and monitoring two specific transitions (350.10 > 105.95 and 350.10 > 113.89 m/z). The technique was fully validated [2], and the results demonstrated good linearity during each day of testing (R^2 always >0.99). Accuracy and precision were excellent, with calculated bias always within ±15% and CV% always below 15% at all (n=3) quality control levels tested. Furthermore, samples stored at - 20 °C proved analyte stability (±15%) for more than 40 days.
A preliminary application of the analytical approach was performed on samples collected (5 min before and 5 min after each administration) from 12 foals receiving 20 mg/kg of ampicillin intravenously every 6 hours, during the first 2 days of treatment. From this initial investigation, the fluctuation of ampicillin concentrations in foals' plasma was determined (mean peak and trough concentrations were in the 245-75,713 ng/mL range), providing the first insight into its PK in these patients. The ongoing inclusion of a larger number of subjects presenting different clinical conditions and the correlation with Minimum Inhibitory Concentration (MIC) values associated with specific pathogens will enable to verify whether the treatment protocols in use are adequate or require adjustments, especially for critically ill patients.

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IVERMECTIN EXCRETION IN STOOLS FROM INJECTED AND ORALLY TREATED SOWS: A PILOT STUDY

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Ivermectin is an anthelmintic drug belonging to the macrocyclic lactones class. It has a wide spectrum of action against endoparasite and ectoparasites. In Italy ivermectin is authorized in medicinal products registered for use in various animal species, including bovines, goats, equines, dogs, cats, sheep, and swine. It is commercially available in several formulations from injectable to edible pastes and tablets [1]. In swine farming, ivermectin is mainly used in breeding animals (sows and barrows) because of its long withdrawal period. This study aimed to assess ivermectin residue production in feces following injectable and oral treatments within a framework of environmental impact. Sampled sows from two swine farms in Emilia Romagna received either injectable or oral ivermectin treatment (1 ml/50 kg b.w.). Fecal samples were collected before treatment (t0), one day after treatment (t1) and ten days post-treatment (t10). Stools were collected from the rectal ampoule of a total of 70 sows and analyzed through LC/MS/MS with an adapted method from Carrillo Heredero et al [2].

Following treatment, orally treated sows exhibited higher median ivermectin concentrations at t1 (996.07 PPB) compared to injected sows (13.10 PPB). Conversely, at t10, injected sows showed higher concentrations (70.06 PPB) compared to orally treated sows (11.50 PPB). Notably, the oral treatment regimen spans several days, in contrast to the single administration characteristic of injectable treatment protocols. Significant differences in ivermectin quantities were observed between the two treatments at all time points (t0 $p<0.01$, t1 $p<0.0001$, t10 $p<0.001$). The oral treatment regimen resulted in a notable and statistically significant elevation in ivermectin levels between t0 and t1 ($p<0.0001$), while no discernible variance was observed with injectable treatments. This discrepancy may be attributed to the comparatively delayed distribution to the gastrointestinal tract associated with the injectable administration route.

In an environmental residue-focused context, our findings highlight substantial differences between oral and injectable ivermectin treatments. Giving its high resistance in the environment and proven toxicity to non-target species, these disparities, both quantitative and in long-term excretion patterns, underscore the importance of considering administration routes in managing ivermectin residues in swine farming environments.

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A Preliminary Study from a One Health Perspective: N-3 Polyunsaturated Fatty Acid Supplementation in Rabbit Diet Assessing Intestinal Homeostasis and Gut Immunity to Enhance Health and Reduce Antimicrobial Use

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Pet rabbits are very sensitive to infections of gastrointestinal and respiratory tract and thus may often undergo antibiotic treatment, consequently concern can arise from the transmission of antibiotic-resistant genes and multidrug-resistant bacteria to their owners. This coincides with a rising interest in nutraceuticals, which can enhance local immunity and maintain homeostasis, thereby preventing diseases, or that can serve as substitutes for antimicrobials. In this context, we assess the effects of fish oil and flaxseed supplemented in rabbit feed on the regulation of intestinal immunity and gut vascular barrier homeostasis, in jejunum and caecum of rabbit. The extruded flaxseeds were added in quantities of 100 g/kg of feed, while the fish oil was added in quantities of 35 g/kg. The feed was provided in pellet form, and the ingredients of the two diets with supplements were rebalanced. To evaluate the effects of these substances, 12 females New Zealand White rabbits were enrolled, and divided in 3 groups: control group (n. 4), flaxseed treated group (n. 4), and with fish oil treated group (n.4) based on dietary requirements. Ninety days after supplementation, the rabbits were slaughtered, and jejunum and caecum samples were collected. RNA was extracted from the tissues, and the expression of β-catenin, PLVAP, TGFβ1, and IL-8 was quantified using qRT-PCR. Fish oil group showed no significant differences compared to control group, only tendency. In the flaxseed treated group, the expression of all genes, except IL-8, was significantly reduced compared to the control in the jejunum samples. In the study by Nowak et al. [1], improvement in digestive health and decreased intestinal permeability following flaxseed consumption is reported. Conversely, we observed downregulation of β-catenin and TGFβ1 coupled with increased PLVAP. These results may indicate a reduction in adherens junctions formed by β-catenin, which could result in bacterial dissemination. Additionally, in other studies, upregulation of PLVAP has shown to be associated with a disease status. Finally, the highlighted downregulation of TGFβ1, hindered its inhibition of the pro-inflammatory activity against commensal bacteria and the intestinal remodelling and wound healing. Considering fish oil, the effects, albeit only as trends, could be opposite to flaxseed and therefore positive. As this is a preliminary and promising study, it would be useful to deepen the knowledge on the action of these two nutraceutical substances rich in N-3 Polyunsaturated Fatty Acids on gut vascular barrier homeostasis and local intestinal immunity by increasing the number of animals and genes analysed.

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77° CONVEGNO SISVET

Stato: INVIATO - ID: 13359

Assessing Ochratoxin A Contamination in Pre-Packaged Grated Cheese: Implications for Food Safety

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Ochratoxin A (OTA) is a mycotoxin produced by fungi, including certain species of *Aspergillus* and *Penicillium* sp., with the ability to contaminate various food products, including cheese. Milk contamination by ochratoxigenic fungi may originate from either primary contamination of the cheese or occur during ripening and storage processes in production facilities. Generally, OTA levels are significantly higher on the rind compared to the inner part of the cheese [1]. The presence of OTA in cheese raises concerns due to its toxicity and adverse effects on human health, including potential carcinogenic and nephrotoxic properties [2]. Europe leads in cheese consumption and Italy plays a significant role in the dairy industry, ranking third in cheese production within the EU, with a substantial portion comprising Protected Designation of Origin (DOP) cheeses, notably Grana Padano (GP) and Parmigiano Reggiano (PR) [3]. These hard cheeses are increasingly used as pre-packaged grated products due to their popularity and quality. About 25% of all grana cheese produced in Italy is processed and sold as grated. In industrial grating processes, the utilization of rinds is regulated, with the percentage sometimes specified by specific DOP regulations. For instance, according to DOP regulations for grated GP and PR cheeses, a maximum of 18% rind is permitted. This study aimed to assess OTA contamination through high-performance liquid chromatography (HPLC) analysis with a fluorescence detector in various types of pre-packaged grated cheese, assessing its occurrence and potential implications for food safety. The limit of detection (LOD) and limit of quantification (LOQ) were determined as 0.05 and 0.1 µg kg^{-1}, respectively. The investigation revealed the widespread presence of OTA in nearly all analysed samples (97.6% incidence), with contamination levels varying significantly (from
<LOD to 19.15 µg kg^{-1}). PR cheese exhibited the highest average contamination level (5.06 ± 0.66 µg kg^{-1}), followed by pecorino cheese (2.25 ± 0.31 µg kg^{-1}), mixed cheese (2.15 ± 0.18 µg kg^{-1}), and GP cheese (1.53 ± 0.21 µg kg^{-1}). The presence of OTA in cheese poses significant challenges to food safety, given its potential health risks. The study underscores the importance of assessing OTA contamination in cheese products, particularly pre-packaged grated cheese, to mitigate health risks and ensure consumer safety. Further research is warranted to understand the factors influencing OTA contamination in cheeses and to establish appropriate regulatory limits to safeguard public health.

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77° CONVEGNO SISVET

SOCIETA´ SCIENTIFICA DI RIFERIMENTO **Società Italiana di Farmacologia e Tossicologia Veterinaria (SIFTVet)**

TITOLO ***Quercetin exerts cytotoxic and proapoptotic effects by influencing cell viability, proliferation and migration of canine mammary tumour cells: a preliminary in vitro study***

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Testo e Riferimenti bibliografici

Human breast cancer (HBC) poses a serious threat to women's health. Remarkable progress has been made in the treatment of HBC over the past decade, and to date several effective anti-cancer drugs are available for the treatment of HBC (1). However, despite these advances, the prognosis of HBC remains poor. Canine mammary tumours (CMTs), the second most common neoplasm in dogs, and HBC share similarities and it has been widely demonstrated that the dog represents an alternative model for comparative oncology research (2). Currently, there are few clinically relevant treatments for CMTs and the drugs that are actually available and used can cause significant side effects (e.g. anorexia, vomiting, alopecia and neutropenia). Quercetin, a traditional medicinal herb, is an important flavonoid and has anti-cancer activity (3). In this study, we investigated whether quercetin could inhibit the viability, proliferation and migration of CMT cells and promote apoptosis. Using the crystal violet assay, we demonstrated that exposure of CMT-U229, P114 and CMT-U309 cells to different concentrations of quercetin inhibited the viability of CMT cells in a concentration-dependent manner. Annexin V/propidium iodide (PI) double staining assay showed that quercetin promoted apoptosis in CMT cells, while DNA staining of ethanol-fixed cells with PI showed that quercetin arrested CMT cells in the G1 cell cycle phase. A wound healing assay showed that quercetin significantly reduced the migration of CMT cells. Western blot analysis showed that quercetin-induced cell death was associated with the cleavage of caspase-9, caspase-3, PARP and BCL-XL. At the molecular level, quercetin-induced inhibition of cell cycle and migration was also associated with a reduction in c-myc and matrix metallopeptidase-9. Thus, we conclude that quercetin may inhibit viability, proliferation and migration and promote apoptosis of CMT cells by suppressing, at least in part, key regulators of these molecular signalling pathways. To summarise, our preliminary results suggest that further *in vitro* studies should be performed before these results might be translated into *in vivo* studies.

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Establishment and Characterization of a Cytochrome P450 1A1 CRISPR/Cas9 Knockout Bovine Foetal Hepatocyte Cell Line (BFH12)

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The cytochrome P450 1A (CYP1A) subfamily of xenobiotic metabolizing enzymes (XMEs) consists of two different isoforms, namely *CYP1A1* and *CYP1A2*, which are highly conserved among species. These two isoenzymes are involved both in the biotransformation of drugs and endogenous compounds as well as in the bioactivation of several xenobiotics into carcinogenic derivatives, thereby increasing the risk of tumour development [1]. Cattle (*Bos taurus*) are one of the most important food-producing animal species worldwide. During their life they are exposed to various xenobiotics (e.g., drugs, feed additives, pesticides, growth hormones, feed and food contaminants and environmental pollutants) that, along with their derivatives, can permeate cattle-derived food products, posing risks to both animals and consumers. Despite the health concern associated to the presence of xenobiotic residues in cattle-derived food products, there is limited research into bovine CYP1A-mediated hepatic metabolism and molecular mechanisms governing its expression and regulation. To fill this gap of knowledge, in the present study the CRISPR/Cas9 system was applied to a bovine foetal hepatocyte cell line (BFH12), showing a hepatocyte-*like* metabolism and a stable expression of several XMEs and drug transporters [2], with the aim of establishing a bovine *CYP1A1* knockout (KO) *in vitro* model. After clonal expansion and the subsequent clonal selection by end-point PCR and Sanger sequencing, *CYP1A1* deletion was assessed at the mRNA and protein levels by quantitative real time RT-PCR, immunoblotting and ethoxyresorufin-*O*-deethylase activity (i.e., EROD assay). In the selected clone, an almost complete loss of *CYP1A1* mRNA, of the corresponding coded protein and its catalytic activity was confirmed. The residual expression and activity were associated to a potential contamination by non-homozygous KO and naïve cells escaping clonal selection, as previously reported [3]. To better characterize the bovine KO cell line, its transcriptome was assessed by using a RNA-sequencing approach. This analysis revealed significant transcriptional changes in KO cells compared to wild type ones. Specifically, the *CYP1A1* deletion affected the mRNA expression of genes involved in cell cycle regulation, cell proliferation and detoxification processes as well as in iron, lipid and mitochondrial homeostasis, suggesting a pivotal role of *CYP1A1* in the maintenance of cell homeostasis further than in well-known biotransformation/bioactivation processes. In conclusion, a new bovine *CYP1A1* KO *in vitro* model was successfully generated and characterized. Based on the present results, this cell line might be used in the future to evaluate the potential toxic effects of xenobiotics in cattle liver, as well as for improving the knowledge on the role played by bovine *CYP1A1* in cell homeostasis.

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Ability of Mycotoxin Binders to Counteract Oxidative Stress Induced by Aflatoxin B1 in Broiler Chickens.

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Aflatoxins are secondary metabolites produced by fungi of the genus *Aspergillus*. Among them, aflatoxin B1 (AFB1) stands out as a well-known hepatotoxic, immunotoxic and IARC group I carcinogenic substance. In farm animals, the most prevalent adverse effects include oxidative stress and decreased zootechnical performances resulting in significant economic losses. Chickens exhibit a moderate sensitivity to AFB1, leading to impaired growth and diminished quality in both eggs and meat [1]. The increasing temperatures and humidity associated with climate change have exacerbated the risk of AFB1 contamination in food and feed [2]. The application of mycotoxin binders is an efficient method to reduce the absorption of mycotoxins in the gastrointestinal tract (GIT). The aim of this study was to investigate the potential ability of two binders (a tri-octahedral smectite mixed with lignocellulose – SeOx –, and an organic adsorbent currently under registration – ADS –) in reducing the bioavailability and mitigating the adverse effects of AFB1 in broilers exposed to concentrations approaching the EU maximum limits of AFB1 in feed (0.02 mg/Kg feed).
A total of 48 male 18-day-old broiler chickens (ROSS 308) was housed in cages (according to Directive 2007/43) and received water and a standard basal diet (BD) *ad libitum*. The cages were placed in an insulated room with devices to control the temperature, light and humidity and supplied with a linear feeder and a nipple drinker. Broilers were divided into 6 experimental groups (n=8 each) and dietary exposed for 10 days to: BD, AFB1 (0.02 mg/kg feed), SeOx (5 g/kg feed), ADS (5 g/kg feed), AFB1 + SeOx and AFB1 + ADS (approval number = 319508/2017-PR). At the end of the treatment, blood samples were collected to evaluate the serum antioxidant capacity (SAC). Liver samples underwent testing for malondialdehyde (MDA) levels, reduced glutathione content (GSH) and glutathione peroxidase (GPx) and total and mu-class glutathione S-Transferases (GSTs) activities. Hepatic gene expression of antioxidant and biotransformation enzymes, and drug transporters was assessed by qRT-PCR. In addition, AF content was measured in the excreta, collected at the end of the treatments, by UPLC-FD according to the ISO standard method 17375:2006.
Animals exposed to AFB1 alone displayed a significant ($p<0.05$) reduction of SAC by approximately 30% and an increase in liver MDA levels by about 8 folds ($p<0.001$). Both binders were able to counteract such effects. Conversely, statistically significant differences in enzymatic activities and GSH content in liver were not observed. Among the investigated genes, AFB1 significantly ($p<0.001$) induced the downregulation of Nrf2 and the upregulation of CYP2A6. However, both binders reverted the modulation of Nrf2 alone. Finally, broilers exposed to combined diet (AFB1 + SeOx or ADS) displayed a higher AFB1 concentration in the excreta.
These results demonstrate that the selected binders are effective in limiting AFB1 absorption, thus mitigating or even reverting the oxidative stress caused by AFB1 in broilers, even following the exposure for a limited period of time (10 days) at concentrations approaching the EU limits in feed.

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Per- and polyfluorinated alkyl substances content in different roe deer tissues for the biomonitoring of a geographic area in Northern Italy

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Per- and polyfluorinated alkyl substances (PFASs) are persistent and bioaccumulative chemicals linked to adverse effects in humans and animals. Among wildlife, the European roe deer has been recognized as a good bioindicator of environmental pollution, particularly concerning PFASs contamination [1]. This study aimed to assess PFASs concentrations in muscle, liver, and hair samples collected from 40 slaughtered roe deer in a specific geographical area in Northern Italy. Perfluorooctanoic acid (PFOA), Perfluorooctanesulfonic acid (PFOS), Perfluorononanoic acid (PFNA), Perfluorohexanesulfonic acid (PFHxS), Perfluorobutanoic acid (PFBA), and Perfluorobutanesulfonic acid (PFBS) were identified using a validated UPLC-HRMS method. Normality of data distribution was assessed with Shapiro–Wilk test, while comparison in PFASs content between the three matrices was performed with one-way ANOVA test. Significance was set with a p-value < 0.05.
Liver PFOA concentrations resulted significantly higher than in hair, while no differences between liver and muscle were detected with mean values of 0.258 ± 0.829 and 0.069 ± 0.101 µg/kg, respectively. For PFOS, hair concentrations resulted the highest among the evaluated matrices, with a mean value of 0.912 ± 2.258 µg/kg. Both PFNA and PFHxS demonstrated a similar pattern, with the highest content in liver, followed by muscle, and hair. In the latter matrix, PFHxS was not detected in any of the samples. For PFBS, the trend presented by other compound was confirmed with the highest content detected in liver, whilst in the case of PFBA the highest concentration was found in muscle with a mean value of 0.051 ± 0.119 µg/kg. Finally, PFBA was never detected in hair samples. The tendency for PFASs to accumulate in liver is a known phenomenon, which was confirmed for most of the compounds evaluated in this study. The PFBA results are in contrast, but it is possible that this compound, which is the shortest carboxylic PFAS, has a greater capacity of distribution and redistribution in the body and thus greater mobility in different biological matrices. Notably, the different PFASs content in the hair demonstrated the feasibility of this alternative matrix for monitoring the two legacy compounds, PFOA and PFOS, and also proved very useful for other compounds such as PFNA and PFBS. The different result between PFBA and PFBS suggests that their presence/absence in the hair is not directly related to the length of the carbon chain, but could possibly be related to the chemical characteristics of the functional groups. In conclusion, this study confirms the suitability of roe deer biological matrices for PFASs biomonitoring. Further investigations are warranted to deepen the knowledge on PFASs distribution in different biological matrices, increasing the available information to correctly assess the environmental occurrence of these persistent chemicals.

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77° CONVEGNO SISVET

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Lead and rare earth elements determination in bones and soft tissues of Griffon Vulture (Gyps fulvus) from Central Italy

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Rare Earth Elements (REEs) are crucial elements playing a strategic role in several field industries, especially in producing high-tech materials. However, data on REE occurrence in environmental matrices are still fragmentary, especially in European countries, and the establishment of environmental background levels to assess the ecotoxicological risk related to REE exposure is still challenging. Griffon Vultures (Gyps fulvus) is a long-lived scavenger bird classified as 'Least Concern' by the International Union for the Conservation of Nature and currently listed as a priority species by the European Union [1]. Vultures, by their position at the top of the food chain and their long-lasting and strict dependence on human activities, are at risk of accumulating and concentrating potentially toxic inorganic elements in their tissues, representing sensitive and valuable bioindicators of the level of environmental contamination. The present study attempts to investigate REEs and lead (Pb) concentrations in hard and soft tissues of Griffon Vulture (Gyps fulvus) living in the Abruzzo region, Central Italy. Between 2021 and 2024, soft tissue (liver and kidney) and samples of both long (femur and humerus), and flat bones (rib) of 19 Griffon Vultures were collected from carcasses sent to "Istituto Zooprofilattico Sperimentale dell'Abruzzo e del Molise" for anatomopathological investigations. Local authorities provided informed consent for the necropsy of dead animals. Sample preparation was performed by a microwave digestion system with a single reaction chamber, and determination of the presence of inorganic elements was performed by Inductively Coupled Plasma Mass Spectrometer (Q-ICP-MS). All sampled animals showed exposure to Pb, with the highest median concentrations found in bones (humerus>rib>femur; 1.9, 1.8, and 1.5 mg/kg wet weight) compared to soft tissues, thus indicating a lifetime accumulation of this element rather than a recent exposure. REE concentrations varied according to the sampled tissue, with bones showing the highest median concentrations of Scandium (Sc), Cerium (Ce), and Yttrium (Y), whereas soft tissue showed the highest concentrations of Ce and Lanthanum (La). Our results reveal the exposure of Griffon vultures living in Central Italy to Pb, with most of the birds exposed to background lead levels probably derived from both direct topsoil exposure and a transfer between trophic levels. Moreover, this study also determines and quantifies, for the first time, REE concentrations in Gyps fulvus.
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Whole-transcriptomic effect of a weight loss diet on overweight female neutered dogs with or without *Arthrospira platensis* supplementation

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Obesity has been defined as a disease in which excess body fat has accumulated such that health may be adversely affected. Obesity is a well-known concern in pets, and considering the large number of comorbidities related to it, nutraceuticals represent a promising strategy to counteract this disease. A limited number of studies evaluating the whole-transcriptomic effect of weight loss programs, with or without nutraceuticals supplementation, on canine obese patients are currently available. In this study, blood samples were collected at three different time points (day 1, 42 and 84) from twelve healthy overweight neutered female dogs (BCS ≥7/9), and whole-transcriptomic effects were assessed as a part of a twelve-week randomized, double-blinded, controlled dietary trial in which dogs were equally divided into two groups receiving a weight loss diet either with the supplementation of the microalga *Arthrospira platensis* (Spirulina) or with a placebo (tablets of the same weight, size and color as the ones used for the treatment). Total RNA was extracted from whole blood using the Tempus™ Spin RNA Isolation Kit (Thermo Fisher Scientific, Segrate, Milan, Italy). Library preparation and sequencing were performed by Novogene Biotechnology (Cambridge, UK), using a 150 pb approach and Ribo-Zero Globin Kit (Illumina, San Diego, CA) to remove ribosomal and globin RNA. Counting, quality check, trimming and rRNA removal was then performed following a validated pipeline. Kallisto was used for the pseudo-alignment building the index on *Canis Familiaris* reference transcriptome (Ensembl). Kallisto outputs were imported in Rstudio with tximport package; EdgeR and ClusterProfiler were used to identify differentially expressed genes (DEGs; False Discovery Rate<0.05 and absolute Fold Change>1.5) and carry out functional enrichment analysis, respectively. The bioinformatics analysis was structured as four-paired comparison experiments, i.e. one for each experimental condition, adjusting the baseline differences among the patients and comparing each condition to the corresponding day 1. Overall, and worth noting, the diet had a prevailing effect on dogs' transcriptome. Iron homeostasis was one of the main biological processes modulated by the effect of weight loss, underlining how important is the fine-tuning of iron levels in obesity. Indeed, iron plays a pivotal role in adipocytes differentiation, in mitochondrial biogenesis, redox balance and inflammation. Furthermore, we noticed that weight loss caused a transcriptional modulation of genes involved in lipid β-oxidation, ferroptosis and thermogenesis (i.e. *CPT1A, UCP2, ALOX15, MTOR, RPTOR*). As to Spirulina, DEGs partially overlapped the ones regulated by the diet, except for 63 DEGs specifically related to this microalga. Thus, encouraging outcomes were obtained mainly for genes linked to anti-inflammatory and pro-apoptotic pathways (i.e. *CXCL8*, *BCL2L1*). Present data confirm that nutrigenomics may have a considerable role in the veterinary clinical practice, providing possible biomarkers and outcome predictors which may be used to beneficially modulate the clinical response of canine patients suffering from metabolic disease such as obesity.

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Determination of glycolic acid, the major toxic metabolite of ethylene glycol in feline plasma

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Ethylene glycol (EG) finds utility in antifreeze blends, industrial solvents, and various products including detergents, cosmetics, and brake fluid. Toxic effects resulting from EG ingestion, such as severe metabolic acidosis, renal impairment, and central nervous system toxicity, stem not from EG itself, but rather from the formation of several organic acids through EG metabolism, notably glycolic acid (GA) and oxalic acid (1). Diagnosis of EG intoxication by measurement of EG may be unreliable due to rapid clearance of EG. GA has been shown to be present in serum even after all EG has been metabolized or excreted. Several studies have shown the value of measuring GA, but detection of GA has been limited by long analytical turnaround times and by instrumentation such as GC-MS which may not be available on a 24h basis for GA analysis. The aim of this study was to describe a colorimetric procedure for quantitation of GA in plasma of cats exposed to EG. GA was determined in feline plasma using the method described by (2) with slightly modifications. The method involves the quantitative determination of GA using spectrophotometric technique. Five hundred µl of serum, standards or water blank were added into microcentrifuge tubes with 500 µl of 5% trichloroacetic acid, mixed and centrifuged for 5 min; then 100 µl of each solution was mixed with 100 µl of chromotropic acid and boiled for 10 minutes. After cooling samples were transferred into 96 wells microplates and read at 580 nm. The standard curve for GA was linear from 1.0 to 10.0 mmol/L in the colorimetric procedure. The limit of detection was 0.67 mmol/L. Plasma samples of 9 cats with suspected EG poisoning were analyzed by using the colorimetric method two times: one when they arrived at the Veterinary Teaching Hospital of the University of Parma and one after 3 weeks. Intra and inter assay coefficient of variation (n = 10) for the colorimetric were 2 and 3%, respectively. The presence of GA was detected in 5 out of 9 samples with concentrations ranging from 1.09 mmol/L to 2.88 mmol/L. One of the 5 cats died shortly after and was not further analyzed. All the other cats tested negative for the presence of GA after 3 weeks. The presence of EG was investigated in the pet food, organs (they were feed with a barf diet), tap water. Positivity to EG and diethylene glycol was found in tap water; a contamination from the boiler was subsequently confirmed. The colorimetric procedure does not require any chromatographic instrumentation and can be performed in less than 1 h upon receipt of a specimen for EG analysis. The method was determined to be specific for GA. Serum GA levels have been directly correlated with clinical symptoms and mortality in poisoning cases (3). Furthermore, GA has been detected in plasma even when EG is undetectable (3). GA has been acknowledged as the primary toxic agent in EG poisoning, but existing methods do not enable analysis within a clinically relevant timeframe. In conclusion, the colorimetric procedure described offers a rapid and reliable method for quantifying GA in plasma, aiding in the timely diagnosis and management of EG poisoning cases, thereby potentially reducing morbidity and mortality associated with this toxic ingestion.

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77° CONVEGNO SISVET

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Effect of Snail Secretion Filtrate in chronic and acute inflammation

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Ulcerative colitis is a chronic inflammatory bowel disease that affects the colon and rectum. This condition is characterized by symptoms such as bloody diarrhea, abdominal cramps, weight loss, and fatigue, which can significantly impact patients' quality of life. In this model, rats are exposed to a solution of dextran sulfate sodium (DSS) in drinking water, causing damage to the intestinal mucosa, and inducing inflammation like that observed in patients with ulcerative colitis.
Moving on to lung inflammation, we encounter a range of conditions involving inflammation of lung tissues, with symptoms such as cough, dyspnea, and chest pain. In these models, LPS is usually administered directly into the animals' lungs, causing rapid and localized inflammation in lung tissues. LPS (lipopolysaccharide)-induced lung inflammation is another experimental model used to study inflammatory lung diseases, such as pneumonia. We decided to use both models to evaluate the effect of snail slime in acute inflammation conditions, such as in the case of lung inflammation, and in chronic inflammation conditions, as in the case of ulcerative colitis (approvation number 294/2021-PR). We have decided to use snail secretion filtrate (SSF) because it contains compounds with anti- inflammatory effects, such as proteins and polysaccharides, which can help reduce inflammation and associated symptoms. Snail slime also contains natural antioxidants, such as vitamins A, C, and E, which can help combat oxidative stress and protect cells from damage caused by freeradicals.
The experimental model of chronic colitis induced by DSS 2% involves administration of this substance in rat drinking water for a duration of 29 days. From day 0 to day 6 we administered 2% DSS; from day 7 to day 19 we administered 1% DSS; from day 20 to day 29 we administered 4% DSS. This model is widely used because it reflects many aspects of human IBD (Inflammatory Bowel Disease) pathology and can be used to evaluate the efficacy of potential treatments. SSF (300uL) was administered from day 20 to day 29 intrarectally (IR). 24h after the induction of the inflammatory condition, the animals were sacrificed to carry out histological, western blot and immunohistochemical analyses.
In this lung inflammation model, LPS is usually administered intranasally or endotracheally to induce rapid, localized lung inflammation characterized by cellular infiltration, inflammatory cytokine production, and lung tissue damage. We induced inflammation by intratracheal LPS administration. After induction at t=4h and t=8h we performed snail slime administration by nebulization. 24h after the induction of the inflammatory condition, the animals were sacrificed to carry out histological and western blot analyses.
SSF was able to counteract the inflammatory process myeloperoxidase (MPO), IFN-γ IL-6, TNF- α) by strengthening the anti-inflammatory barrier (IL-10).
The results obtained have allowed us to assert that snail slime may be used to attenuate chronic and acute inflammatory processes such as chronic colitis and lung inflammation, respectively.

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Platelet Contribution in Secondary Damage: Insights from an Experimental Rat Model of Ischemia-Reperfusion Injury

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Ischemia, stemming from diminished blood flow and subsequent tissue oxygen deprivation, triggers a cascade of events culminating in ischemia/reperfusion injury (IRI), a condition marked by oxidative stress and inflammation upon reperfusion. This phenomenon, affecting both humans and animals, is characterized by the production of reactive oxygen species (ROS) during ischemia, leading to cellular damage and death. Upon reperfusion, inflammatory reactions escalate, fueled by the release of inflammatory cytokines and mediators. Notably, platelet activation emerges as a pivotal contributor to this process, with platelet activating factor (PAF) playing a prominent role in vascular permeability alteration, neutrophil and platelet activation, and the ensuing reperfusion injury pathology. The action of acetylsalicylic acid (ASA) on cyclooxygenase (COX) determines the inhibition of thromboxane, which consequently prevents platelet aggregation. Anesthesia can also have an impact on intestinal ischemia because it reduces intestinal blood flow, slows or stops intestinal contractions, and increases the risk of intestinal ischemia. In fact, the impact of different anesthetic protocols on the reperfusion damage of intestinal ischemia has been evaluated. The objective of our study was to evaluate the potential of using ASA in the treatment of intestinal IR induced damage in a cohort of XXX rats (Ethics committee approval number:499/2018-PR).

The occlusion lasted 60 minutes, after which the animals were reperfused for 2 hours. ASA was subsequently administered at a dose of 70 mg/kg, then the animals were sacrificed and blood, intestinal and lung tissues were collected. Histological and biochemical analyzes were performed on these samples, measuring the levels of PAF, lactate and inflammatory cytokines such as TNF-a, IL-6, IL-10. The antioxidant action was evaluated by measuring the activity of MPO and MDA. In this study, we demonstrated the potential of ASA as a valuable strategy in treating damage induced by intestinal IR. Our analyses shown that ASA effectively reduces the damage induced by intestinal IR, particularly in both the intestine, lungs and cardiac tissues , by acting on inflammation and oxidative stress and platelet activation. Further studies are needed to explore the potential clinical applications of ASA in IRI, both in human and veterinary medicine.

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Immunotoxicology of endocrine disruptors: effect of chronic exsposure of PFOS

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Endocrine disruptors (EDs) represent a significant concern due to their potential to interfere with the delicate balance of hormonal systems in living organisms. These substances, found in various everyday products such as plastics, pesticides, and industrial chemicals, have been linked to a myriad of health issues, ranging from reproductive problems to metabolic disorders. We aimed to delve deeper into the impact of one such endocrine disruptor, perfluorooctane sulfonate (PFOS), on the immune system. Specifically, we focused on the effects of orally administered PFOS exposure on primary and secondary immune organs, (spleen, thymus, lymph nodes) in CD1 mice (Approvation number P.R. 904/2021). Upon exposure to PFOS, we analysed the alterations occurring in the spleen and thymus and peripheral lymphoid tissues. These organs play pivotal roles in immune function, with the spleen serving as a site for immune cell maturation and the thymus crucial for T cell development. Our results revealed significant tissue damage and structural alterations in the spleen, thymus and peripheral lymphoid tissues, following exposure to PFOS. In addition, we identified alterations in lymphocytic populations (primarily CD3, CD4, CD8, CD25, FOXP3 cells) via flow cytometry. These findings underscore the detrimental effects of PFOS on immune organ integrity and functionality, highlighting the susceptibility of the immune system to endocrine disruption. Such disruptions can potentially compromise immune surveillance, leading to increased susceptibility to infections, autoimmune disorders, and other immune-related pathologies.

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Immunohistochemical analysis of interleukin-17A protein expression in duodenal mucosa of dogs with inflammatory bowel disease

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Both humans and dogs suffer from inflammatory bowel disease (IBD), a chronic nonspecific inflammatory condition of the gastrointestinal tract, in the pathogenesis of which dysregulation of the mucosal immune system plays important role [1]. In human IBD-affected patients, a strong association with increased intestinal expression of interleukin 17A (IL-17A) has been demonstrated [2], to such an extent that a therapeutic approach based on the neutralization of this cytokine has been proposed and developed. Data on the association between canine IBD and IL-17A are, by contrast, conflicting. Indeed, previous studies analyzing the cytokine expression at the protein level in the intestinal mucosa of IBD-affected dogs reported either increased [3] or unchanged [4] IL-17A protein expression in comparison with healthy controls.

With the aim to shed light on the involvement of IL-17A in canine IBD, the present study used an immunohistochemical procedure to re-evaluate the expression and localization of IL-17A protein in archived duodenal mucosal biopsies that had been collected, after informed owner consent, from 49 canine IBD patients during routine diagnostic endoscopic procedures. Specimens of normal intestinal mucosa obtained post-mortem from 12 dogs euthanized for other medical reasons served as controls. Sections cut from paraffin blocks of formalin-fixed biopsies were stained with a primary antibody directed against human IL-17A (Abcam). Immunoreactivity was detected using a biotinylated secondary antibody, an avidin-biotinylated peroxidase complex and the peroxidase substrate diaminobenizidine (Vector Laboratories). By means of a computer-aided imaging system (Leica Application Suite), immunopositive cells were counted in properly selected digital fields of the immunostained sections, and cell counts were compared between the two study groups by the non parametric Mann-Whitney *U* test (with level of significance set at $P<0.05$).

In all of the duodenal samples examined, mononucleated IL-17A expressing cells were present, showing cytosolic positivity, plasmacytoid morphology (consistent with lymphocyte and macrophage phenotype) and scattered distribution mainly in the connective tissue of the lamina propria. However, these cells were very few in numbers, with no significant differences between diseased and control dogs.

In conclusion, our results provide evidence that canine IBD, unlike human IBD, is not associated with increased intestinal mucosal expression of IL-17A, corroborating the existence of differences in the immunopathological basis of the two conditions and the preference accordingly assigned to the term immunosuppressant-responsive enteropathy (IRE) when referring to IBD in dogs [1]. Moreover, given the recent demonstration that IL-17A may exert a protective role in human IBD [5], our findings inspire new potential pharmacological approaches to the treatment of canine IBD (IRE).

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SIRA

77° CONVEGNO SISVET

Stato: INVIATO - ID: 13253

Recurrence of congenital malformations in Bernese Mountain Dogs

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The Bernese Mountain Dog (BMD) is a large breed which was selectively bred in the early 1900s. This breed is predisposed to several diseases, including histiocytic sarcoma and degenerative myelopathy (1). Reproductive challenges, such as dystocia and hypofertility, have been documented (2,3). Although not reported in the literature, breeders are aware of the common occurrence of malformations in BMD puppies. The aim of this work is to gather preliminary information on the incidence and characterization of these malformations in Italy.
Clinical cases: Six Italian BMD breeders reported malformations in newborn puppies across different litters. Dams had to be healthy and previously screened for breed-specific pathologies. The study included 6 litters, born in 2013 (n=1), 2021 (n=1) and in 2023 (n=4), with a total of 38 puppies, 39,5% of which (n=15) presented malformations at birth. All but one malformed puppies (9 males and 6 females) were born alive. Among these, 13 (86.7%) exhibited cleft palate, which was associated with labioschisis in 11 of them (73.3%). Absence or hypoplasia of the external auditory canal was reported in 13 cases (86.7%), which was unilateral in 4 cases and bilateral in 9. Fissures at the level of the nose were reported in 6 cases (40%). Seven puppies (46,7%) were euthanized at birth, five (33.3%) died within the first 24 hours, one puppy died at 15 days and one puppy is alive, 8 months, with reported external auditory canal hypoplasia and deafness. No post-mortem analyses were conducted. The birth weights were not available. The study of the pedigrees provided an inbreeding coefficient (COI) of 1.32% ± 1.30%.
Discussion: This study shows that puppies with characteristic and common malformations are being born in the BMD breed. The breed shows low genetic variability due to the use of few sires (sire effect) and increased inbreeding rates (4), which could predispose to the development of congenital diseases. In this study, the COI, conducted through pedigree examination, is low but the pedigree derives largely from a few male ancestors (n=4) who were mated multiple times. To date, there is no information on the presence of congenital malformations in BMDs. The macroscopic lesions described partially overlap with those seen in humans with Goldenhar syndrome (5), especially the common presence of cleft palate and/or lip, absence/hypoplasia of the external auditory canal, and fissuration at the terminal part of the "nasal rod." This lesions complex has not been described in other dog breeds, except for a resemblance to lesions described in St. Bernards (6). The lack of data such as birth weight and post-mortem examinations does not allow for a complete picture of the condition but this investigation is the beginning of a future study.
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77° CONVEGNO SISVET

Stato: INVIATO - ID: 13311

Testosterone and 17-β-estradiol concentrations in hair of male and female adult dogs

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In veterinary reproduction, hormonal investigations are of pivotal importance. Males and females are characterized by different hormonal patterns, especially for Testosterone (T) and 17β-estradiol (E2), the main sexual steroid hormones. In dogs, the use of alternative matrices like hair instead of classical blood, urine, or feces in hormonal studies was already reported [1], however, less was published about sexual hormones like T and E2 [2]. The present study aimed to evaluate the usefulness of hair, collected only once, to distinguish between male and female adult dogs, on the basis of T and E2 hair concentrations. The study was approved by the Università degli Studi di Milano Ethical Committee (OPBA) with protocol OPBA_117_2022. Hair of 10 male and 10 female Golden Retriever adult dogs (2-6 years old) was collected only once, by shaving an area of about 4 cm2 on the dorsal surface of the forearm and stored in paper envelopes until RIA analysis [2]. In all the females, hair was collected in diestrus (about 40 days after the last estrus). Mean T concentrations in hair were 5.60±1.77 pg/mg in males and 2.22±0.63 pg/mg in females, with a statistically significant difference ($p<0.01$). Mean E2 concentrations were 2.29±0.52 and 2.26±0.42 pg/mg in males and females, respectively, without significant difference. Results agree with previous data from hair in peripubertal dogs [2] and from postpubertal cats [3], in which higher concentrations of T were found in males when compared to females, confirming the usefulness of hair to distinguish between the two sexes on the basis of T concentrations. Another common finding with the previous above-mentioned studies is the lack of significant differences when comparing E2 hair concentrations between male and female subjects. Even if results of the present study were drawn from a small number of dogs, it is possible to suggest that the single T analysis on hair can be useful to distinguish between male and female adult subjects. On the opposite, further investigations are needed to understand the lack of differences between the two sexes on the basis of E2 hair concentrations.

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In vitro effect of steroidal and non-steroidal anti-inflammatory drugs on bovine granulosa cells and impacts on oocyte maturation

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Granulosa cells play a key role in the female reproductive system. These cells are located within the ovarian follicles and are essential for follicular and oocyte development [1].
The ovarian microenvironment lays a delicate equilibrium for reproductive processes, and any disturbances could have profound consequences on fertility.
In veterinary medicine, nonsteroidal anti-inflammatory drugs (NSAIDs) and steroidal anti-inflammatory drugs (SAIDs) are used for several diseases, but the impacts of these drugs on granulosa cells are not well studied despite the need to know in depth their mechanism of action to avoid side effects on the delicate reproductive processes.
Granulosa cells were isolated from follicular fluid aspirated from bovine ovaries collected at the slaughterhouse. Cells were seeded in 24 well plates at the density of 10000 cells/cm^2 and dose-response curves were studied to identify concentration and time of onset of cytotoxicity of Meloxicam (NSAID) and Dexamethasone (SAID). The effects of these drugs were evaluated at 0.5, 5 and 60 µM for Meloxicam and at 0.01, 0.1 and 1.2 µM for Dexamethasone at 4, 8, 12, 24 and 48h of treatment, examining viability by 3-2,5-diphenyl tetrazolium bromide (MTT) and acridine orange (AO).
For Meloxicam, a peak of apoptosis was highlighted at the 4 and 8th hour with the most drastic effects detected at 5 µM. From the analyses carried out with Dexamethasone, cells showed damage at the 4 and 8th hour of exposure to a concentration of 0.1 µM that induced the formation of cellular debris, visible as vacuolar cells of which only the cytoplasmic membrane remained.
To study the effect of these drugs on the rate of *in vitro* oocyte maturation, 4 days before the oocyte maturation, granulosa cells were seeded in 3D at a density of approximately 20000 cells/filter on 1 µm pore size 12-well plates polyethylene terephthalate inserts with standard culture medium. Then, 4-8 hours before the start of *in vitro* maturation, granulosa cells were treated with Meloxicam or Dexamethasone in the apical compartment to allow the release of soluble and non-soluble factors of stressed granulosa cells in the basolateral compartment, where oocyte were cultured in *in vitro* maturation medium for 24 hours. The controls (CTR) were carried out in 12-well plates with granulosa cells not treated with drugs, and in classical 4-well plates without granulosa cells. The rate of maturation was evaluated by emission of polar body after oocytes denuding. Every experiment was carried out in triplicate. Statistical analyses were performed by chi square. Differences were considered statistically significant at $P<0.05$.
The maturation rate of CTR was 84.2±0.04% with or without granulosa cells. The rates of maturation fell to 24.7±0.07% at 4h and 24.3±0.01% at 8h, after treatment with Meloxicam, while to 20.7±1.3% at 4h and 23.5±2.1% at 8h with Dexamethasone, with statistically significant differences compared to the CTR.
These preliminary data, that will be completed with the subsequent embryonic *in vitro* development, are underway to to deepen the understanding of the impact of anti-inflammatory treatments on the reproductive efficiency of cows.
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77° CONVEGNO SISVET

Stato: INVIATO - ID: 13370

Evaluation of the effects of dietary supplementation with Lepidium meyenii (Maca) on the quality of fresh and cooled canine semen

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The use of cooled semen for artificial insemination is significantly increasing. Compared to frozen semen, the use of cooled semen shows high efficiency in breeding management. However, the fertility of cooled sperm is maintained for a maximum of 24-48 hours. Storage at refrigeration temperatures leads to a decrease in semen quality due to an increase in reactive oxygen species (ROS). At high concentrations, ROS can be devastating to cell function due to excessive peroxidation of membrane phospholipids. A promising option to improve long-term semen preservation is the use of antioxidants that regulate ROS levels. Lepidium meyenii (Maca) is a plant that has become popular due to its antimicrobial, antioxidant, and anti-inflammatory activities [1]. Maca is rich in valuable nutrients and secondary metabolites such as macamides, alkaloids and glucosinolates. Macamides and glucosinolates reduce free radicals and protect cells from oxidative stress [2]. The aim of this study was to evaluate the effects of oral Lepidium meyenii supplementation in improving the quality of canine semen and its preservation in refrigerated conditions at 5°C. Forty male dogs were included in the study. The subjects were divided into four groups of 10 dogs each: Subfertile control group, Subfertile treatment group, Normofertile control group and Normofertile treatment group. The dogs in the treatment groups received Maca in their diet in a capsule formulation (75 mg/kg), while the control groups received placebo (starch) capsules. Three semen samples were collected from each subject at three time points of the sperm cycle: immediately before the start of oral supplementation (T0), after 31 days (T31) and after 62 days (T62). Blood samples were taken on each day of semen collection and used to assess testosterone concentrations. Sperm collection was performed after removal of the extragonadal reserve to minimize defects of sperm stored in the epididymis. The ejaculate was fractionated by discarding the third fraction and immediately examining the first two fractions. The semen was assessed for concentration, motility, morphology and membrane integrity. An aliquot of the previously analysed fresh semen was centrifuged and the supernatant seminal plasma was removed to optimise storage at 5°C. The remaining pellet was diluted appropriately with CaniPRO™Chill10 extender. The samples were immediately refrigerated and stored at 5°C. Total motility, progressive motility and membrane integrity were measured at 3 (T0), 24 (T24), 48 (T48) and 72 hours (T72). This study indicate that oral supplementation of 75 mg/kg of Maca extract in dogs can improve sperm parameters, including ejaculate volume, total sperm count, motility, morphology and membrane integrity, leading to improved reproductive capacity. The semen of subjects treated with oral Maca supplementation conserved its parameters for a longer period of time when stored at 5°C compared to the semen of control subjects, demonstrating the beneficial effect of the use of this extract on male fertility.

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77° CONVEGNO SISVET

Stato: INVIATO - ID: 13378

Isolation, in vitro expansion and preliminary characterization of mesenchymal stromal cells from fat tissue of pond sliders (*Trachemys scripta*)

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Mesenchymal stromal cells (MSCs) are of strong clinical interest in veterinary regenerative medicine. Currently, there are no published studies about their isolation and characterization in reptiles. The aim of this work was to evaluate the feasibility of the isolation of adipose tissue-MSCs obtained from male and female pond sliders (*Trachemis scripta)* during a routine neutering. Five fat tissue samples (obtained from five animals) were collected during elective neutering. Fat fragments were processed, and the cell suspension was harvested and incubated at 28°C in a professional humidified incubator. Cell growth rate was characterized by direct cell counting. The cells were successfully differentiated into adipogenic, chondrogenic and osteogenic lineages. Cell phenotype was characterized by RT-PCR and amplicon sequencing of a panel of markers routinely used for mammalian MSCs characterization. Blood cells were used to validate primers for genes not expressed in MSCs. CFU forming ability was evaluated in low density cell cultures. Cells exhibited the capacity to undergo differentiation into adipocytic, chondrogenic, and osteogenic lineages. The cells exhibited the expression of CD105, CD73, CD44, and CD90, while lacking the expression of CD34 and HLADRA. The sequence homology exceeded 98% with sequences reported for the *Trachemys scripta* genome. This is the first study regarding the isolation, in vitro expansion and characterization of reptile MSCs obtained from fat tissue. These preliminary data strongly suggest that the isolation of MSCs-like cells from chelonian fat tissue is feasible, opening new prospective for their potential application in the field of regenerative medicine, for both pet and wild endangered reptile species.

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77° CONVEGNO SISVET

Stato: INVIATO - ID: 13404

Evaluation of canine cryptorchidism using computed tomography

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Cryptorchid testes, characterized by the failure of one or both testicles to descend into the scrotum, pose diagnostic challenge in veterinary medicine. Although ultrasound remains a reliable method for detecting cryptorchid testes, precise localization may often prove challenging. The use of computed tomography (CT) imaging offers a non-invasive and comprehensive evaluation of the anatomical location and characteristics of retained testes, aiding in accurate diagnosis and surgical planning. This study explores the potential of CT in the assessment of retained testes in dogs.
Nineteen CT scans of intact dogs affected by cryptorchidism and presenting no testicular abnormalities were retrospectively enrolled and analyzed. Multidetector CTs (BrightSpeed GE and Optima 540 GE, Milwaukee; WI, USA) were employed for all scans, with the gantry remaining untilted and a slice thickness ranging between 1.25–2.5 mm. CT scan was repeated forty seconds following intravenous administration of a contrast agent (Optiray, Guerbet, Roissy, France), performed with a contrast injector at a dose of 600 mg/kg. Soft tissue settings (WW 300–350, WL 35–40) were utilized for review. Evaluation criteria of retained and scrotal testes included size, shape, margins, and radiodensity, measured with Hounsfield units for both pampiniform plexus and parenchyma before and after contrast agent injection. A ratio was calculated for each dimensional measurement to describe the difference between retained and scrotal testes in unilateral cryptorchid dogs. Statistical analyses, including the Shapiro-Wilk test to assess data distribution, were conducted. Quantitative data of radiodensity were expressed as median and interquartile range (IQR) and comparison between scrotal and cryptorchid testes was performed using the Kruskal-Wallis test followed by Dunn's post-hoc test. Size measurements were compared using the Wilcoxon signed- rank test to detect any differences in dimensions between scrotal and retained testes in unilateral cryptorchid dogs.
CT scan successfully detected cryptorchid testes in all cases. Nineteen cases, fifteen unilateral and four bilateral cryptorchid dogs were identified, with thirteen abdominal and ten inguinal retained testes detected. Notably, in unilateral cryptorchid dogs, the right testicle was more commonly affected (60%). All testicles exhibited ovoid or ellipsoid shape, smooth margins and, in abdominal ones, the localization corresponded to the level between L6-S1 vertebra. No discernible differences were observed in terms of pre- and post-contrast enhancement between scrotal and cryptorchid testes. However, a statistically significant difference was noted in individual size measurements, including length (p=0.001), height (p=0.002), and width (p=0.004), with the retained testes resulting consistently smaller in all dimensions. The height, length, and width ratios were calculated as 0.7 (IQR=0.6 – 0.83), 0.64 (IQR=0.52 – 0.78), and 0.71 (IQR=0.59 – 0.88), respectively.
CT imaging offers valuable insights into the size, shape, and position of retained testes, aiding veterinarians in treatment decisions. Furthermore, CT imaging aid in detecting associated complications that may impact surging planning. Integrating CT into the assessment of retained testes in dogs enhances diagnostic accuracy and improves patient outcomes.

77° CONVEGNO SISVET

Stato: INVIATO - ID: 13412

Antimicrobial resistance in Escherichia coli and Staphylococcus pseudintermedius isolated from breeding bitches housed in kennels compared to household bitches

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Escherichia coli and Staphylococcus pseudintermedius are part of the normal bacterial flora of dogs. E. coli normally lives in the intestine while S. pseudintermedius is a commensal of the dog's skin. However, both can cause diseases which are treated with antibiotics. Their high prevalence in healthy animals makes them suitable for studying antimicrobial resistance. As antimicrobial misuse can occur in breeding kennels, bacteria may develop resistance to the most commonly administered antimicrobials [1]. The aim of this study was to take the two bacteria species as indicators of antibiotic administration degree in dog breeding kennels, by comparing their resistance profiles with those of bacteria strains isolated from a population of privately owned dogs living in a domestic environment.
The investigation included two groups of healthy animals (25 bitches from 5 Piedmontese (Italy) breeding kennels and 25 privately owned bitches selected from patients of the Veterinary Teaching Hospital of the University of Turin (Italy) or from private practices), that had not received any pharmacological treatment at least in the last month. A perivulvar skin swab and a rectal swab were taken from each animal to maximize the chances of isolating the two bacterial species. The samples were sent to the Istituto Zooprofilattico Sperimentale delle Venezie for processing. Bacteriological exams included: i.standard and selective media to highlight methicillin-resistant staphylococci and cefotaxime-resistant E.coli (suspected Extended Spectrum Beta-Lactamase-producing strains, ESBL); ii. species identification by MALDI-TOF MS; iii.Minimum Inhibitory Concentration (MIC) to evaluate sensitivity to a range of antimicrobials ;iv. phenotypic confirmation of ESBL E.coli. Comparison between antibiotic resistance rate, presence of ESBL and MR S.pseudintermedius isolates, as well as presence of multidrug-resistant strains in breeding kennel and privately owned animals was performed using Fisher's exact test.
From the 25 rectal and 25 cutaneous swabs of breeding bitches, 41 Escherichia coli and 9 Staphylococcus pseudintermedius strains were isolated; 32 and 11, respectively, from the swabs of household animals. The percentage of resistance towards the tested antimicrobials was generally higher in dogs from breeding kennels than in owned animals, with statistically significant differences in E. coli for doxycycline ($P=0.0026$), tetracycline ($P=0.0004$), trimethoprim/sulfamethoxazole ($P=0.003$), cefazolin ($P=0.0002$), cefpodoxime ($P=0.0005$) and cefovecin ($P=0.0005$). Furthermore, the prevalence of ESBL E. coli strains was significantly higher in breeding dogs ($P<0.0001$), as was the prevalence of multidrug-resistant (i.e resistant to 3 or more antimicrobial classes) E. coli isolates ($P=0.0136$). Similarly, breeding kennels showed a higher number ($P=0.0498$) of methicillin-resistant strains of S.pseudintermedius, although there was no corresponding increase in the presence of multidrug-resistant S.pseudintermedius strains.
The generalized higher resistance profile of E.coli and S.pseudintermedius strains isolated in breeding kennels reveals a wide use of antimicrobials, although the distribution of resistant strains suggests a disomogeneous situation among kennels. Antibiotic administration in breeding facilities should not be a substitute for good management and appropriate structures.
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USE OF AN INNOVATIVE DEVICE FOR ENDOMETRIAL CYTOLOGY AND INTRAUTERINE INFUSION

Di Spigno F. [1], Madrigali A. [1], Paoli D[1], Rota A. [1], De Maria C. [2,3], Coro F. [2,3], Fortunato G. M. [2,3], Gori E.[1], Fanelli D. [1], Panzani D.[1,3]
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The aim of this study was to evaluate the cervical catheterization time and the endometrial cell smears quality obtained using cytobrush in dairy cattle by comparing three different instruments. This study was conducted at the AgroEnvironmental Research Center 'Enrico Avanzi' of the Pisa University as a non-experimental clinical trial (resolution 39/2022) within the framework of the Agritech National Research Center Project (PNRR) and the Call for Technological Demonstrators (Rectoral Decree UNIPI 53/23 of 01/12/2023, protocol 3421). Eight pluriparous Holstein cows, beyond 150 days postpartum, were subjected to endometrial cell sampling during diestrus for three cycles randomly using one of three different instruments each cycle: a Minitube commercial device (Minitube®, Germany; M), a modified insemination catheter (IA)[1], and the CatCet (patent application number: 102024000000165 of 01/05/2024). After confirming diestrus stage through ultrasound examination (IMV Easy Scan Go®, IMV Technologies, France), sampling was performed by inserting one of the 3 devices into the vagina until the vaginal cervical os, after careful perineal washing with povidone iodine,. From this point, the attempt to catheterize the cervix via transrectal taxis was timed for a maximum of 5 minutes. After sampling, the cytobrush was immediately smeared on a slide, stained (Diff-Quick®), and microscopically evaluated using quality parameters (cell density, cell morphology quality, and red blood cell contamination) as described and scored by Kusaka et al. 2020 [2]. When using the CatCet, before retracting it from the cervix, 60 ml of fluid were infused into the uterus, immediately followed by ultrasound to ensure successful uterine repletion, which always occurred successfully. Catheterization times for the three groups were evaluated using repeated measures ANOVA GLM with Tukey's post hoc test, employing the catheter type as a factor and the animal as a random factor. The smear quality parameters were assessed using the Wilcoxon Signed rank test.
Contrary to the CC and IA methods, it was never possible to catheterize the cervix within 5 minutes and perform sampling with the M method. In 1/8 and 0/8 IA and CatCet samplings respectively, the cytobrush remained inside the cervix and had to be recovered so the procedures was repeated the next diestrus. The results obtained for the time variable were 70±77 and 56±55 seconds for the CatCet and IA groups, respectively (Mean ± SD; $P>0.05$). The median (IQR) of the evaluations regarding cell density, morphological quality, and red blood cell contamination were: 3.5 (4-4) and 4.0 (2-4); 2 (2-2)^{a} and 3 (3-3)^{b}; 1.5 (1-3.5) and 2 (1-3.5) ($^{a\neq b}$: $P<0.03$), respectively.
Catheterization of the cervix was possible with the IA and CC methods without significant differences in sampling times, probably thanks to their rigidity, reduced diameter (5mm and 6mm respectively), and shape.
Cytological evaluation was possible in both cases although differences in cell quality were observed between the two techniques [2]. Therefore, the CatCet proved to be a valid tool for atraumatic catheterization of the uterine cervix and useful for both assessing endometrial health and infusing fluids into the endometrial lumen.

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Incidence of spontaneous ovulation in an intact female cat population

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The queen is classified as an induced ovulation species. Spontaneous ovulation (SO) consists in ovulation without male contact and therefore without mating. The incidence of SO in previously published studies varies between 35% and 87% (1,2). SO may lead to an increased risk of development of progesterone (P4)-dependent conditions (cystic endometrial hyperplasia – pyometra complex, mammary hypertrophy and mammary neoplasia), as the queen will enter a non-gravidic luteal phase post-SO. With this study we set to provision an estimate of the rate of SO in a population of intact queens presented to a veterinary care facility, for both reproductive and non-reproductive reasons.

Blood was collected from adult intact queens presented between January 2020 and June 2023 to the Veterinary Teaching Hospital of the University of Padova, on which serum P4 was assayed AIA-360™ (TOSOH Bioscience, Japan). Serum P4 above 2.0 ng/mL was considered as proof of ovulation. Owners were contacted to obtain information on housing conditions and the possibility of male contact. Serum P4 ≥ 2 ng/mL without male contact was classified as SO. Age, breed and body weight of the queens were analysed.

The population was composed of 29 intact post-pubertal queens, from which 31 serum samples were obtained, presented for non-reproductive (n=16) and reproductive reasons (n=15). Nine/29 queens (31.0%) ovulated spontaneously. From these 9 queens, 2 were presented for pyometra and 1 for mammary hypertrophy. The mean age and weight of the SO queens were 4.3±5.7 years and 3.7±0.8 kg, respectively. One SO was registered at 6 months of age (in the first heat of the queen), making it the earliest SO ever reported. Comprised breeds were European shorthair (n=4), Maine coon (n=3), Bengal (n=1) and Scottish Fold (n=1). The cycle phase of the queens was unknown at presentation. Some queens might have been in anestrus being unable to ovulate, which possibly underestimates the incidence of SO in the intact female cat population.

SO is likely to occur in approximately 1/3 of all intact queens seen in a veterinary clinic while this percentage may increase if only cycling queens are considered. When studied, SO rate is found to be consistently over 30%. Thus, cats should no longer be classified as solely induced ovulators, but as a species in which ovulation can either be spontaneous or induced. Age is not a consistent influential factor, since both young and old queens can ovulate spontaneously. Yet, there is no minimum age, beyond puberty, for it to occur. Weight (heavier queens) and breed (oriental breeds and Maine Coons) seem to be predisposing factors for SO, although further and directed research is needed. Physical and sensorial interaction with conspecifics triggers SO, although these circumstances are not essential for its occurrence. As P4-dependent conditions' risk is increased in queens who ovulate spontaneously, veterinarians should be aware and advise breeders and clients accordingly.

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Clinical use of low-dose megestrol acetate treatment for reproduction control in cats: efficacy, safety and interval from treatment end to resumption of reproductive activity

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Megestrol acetate (MA) is a synthetic progesterone analog marketed in Italy under the name Estropill™, MSD (syrup formulation) for short-term and extended suppression of estrus[1]. This study aims to verify the efficacy and safety of a low-dose MA protocol in preventing estrus in queens and suppressing reproductive behaviors and fertility in tomcats. Furthermore, we set out to investigate the interval between the end of treatment and the resumption of reproductive function in treated cats.
Twenty-one, intact, healthy, adult privately-owned cats (18 females and 3 males) were presented to the veterinary teaching hospitals of the universities of Padova, Italy and Ljubljana, Slovenia for short-term control of reproductive function. Subjects were enrolled in the study and treated daily with 11.5 µgMA/kg orally, corresponding approximately to 5 drops/kg/day. Animals were divided into groups according to treatment duration, decided based on owners' request: 4 (G4: 4 females, 1 male), 5 (G5: 3 females, 2 males) and 6 (G6: 11 queens) months.
The health status of the animals was assured pre-treatment through a complete clinical and reproductive examination, hematology, biochemistry, urinalysis, and reproductive ultrasound. Queens further underwent vaginal cytology and progesterone assay, while tomcats were checked for the presence of penile spikes. All examinations were performed again post-treatment for safety assessment purposes. Monthly check-ups were conducted during treatment, repeating physical examinations, reproductive ultrasound and vaginal smears, to monitor both the efficacy and safety of the protocol. Time until ovarian resumption and weight gain were analyzed through a one-way ANOVA and a Student *t* test for paired samples, respectively (α=0.05).
Seventeen/18 treated queens consistently exhibited behavioral and cytological anestrus while one queen, who showed significant weight gain during treatment, displayed vocalization, lordosis, rubbing and increased affection, probably due to underdoing. Two/3 of tomcats' penile spikes and marking behavior disappeared completely 3 months after treatment with spikes reappearing 100 days post-treatment cessation. The third male showed only reduction in the size of the spikes and continued displaying mounting behavior, successfully impregnating a queen during treatment.
Laboratory and ultrasonographic parameters during monthly checkups and after treatment remained unaltered. All subjects exhibited increased appetite, resulting in weight gain. Weight gain was observed in all treatment groups, being statistically significant in G4 (*p*-value=0.021) and G6 (*p*-value=0.0004). Most queens and all tomcats experienced non-significant weight loss following the end of treatment.
The time until resumption of ovarian activity post-treatment was 50.12±17.08 days, not being significantly different between treatment groups (G4: 42.33±30.08, G5: 49.3±10.21 and G6: 52.45±15.5 days). One queen was considered an outlier due to a prolonged delay in heat return.
The low-dose MA treatment is a safe option for controlling reproduction activity in male and female cats and is highly efficacious in suppressing cyclicity in adult queens for up to 6 months; ovarian function resumes on average about 7 weeks after treatment ends. Efficacy in tomcats needs further investigation. No side effects were observed besides the increase in body weight, that tends to reduce after treatment. The possible case of underdosing due to weight gain suggests that the dosage employed impends on the minimum effective dose of the drug, contributing to a greater safety of treatment.
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OBSTRUCTIVE AZOOSPERMIA IN A MURGESE STALLION: A CASE REPORT

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The breeding soundness evaluation (BSE) aims to evaluate the stallion's mental and physical attitude to deliver viable spermatozoa to the female reproductive tract. This procedure should be performed before the first mating or breeding season, in case of known or suspected subfertility, and yearly to evaluate any change [1]. A thorough BSE should include history, physical examination, libido assessment, and semen evaluation. Further investigation, such as ultrasonography, hormonal analysis, and biopsies may be necessary, depending on the circumstances. Azoospermia is the absence of sperm in the ejaculate. This condition can arise from a defect in spermatogenesis (alterations of the hypothalamic-pituitary-gonadal axis or testicular neoplasia) or ejaculation (occlusion of the efferent ducts). Alkaline phosphatase (AP) is a dephosphorylating enzyme, found in seminal plasma of various species. In horses, ampullae, testes, and epididymis produce AP [2]. AP dosing helps to confirm occlusive azoospermia, as levels above 2500 U/L are reported in the ejaculate of normal stallions. This report aims to present a case of obstructive azoospermia in a stud stallion, diagnosed with AP assessment and treated endoscopically. A 20-year-old Murgese horse is referred for acquired azoospermia. History is negative for reproductive disorders, and clinical examination and ultrasonography of internal and external genitalia do not reveal significant alterations. Semen collection is then performed twice: mating behavior and libido are normal for the species; the volume of the ejaculate is adequate, but the appearance is transparent, and the microscopic analysis highlights the absence of spermatozoa in both samples. The stallion also undergoes bladder catheterization before and after semen collection, and the urinalysis is normal and negative for spermatozoa. AP assay is then performed on seminal plasma, with a value of 30 U/L, compatible with an obstruction of the efferent ducts [2]. A biopsy is performed, detecting the presence of complete germ lines in both testes. Attempts are made to obtain the emission of sperm by performing a transrectal massage of the ampullae and administering 20 IU of oxytocin, before semen collection, with negative results. A resolution is then attempted endoscopically: the horse is sedated and the openings of the vas deferens are visualized using a flexible video-endoscope with a maximum diameter of 10 mm. Ampullae are gently insufflated, and a further transrectal massage is performed, followed by the emission of the spermatic fraction. In the following days, the stallion again shows azoospermia and, therefore, is declared functionally sterile and excluded from breeding. Time afterward, the stallion dies of natural causes, and necropsy and histological analysis of the genital tract are performed. The complete germinal line is evident in both testes, with the presence of spermatozoa in the seminiferous tubules; corpora amylacea are present in both seminal vesicles; the right and left ampullae show ectasic lumen, with the diffuse presence of amorphous and hyaline protein material, multifocal inflammatory aggregates are also evident. Histological findings confirm obstructive azoospermia. Ampullae obstruction is an uncommon pathology, which can affect stallions and jacks, generally caused by the accumulation of spermatozoa. This report highlighted an accumulation of protein and hyaline material which probably did not make resolution possible, as instead in the cases of sperm accumulation described in the literature.

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77° CONVEGNO SISVET

Stato: INVIATO - ID: 13484

Paracrine action of endometrial cells cultured in 3D system in normal or stressed condition on bovine in vitro embryo production

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During preimplantation period, in vivo, the embryo develops in absence of a direct cell contact with the reproductive tract and is dependent on luminal secretions of uterus for its nutrition. The importance of normal embryo–maternal interaction is evidenced by the finding that exposure of ruminant embryos to a suboptimal environment can lead to abnormal embryonic, fetal and neonatal development, illustrating the pressing need to investigate how stressful related events at the time of fertilization and during the first days of life impact on embryo survival. Cows affected by endometritis show a 16% reduction in pregnancy rate and the most significant pathogenic bacteria responsible for uterine infections are E. coli, which produce a lipopolysaccharide endotoxin (LPS) that is present in their cell wall [1].The aim of this study is to evaluate the rate of embryo production analyzing the paracrine communication between healthy or LPS-stressed bovine endometrial cells seeded in an in vitro 3D model. A previous study revealed that exposure of endometrial cells to 10 ng/ml of LPS for 1h represents the most effective dose to obtain an inflammatory response with a significant increase in the expression of IL-1β, iNOS and COX2 compared to untreated cells [2].In our study, embryos were produced with an established protocol of oocyte IVM-IVF and embryo in vitro culture in synthetic oviductal fluid (SOF) based on our previously published procedure [3]. Embryos were generated from 960 oocytes and presumptive zygotes were transferred in SOF in standard 4-well plates until day 5 post fertilization. The day of fertilization, endometrial cells (previously obtained by collagenase digestion from slaughterhouse collected uteri and then cryopreserved at passage 1) were thawed and seeded at a density of 20000 cells/filter on 1 μm pore size 12-well plate polyethylene terephthalate inserts. In this 3D system, endometrial cells located in the apical compartment release soluble and non-soluble factors in the basolateral compartment. At 5 days of embryo culture, one hour before the transfer of embryos in the basolateral compart, endometrial cells were treated with 10 ng/ml of LPS. Then, the LPS was removed by washing cells with fresh culture medium. After that, embryos already cultured in SOF were randomly transferred into the basolateral compartment together with their own culture. Embryo culture continued until the seventh day, when the embryo developmental rate to the blastocyt stage was evaluated. The control (CTR) was represented by culture of endometrial cells without LPS.Statistical analyses were performed by chi-square. Differences were considered statistically significant at $P<0.05$.Our results showed a rate of 34.21±2.83% of blastocysts in CTR, while with LPS, embryo rate fell to 16.67±2.53%, with a statistically significant difference.These results highlight the existence of paracrine communication between endometrial cells and embryos and that exposure of embryos to normal or adverse culture conditions (corresponding to an in vivo suboptimal environment) is crucial in the embryo–maternal dialogue.[1] Sheldon et al. Defining postpartum uterine disease and the mechanisms of infection and immunity in the female reproductive tract in Cattle. BIOLOGY OF REPRODUCTION 81:1025–1032, 2009.[2] Marini et al. Effects of platelet-rich plasma in a model of bovine endometrial inflammation in vitro. REPRODUCTIVE BIOLOGY AND ENDOCRINOLOGY 14, 58, 2016[3] Perrini et al. Secretome derived from different cell lines in bovine in vitro embryo production, REPRODUCTION FERTILITY AND DEVELOPMENT 30, 658-671, 2018.

77° CONVEGNO SISVET
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Presence and localization of the CB1 receptor in healthy canine testis Preliminary results

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The endocannabinoids are natural lipidic molecules that along with their major receptors (Cannabinoid receptor type 1, Cannabinoid receptor type 2 and Transient vanilloid receptor potential) and biosynthetic and degradative enzymes represent the endocannabinoid system (ECS) [1]. Over the years, human and animal studies have provided the presence of ECS in testis [2,3]. In mammalian reproduction, this system plays a role in different physiological and pathological events. [1] and any modification to this system has a negative effect on male reproduction, from germ cell differentiation to sperm functions. In particular, the cannabinoid receptors were demonstrated in Leydig cells, Sertoli cells and germ cells (spermatozoa) implicate their potential in regulating pathways such as spermatogenesis and steroidogenesis [4,5]. In fact, immunohistochemical localization of CB1 receptors in mature and differentiating Leydig cells, spermatids (SPT), spermatozoa (SPZ), and spermatogonia (SPG) of rat testis have also been reported [4,5] with their active involvement in processes like steroidogenesis [6]. The aim of the study was to evaluate the eventual presence and localization of cannabinoid receptor of type 1 (CB1) in dog testis. Testicular tissues were collected from 7 healthy mixed breed dogs (aged 2 to 5 years) admitted to the Veterinary Teaching Hospital (VTH) of the University of Perugia for elective orchiectomy. Testicular tissues were analized by Western Blotting and immunohistochemical techniques. In all of the samples, immunoblot indicated that the anti-canine CB1 antibody tested cross-reacted with the corresponding canine proteins (MW 55 kDa). Immunohistochemical reaction showed moderate to strong diffuse and cytoplasmic positive staining for CB1 receptor of the germinal cells, through the epithelium of the convoluted seminiferous tubules. These results could provide valuable insights to study the possible role of the ECS system under normal and pathological conditions in dogs, which could represent an alternative animal model for comparative human pathology.
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77° CONVEGNO SISVET

Stato: INVIATO - ID: 13504

Evaluation of canine cryptorchidism using computed tomography

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Cryptorchid testes, characterized by the failure of one or both testicles to descend into the scrotum, pose diagnostic challenge in veterinary medicine. Although ultrasound remains a reliable method for detecting cryptorchid testes, precise localization may often prove challenging. The use of computed tomography (CT) imaging offers a non-invasive and comprehensive evaluation of the anatomical location and characteristics of retained testes, aiding in accurate diagnosis and surgical planning. This study explores the potential of CT in the assessment of retained testes in dogs.

Nineteen CT scans of intact dogs affected by cryptorchidism and presenting no testicular abnormalities were retrospectively enrolled and analyzed. Multidetector CTs (BrightSpeed GE and Optima 540 GE, Milwaukee; WI, USA) were employed for all scans, with the gantry remaining untilted and a slice thickness ranging between 1.25–2.5 mm. CT scan was repeated forty seconds following intravenous administration of a contrast agent (Optiray, Guerbet, Roissy, France), performed with a contrast injector at a dose of 600 mg/kg. Soft tissue settings (WW 300–350, WL 35–40) were utilized for review. Evaluation criteria of retained and scrotal testes included size, shape, margins, and radiodensity, measured with Hounsfield units for both pampiniform plexus and parenchyma before and after contrast agent injection. A ratio was calculated for each dimensional measurement to describe the difference between retained and scrotal testes in unilateral cryptorchid dogs. Statistical analyses, including the Shapiro-Wilk test to assess data distribution, were conducted. Quantitative data of radiodensity were expressed as median and interquartile range (IQR) and comparison between scrotal and cryptorchid testes was performed using the Kruskal-Wallis test followed by Dunn's post-hoc test. Size measurements were compared using the Wilcoxon signed- rank test to detect any differences in dimensions between scrotal and retained testes in unilateral cryptorchid dogs.

CT scan successfully detected cryptorchid testes in all cases. Nineteen cases, fifteen unilateral and four bilateral cryptorchid dogs were identified, with thirteen abdominal and ten inguinal retained testes detected. Notably, in unilateral cryptorchid dogs, the right testicle was more commonly affected (60%). All testicles exhibited ovoid or ellipsoid shape, smooth margins and, in abdominal ones, the localization corresponded to the level between L6-S1 vertebra. No discernible differences were observed in terms of pre- and post-contrast enhancement between scrotal and cryptorchid testes. However, a statistically significant difference was noted in individual size measurements, including length (p=0.001), height (p=0.002), and width (p=0.004), with the retained testes resulting consistently smaller in all dimensions. The height, length, and width ratios were calculated as 0.7 (IQR=0.6 – 0.83), 0.64 (IQR=0.52 – 0.78), and 0.71 (IQR=0.59 – 0.88), respectively.

CT imaging offers valuable insights into the size, shape, and position of retained testes, aiding veterinarians in treatment decisions. Furthermore, CT imaging aid in detecting associated complications that may impact surging planning. Integrating CT into the assessment of retained testes in dogs enhances diagnostic accuracy and improves patient outcomes.

77° CONVEGNO SISVET

Stato: INVIATO - ID: 13561

Beneficial effect of a biphasic culture system in 3D Liquid Marble micro-bioreactors on *in vitro* maturation of prepubertal sheep oocytes.

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In vitro embryo production using oocytes recovered from prepubertal donors (juvenile IVEP) has great potential to increase the rate of genetic gain by reducing the generation interval in the logic of sustainable productivity and mitigation of environmental impact in livestock systems. However, in ruminants, the developmental competence of prepubertal oocytes after *in vitro* fertilization (IVF) is lower in comparison to oocytes derived from their adult counterpart. The two main factors limiting the success of the juvenile IVEP are the intrinsic quality of the oocytes and the suboptimal culture systems for *in vitro* maturation (IVM).
In prepubertal goats, maintaining meiotic arrest during a pre-maturation culture phase (pre-IVM) using the physiological meiosis-inhibiting regulator, C-type natriuretic peptide (CNP) [1] plus Estradiol (E_2) prior to conventional IVM enhanced the embryo developmental competence of oocytes after IVF [2]. It has been proved that the use of a polytetrafluoroethylene (PTFE) microbioreactor, which consists of a drop of IVM enclosed in hydrophobic PTFE (Liquid Marble, LM) provided a suitable environment for IVM of prepubertal ovine oocytes [3].
The objective of this study was to evaluate the effect of a biphasic IVM culture system using LM micro-bioreactors on in vitro maturation of prepubertal ovine oocytes.
Cumulus oocytes complexes (COCs) collected from ovaries of slaughtered prepubertal ewes were submitted to IVM in four well Petri dishes for 24h (Control, Ctr) or in a biphasic LM system (6 h pre- IVM with 200 nM CNP and 10 nM E2, plus 18 h IVM) in 30 µL (10 COCs/drop) TCM 199 containing 10% FBS, 0.36 mM sodium pyruvate, 100 µmol/L cysteamine, 2.2 mM calcium lactate, 1 IU/mL FSH, 1 IU/mL LH and antibiotics, at 5% CO2 and 38,5°C. After 6h and 24 h of culture, oocyte nuclear stage was assessed by Hoechst-33342 staining (10 µg/mL in glycerol) and the evaluation of cumulus cells-oocyte communications was performed by analysis of the density of Transzonal projections (TZPs) by phalloidin-rhodamine staining. In matured oocytes reactive oxygen species (ROS) levels and the distribution of mitochondria were quantified, respectively, by H_2DCFDA and MitoTracker Orange staining under a confocal microscope.
Data on oocyte nuclear maturation and mitochondria distribution were analysed by Chi-square test; ROS levels and TZPs density by parametric analysis of variance (ANOVA) using Stata/IC 11.2.
Pre-IVM allowed maintaining oocytes at GV stage at a higher rate as Ctr (25/27, 92.6% vs 6/32, 18.8% respectively;$P<0.05$). After pre-IVM, COCs presented the same density of TZPs than uncultured COCs (0 h), whereas, after 6 h of IVM, the TZPs density decreased ($P<0.05$).
The biphasic LM system increased the percentage of oocytes at MII stage compared to conventional IVM (39/42, 92,9% vs 28/36 77,8% respectively;$P<0.05$) after 24h culture.
ROS did not differ among groups. Mitochondria were organized in small granulations spread throughout the cytoplasm in Ctr MII oocytes whereas heterogeneous medium-large granulations located in the pericortical region of the oocytes were observed after IVM in biphasic LM system.
In conclusion, the biphasic LM system exerted a positive effect on IVM of prepubertal sheep oocytes. Further studies are ongoing to evaluate the influence of pre-IVM and LM culture on oocyte developmental competence.

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Evaluation of uterine pH, temperature and intraluminal pressure in normal and Repeat Breeder dairy cows

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Abstract

Repeat Breeder (RB) syndrome is a major cause of economic loss in the dairy industry and its aetiopathogenesis remains unclear. An alteration of the uterine microenvironment affects conception [1]. The aim of this study was to measure uterine pH, temperature and pressure in both repeat breeder and normal cows, using SmartPill® motility testing system (Medtronic-Milano-Italia), a novel device normally used in human gastroenterology diagnostics. The device is a wireless motility capsule that consists of a rigid polyurethane shell and measures 26.8 mm in length and 11.7 mm in diameter. It contains sensors for pH, temperature and pressure. The capsule was manually inserted into the uterus through the cervix of experimental cows, 10 RB and 10 normal (NB) cows, during the estrous phase, and monitored for 15 minutes. The receiver was positioned over the hindquarter and real-time measurements of temperature, pH and intraluminal pressure were recorded. Potential behavioral signs of pain were also monitored during the experiment. Each parameter was tested for its normal distribution applying both Shapiro-Wilk and Kolmogorov Smirnov tests (SAS, 2011). All data was shown to be normally distributed. The data set was subjected to analysis of variance (ANOVA) using the Generalized Linear Model (GLM) by SAS software. No differences in temperature were observed between groups. NB cows had higher pH values of mean, while the median data, the first and third quartiles, minimum and maximum were higher in RB than NB cows ($p<0.05$). For intraluminal pressure values, the statistical analyses detected significant differences for minimum and first quartile, with values in NB higher than RB ($p< 0.0001$). The device is non-invasive and not stressful for the cows: at no time the pain score exceeded the threshold requiring analgesic intervention. The in vivo study confirmed that RB syndrome is associated with an alteration in the uterine microenvironment, in particular pH and intrauterine pressure levels [2,3]. pH changes in RB cows can be found in subclinical endometritis, which alters the physio-chemical properties of the uterine microenvironment. The RB cows had a higher frequency of contractions, tendentially even stronger, but of shorter duration, than NB cows. These alterations of contractility could be not functional for sperm transport. Further studies should clarify whether these changes are primary or secondary causes for the development of the syndrome.

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Evaluation Of Oxidative Balance And Sperm Quality Following Heat Stress In Dogs

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Abstract:

Heat stress (HS) has negatively influenced animal reproduction performance, in addition to animal well-being and welfare. Among the most significative changes, there are the increase of reactive oxygen species (ROS) causing lipid peroxidation and sperm vitality impairment. In other species is known that oxidative damage can cause sperm dysfunction, such as loss of motility and structural malformation [1]. This study aimed to evaluate the effects of environmental HS on canine quantitative and qualitative ejaculate parameters. Twenty dogs were enrolled for this experiment, in 2022, precisely from May to August. All dogs were owned mixed breeds; 10 were consistently kept indoors with functioning air conditioning systems (thermoneutrality group, TN), while 10 were consistently kept outdoors (heat stress group, HS). All owners were provided with a temperature and humidity monitoring system placed within the indoor space for the TN group and in the outdoor area for the HS group, for the duration of the experiment. Data loggers were set to record temperature and humidity on an hourly basis. Semen and blood samples were collected at 30-day intervals, starting from May (T0). During each control time for semen and blood sampling, the data logger were downloaded to calculate the Temperature Humidity Index (THI). All parameters were subjected to analysis of variance (ANOVA) and significance was set at $p < 0.05$. ROS level and sperm quality parameters indicated important variations due to the effects of environmental heat stress. The parameters of oxidative stress were significative increased in HS groups(TN 75.88 vs HS155.00 U/CARR) $p<0.01$, while antioxidants were reduced (T90 TN 2281.11 vs HS 1445.99 mmol/L) $p < 0.01$. This is indicative of oxidative stress, that is the unbalance between the production of oxidation byproducts and the reduction of antioxidant enzymatic activity [2]. As ejaculate parameters, a significant increase in tail abnormalities (bent and coiled tails) in seminal samples from HS dogs was found. Indeed, cell apoptosis is considered the major consequence of HS, followed by sperm metabolic and structural abnormalities [3] Currently, there are no studies that can substantiate such evidence because, based on our current knowledge, no researcher has yet studied the oxidative stress and the sperm alteration during HS in dogs.

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77° CONVEGNO SISVET

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EXPRESSION OF L-TYPE VOLTAGE CALCIUM CHANNELS IN EQUINE GRANUOSA CELLS: A MOLECOLAR AND FUNCTIONAL STUDY

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The ovary is dependent on pituitary gonadotropins to ensure folliculogenesis which in turn relies on intracellular calcium signaling, induced by follicular stimulating hormone to promote oocyte activation and maturation [1]. The intracellular calcium concentration $[Ca^{2+}]_I$ rise is due in part to L-voltage-gated calcium channels (L-VOCCs). The role of L- VOCCs in the ovarian follicular dynamic has been assessed in the granulosa cells (GCs) of several animals [2] but not in the mare, whose follicular growth is primarily controlled by the changing daylength that drives the reproductive seasonality. This study aims to evaluate the presence, quantification, and localization of L-VOCCs in equine GCs recovered from follicles of different diameters and their involvement in modulating $[Ca^{2+}]_I$ during follicles development. GCs were recovered from follicles of ovaries obtained during the year from a local slaughterhouse. An average of 5±1 mares were analyzed each month. Based on the cyclic periodicity, the number of follicles varied. Follicles were categorized as small, medium, and large [3]. GCs from each type of size-follicle were pooled and processed by a) western blot, to assess the expression and quantify L-VOCCs employing a rabbit affinity-purified primary antibody against the α_1 subunit of L-VOCCs and a biotinylated universal secondary antibody (Vectastain Elite ABC Universal kit, Vector- Laboratories, Burlingame, CA, USA). The positive hybridization was revealed by DAB substrate kit (Vector-Laboratories, Burlingame, USA); b) immunocytochemistry, to localize L-VOCCs by the same primary antibody used in the western blot procedure and a FITC- conjugated secondary antibody. c) fluorescent measurements of L-VOCCs activity and cytosolic Ca^{2+} dynamics employing Fura-2-AM fluorescent dye and Bay K-8644 and Nifedipine, which are agonist and antagonist, respectively, of the L-VOCCs using a QuantiCell 900 integrated image system (VisiTech International, Sunderland, UK). Data were analyzed by parametric tests. Results showed that all categories of GCs expressed L-VOCCs, which, as expected, appeared as a doublet of bands at different molecular weights. Fluorescence immunocytochemistry evidenced positive signals at the surface of GCs. The quantification of the L-VOCC levels showed that small follicles increased their expression in February (January *vs* February, $P<0.05$), just before the beginning of the equine breeding season. Moreover, it has been found that the used agonist and antagonist of the L-VOCCs induced a significant ($P<0.001$) change in the $[Ca^{2+}]_i$ in follicles of all sizes. Large follicles resulted in a greater change in $[Ca^{2+}]_I$ after addition of Bay K-8644 or Nifedipine when compared to the basal condition. The results obtained indicate the existence of a regulatory system of the function of the L-VOCCs associated with the functional state of the follicle during its growth and maturation.

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Effect of intrauterine infusion of Wharton's jelly mesenchymal stromal/stem cell–derived conditioned medium (WJMSCs-CM), after insemination on uterine response in problem mares

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Regenerative therapies have been proposed as a treatment option for endometritis, which is one of the primary causes of impaired fertility in mares.

This study tested the effect of WJMSCs-CM on uterine response in problem mares. Equine WJMSCs were isolated from 3 samples and frozen at passage 3. WJMSCs were thawed and maintained in culture medium (DMEM + 10% FBS) until 80-90% confluence, then cultured with serum-free Ringer Lactate. CM was collected after 24 hours of starvation and cellular debris were removed by centrifugation and then stored at −80°C until use. Twelve mares, aged between 4 and 20 years, that had failed to conceive in the previous breeding season or after at least two insemination attempts within the same season were included. The mares were randomly assigned to control (CTR) or CM group.

Estrous progression was tracked via transrectal ultrasound; when a pre-ovulatory follicle appeared, ovulation was induced and, 24h later, mares were inseminated with cooled semen. The CM group received 7/8 h after insemination an intrauterine infusion of 20 ml of WJMSCs-CM, whereas CTR group was treated with the same volume of Ringer Lactate. A low-volume flush (LVF) was conducted immediately before (PRE) and 12h after (POST) the infusion. An aliquot of LVF was sent for bacteriology, by inoculation in Brain Heart Infusion broth and incubated aerobically at 37°C for 24h. In case of turbidity, broth sub-cultivation in Agar plates was performed for additional 24h at 37°C; arisen colonies were firstly screened by standard rapid techniques and then identified by Maldi Tof Ms. The LVF was centrifuged at 400 g for 5 min, and interleukin (IL)-10 concentration was evaluated by ELISA in supernatants; pellets were smeared and stained with Diff-Quick for cytology: presence of 0–2, 2-5 and >5 polymorphonuclears/high power fields (PMNs/HPF) indicate no, moderate and severe endometrial inflammation, respectively. Pregnancy diagnosis was performed at 14 d and confirmed at 60 d after ovulation.

Positive bacteriology was detected in 5/6 and in 4/6 mares of CTR and CM groups, respectively; no differences were found between PRE and POST samples in both groups. The inflammation score ranged between moderate and severe in both groups before and after treatment, with no significant differences observed (CTR: PRE 2.3 ± 0.8 and POST 2.7 ± 0.5; CM: PRE 2.3 ± 0.8 and POST 2.7 ± 0.5). The IL-10 concentration in LVF differed ($P<0.05$) between PRE and POST only in CM group (1023 ± 158 vs. 1427 ± 188 pg/mL), and a difference between CTR and CM was found ($P<0.05$) in POST samples (1085 ± 342 vs. 1427 ± 188 pg/mL). No differences were found for pregnancy rates of the CM (4/6) and CTR (2/6) groups; while among the mares that were positive for bacteria, a difference ($P<0.05$) was found between CTR (1/5) and CM (3/4) groups.

The uterine infusion of WJMSCs-CM in mares 7/8 h after artificial insemination with chilled semen resulted in an increased concentration of IL-10, an essential cytokine for modulating the immune response. Although no differences were found in bacteriology results 12h after treatment, the higher pregnancy rate after CM treatment in the mares positive for bacteria suggests a potential antimicrobial effect of WJMSCs-CM. Further studies are necessary to confirm these results.

Ethylene glycol intoxication in a pregnant queen.

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Ethylene glycol (EG) exposition is associated with increased risks of spontaneous abortion and subfertility in women and rats. To date, there are few reports of queens intoxicated at the end of gestation (1;2).
A six-year-old Norwegian Forest queen was presented to the emergency room for severe hyperazotemia. In the previous days, 3 other cats living in the same house were admitted to the emergency room with a diagnosis of AKI, hence EG intoxication was suspected.
Besides a significant dehydration, clinical examination was normal.
Venous blood gas analysis showed severe metabolic acidosis with increased anion gap.
CBC was unremarkable, serum chemistry showed severe hyperazotemia (creatinine 11.29 mg/dl; urea 382 mg/dl), hyperphosphatemia (15.03 mg/dl) and increase in serum amyloid A (84.39 µg/dl).
Urinalysis showed isosthenuria (urine specific gravity 1018) and crystalluria of calcium oxalate monohydrate at sediment. Urine culture was negative.
An abdominal ultrasound was performed, which highlighted the presence of two vital embryos approximately 20 days old, with a heart rate of 205 heartbeats/minute. The queen's kidneys were increased in size, with hyperechoic cortices and no signs of pyelitis.
The cat was hospitalized and treated with Ringer Lactate fluid therapy, maropitant, mirtazapine, omeprazole, phosphorus binders. Marbofloxacin was prescribed until urine culture results.
After 9 days the cat was discharged with creatinine of 12.33 mg/dl, urea of 346 mg/dl and phosphorus of 8.58 mg/dl. Therapeutic abortion was refused by the owner.
EG intoxication was confirmed by the presence of glycolic acid in the cat plasma. EG was found in tap water.
Glycolic acid was determined by a quantitative determination using spectrophotometric technique by chromotropic acid reaction.
The cat was serially monitored throughout the pregnancy every 3 weeks.
On the 38th day of gestation, an abdominal ultrasound was performed. Fetuses appeared normal-structured for the estimated age, the kidneys were visible without signs of ultrasound-detectable alterations.
On the 50th day of gestation serum chemistry showed creatinine of 3.3 mg/dl, urea of 141 mg/dl, phosphorus of 8 mg/dl
On the 72nd day of gestation, the cat naturally gave birth to two stillborn fetuses. Labour occurred within the standards.
Necropsy of both fetuses was performed 24h post mortem. Fetuses appeared macroscopically normal. Besides important autolytic processes there were no lesions in histopathology, in particular no lesions or oxalate crystals in kidneys were reported.
Cases of EG poisoning in pregnant cats are rarely reported in the literature. In reported cases, intoxication occurred at the end of pregnancy and the fetuses had ultrasonographic renal lesions and presence of renal oxalate crystals on histopathological examination.
These fetuses had none of the above alterations.
Development of the metanephros starts around day 21 of gestation but the kidney is not fully formed and functioning until the 50th day of pregnancy. Therefore, the absence of renal injury is suspected to be associated with intoxication prior to the period of renal development and functioning.
Although intoxication did not lead to immediate abortion or teratogenic effects, pregnancy was carried out in a hyperazotemic patient with consequent conditions on fetal vitality.

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The endometrial microbiome of the healthy mare during estrus and diestrus

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In veterinary clinical literature, the uterus of a healthy mare has been usually considered sterile or to have a transient non-resident microbiota [1]. However, this opinion was based on the classical culture-based approach, which can miss the great microbial diversity present in a specific environment focusing only on culturable bacteria. With the advent of the non-culture-based sequencing techniques targeting the 16S rRNA gene, the dogma of a sterile uterus has been challenged and it has been shown that the uterus of healthy mares hosts a resident microbiota [2].
The aims of the present study were to describe the uterine microbiome of healthy mares and compare the microbiome between follicular (estrus) and luteal (diestrus) phase.
The study was performed between June and September 2022. Eleven healthy mares were included in this study. During the follicular phase, when a follicle >35 mm, uterine edema, estrus behavior and a relaxed cervix were detected, a double-guarded cytobrush was collected for the analysis of microbiome. The sampling was repeated during diestrus, 6 to 8 days after ovulation. For microbiome analysis, DNA was extracted from cytobrush samples using E.Z.N.A. Soil DNA Kit (Omega Bio-Tek). PCR amplification of the V1–V2 variable region of the bacterial 16S rRNA gene was performed. Amplicons were sequenced on the Illumina MiSeq platform and DNA sequencing data were then analysed to assess the equine microbiome composition. According to metagenomic sequencing, bacteria belonging to 24 Phyla and over 500 Genera were found.
The most abundant Phylum was *Firmicutes* (39%), followed by *Proteobacteria* (26.2%), *Bacteroidota* (15.3%) and *Actinobacteriota* (14.1%). In total, these Phyla accounted for almost 95% of relative abundance. The most abundant genera were *Staphylococcus* (8%), *Acinetobacter* (6.3%), *Sphingomonas* (5.7%), *Corynebacterium* (5.6%), *Streptococcus* (5.5%), *Rikenellaceae* (3.5%), *Clostridium* (3.1%) and *Pseudomonas* (2.5%). Regarding differences between estrus and diestrus, it was observed that during estrus a higher number of bacterial species is present in the uterus and the alpha diversity, measured with Shannon index, is higher (p=0.02), indicating a more diverse microbiome.
The present work is one of the firsts to describe the uterine microbiome of the mare. Interestingly, bacterial genera that are usually considered pathogenic according to classical culture-based technique are actually normal constituents of the uterine microbiota of the healthy mare (e.g. *Staphylococcus, Streptococcus, Pseudomonas*). Furthermore, a higher diversity was observed during the follicular phase. This may be due to the cervix, which is a dynamic organ that relaxes during estrus to allow the entrance of semen and the drainage of uterine fluid while it closes tightly during diestrus. Furthermore, the influence hormones, especially estrogens, that fluctuate during the cycle could affect the reproductive tract microbiome, as observed in other species [3].

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ORCHIECTOMY IN MOUFLONS (*OVIS ARIES*) IN THE FIELD: DESCRIPTION OF THE ANESTHETIC PROTOCOL, SURGICAL TECHNIQUE AND HISTOLOGICAL ANALYSES OF THE TESTIS

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The overpopulation of wild animals may alter the soil and the vegetation composition. Orchiectomy is a useful intervention for birth control and may help in controlling animal populations (1). This study (approval number 20/2022) describes an innovative technique of orchiectomy in mouflons, using a suitable anesthetic/analgesic protocol and an innovative device for the surgical orchiectomy, in field conditions. This study compares two surgical techniques: the first using the classic ligation with absorbable suture thread for ligation of the spermatic cord; the second using Aesculap Caiman® Seal and Cut device, to cut and cauterize the spermatic cord. Eight mouflons, during pre-breeding period were neutered, at Natural Park of the Tuscan Archipelago. For all animals, the anesthesiologic protocol was performed using xylazine (0.1 mg/kg) and an association of Zoletil (4 mg/kg, mixed) injected in the brachiocephalic muscle. After 10 minutes, propofol (2 mg/kg) was administered intravenously and anesthetic maintenance was performed with isoflurane (2). The animals were continuously monitored through multiparametric monitor and all surgeries were performed by the same surgeon. At the level of the scrotal neck an anterior approach was performed using a 3 cm incision of the median raphe of skin and dartos, using a proximal-distal approach. Then the tunica vaginalis proper was incised and the testis were exteriorized. Orchiectomy was performed in the Control Group (C=4 animals) using ligatures with absorbable suture and testicular resection with a scalpel blade. In the Group E (=4 animals), resection of testicular structures was performed with Caiman®. Subsequently, the skin was sutured with detached U-shaped stitches. For each mouflon, the surgical times, the intraoperative nociceptive response, and the intraoperative complications were detected to compare the effects of the two techniques. All the animals were observed for 5 h post-surgery and the day after by the veterinary staff evaluating behavioral changes, as reluctance to move, reduced feed intake, and changes in posture. After that, the animals were released in a large enclosure and monitored by park operators for one week. For histological observations, the testes and spermatic cord of the animals of the two groups were weighed and fixed in 10% neutral buffered formalin, cut transversely along the long axis approximately 3 mm thick, embedded in paraffin wax using an automatic tissue processor. Serial 5 µm sections from all specimens were cut with a 2030 Biocut rotary manual microtome, mounted on Super Frost glass slides and then stained with standard hematoxylin and eosin, PAS, Mallory's Trichrome and Verhoeff. Surgical procedures lasted approximately 13 minutes (± 2 min) for both groups. For all patients, no intra or post-operative complications were reported, and all animals were orchiectomized without side effects. As demonstrated by histological analyses, use of Caiman® has boosted uniform compression of the spermatic cord and a steady hold of the proximal and distal part of the thermal suture without damage the testis. The seal created by the Caiman® allowed an optimal sealing of the spermatic cord, which is very useful in wild animals orchiectomized in the field.

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ANTIOXIDANT ADMINISTRATION IN BOVINE PRE-PARTUM: FINDINGS IN POST-PARTUM

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During transition period, the intense metabolic pressure results in a considerable production of Reactive Oxygen Species (ROS), which is not adequately neutralized by organic antioxidant systems (1).
The aim of the study was to evaluate the efficacy of the administration of an association containing vitamin A, vitamin D3, vitamin E, during the last 3 weeks of pregnancy, to improve the reproductive efficiency and oxidative status of cows in the postpartum period. Furthermore, it was evaluated whether this treatment can influence the colostrum quality and the immune status of calves.
The study (ethics committee number 1/19) involved 20 Italian Fresian cows at the end of pregnancy. The cows enrolled were multiparous, aged between 3 and 8 years and with an inter-partum period between 365 and 385 days. The animals were randomly divided into 2 groups: Treated Group (T) composed of 10 cows treated with 10 ml/head of an association containing retinol acetate 100,000 I.U., cholecalciferol 25,000 I.U., alpha-tocopherol acetate 100 mg (Adecon® - Fatro, Italy) and Control Group (C) composed of 10 cows, treated with 10 ml saline (NaCl 0.9%). In all cows the administrations were performed at -21, -14, -7 days before calving.
The calving, pathologies in the immediate postpartum and the reproductive parameters were evaluated. Calves underwent a clinical examination to assess their health status.
An aliquot of colostrum was taken within 2 h after birth to evaluate the quality by means a refractometer.
Blood samples from the coccygeal vein were taken in serum vacutainer tubes at 21 days before calving date (T-21) and 21 days after calving (T+21) for all cows, to evaluate oxidative status and 24 h after birth for calves, to evaluate oxidative status and IgG concentrations, by means a refractometer. The serum obtained was stored in 1.5 mL eppendorf at -20°C until analytical determinations were made. ROS were assayed by colorimetric reaction capable of assaying reactive oxygen metabolites (dROMs) (2). BAP (Blood Antioxidant Potential) was determined with the BAP test using a photometric system (3).
The treated cows showed the first postpartum heat earlier (40.29±15 days) than the C group (46± 13.78 days) and consequently they had a reduced calving interval conception (100.14±42.22 vs 107.86±56.47 days); however these data were not statistically significant. A statistically significant difference ($p<0.05$) was observed for both BAP and dROMs concentrations, in cows Group T compared to Group C, at T+21. The same result was obtained in calves at 24 h after calving. The concentrations of IgG in the serum of calves showed statistically significant differences in Group T than C, while in the colostrum there were no differences.
In conclusion, the administration of antioxidants in the prepartum provides useful support to both cows and calves, to improve oxidative status and the immune system.

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77° CONVEGNO SISVET

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Factors affecting uterine artery Resistivity Index at pregnancy diagnosis in bitches

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The evaluation of uterine artery Resistivity Index (RI) has become common for the assessment of uterine vascularization and canine foetal and placental conditions [1]. During ultrasonographic pregnancy diagnoses, embryonic resorptions can be often observed and were affected by aging of the dam [2]. RI was also studied during induced canine abortion [3], however, the relationship between embryo resorptions and this index remains unclear. The aim of this study was to evaluate if uterine artery's haemodynamic at pregnancy diagnosis is related to embryo resorption rates and other parameters of the mother or pregnancy. This study was approved by the Ethical Committee of Pisa University (no. 36/2022). The ultrasound examinations were performed 25±4 days post-ovulation (between days 21 and 29) with a Toshiba Aplio 400, equipped with linear (7-14 MHz) and microconvex (4,2-10 MHz) probes. During the examinations, animals were kept in lateral recumbency and both uterine horns were scanned to determine the number and the viability of the embryos and the eventual presence of resorption sites. Left uterine artery blood-flow and RI were assessed according to the literature [1, 3]. Breed, age, weight, and reproductive history of the animals were recorded. Forty-four bitches between 18 and 88 months of age, weighting 2.8-39.2 kg, were included for a total of 50 pregnancy diagnoses. Bitches were allocated to different size groups according to their body weight and breed: Medium (M, 10-40.0 kg), Small (S, 5-9.9 kg), Toy (XS, <5 kg). A linear regression was used to model Resistivity Index as outcome and the size group and age of the dam (above or below the median value), litter size (including viable embryos and resorptions), gestational age (days post-ovulation), parity (multiparous vs primiparous), endometrial cystic hyperplasia (present or not) and percentage of resorptions (on litter size) as predictors. Analyses were performed with IBM SPSS Statistics (version 29.0.2.0). Embryo resorptions were observed in 17/50 (34.0%) pregnancies. The overall model was statistically significant (P=0.029) with an R2 index of 0.296. A significant relationship with the response was found for gestational age (P=0.012) and percentage of resorptions (P=0.045). No significant relationships were found for the other parameters. As already reported in the literature, with increasing gestational age RI decreased [1, 3], this despite evaluations were all done in a 9-days window. As the percentage of resorbed embryos in the litter increased, the RI increased as well: mean RI was 0.74, 0.76 and 0.81 with 0%, 10-25% and >25% resorbed embryos. In this study, for the first time a significant relation between embryonic resorptions and RI evaluated at pregnancy diagnosis was found.

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Implementation of bull breeding soundness examination during emergence of besnoitiosis

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Bull breeding soundness examination (BBSE) is a routine practice to evaluate the reproductive fitness of a potential sire. The BBSE includes physical examination and evaluation of internal and external genitalia, evaluation of scrotal circumference, and semen quality as assessed by sperm progressive motility and morphology. The breeder is evaluated as satisfactory, unsatisfactory, or deferred [1]. Different variables may influence overall BBSE satisfaction rates include geographic location and climate, operator, diet, herd environment and management, season, and infectious diseases [2]. Bovine besnoitiosis is a re-emerging parasitic disease in Europe It is a chronic and debilitating disease characterised by both local and systemic clinical signs of varying severity, including reproductive failure. In the last years, 3 outbreaks of bovine besnoitiosis (*Besnoitia besnoti*) have been reported in Sicily, Southern Italy [3]. In 2023, during routine BBSEs, besnoitiosis was diagnosed in 10 bulls in 10 different Limousine farms in central Sicily, within 40 km from each other. In these farms, the herd history reported many cows returning to heat and failure to conceive, despite normal mating behaviour. The bulls were from 2 to 6 years old. At the time of the clinical exam (BBSE), each bull had good health, and a normal body condition score (BCS 3.5- 3.75). Two bulls had small alopecic lesions on the skin. The libido was maintained in each bull. All 10 bulls had a low (30-32 cm) scrotal circumference. The scrotal skin was thickened, and some bull had small crusty lesions. Testicular mobility was maintained. On ultrasound examination, the testis showed several hyperechoic spots, from the parenchyma to the mediastinum classified as low to highly fibrotic. The semen was collected with electroejaculation, and there was azoospermia in 8 bulls and oligospermia in the other 2. Based on these findings, besnoitiosis was suspected as the main differential diagnosis, therefore, a scrotal biopsy punch was performed, and the samples were processed routinely for histopathology. Numerous cysts containing bradyzoitis (compatible with *B. besnotii*) were detected in the subcutis associated with inflammatory infiltrates. Considering the lack of effective drugs or vaccines, bulls were culled and biosafety measures, like treatment against vectors, were reinforced in the farms. In conclusion, within this case series, the integration of BBSE results observations (oligo/azoospermia, reduced scrotal circumference, thickening of scrotal skin, scrotal ultrasound findings) suggested besnoitiosis, and scrotal biopsy, an easy and safe test, confirmed the infection. As the scrotum is an elective site for parasites, it is proposed as a complementary test when besnoitiosis is suspected. Vascular injuries and alteration of thermoregulation are at the basis of the testicular lesions with reproductive failure in the bull possibly being the sole evident symptom within the herd during the onset of a *B. besnotii* infection.

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77° CONVEGNO SISVET

Stato: INVIATO - ID: 13649

Antioxidant effects of the addition of olive extracts in buffalo frozen semen

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The damage to spermatozoa during cryopreservation is attributed to the increase in oxidative stress, due to the thermal and osmotic insults. Therefore, the enrichment of semen extender with antioxidants before freezing has become a common strategy to mitigate cryopreservation-induced oxidative stress in various domestic species. The interest in the use of herbal natural antioxidants has recently increased, attributed to their reduced toxicity, minimal residue, and high content of beneficial compounds such as polyphenols, flavonoids, carotenoids, gallic acid, tannins, and essential oils. The olive fruit extract (OFE) is a blend of natural antioxidants that can synergistically enhance cellular function. This product can be readily obtained from discarded olives, typically wasted during commercialization processes. The OFE contains several phenolic compounds in particular the hydroxytyrosol (HT), that is known for its antioxidant properties, acting as a natural ROS scavenger, reduces oxidation of low-density lipoproteins, protects from hydrogen peroxide-induced cytotoxicity and minimizes the lactate dehydrogenase activity [1]. The composition of buffalo spermatozoa renders them highly susceptible to oxidative damage induced by cryopreservation. In a previous work we demonstrated that the enrichment of the buffalo extender with the addition of D50 OFE, containing 50 μM of hydroxytyrosol (HT), improved post-thaw sperm membrane integrity, the percentages of total live and acrosome-intact live sperm, as well as total and progressive sperm motility [2]. The aim of this study was to investigate whether the observed positive effect on post-thaw semen quality is attributed to the antioxidant properties of OFE. Specifically, we aimed to evaluate the impact of OFE supplementation on oxidative stress markers, such as the levels of biological antioxidant potential (BAP) and Reactive Oxygen Metabolites (ROMs). The OFE was obtained by hot extraction with acidified water and the HT content of 10.8% was obtained by high-pressure liquid chromatography analysis with a calibration line. Ejaculates from 16 buffalo bulls were collected by electroejaculation and each ejaculate was split in 2 aliquots and diluted at 37°C with Triladyl extender, without (control) and with the addition of 50 μl OFE, to a final concentration of 30 × 106 spermatozoa per mL. The semen was frozen in two steps: diluted samples were cooled from 37°C to 5°C (cooling rate 2°C/3min), equilibrated at 5°C for 6 h, exposed to the liquid by nitrogen vapor for 10 min and then plunged into liquid nitrogen. At thawing, BAP and ROMs were evaluated in control and treated semen. Differences between groups were analyzed by Student's t-test. The enrichment of the extender with D50 OFE increased the post-thaw biological antioxidant potential (1616 ±132 vs. 2705 ± 218 μmol/L HClO, in control and D50 OFE groups, respectively; $P<0.01$) and reduced the ROMs levels in sperm seminal plasma (88 ±18.1 vs. 34 ± 6.2 UCARR, in control and D50 OFE groups, respectively; $P<0.05$). In conclusion, this study demonstrates that the improvement in semen quality observed with the addition of OFE to the semen extender before cryopreservation is attributed to its ability to counteract cryopreservation-induced oxidative stress.[1] Cicerale et al. Biological activities of phenolic compounds present in virgin olive oil. International journal of molecular sciences, 11:458-479, 2010.[2] Benítez Mora et al. Influence of olive extracts on buffalo semen quality following cryopreservation. Reproduction, Fertility and Development, 36: 173-174, 2023.

THE IMPACT OF OZONE SUPPLEMENTATION ON MOTILITY PARAMETERS AND BACTERIAL GROWTH ON CRYOPRESERVED EQUINE SEMEN

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Ozone (O_3) is an energy-rich, unstable gas that has been reported to increase the antioxidant response of the treated cells [1]. Ozone generators can produce different concentrations from 1 to 100 µg/ml, however, typically for medical purposes 10 to 40 µg/ml concentrations are used. From a practical point of view, ozone therapy has been proposed as a primary or adjunct therapy for various diseases in both humans and veterinary medicine. Regarding male reproduction, the scientific literature on the use of O_3 supplementation in semen is scarce, and results are still very controversial.
Two studies were conducted to evaluate the use of medical ozone (O_3) as a supplement in commercial extenders for equine semen cryopreservation aiming to evaluate the effects of supplementations of medical O_3 on motility parameters and to determine whether low-concentration O_3 supplementation can reduce bacterial overgrowth in chilled semen in the absence of antibiotics.
The studies were approved by the Body for the Protection of Animals of Pisa University (number 43/2022). In Study 1 (ST1), 0, 5, and 15 µg/mL of O_3 were added to the diluents for chilled and frozen semen of five stallions of different breeds. In Study 2 (ST2), semen was collected from six Standardbred stallions and extended and chilled in an antibiotic-free diluent supplemented with 0, 5, and 10 µg/mL of O_3 or in the same extender containing antibiotics. In both studies, semen samples were evaluated by the Androvision CASA system for sperm kinematics at different time points (H0, H24, and H48) for the chilled samples and after a thermoresistence test for the frozen/thawed samples (after 5-, 15-, and 60-minutes incubation at 37°C). In ST2, semen samples, among the different groups, were also evaluated for bacterial growth after 48 hours of chilling. In ST1 and ST2 no differences were found comparing all the kinematic parameters analyzed among treatments ($P>0.05$) at any time point. In ST2, when antibiotics were added, a smaller number of bacterial colony-forming units were detected compared to samples without antibiotics and without or with different O_3 supplementations ($P<0.05$).
In conclusion, O_3 treatment at low dosages did not affect the semen kinematics but was ineffective in preventing bacterial overgrowth confirming that to date, the addition of antibiotics to the diluent is the only method that can reduce the risk of disease transmission when shipping equine semen for AI. Higher O_3 concentrations should be investigated, to explore the possibility of reducing the use of antibiotics in equine sperm preservation. In case of positive results, an *in vivo* test should be carried out to further investigate the effect of O_3 supplementation of semen diluent on fertility.

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77° CONVEGNO SISVET

Stato: INVIATO - ID: 13663

Ovarian torsion in a pet rabbit (Oryctolagus cuniculus): a case report

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Genital tract disorders rank among the most prevalent conditions affecting female pet rabbits (Oryctolagus cuniculus). However, ovarian diseases are relatively rare in this species, accounting for only 3.3% of reproductive conditions, which include ovarian cysts, neoplasia, and necrosis. Ovarian torsion (OT) is a condition that can affect both humans and animals, although it is less common in the latter, with very few cases documented in the literature. To our knowledge, no previous reports have documented the occurrence of this condition in rabbits. This study presents the first documented case of spontaneous ovarian torsion in rabbits.A female pet rabbit was presented for routine clinical examination, exhibiting mild lethargy but no other remarkable clinical signs. Upon abdominal palpation, a large, nodular, painful mass extending from the right kidney to the pelvic cavity was detected. Blood analysis revealed values within normal ranges. Subsequent abdominal ultrasound (8 MHz) identified a 5.5 x 6.7 cm hypoechoic, non-vascularized mass occupying the majority of the caudal abdomen in the region of the right ovary. No signs of peritoneal reactivity or effusion were observed in the adjacent area of the mass. The uterine horns and the contralateral ovary displayed normal morphology and echotexture. A CT scan further confirmed the presence of a heterogeneous mass exhibiting the Whirlpool sign, characteristic of organ torsion. Given the potential risks and high malignancy of neoplastic conditions of the reproductive tract in rabbits, surgical removal of the mass was deemed necessary.The anesthesia regimen consisted of dexmedetomidine, ketamine, and methadone administered at dosages of 0.025 mg/kg, 20 mg/kg, and 0.2 mg/kg, respectively. Anesthesia maintenance was achieved using 0.5l/h of isoflurane.A midline surgical approach was performed prepubically, and a voluminous brown mass with smooth margins and parenchymatous consistency was discovered. Anatomically, the mass corresponded to the right ovary, exhibiting severe torsion of the ovarian pedicle and ligament, along with twisting of the ovarian vessels. The mass was meticulously excised, followed by neutering. The rabbit underwent a smooth recovery within 24 hours following the surgery. Seven days post-surgery, the rabbit presented again to the clinic in good general condition. Histopathological examination of the right ovarian mass revealed diffuse and extensive necrosis and few epithelial cells, with no evidence of neoplastic transformation. The vessels showed thrombi and necrosis, with fibrin accumulation within the interstice. The contralateral ovary appeared normal, with follicles at various stages of maturity and corpora lutea without mitotic activity.While no neoplastic tissue was identified upon histological examination due to extensive necrosis, a tumoral etiology of the condition cannot be entirely ruled out. Notably, the rabbit did not exhibit any clinical signs of discomfort. However, the torsion involved both the right ovarian pedicle and uterine tubes along the same axis, likely compromising both inflow and outflow from the affected structures. This may have contributed to the absence of overt clinical symptoms despite the severity of the torsion. This study represents the first report of spontaneous OT in rabbit species, even though the primitive cause of the condition in this case remains unknown.

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77° CONVEGNO SISVET

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The uptake by equine endometrial cells of extracellular vesicles of amniotic origin is mediated by fucosylated and sialylated glycans

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Mesenchymal stromal/stem cells (MSCs) secrete extracellular vesicles (EVs) which can transfer proteins, messenger RNAs and microRNAs into target cells. By this mechanism, EVs are involved in cell-to-cell communication during tissue regeneration through the paracrine action of MSCs. Equine amniotic mesenchymal/stem cell EVs (eAMC-EVs) bud from cell membrane [1] that is coated with a glycocalyx that can be transferred to secreted EV surface. It has been demonstrated that eAMC-EVs are internalized by equine endometrial cells (eECs) in vitro and may contribute to prevent persistent post-breeding endometritis in vivo [2]. Since EV internalization mechanism results from their direct fusion or endocytic uptake by target cells, the interaction between EV surface glycoproteins and recipient cell's surface receptors is essential for this process [3]. Lectins are carbohydrate-binding proteins that recognize specific glycans. Reports have highlighted the involvement of several sugar residues, such as sialic acid (NeuNAc) and fucose (Fuc), in the uptake of EVs. This study aimed to investigate the effects of de-fucosylation and de-sialylation on the glycopattern of eECs and eAMC-EVs on the uptake of these EVs. Equine AMC-EVs were isolated by ultracentrifugation of conditioned medium obtained by culture of eAMCs [2]. Equine ECs were derived by collagenase digestion of slaughterhouse-collected uteri and used at passage 1. Aliquotes of eAMC-EVs were incubated with fucosidase or sialidase for 1h, then ultracentrifuged and the resulting pellet was resuspended in DMEM. On the day of the experiment, different treatment conditions were prepared: 1) endometrial cells + EVs; 2) endometrial cells reacted with fucosidase or sialidase + EVs reacted with fucosidase or sialidase with all different combinations. Equine AMC-EVs were used at the concentration of 100x106/ml and, after 24h incubation, the samples were fixed with 4 % (w/v) phosphate buffered paraformaldehyde. Then, the incubation with FITC-conjugated Con A, AAL, MAL II, and SNA lectins was performed. Results show that eECs displayed binding sites for Con A, AAL, MAL II, and SNA evidencing the presence of high-mannose Nglycans, Fucα1,4/l,6N-acetilglucosamine, NeuNAcα2,3galactoseβ1,3N-acetylgalactosamine, and Neu5Acα2,6galactose/N-acetilgalactosamine, respectively. The incubation of eECs with eAMC-EVs resulted in the internalization of these EVs. Desialylated eECs incubated with eAMC-EVs highlighted this vesicle uptake only in eECs containing Con A binders, suggesting Con A-reactive high-mannose N-glycans did not affect the internalization of eAMC-EVs. The removal of sialic acid from eAMC-EVs inhibited the AMC-EV uptake. Defucosylation of eECs provided a negative effect on the uptake of these EVs, as they were never observed within the cells. Concurrently, no signs of internalization were observed when eECs were incubated with defucosylated eAMC-EVs. In addition, the removal of sialic acid and fucose reduced the adhesion properties of the cells. These findings indicate that sialylated and fucosylated glycans are involved in the uptake of eAMC-EVs by eECs.[1] Lange-Consiglio et al. Equine amniotic microvesicles and their anti-inflammatory potential in a tenocyte model in vitro. STEM CELLS DEV., 25: 610-621, 2016. [2] Lange-Consiglio et al. Amniotic mesenchymal-derived extracellular vesicles and their role in the prevention of persistent post-breeding induced endometritis. INT. J. MOL. SCI., 24: 5166, 2023.[3] Islam et al. Lectins as potential tools for cancer biomarker discovery from extracellular vesicles. BIOMARKER RES., 11: 85, 2023.

COMPARISON OF DIFFERENT SITES OF FAT SAMPLING DURING OVARIOHYSTERECTOMY FOR STEM CELL PRODUCTION IN RABBITS.

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Stem cells are derived from a variety of sources: embryonic, bone marrow, and adipose tissue. Adipose derived stem cells (ADSC) are the most accessible cells for the average veterinary clinic. Stem cells stimulate regeneration in bone, joint, tendon, and ligament injuries. [1] Stem cells also act as drug factories churning out potent biomolecules to reduce inflammation and pain. Stem cells can home to areas of injury and inflammation to begin reducing that inflammation and working to heal damaged tissue.[2] Orthopaedic indications are the most common use of stem cell therapy. Stem cells can also be used in corneal lesions and non-healing wounds [3] and can be delivered IV and are therefore a potential therapy for conditions such as inflammatory bowel disease and kidney disease. The aim of this study is standardizing fat sampling during ovariohysterectomy in rabbit and evaluate which is the best sampling site between ovarian fat pad, uterine ligament, and visceral fat. In this pilot study we have taken sample from 6 female rabbits during elective ovariohysterectomy. Each animal was given a standard laparotomy access with an incision on the linea alba. After externalizing the right ovary, a fat sample was immediately taken and and a second sample was taken from uterine fat along mesometrium. At the end of surgical procedure, before closing the abdomen, another sample was taken from the visceral abdominal fat. All samples were placed in a tube containing Dulbecco's Modified Eagle Medium (DMEM) and immediately processed. Results show that ovarian samples were 0.11±0.02 gr, uterine fat samples 0,22± 0.05 gr and abdominal visceral fat samples 1.45 ±0.27 gr. Stem cells were isolate from 6 visceral fat samples and from 2 uterine fat samples. In 4 uterine samples and 6 ovarian samples stem cell culture was not possible due to epithelial cell contamination. In this study, the cut-off for considering a sample positive was to have more than 1000000 cells within 2 weeks of collection. All abdominal visceral fat samples reached cut off but only one uterine sample reached it. In our study, visceral fat proved to be the best sampling site for stem cell production. Uterine fat samples can also be used to produce stem cells only in particularly overweight subjects. The achievement of therapeutic dose of stem cells is also directly proportional to the size of the sample. In our case series, the most frequent complication encountered was the contamination of the stem cell culture by epithelial cells, probably related to the nature of sample taken. In conclusion, fat sampling from the abdominal visceral fat during elective ovariohysterectomy in rabbits is simple and effective as long as the sample is not contaminated from epithelial cells and adequate in size.

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77° CONVEGNO SISVET

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Effect of laparotomic and laparoscopic elective ovariectomy on inflammatory response in dogs

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Gonadectomy is the most common option for spaying female dogs. After any tissue injury, including surgical procedures, the acute phase response occurs, characterized by several systemic effects. This includes changes in the concentrations of blood proteins known as acute phase proteins (APPs). The major APPs, such as C-reactive protein and serum amyloid A, typically exhibit an early and significant increase in concentration followed by a rapid decline in dogs. Most positive acute phase proteins are glycoproteins primarily synthesized by hepatocytes in response to stimulation by proinflammatory cytokines, subsequently released into the bloodstream [1]. Cytokines play a pivotal role in orchestrating the inflammatory response across various conditions. Interleukin (IL)-6 is recognized as one of the most important pro-inflammatory cytokines, released in response to infection, trauma, and neoplasia. In dogs, serum levels of IL-6 have been found to be markedly increased during an acute phase response [2]. It has been hypothesized that cytokine assays could be utilized to quantify the induced systemic response to infection or inflammation. The objective of this study is to objectively evaluate the inflammatory response in laparoscopic ovariectomy (LO) and conventional open ovariectomy (OO) in dogs by measuring the serum levels of IL-6. Twenty adult healthy intact female dogs in need of elective gonadectomy were divided into two groups: 10 underwent LO surgery using a 2-portal technique with linear abdominal access, while the other 10 were subjected to conventional OO. For each animal, blood samples were collected from the cephalic vein using a 22-gauge needle and then placed into plastic serum tubes. The samples were allowed to clot at room temperature for 15 minutes before being centrifuged at 3000×g for 10 minutes. Serum levels of IL-6 were evaluated by ELISA test at four time points: 1 hour before ovariectomy (T0), 2 hours after surgery (T1), 24 hours after surgery (T2), and 7 days after surgery (T3). The analysis involved plate preparation and assay procedures conducted in accordance with the manufacturer's ELISA protocol. Subsequently, the microtiter plate was read using a microlitre reader at 450 nm (with a correction wavelength of 540 nm).The serum concentration of IL-6 did not show significant differences between the LO and OO groups at T0 and T1. However, at T2 and T3, the IL-6 serum concentration significantly increased ($p < 0.05$) in the OO group compared to the LO group. The obtained results confirm that IL-6 can serve as a valid biomarker of the acute inflammatory response after surgery in dogs. Despite the recognized inflammatory effect of CO2 in the pneumo-peritoneum, the inflammatory response appears to be lower in the laparoscopic surgical approach compared to open surgery. These findings validate mini-invasive ovariectomy as the preferred procedure, given its ability to minimize the inflammatory perioperative response.1) Rajabi et al. Effect of direct therapeutic ultrasound exposure of ovaries on histopathology, inflammatory response, and oxidative stress in dogs. BMC Veterinary Research, 19(1), 88. (2023). Med. 1999;41:643–55.2) Ceciliani et al. The systemic reaction during inflammation: the acute-phase proteins. Protein Pept Lett. 2002;9(3):211–23

77° CONVEGNO SISVET

Stato: INVIATO - ID: 13724

Containment of hybrid wolf through surgical infertilization in Life Wolfalps EU project

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Anthropogenic hybridization is widely considered a threat to the conservation of biodiversity[1]. Regarding free-ranging wolves, hybridization with dogs is a condition reported in Europe and Central Italy. In the Alpine area, the phenomenon is potentially manageable for the low number of cases, and prompt intervention is needed to control the presence of hybrid individuals. The dissensus on lethal removal of hybrids represents a clear issue within the scientific community [1]; therefore, action C5 of the Life Wolfalps EU project approved by ISPRA, provides guidelines for fertility control of these subjects. The infertilization procedure, performed at the Centro Animali Non Convenzionali (CANC) of the University of Turin, consisted of the interruption of the reproductive tract (vasectomy or salpingectomy).
Four hybrid-wolves, two males and two females, were catched during the three capture sessions planned in October 2022, April 2023, and October 2023. Premedication on all the subjects was performed by inoculation of thyletamine mixture and zolazepam (Zoletil 100®, 50/50 mg/ml, Virbac S.r.l, Milan) intramuscularly 8.7 mg/Kg, while the induction was performed by intravenous inoculation of Propofol (Proposure®, 10 mg/ml, Boehringer Ingelheim Animal Health Italia S.p.a) 2.5 mg/kg. Maintenance was carried out by isoflurane via tube in pure oxygen and throughout the procedure the animal is remained connected to a multi-parametric monitor (Infinity Delta®, Dräger Italia S.p.A., Corsico, Italy) for intraoperative monitoring of vital signs.
On male wolves laparotomic vasectomy was performed given its minimal invasiveness and very short lead times. On the two females, on the other hand, the minimally invasive salpingectomy method was opted for, involving the opening of three surgical breaches of 3-5 mm, which were then left to heal by second intention, without the application of sutures. The intervention time was longer than the laparotomy technique, but the less invasive allowed a faster post-surgical recovery and a faster release.
At the end of the surgery, a dose was administered cefovecin (Convenia®, Zoetis Italia S.r.l., Rome) at 8 mg/kg, for ensure two-week antibiotic coverage; analgesia was subcutaneously administration of Carprofen (Rimadyl®, Zoetis Italia S.r.l., Rome). The males, equipped with a GPS collar positioned during anesthesia, returned to the center of the territory beaten by the pack within 3 hours of being liberated and he was reunited with the other subjects without being attacked or chased away. The two females are still hospitalized at the “Uomini e Lupi” center of Entraque (CN) for monitoring the sexual hormonal patterns after salpingectomy.
Surgical fertility control is considered an acceptable method for managing wildlife populations, avoiding euthanasia. As wolves show relevant hormone-induced social and hierarchical characteristics, the depletion of hormonal stimuli following gonadectomy would provoke profound alteration in the pattern of the pack. Therefore, orchiectomy and ovariectomy would not be acceptable methods [2]. On the other hand, the interruption of the reproductive tract, preserving the gonads, allows to control the fertility of captured subjects without altering their behavioral characteristics [3], as confirmed by the return to the packs. As the results of this initial phase of the project were encouraging, further capture sessions for the management of hybrids through infertilization will be planned.

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77° CONVEGNO SISVET

Stato: INVIATO - ID: 13849

Preliminary evaluation of the factors affecting lymphocytes in peripheral blood and endometrial tissue of ovariohysterectomized bitch

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The aim of this study was to investigate the trend of lymphocyte subsets in the peripheral blood and the lymphoid phenotypes in the uterine mucosa of bitches that underwent elective or therapeutic ovariohysterectomy (OHE). In veterinary practice, indications about the optimal age for elective OHC it's a long-time topic. It has been a source of debate, and emerging indications are variable: prepuberal-pubescent-adult, due to the importance of sexual hormones during body development, and perhaps because of their influence on the immune system. Local uterine immunity takes part in the development of pyometra, and it is influenced by sex hormones. Little is known about the prevalence of immune cells in the canine uterus compared to the other species in the same conditions (1) and the consequences of ovariohysterectomy on cells of the peripheral blood.
25 bitches of different breeds and ages that underwent elective or therapeutic ovariohysterectomy (pyometra) were enrolled in the study and investigated for the following lymphocyte subsets: B lymphocytes (CD20, CD21), T lymphocytes (CD3, CD4, CD8, TCRγδ) and regulatory T lymphocytes (Tregs) both in peripheral blood and on excised uterine tissue of the same subject and for the % lymphocytes/PBMC in blood samples. Blood samples withdrawn before (T0) and after (T1-T2) surgery were analyzed by flow cytometry (Cytomics FC500©). Healthy and pathological uteri were investigated by immunohistochemistry on frozen sections (the third part of the uterine horns proximal to the ovaries) to evaluate mucosal lymphocytes subsets. For statistical purpose data (%±SD) reported in blood samples and uteri were grouped as follow: T0-T1-T2; healthy (group H; n=19) and pathological (group P; n=6); young (n=5; < 1y) adult (n=15; 2y>age<6y elderly (>7y; n=5) as proposed by Harvey (2021). Statistical analysis (T test) revealed a significative increases (P<0.05 for T2 in %lymphocyte/PBMC (T2=65.87±8.5; T0=46.84±21.05), CD3+ (T2=73.74±3.98; T0=66,55±7.43), while CD21+ decreased between T0 and T2 (T0= 10.88±2.77 T2=16.8±6.1). The comparison between H and P groups revealed statistically significant increases for % lymphocytes/PBMC (T0H= 52.5±19.4 - T2H=67.9±7.2; T0P= 31.83±19 - T2P= 51,13±18); CD3+ (HT0= 67.85±7, T2H=72.80±3; T0P= 63.01±7.8, T2P=79.4), CD8+ (HT0= 21.3±7.06, T2H=27.35±7; T0P= 25.08±6.98, T2P=38±12) and CD21+ (HT0= 16.67±6, T2H=11.82±1; T0P= 17.15±7, T2P=5.3±3). The data comparison by age showed statistically significant differences in %lymphocyte/PBMC with T2>T0 (70.26±4.84 and 51.52±18.94 respectively) for group A , in CD8+ with T1>T0 (55±8 and 24.3±7.5 respectively) for group S and the CD20+ percentage is low in T1 for group S compared with group J and T1 with lower percentage for group J compared with group A. Uterine tissue immunohistochemistry reveled increased values in pathological than in healthy group (P>H) for CD3+, CD8+ and CD20+.
Flow cytometric analysis-age related changes, of CD8+ (T2>T0) lymphocytes are in contrast with literature (2), while for CD3+ (T2>T0) also other author reported the same results (3). In pyometra lymphocytes were upregulated, confirming the inflammatory status, as reported by Bartoskova (1). Based on these results we can affirm that OHE surgery influences the immune system of the bitch in relation with age/health of the patient, but further must be investigated.

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SOFIVET

77° CONVEGNO SISVET

SOCIETA´ SCIENTIFICA DI RIFERIMENTO **SOFIVET**

TITOLO **Exogenous melatonin strengthens fetal-maternal cross-talk by ameliorating uterine microenvironment during the early stage of pregnancy in sheep**

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Testo e Riferimenti bibliografici

Early pregnancy loss is still the current cause of 25% of pregnancy failure in small ruminants due to asynchrony between conceptus and uterine signals [1]. In this context, melatonin plays a crucial role in sheep reproductive dynamic exerting its action by binding MT1 and MT2 receptors. Melatonin implants lessen oxidative stress and increase corpus luteum competence, which prolongs the breeding season [2]. However, little is known about its effect during the peri-implantation period. Thus, we hypothesized that melatonin supports embryo implantation by affecting the uterine microenvironment. This study aims to elucidate the exogenous melatonin effects on the endometrium and early placenta rearrangement, allowing pregnancy proceeding in sheep.
The experiment followed a protocol (PI47/21) approved by the Ethics Committee of the University of Zaragoza, Spain. Ten multiparous Rasa Aragonesa ewes were selected, 5 of which were treated with subcutaneous melatonin implants (18 mg, Melovine, CEVA) 50 days before synchronized and controlled natural mating (CTR: control; MEL: melatonin implanted). On Day 21 of pregnancy, pregnant status was confirmed by transrectal ecoscan, and sheep were euthanized. Blood, endometrium, and conceptus were collected. Plasma progesterone concentration (P4) was evaluated. Embryo viability was immediately checked, and crown-rump length was measured and placenta was fixed for histology and histochemistry. Moreover, placenta and endometrium were stored at -80°C for further gene (angiogenic factors) and protein (MT2) expression analyses.
MEL ewes showed a higher prolificity rate (1.8 vs 2.8 embryos/ewe) and an increase of P4 (2,9 vs 3,6 ng/mL; $p<0.05$) compared to the CTR group. No difference was observed in crown-rump length, notwithstanding the distribution of MEL embryos measurement appeared more restricted ($\sigma 2$ 1.32 vs 0.36). Immunocytochemistry revealed the expression of epithelial-cadherin at the chorionic level in both MEL and CTR placentas. Alpha-smooth muscle detection evidenced placental blood vessels only in the allantois part and no difference in the stage of vessel formation was observed between the two groups [3]. However, MEL placenta consisted of more binucleated trophoblast cells in the chorion region than CTR ($p<0.0001$), and ovine placental lactogen was upregulated in MEL placenta ($p<0.05$). Exogenous melatonin led to higher levels of angiogenetic factors expression (VEGFA, VEGFR1, IGF1R, $p<0.05$) in the caruncular endometrium, whereas no difference was observed either in the intercaruncular region and the placenta. Moreover, IFNAR2 was upregulated in MEL endometrium ($p<0.05$). Lastly, an increase of MT2 receptor mRNA was observed in the endometrium of MEL sheep, as well as in western blot analysis in placenta tissue ($p<0.05$). These findings suggest that melatonin implants differentially act on the uterus and placenta rearrangement. We propose that melatonin drives placenta toward differentiation while promoting vessel maturation at the endometrium level. In conclusion, exogenous melatonin seems to enhance the uterine microenvironment, improving the success of embryo implantation during the early stage of pregnancy in sheep.

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77° CONVEGNO SISVET

Stato: INVIATO - ID: 13119

The circulating microRNAs enter the reproductive landscape of a threatened sheep breed: a sequencing data analysis

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The preservation of locally endangered breeds during the current climate change is essential to the conservation of ecosystems, livestock biodiversity, and typical local products [1]. From this perspective, reproductive fitness becomes a crucial consideration. MicroRNAs (miRNAs) are small noncoding RNA molecules with a key role in the post-transcriptional regulation of a wide range of cellular processes, including development, immune responses and homeostasis [2]. When they are released into extracellular fluids from tissues, where they are synthesized, they are termed circulating miRNAs (c-miRNAs). C-miRNAs might be potential biomarkers, whose profile changes under conditions such as viral and bacterial infections, physiological states (eg. pregnancy) and environmental stressful stimuli [3].
There are several studies on tissue miRNAs' expression variations in ruminant oestrous cycles in the literature, but not many on c-miRNAs. The purpose of this work is to establish a connection between distinctive variations in c-miRNAs' expression and specific oestrous cycle moments in Frabosana-Roaschina sheep, an endangered Piedmontese sheep breed.
For this study, twenty Frabosana-Roaschina pluriparous ewes were synchronized with a standardised protocol, blood was sampled and ultrasound scans were performed during the induced oestrus cycle. Based on plasma hormonal trends (17β-oestradiol and progesterone) and ultrasound images, two moments, the beginning of the follicular phase and the beginning of luteal phase, were identified for analyses. C-miRNAs of 6 selected animals were extracted from blood and sent to be sequenced (Illumina platform, single end 150-bp mode, smallRNA-seq analysis).
All sequenced samples shared seven miRNAs among the most expressed per sample: oar-miR-16b, -191, oar-let-7a, -7b, -7f, -7g and -7i; seven out of twelve samples shared also oar-miR-26b, -29a and -103 among the most expressed per sample. Further analyses on the sequencing data have highlighted twelve c-miRNAs differentially expressed: five miRNAs resulted upregulated, whereas seven miRNAs are downregulated. Some of these miRNAs are reported in literature to be expressed in ovarian cells and related to their endocrine regulation, development, growth and differentiation. Through TargetScan, a list of predicted target genes of these miRNAs was created. Since there isn't an archive for Ovis aries, orthologous miRNAs from Bos taurus and Homo sapiens have been used to perform an enrichment analysis using the Kyoto Encyclopedia of Genes and Genomes (KEGG) and Gene Ontology (GO) databases. The objective of this analysis was to identify putative pathways that might be regulated by the identified c-miRNAs. Numerous different pathways were revealed by this investigation. Several of these can be linked to the regulation of the reproductive sphere: pathways of hormones' signalling (prolactin, oestrogen, oxytocin) from KEGG analysis, response pathways (response to hormone, response to endogenous stimulus) and behavioural pathways as biological processes from GO, pathways related to the circadian rhythm from both KEGG and GO databases.
The investigation of these miRNAs' involvement in the regulation of the oestrous may prove to be useful for deepening the knowledge of physiology and reproductive efficiency in the Frabosana-Roaschina and other breeds.

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77° CONVEGNO SISVET

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Molecular biomarkers and subcutaneous bio-loggers to assess the lambs' adaptive physiological response during transport

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Transportation is one of the most stressful conditions for farm animals [1]. Handling and management of lambs during transport are challenges that perturb homeostasis. The consequent adaptive response with the hypothalamic-pituitary-adrenal axis (HPA) activation attempts to restore balance and welfare conditions. Thus far, the digital revolution has enabled the quantification of animal welfare through the use of parameters analysis gathered by sensors, and this has led to innovative approaches to define the welfare condition of food-producing animals. On the other hand, from molecular biology comes identifying new molecules as biomarkers. Stressors that alter behaviour, physiological parameters, and molecular expression—such as those of microRNAs—quickly activate the HPA axis. These molecules play a role in the post-transcriptional gene regulation of several cellular processes. Expressed by tissues, miRNAs are released in body fluids and therefore called circulating miRNAs (c-miRNAs) [2]. They can be used as minimally invasive biomarkers by changing their profile under physiological, pathological, and psychological conditions [3]. This study aims to evaluate the effects of transport on the Aragonesa breed lambs' adaptive response and welfare status, evaluated in terms of physiological (Heart rate and Body temperature) and molecular (saliva cortisol and c-miRNAs) parameters.
Plasma and saliva samples of fourteen lambs, implanted with subcutaneous temperature (BT) and heart rate (HR) bio-loggers (DST micro-HRT, Star Oddi, Iceland), were collected five times/animal: 2 PRE (T0-24h; T1-4h before loading) and 3 POST (T2-immediately after unloading; T3-4h; T4-24h) a transport of 75 min.
Salivary cortisol concentration was determined with an enzyme immunoassay. Based on their involvement in the adaptive response, 17 c-miRNAs were selected from literature and extracted from plasma and saliva. C-miRNAs' expression analysis was performed in real time q-PCR. BT and HR data were analysed with the Star-Oddi HRT Analyzer software.
Cortisol analysis showed a significant concentration immediately after the unloading procedure (T2) (ANOVA one-way test; $p<0.05$) when compared with T0, T1, T3 and T4 identified as basal undisturbed points (rest time). At T2, lambs also presented a significant drop of BT (38.72±0.01 °C) ($p<0.05$) and a peak of HR (155.14±5.44 bpm) ($p<0.05$).
Out of the 17 c-miRNAs analyzed, 5 were found expressed in plasma and saliva matrices of all sampling points. MiR-17, -23a, and -27a were differentially expressed in T2 only in saliva samples, while miR-24 in both matrices.
Lambs presented the same BT at basal undisturbed points (mean 39.02±0.19), reaching a significant drop at T2 (38.72±0.3) ($p<0.05$), while they reached the maximum HR (bpm) during loading (150.09±4.89) and unloading procedures (T2: 155.14±5.44).
In conclusion, detecting the most stressful times during routine farm procedures can be accomplished by combining molecular and classical biomarkers with physiological data collected by innovative devices like as bio-loggers.

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77° CONVEGNO SISVET

SOCIETA´ SCIENTIFICA DI RIFERIMENTO **SOFIVET**

TITOLO **THE PHYSIOLOGICAL CONDITION OF PIGS IN RESPONSE TO LOADING AND UNLOADING THROUGH RAMPS OF DIFFERENT CONFIGURATION**

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Testo e Riferimenti bibliografici

The pig market weight is increasing in North America due to the improved genetic selection for leaner and faster-growing pigs as well as the benefit of diluting the fixed production costs through the decrease in the total number of pigs needed to produce the same amount of meat [1]. However, this production strategy may result in animals being more vulnerable to the stress of handling around transportation to slaughter, with effects lasting for a long time before animals can return to a complete recovery of their homeostasis conditions [2]. This study aimed to evaluate the physiological condition of 144 pigs of two slaughter weights, i.e., lighter (122 kg on average) and heavier (153 kg on average), during simulated loading and unloading procedures using four different ramp configurations (0° or floor level, 15° and 1.66 m and 2.71 m length, and 25°). The physiological measures included blood lactate concentration, measured immediately pre- and post-handling, and heart rate (HR) values, recorded through an HR monitor (Polar Team 2, Polar Electro Canada, Lachine, Canada), before and during handling, and upon return in the home pen (15, 25, 35 min after the return to their pen). All experimental procedures performed in this study were approved by the institutional animal care committee (approval #565) of the Agriculture and Agri-Food Canada Sherbrooke Research and Development Centre (Sherbrooke, QC, Canada). For statistical analyses, the SAS software (SAS Inst. Inc., Cary, NC) was used. The animal within a group was considered as the sampling unit and the group of pigs was the experimental unit. No effects of the interaction between ramp configuration and slaughter weight was found on the physiological parameters ($P > 0.10$). The greatest blood lactate concentrations were found in pigs negotiating the steepest ramp (25°) and the lowest values in pigs walking at the floor level ($P = 0.02$). Although before the handling test, blood lactate concentrations were not different between the two slaughter weight groups ($P > 0.10$), the lighter pigs presented greater post-handling blood lactate levels ($P = 0.04$). Ramp configuration did not affect the pigs' heart rate increments (ΔHR) during handling and during the post-handling recovery period ($P > 0.10$). The ΔHR during handling were greater in the heavier pigs ($P = 0.03$). Although the HR of lighter and heavier pigs recovered during the post-handling rest period ($P < 0.001$), heavier pigs presented a faster recovery compared with lighter pigs ($P = 0.04$). These findings showed that the use of steep ramps and of pigs of heavier weight may have an impact on the animal physiological condition during handling, increasing the risk of physical fatigue and reduced fitness for transport and slaughter.

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77° CONVEGNO SISVET

SOCIETA´ SCIENTIFICA DI RIFERIMENTO **SOFIVET**

TITOLO **Preliminary results on the alteration of microbiota in lactating Göttingen Minipig sows treated with metformin**

Autori

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Testo e Riferimenti bibliografici

Dysbiosis is a disruption in the composition and function of the microbiota and, particularly, Gut Microbiota (GM) dysbiosis has been associated with the onset and development of several pathologies. Gut dysbiosis in early life is linked to a higher risk of developing immune disease and, as breast milk is the main nourishment of newborns with its own microbiota [1], it is interesting to study whether maternal drug treatment could affect milk microbiota composition directly or via gut microbiota contamination. Metformin is a commonly prescribed drug for gestational diabetes, but it is still unknown whether its use could influence the milk microbiota. The Göttingen Minipigs are the translational model of choice because of their metabolic and physiological similarities to humans and their suitability for lactation studies [2]. The aim of the research is to evaluate changes in the GM and milk microbiota of lactating Göttingen minipigs sows after metformin treatment.
Metformin was administered orally to 4 Göttingen Minipig sows daily from the second week of lactation (authorization n° 35/2023-PR, prot. 2216A.26). The sows received a dose of 500 mg/day during the first two weeks, and a higher dose of 850 mg/day during the third week. Milk and feces were collected together with a cutaneous brush from the mammary gland (as a control for environmental contamination of the milk) and a fecal swab of piglets before the start of treatment and at the end of the third week. DNA was extracted using a previously described protocol with few modifications [3]. The V3-V4 hypervariable regions of the bacterial 16S rRNA gene were sequenced using the Illumina MiSeq platform. Raw sequences were imported into QIIME 2 (v2024.02) after leftover primers were trimmed, and the DADA2 plugin was used to denoise and quality filter the reads and obtain the amplicon sequence variant (ASV) table. Then, ASVs were assigned a taxonomic classification based on the Greengenes2 database (v2022.10). Biostatistical analysis was performed using R software.
The results of the GM analysis showed that metformin treatment tended to increase the phylum Verrucomicrobia and the genera Alloprevotella, Enterenecus, Eubacterium_R and Ornithospirochaeta, while the genera Peptococcus and Ruminococcus_F tended to decrease, although they do not reach statistical significance ($p<0.2$). Despite the small sample number, the observed modifications suggest a possible effect of metformin on the GM. These results will be completed by the analysis of the milk and skin microbiota of the sows and the GM of the piglets, which is in progress.

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77° CONVEGNO SISVET

Stato: INVIATO - ID: 13188

SET UP OF A COMPLEX AND STABLE 3D CELL CULTURE MODEL TO PRODUCE IN VITRO MAMMARY MULTICELLULAR SPHEROID

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Mammary gland is a complex secretory organ consisting of a branched alveolar structure that produces milk, the source of nutrients for newborn mammals. The secretory tissue is composed of different cellular components: the mammary epithelial cells and the stromal/vascular cells, which are arranged in a highly organized three-dimensional (3D) structure for guaranteeing specific functions. Over the past decade, the interest on 3D cell culture models has grown for study the mammary gland in biomedical and veterinary fields [1], but a fully defined in vitro model of mammary gland is lacking. The Göttingen Minipigs is a common breed of minipig widely used for biomedical research, due to small size, genetic stability, good behaviour. The present work is aimed to set up a 3D cell culture protocol to create a mammary multicellular spheroid, using different primary cell cultures isolated and characterized from Göttingen Minipigs (mp): mammary epithelial cells (mpMECs), aortic endothelial cells (mpAECs) and vascular-wall mesenchymal stem cells (mpVW-MSCs) [2,3]. Cells were thawed and cultured in serum-free medium (37°C, 5% CO2) using two different culture methods: the hanging drop (HD) and the low adherence plate (LAP). Cells were seeded at different density (from $5x10^3$ to $20x10^3$ cells/well), in different cell type combinations; the resulting cellular aggregates were observed by means of inverted optical microscope for cells cultured in HD, while for cells cultured in LAP by means of an automated live-cell imaging system (the Incucyte® S3-Sartorius), and experiments were conducted in triplicate. After 24h the cells, cultured in HD, showed the formation of multiple aggregates of various sizes that degenerated after 48h for all the cell density and mixed cell combination tested. The live-cell imaging system pointed out that cells started to self-assembly by forming irregular shape aggregates in mpMECs monocultures, while in co-culture (mpMECs–mpVW-MSCs) or triple co-culture (mpMECs–mpVW-MSCs–mpAECs), the cells formed one spherical compact shape structure that remain stable for at least 7 days as indicated by viability test (Alamarblue® assay). Quantitative analysis of brightfield objects area and eccentricity revealed significant differences between mpMECs monocultures and co/triple cultures confirming the spheroids' formation only with multiple cell types in co-culture. Moreover, viability test resulted in a higher metabolic activity of the cells in co- and triple co-cultures with respect to mpMECs monocultures. In conclusion, in the present research a complex and stable 3D cell culture model was set up to create a mammary multicellular spheroid viable for at least 7 days in the LAP culture, further investigations will be necessary to study hormonal modulation to produce a usefull in vitro model.

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77° CONVEGNO SISVET

Stato: INVIATO - ID: 13201

iNOS AND TSHR IMMUNOLOCALIZATION IN FOLLICLES AND CORPUS LUTEUM DURING THEIR DIFFERENT DEVELOPMENTAL PHASES IN THE BRUNA COWS' OVARIES

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Several researches carried out on both animal and human ovaries have shown the involvement of thyroid-stimulating hormone receptor (TSHR) and inducible nitric oxide synthase (iNOS) in modulating ovarian activity, as folliculogenesis, oogenesis, ovulation and in the corpus luteum (CL) lifespan control [1,2,3]. This study aims to investigate the TSHR and iNOS immunolocalization in both the ovarian follicles and CL, to evaluate whether their developmental phase could modify their expression in cow's ovary. Ovaries were collected from 5 Bruna cows. The estrous cycle phases have been determined post-mortem. Three of them were cyclic (follicular, early luteal and late luteal phases), aged between 12-24 months, two were acyclic (prepubertal and in anaestrous, 10 months and 8 years old, respectively) and the ovaries have been classified based on the presence or absence of the CL, in CL+ and CL- ovaries. The evaluation of the immunohistochemical reaction has been done by quantifying the intensity of staining and classifying it by gradation. The results confirmed the presence of TSHR and iNOS in various structures of the bovine ovary in their different developmental phases. Also, the same structures within the same ovary showed different immunolocalization. Specifically, the presence of follicles in the same developmental phase from the same ovary expressing or not the TSHR and iNOS demonstrated the existence of expression-silencing phases. This suggests for these proteins a pivotal role in the exit from dormancy and, therefore, in the follicular and oocytic development/atresia. The study also demonstrated the presence of the TSHR and iNOS in the CL, in which the expression changed based on the developmental phase, suggesting their role in the growth, maintenance and regression of the bovine luteal tissue. These findings confirmed the involvement of the TSHR and the iNOS-nitric oxide (NO) axis in modulating the cyclic morpho-functional characteristic events of the bovine ovarian structures.

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77° CONVEGNO SISVET

SOCIETA´ SCIENTIFICA DI RIFERIMENTO **SOFIVET**

TITOLO **CAN THE ESTRUS CYCLE PHASE AND THE CORPUS LUTEUM AFFECT THE iNOS AND TSHR EXPRESSION IN THE VASCULATURE OF THE BRUNA COWS' OVARY? A PRELIMINARY STUDY.**

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Testo e Riferimenti bibliografici

Many authors reported the presence of the thyroid-stimulating hormone receptor (TSHR) and inducible nitric oxide synthase (iNOS) in the vessel walls and their putative role in the vasomotor control, proliferation, homeostasis and protection [1,2,3]. The aim of this preliminary study was to investigate the ovarian vascular iNOS and TSHR immunolocalization in order to evaluate whether their expression in the bovine ovary vasculature could be modified during the estrus cycle phases and by the presence or absence of the *corpus luteum* (CL).

Ten ovaries were collected from 5 Bruna cows. The estrus phase has been determined *post-mortem*. Three of them were cyclic (follicular, early luteal and late luteal phases), aged between 12-24 months, two were acyclic (prepubertal and in anestrus, 10 months and 8 years old, respectively) and the ovaries have been classified, based on the presence or absence of the CL, in CL+ and CL- ovaries. The evaluation of the immunohistochemical peroxidase reaction has been done by quantifying the intensity of staining, and classifying it by gradation (from negative to strong positive staining).

Ours results confirmed the presence of iNOS and TSHR in the vessel walls of the bovine ovary, in which the expression changed based on the estrus cycle phases and with the presence or absence of the CL. Also, the vessels within the same ovary showed a different immunostaining pattern. During the follicular phase, in both the CL- and CL+ ovaries, the media of arterial vessels exhibited a different immunolocalization for the iNOS and TSHR, characterized by a stronger expression in the vessels close to the follicles until an absence of immunolocalization in those far away. In addition, the percentage of positive arteries was higher for the perifollicular vessels compared to those farther away, which appeared negative. During the late diestrus, the artery walls in the CL+ ovary did not show any expression for iNOS compared to the CL- ovary vessels, which were positive, suggesting a local negative effect of the ipsilateral mature CL. Instead, the CL effect on the TSHR expression was unclear. During the metestrus, a TSHR vascular expression was present in the CL+ ovary containing the hemorrhagic CL, unlike the CL- ovary vessels which appeared negative, indicating a putative positive influence of this ipsilateral structure.

These results confirmed the involvement of the pathways initiated by the iNOS-nitric oxide (NO) and TSHR axis in modulating the dynamic vascular events accompanying the bovine ovarian morpho-functional changes.

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77° CONVEGNO SISVET

SOCIETA´ SCIENTIFICA DI RIFERIMENTO **SOFIVET**

TITOLO **BIOENGINEERING OVARIES: A LEAP IN VETERINARY FERTILITY**

Autori

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Testo e Riferimenti bibliografici

Utilizing cutting-edge tactics to improve domestic animal reproductive performance or protect biodiversity by halting the extinction of mammalian endangered species is still one of the challenges of the assisted reproductive technologies/ART. *In vitro* recapitulation of early phases of folliculogenesis/*iv*F is one of the most challenging ART perspectives for attracting immature and low competent female gametes for use in reproduction. Although early-stage ovarian follicle cultures were originally developed, their limited translation to *in vivo* systems has constrained their broader use. However, current advances in reproductive tissue engineering/REPROTEN [1] have opened the potentiality of mimic the ovarian stroma environment on which engraft multiple isolated ovarian early follicles by generating transplantable artificial ovary.
The purpose of this study was to assess the ovarian biomimicry of the Poly(epsilon-caprolactone)(PCL) electrospun patterned scaffolds in reproducing a suitable long-term (18 days) microenvironment supporting the transition from preantral (PA) to early antral (EA) follicle stage by recruiting incompetent oocyte towards the final step of specialization (fully grown dimension, ability to resume meiosis and embryo development).
Ovine preantral (PA) follicles transition into early-antral (EA) stage was assessed through a stepwise refinement of the current *iv*F. Firstly, PCL scaffolds were introduced to provide microarchitectural 3D guidance to PA for single-follicle *iv*F (SF-PCL). Then, a multi-follicle culture system with PCL scaffolds (MF-PCL) was tested with the aim to better mimic the native ovarian microenvironment. Morphological and functional endpoints were evaluated, including follicle growth, antrum differentiation, steroidogenic switch off, chromatin configuration, oocyte maturation, and parthenogenetic activation rate.
The synchronous growth of follicles and oocytes was supported by both SF- and MF-PCL systems. However, the MF-PCL exhibited a slower follicular growth rate, a delayed antrum differentiation but a prolonged resilience in long-term *iv*F. Indeed, MF-PCL showed consistently lower degeneration rates (5% vs. 28% of SF-PCL; $p<0.001$) and a higher percentage of antrum cavity differentiation (95% vs. 72% of SF-PCL; $p<0.001$). Furthermore, the follicles transited from PA to EA on MF-PCL group displayed a significant upregulation of the aromatase enzyme (3-fold change PA vs. EA; $p<0.0001$), which was similar to that recorded in EA follicles developed physiologically (EA in vivo).
Notably, MF-PCL 3D long-term culture also improved the developmental quality of oocytes that displayed a more advanced chromatin configuration (53% Chromatin Surrounding-Nucleolus; 27% Surrounding-Nuclear-Envelope vs. 100% Non-Surrounding-Nucleolus of SF-PCL; $p<0.001$). The more advanced specialization of MF-PCL incubated oocyte was finally confirmed for their greater ability to resume meiosis after hormonal stimulation (Metaphase II stage oocyte: 62% vs. 50% for SF-PCL; $p<0.05$), and to be activated by parthenogenesis (> 8 cells embryo: 82% vs. 28% for SF-PCL; $p<0.001$). Of note, MF-PCL was effective in bypassing the embryo blockage (8 blastomeres stage).
PCL-scaffolds have demonstrated their capability to effectively mimic the natural ovarian environment, fostering the growth and development of multiple follicles.
This innovation, coupling the use of biomaterials and the ovary reconstruction, could lead to substantial advancements in the fields of animal reproduction and wildlife conservation, offering new strategies to enhance fertility and to preserve and use diverse genome materials.

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The inhibition of Epithelial-mesenchymal transition enhances pro-regenerative properties of ovine amniotic-derived stem cells

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Amniotic epithelial cells (AECs) represent a promising subset of placental stem cells renowned for their remarkable regenerative and anti-inflammatory capabilities. However, AECs physiologically undergo significant changes during pregnancy under the dynamic influence of reproductive hormonal framework. Specifically, in the ovine species a favorable endocrine influence for the preservation of AECs function during the five months of gestation is exerted by the high levels of progesterone (P4). Yet, as pregnancy ends, the decline in P4 levels and the subsequent rise in estrogens and TGF-ß contribute to weakening the amniotic epithelial layer, triggering the mechanism leading to delivery. Notably, the inversion of systemic steroids' ratio and the local release of TGF-ß are both responsible for the epithelial-mesenchymal transition (EMT).
Recent studies from our group have also demonstrated that AECs spontaneously undergo EMT and that P4 supplementation was able to prevent epithelial cells to shift towards mesenchymal phenotype [1, 2]. Following these premises, the aim of the present study is to assess whether the plasticity to undergo EMT that AECs showed during pregnancy could also be exploited in clinic to activate *in vitro* and *in vivo* pro-regenerative mechanisms.
To this aim, we compared P4-treated AECs – in which epithelial phenotype was retained (hereafter referred as eAECs) – with AECs spontaneously underwent EMT that have acquired mesenchymal phenotype (hereafter referred as mAECs). In particular, we assessed stemness, motility abilities, and anti-inflammatory properties of these two phenotypes of AECs. As a result, P4 exposure induced an increased expression of stemness genes *Oct4*, *Sox2* ($p < 0.05$) and *Nanog* ($p < 0.01$) in eAECs, evaluated by RT-qPCR. The increased stemness genes expression was also correlated to an enhancement of eAEC differentiation towards osteogenic lineage *in vitro*, as demonstrated by the Alizarin red staining. Moreover, P4-treated eAECs exhibited a collective, rather than individual type of migration in Wound healing assay, which has been associated to an improved regeneration ability. In addition, P4 potentiated the anti-inflammatory properties of eAECs compared to mAEC, as evidenced by an increased inhibition of PHA-induced leukocyte proliferation and macrophage activation both subjected to the culture with eAECs- and mAECs-derived conditioned media. In particular, leukocyte proliferation was assessed by MTS assay while and macrophage activation by dosing IL-6 release with ELISA. Finally, the enhanced pro-regenerative attitude of P4-exposed eAEC was further confirmed by using an ovine preclinical model of experimental-induced Achilles tendon lesion [3]. In fact, tendon explants transplanted with eAECs showed an advanced tissue regeneration and a minor involvement of inflammation compared to the tissues transplanted with mAECs.
In conclusion, our data demonstrate that P4 supplementation not only recreates a physiologically suitable environment for *in vitro* AECs cultures and expansion but also preserves and enhances their regenerative properties which can be effectively exploited *in vivo*. These findings hold significant implications for advancing AECs-based therapy and a proof of concept for translational medical applications.

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77° CONVEGNO SISVET

Stato: INVIATO - ID: 13224

Catalase Inhibition Enhances Protein Phosphorylation during Horse Sperm Capacitation

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Sperm capacitation, a crucial process for successful fertilization, is modulated by reactive oxygen species (ROS) and involves various molecular transformations including membrane fluidity enhancement, cholesterol depletion, lipid raft aggregation, and protein tyrosine phosphorylation [1, 2]. An effective equine in vitro fertilization (IVF) protocol has been recently described, but the 22-hour capacitation requirement limits its application to fresh spermatozoa from highly fertile stallions [3]. Stallion spermatozoa are endowed with endogenous antioxidants like catalase, superoxide dismutase and glutathione peroxidase, which can mitigate ROS and therefore delay ROS-mediated capacitation. Here, we investigate whether 3-amino-1, 2, 4-triazole (3-AT), a specific catalase inhibitor, can increase sperm capacitation rates by enhancing endogenous ROS production. Stallion ejaculates (n=8) were collected and processed by colloidal centrifugation, with pellets resuspended to 30 x 106 sperm/mL in either: control (CTR: Tyrode's Albumin Lactate Pyruvate [TALP]-based media), capacitation medium (CAP: TALP supplemented with 18 μM penicillamine, 9 μM hypotaurine and 1.8 μM epinephrine), or CAP supplemented with either 10 mM or 25 mM 3-AT. After a 22-hour incubation at 37 ºC in a humidified atmosphere of 5% CO2 in air; sperm viability, cytoplasmic ROS, mitochondrial ROS, and tyrosine phosphorylation were evaluated using flow cytometry (BD FACS Canto IIA) and compared with CTR at t=0. Statistical analyses were performed using GraphPad Prism 10.2.0 software; normality was assessed using the Kolmogorov–Smirnoff test, followed by one-way ANOVA and Dunnett's test. Results are presented as mean ± SEM. Our analyses revealed a significant decrease in viable spermatozoa post-incubation ($p \leq 0.0001$) across all treatments. While there were no significant changes in cytoplasmic ROS, mitochondrial ROS increased in all samples over time, with significance level increasing with catalase inhibition. Flow cytometry data are presented as percent of the pre-incubation values for CTR (CTR: 232.6 ± 27.3%, $p \leq 0.001$; CAP: 234.5 ± 40.4%, $p \leq 0.001$; 10 mM 3-AT: 263.3 ± 33.5%, $p \leq 0.0001$; and 25 mM 3-AT: 291.1 ± 27.1%, $p \leq 0.0001$) versus CTR pre-incubation (100 ± 27.8%). Furthermore, capacitation induction resulted in a significant increase in the percent of live spermatozoa with tyrosine-phosphorylated proteins (CAP: 52.4 ± 9.98%, $p \leq 0.05$) compared to CTR pre-incubation (23.39 ± 5.43%). The addition of 3-AT further increased tyrosine phosphorylation (10 mM: 61.52 ± 9.78%, $p \leq 0.005$; and 25 mM: 61.81 ± 8.45%, $p \leq 0.005$), suggesting a potential role of catalase inhibition in enhancing tyrosine phosphorylation during capacitation. These findings imply that catalase inhibition during the capacitation process in equine spermatozoa may permit an accumulation of hydrogen peroxide, subsequently increasing mitochondrial ROS levels. This increase in ROS may inactivate tyrosine phosphatases, thereby facilitating the upregulation of tyrosine phosphorylation, a critical event in signaling pathways involved in sperm activation and fertilization. Further studies are warranted to elucidate the precise molecular mechanisms underlying these observations and to explore the potential applications of catalase inhibition in enhancing equine IVF.

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77° CONVEGNO SISVET

SOCIETA´ SCIENTIFICA DI RIFERIMENTO **SOFIVET**

TITOLO **NON-INVASIVE ASSESSMENT OF THYROID HORMONES METABOLITES FLUCTUATIONS IN RELATION TO ENVIRONMENTAL AND INDIVIDUAL VARIABLES IN DOMESTIC AND WILD RUMINANTS**

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Testo e Riferimenti bibliografici

Environmental changes occurred in the last decades are triggering an adaptative response in homeotherms, as evidenced in both wild and farm animals [1,2]. Seasonal variations in temperature, humidity, rainfall, food availability etc. induce animals to modify their behaviour and metabolic patterns to adapt to the new conditions and maintain homeostasis. Environmental adaptation is crucial for the survival of wild animals as well as for the productivity of farm animals. Endocrine functions are physiologically susceptible to fluctuations to face these changes. The thyroid gland with its hormones (THs), has a key role in thermoregulation, as in energy balance, modulating the expression of genes involved in glucose and lipid metabolism. It also promotes protein synthesis, brain development and heart function. Therefore, monitoring thyroid hormone variations is of great importance to understand the adaptation mechanisms of animals to specific environmental conditions, to prevent stress and other health problems that could compromise their life quality and their productive performances [3]. Furthermore, we can collect indirect information about the effect of climate changes on animal's physiological functions. The predominant excretion of THs in bile offers the possibility of assaying their metabolites in faeces. The collection of biological samples from wild and farm animals is often associated with issues related to handling and containment procedures and may induce stress, altering animal welfare. Thus, collecting faecal samples represents a more ethical and welfare-friendly method to obtain information on the animals' physiological status. This work aims to evaluate the fluctuations of faecal thyroid hormone metabolites (FTMs) in three different small ruminant species, sheep, mouflon and roe deer, in relation to individual (sex, age, productive status) and environmental variables (temperature, elevation, local density). FTMs have been quantified in faecal samples collected from Sarda sheep (n=50: lambs=10; adult ewes=30; rams=10), in December (average ambient temperature=9◦C), European mouflon (n=10: ewes=5; rams=5) during two different annual periods: July (average ambient temperature =26◦C) and March (average ambient temperature= 11◦C), and roe deer (n=160; doe=42; buck=118), in the months between September and December (average ambient temperature=18 and 2◦C, respectively), under different physiological and environmental conditions. The results obtained confirmed that external temperature is a main driver in T3 fluctuations, since in both European mouflon and roe deer higher values were recorded in response to lower temperatures as those registered in March and December respectively ($p<0.001$). Moreover, as expected, young ewe lambs showed higher FTMs levels than adults ($p < 0.001$) confirming the key role played by THs in growing animals. Among adult ewes, pregnant ones showed a lower concentration of FTMs than cyclic ones, this is important for animals that support pregnancy in the warmer months [2]. On the other hand, FTM concentrations were not affected by sex in all the species considered. In conclusion, the evaluation of FTMs in livestock, as in wild animals, being non-invasive and related both to animal's physiological status and environmental conditions, can represent a promising marker to study the response of animals to emerging environmental changes and their ability to adapt.

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77° CONVEGNO SISVET

Stato: INVIATO - ID: 13296

Comparative Analysis of Long In Vitro Culture of COCs collected from early antra follicles from Adult Sheep and Prepubertal Lambs

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The long in vitro culture (LIVC) of cumulus-oocyte complexes (COCs) derived from early antral follicles (EAFs), has yielded promising results in obtaining meiotically competent oocytes in adult sheep. This offers opportunities for preserving valuable and endangered animals. However, the value of this method could be further enhanced by utilizing prepubertal females. This approach bypasses the issue of the low number of EAFs in adult females, leading to shortened generation intervals and increased genetic gain. With this in mind, our objective was to compare the efficacy of LIVC of COCs collected from EAFs in adult sheep and prepubertal lambs to gain insights into the broader applicability of this technique in sheep. To achieve this, ovaries were collected from both adult sheep and prepubertal lambs, and COCs were retrieved from EAFs (350-450 μm) by rupturing the follicle wall using a 21-gauge needle.Subsequently, they were individually cultured (38.5°C, 5% CO2) in a 96-well plate containing TCM199 supplemented with 0.15 μg/mL zinc sulfate, 10-4 IU/mL FSH, 10 ng/mL estradiol, 50 ng/mL testosterone, 50 ng/mL progesterone, and 5 μM Cilostamide (1). After 5 days of LIVC, the following parameters were evaluated: COC morphology, oocyte diameter, chromatin configuration, gap junction communications, meiotic competence, levels of reactive oxygen species (ROS), mitochondrial activity, and distribution.Adult oocytes reached a higher diameter following LIVC compared to prepubertal ones (115.4 vs. 113.4 μm, p<0.000), and showed a higher rate of high-quality COCs, as determined by morphological evaluation (61.8% vs 42.6 %; p<0.000). Although no significant difference in chromatin configuration was found between groups before LIVC, lamb oocytes demonstrated spontaneous meiosis resumption following LIVC compared to adult sheep (MI/MII, 0% vs. 26.7%; p<0.000). The meiosis resumption rate after IVM was higher in sheep compared to lamb oocytes (MI/MII, 59.4% vs. 21.0%, respectively; p<0.000), accompanied by significantly active (open) gap junctions ($p < 0.001$). In contrast, the majority of COCs from lambs, exhibited closed gap junction ($p < 0.01$), indicating defective cell coupling between oocytes and cumulus cells. Reactive oxygen species (ROS) levels and mitochondrial distribution patterns (fine or granular) exhibited no significant differences between the groups, while lambs demonstrated higher mitochondrial activity (p<0.000).In conclusion, our study highlights the higher efficacy of LIVC of COCs derived from EAFs in adult sheep compared to prepubertal lambs. Adult sheep exhibited higher outcomes in terms of final oocyte diameter, morphological quality, meiotic competence and gap junction communication, essential for efficient meiosis resumption. These results highlight the importance of considering age-specific variations in oocyte quality and response to culture conditions, while offering insights for optimizing assisted reproductive techniques for genetic preservation and breeding programs. Further research is needed to understand the underlying mechanisms and refine protocols for broader applicability in reproductive biotechnology.

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77° CONVEGNO SISVET

SOCIETA´ SCIENTIFICA DI RIFERIMENTO **SOFIVET**

TITOLO **Evaluation of oxidative stress in *Tursiops truncatus*: preliminary study.**

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Testo e Riferimenti bibliografici

The adaptation of marine mammals to aquatic life is a result of evolution. Being vertebrates, they frequently experience apnea, which can result in hypoxia and repeated cycles of ischemia/reperfusion when diving. Underwater vertebrates have developed a set of mechanisms that enable them to endure long periods of apnea (breathing) and enhance their ability to dive [1]. Recent studies have focused on marine mammals defenses against oxidizing by products and inflammation caused by ischemia, hypoxia, and reperfusion at the molecular level. The importance of highly adapted antioxidant systems in marine mammals for preventing oxidative stress has been underlined in numerous studies [2]. It is thought that there is a strong connection between oxidative stress and health and well-being. Assessing the health status of marine mammals is challenging because, unlike humans and domestic animals, the typical clinical signs of diseases in these species are difficult to recognize or interpret. Identifying the multiple aspects of the response to oxidative stress is crucial for marine mammal management and conservation. Our research is focused on investigating oxidative stress in *Tursiops truncatus* using the d-ROMs test, which determines the pro-oxidative state of measurement of hydroperoxides in serum, and the OXY-Adsorbent test, which measures the antioxidant barrier in serum samples [3]. d-ROMs and OXY ratio (x 100) was used to measure the degree of oxidative stress.
At Zoomarine Italia in Torvaianica, RM, Italy, 11 clinically healthy bottlenose dolphins were examined and 11 samples of fasting blood serum were taken, with 6 representing males and 5 representing females between the ages of 9 and 42, and of 5 and 19, respectively. All dolphins kept in the facility were trained to participate voluntarily in veterinary and husbandry procedures to ensure regular routine diagnostic analysis. In particular, the animals were trained to perform voluntary venipuncture by presenting the fluke for blood collection without any physical restraint. The results showed that there was no correlation between d-ROMs and OXY values, while there was a weak and positive correlation between age and OXY values, and a weak and negative correlation between age and d-ROMs and between age and OSi, respectively.
The results of this preliminary study are valuable in determining the health and well-being status of these marine mammals, which is essential for their management and conservation. To gain a better understanding of the pro-oxidant and antioxidant status of *Tursiops truncatus* and establish reference values, more studies are needed.

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77° CONVEGNO SISVET

SOCIETA´ SCIENTIFICA DI RIFERIMENTO **SOFIVET**

TITOLO **Sperm methylome profiling revealed DNA methylation variations in Piedmontese bulls with different reproductive performances**

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Testo e Riferimenti bibliografici

Artificial insemination (AI) has revolutionized the breeding of livestock species, becoming the most used method in bovine reproduction. Nowadays, early prediction of bull fertility is one of the main challenges for AI-centers as the entire process of bull breeder selection is considered time and money-consuming. It is based on animal selection (genotypic and phenotypic traits), field trial inseminations, and conventional laboratory semen analysis. However, notwithstanding the advance of genomic selection allowed reliable identification of genetically elite bulls, selected bulls with apparently normal semen quality can vary significantly in their field fertility. This suggests that conventional semen evaluation methods are not fully foolproof and other sperm characteristics need to be investigated to explain the remaining variation. Emerging data about abnormal sperm DNA methylation and infertility suggest that epigenetic traits are promising candidates for this purpose [1,2]. Therefore, this study tested the hypothesis that sperm-specific epigenetic characteristics, such as DNA methylation, could discriminate Piedmontese bulls with different reproductive performances. Using a genome-wide approach we have characterized sperm methylome from normal (NF) and high fertility (HF) Piedmontese bulls, by reduced representation bisulfite sequencing (RRBS). Bulls (20) were assigned to either the NF or HF group (10/group) based on an adjusted fertility index provided by the Piedmontese AI center. Reproductive efficiency was predicted by a direct measure of field fertility, calculated as the ability of bulls to make cows pregnant through AI corrected for a wide range of factors (year, season, female genetic, farming). NF fertility index ranged from 0.531 to 0.631 while HF bulls showed values between 0.973 and 0.706. As expected, Computer Assisted Sperm Analysis confirmed that motility parameters were comparable within groups (63,7% NF vs 67,4% HF). RRBS identified a total of 348889 cytosines (10X coverage in all samples) able in part to separate NF or HF animals. We identified 968 differentially methylated cytosine DMCs (FDR<exp-6, delta met 10%, at least 2 near C below 2000bp) between HF and NF bulls. The DMCs were found to be close to 294 differentially methylated genes DMGs. Gene ontology analysis of DMGs identified variations in pathways related to protein glycosylation. Interestingly, the mature coating of glycans on the surface of sperms is a prerequisite to gaining fertilizing capability, and aberrant sperm glycosylation is highly associated with disturbed fertility [3].
Our results suggested that bulls with different fertility exhibited distinct regulation of genes related to sperm glycosylation, making them good candidates to serve as epigenetic markers for bull selection. Overall, this study demonstrated that the sperm methylome of Piedmontese bulls displayed DNA methylation characteristics partially capable of discriminating bulls with different reproductive performances. Additional experiments are still ongoing to explore the interaction between DNA methylation and other epigenetic characteristics, such as micro-RNA, in the regulation of fertility. In conclusion, sperm methylome offers a promising source of information about bull fertility which will complement the genetic data currently employed to enhance reproductive efficiency.

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77° CONVEGNO SISVET

SOCIETA´ SCIENTIFICA DI RIFERIMENTO **SOFIVET**

TITOLO **Study of gene expression of angiogenesis and oxidative stress pathways in testicles of roe deer in different reproductive stages**

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Testo e Riferimenti bibliografici

Many mammals which have been evolving in non-equatorial regions have developed mechanisms to synchronize reproduction with the environmental cycle to optimize reproductive success. Animals with a seasonal reproductive cycle, as roe deer (Capreolus capreolus), respond to environmental signals through melatonin, responsible of pulsatile secretion of GnRH which regulates the secretion of gonadotropic hormones (LH and FSH) and the initiation of reproductive activity. During the mating season (mid. July-mid. August), there is an increase in sperm production, testosterone, and spermatic plasma [1]. The regulation of seasonal reproduction is influenced by testosterone, gonadotropins, and plasma proteins, which modulate sperm maturation and sperm integrity.
Angiogenesis and the Vascular Endothelial Growth Factor (VEGF) play a fundamental role in spermatogenesis, promoting the growth and development of testicular tissues. Oxidative stress, instead, mediated by ROS can negatively affect male fertility, causing DNA damage, apoptosis, and epigenetic alterations. This can compromise the quality of sperm, the survival of germ cells and furthermore can activate apoptotic pathways, compromising spermatogenesis [2].
With the purpose of deepen the knowledge of the roe deer's seasonal reproductivity, this study analyzed 18 samples of mature male roe deer testicles, obtained during the 2018 hunting season in the Bolognese Apennines: 9 have been hunted between June 1st and July 15th (pre-mating period); the remaining half between August 15th and September 30th (post-mating period). The aim of the study was to investigate the variation in the expression of genes associated to the oxidative stress and angiogenesis pathways by quantitative Real Time PCR (qPCR) allowing the simultaneous analysis of the expression of 84 genes (RT2 Profiler PCR Array, Qiagen) for each pathway (Caw Angiogenesis or Caw Oxidative stress) in order to assess variations of one group pool compared to the other. Then to validate data, a qPCR of selected genes was performed on each animal.
Genes, whose expression varied by at least 3 times, have been identified to verify the array data on individual animal. In particular, among genes involved in oxidative pathway, extracellular superoxide dismutase (SOD3) and scavenger receptor class A member 3 (SCARA3) are up-regulated about 8 times for the former and about 3 times for the latter in the post-mating samples. They both play an important role in protecting cells from oxidative stress as reported in the epididymis in the same species [3]. Instead in the angiogenesis pathway there are evidences of downregulation of expression of Leptin (LEP) and Thrombospondin2 (THBS2) that require validations on each individual.
This study is still ongoing, nevertheless provides a basis for a deeper understanding of reproductive activity of the roe deer, a poorly studied wild species.

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77° CONVEGNO SISVET

Stato: INVIATO - ID: 13466

Dynamics of PLCζ During Acrosome Reaction in Frozen Ram Spermatozoa

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Despite advancements in assisted reproductive techniques, intracytoplasmic sperm injection (ICSI) success remains limited in ruminants, notably in sheep, suggesting underlying factors negatively affecting early embryonic development [1]. Recent investigations into Ca2+ response dynamics post-ICSI in sheep oocytes revealed aberrant patterns potentially responsible of impairing critical developmental processes beyond initial activation, and highlighted avenues for improving ICSI outcomes in these species [2]. Phospholipase C zeta (PLCζ) is a pivotal molecule in initiating oocyte activation and embryo development [3], yet its characterization in sheep remains lacking. In this study, we aimed to elucidate the localization of PLCζ in ram spermatozoa. Employing ionomycin-induced acrosome reaction and acrosome staining, we sought to investigate the correlation between the acrosome reaction and PLCζ localization, providing insight into its role in sheep reproduction. In a preliminary examination, anti-PLCζ immunocytochemistry and acrosome staining were conducted on thawed spermatozoa (CTR). Results revealed that among the analyzed spermatozoa (n=153), 13% exhibited PLCζ localization in the acrosomal region and 74% in the subequatorial position, with only 23% displaying an intact acrosome. Upon treatment with ionomycin, a progressive translocation of PLCζ was observed, aligning with the temporal and dynamic changes associated with the acrosome reaction. Within just 3 minutes of exposure to ionomycin, PLCζ exhibited a notable shift in localization, with 8% (n=174) found in the acrosomal region, and 35% in subequatorial position ($P<0.05$ vs CTR), accompanied by a significant reduction in intact acrosomes (14.4%, n=174, $P<0.05$ vs CTR). By 5 minutes of ionomycin exposure, spermatozoa demonstrated nearly complete reactivity (intact acrosome <1%, n=162), while PLCζ localization showed a further decrease in the acrosomal position (6%, $P<0.05$ vs CTR) and an increase in the subequatorial position (38.3%, $P<0.05$ vs CTR). The experimental procedures conducted are evidently preliminary, necessitating further analysis. However, the results highlight a clear relationship between the acrosome reaction and the location of PLCζ, suggesting its probable involvement in oocyte activation. Therefore, a comprehensive review of the artificial oocyte activation process would be warranted.

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77° CONVEGNO SISVET

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Expression of metabolic genes in the liver, proximal- and distal intestine of European eels (Anguilla anguilla) in different stages of development.

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The European eel is a catadromous species with a high potential market in aquaculture, but the population has declined by 98% between 1980 and 2010, leading to its “critically endangered” status. During the continental phase of the species’ life cycle, they feed, grow and undergo a maturation process called silvering as a preparation for the following oceanic phase [1]. Once eels are reaching sexual maturation they stop feeding, the digestive tract starts to atrophy, leaving space for the development of the gonads. The objective of this study was to compare the metabolic parameters of the blood plasma and the tissue specific gene expression of metabolic enzymes of eels in different developmental stages to gain insights into the changes of metabolic characteristics during the silvering process. Therefore, 5 yellow, 5 resident, and 5 migrant eels, classified as previously reported [2] were blood sampled for clinical chemistry analysis, while liver, proximal and distal intestine were collected to relatively quantify the gene expression of lipase (LYP), amylase (AMY), two trypsin isoenzymes, trypsin-a (TRYa), trypsin-b (TRYb) and CCK. Clinical blood chemistry profiling revealed statistically significant differences in cholesterol content ($p = 0.0057$), with resident and migrant eels having higher concentrations than yellow ones. Significant differences in AMY expression were found between yellow and migrant eels in the liver ($p = 0.0296$) and proximal intestine ($p = 0.0014$) with yellow eels having higher expressions in both tissues. A gradual increase in TRYa expression was highlighted, with migrant eels having significantly higher expression ($p < 0.05$) in all 3 tissues than yellow eels, while the TRYb expression decreases in the proximal ($p = 0.0142$) and distal intestine ($p = 0.0184$). These results are in accordance with our current understanding regarding a constant lipid metabolism as principal energy source during the migrating period, also underlining a shift between the gene expressions of the two trypsin isoenzymes towards the end of the life cycle. The significantly higher AMY expression in the yellow phase of the animal can be indicative of the importance of carbohydrates in the early life stages both as an energy source and as building materials for other metabolic pathways. Although glucose turnover rates in fish are known to be lower than in mammals or birds, highly active fishes, among others the American eel (Anguilla rostrata) are reported to be an exception [3]. [1]Durif et al. The silvering process of Anguilla anguilla: a new classification from the yellow resident to the silver migrating stage. J Fish Biol 2005;66:1025–43. [2]Casalini et al. Silvering process of female European eel in the north Adriatic: Who is really ready to migrate?. Estuarine, Coastal and Shelf Science 2024; 108660. [3]Polakof et al. Glucose metabolism in fish: a review. J Comp Physiol B 2012;182:1015–45.

77° CONVEGNO SISVET

SOCIETA´ SCIENTIFICA DI RIFERIMENTO **SOFIVET**

TITOLO **Can resilience markers forecast reproductive functionality in heifers?**

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Cortisol is a catabolic hormone and can be considered as a biomarker of allostatic load [1]. It plays a role in the metabolism of proteins, lipids and carbohydrates and influences the concentration of glucose in the blood. DHEA(S) is an anabolic steroid and has a prohormonal role for its conversion into sexual hormones. Moreover, DHEA(S) can counteract the cortisol concentration and represents a marker of resilience [2]. The allostatic load influences reproductive functionality and resilience is pivotal for maternal physiological asset in cattle [2]. To the best of our knowledge this is the first study investigating the relationship between these two steroids and reproductive efficiency in cattle. In humans, these two hormones appear to be involved and have an effect in female reproduction [3]. Therefore, aim of the present study was to investigate these two steroids in relation to reproductive efficiency in dairy cows. The experiment was conducted in a dairy farm in Friuli Venezia Giulia and involved 54 Holstein Friesian peripubertal heifers (13 months old). This study had institutional approval from the Ethical Animal Care and Use Committee of the University of Naples Federico II (protocol no. PG/2021/0130478). All experimental procedures complied with the Italian legislation on animal care (Legislative Decree n.26 of 04/03/2014). Steroids concentrations were measured in hair, a non-invasive matrix that allows a retrospective measurement of hormones. The hair sampling was collected at heifers' 13 months of age, close to puberty. Hormone concentrations were measured by a validated in-house RIA method [1]. The age at first conception (AFC) was positively correlated to cortisol and cortisol/DHEA(S) ratio (0.42 and 0.49, $P < 0.01$) and negatively to DHEA(S) (-0.308, $p < 0.05$). Similarly, the number of artificial insemination (NAI) per conception showed a positive correlation to cortisol (0.258, $p < 0.10$) and cortisol/DHEA(S) ratio (0.359, $p < 0.05$) and a negative correlation to DHEA(S) (-0.244, $p < 0.10$). The positive correlation between cortisol, cortisol/DHEA(S) ratio and AFC and NAI underlined the significant role of allostatic load and resilience on the reproductive functions of cattle, since higher allostatic load delayed the AFC and led to higher NAI. Conversely, the negative correlations between AFC, AI and DHEA(S) showed that a higher resilience could lower the AFC and fewer NAI. Since the reproductive efficiency plays a pivotal role in the environmental impact and profitability of dairy farm, the inclusion of steroids measurements into the reproductive management of cattle could be practical and would provide a more comprehensive assessment of reproductive functionality of heifers. Hence, strategies aimed at reducing allostatic load and increasing resilience in peripubertal period could improve the reproductive efficiency of the farm.

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77° CONVEGNO SISVET

SOCIETA´ SCIENTIFICA DI RIFERIMENTO **SOFIVET**

TITOLO **HAIR CORTISOL/DHEA(S) RATIO AND CLINICAL SYNDROMES EXPERIENCED BY PIGLETS EARLY IN LIFE**

Autori

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Testo e Riferimenti bibliografici

The ratio between cortisol and dehydroepiandrosterone (sulphate) (cortisol/DHEA(S) ratio) is a biomarker that combines into a single piece of information both the stress response of mammals due to the activation of the hypothalamus-pituitary-adrenal (HPA) axis to restore homeostasis, and the resilience which is the ability to be minimally affected by a disorder or to quickly return to the state before exposure to the stressor [1]. So far, in studies conducted in swine species it has never been investigated in relation to inflammatory and infectious/clinical statuses. Hence, aim of this study was to investigate the effect of some common clinical syndromes in lactating piglets on hair cortisol/DHEA(S) ratio later in life. The research hypothesis was that piglets experiencing clinical syndromes during weaning displayed a different endocrine setting later in life compared to healthy ones. All the animals (n = 732) were clinically scored at 28 days of age based on clinical signs classified as: enteric, neurologic, cutaneous, and locomotor syndromes. The threshold to group the batch as clinical (score 1) was the classification of that batch at least in the first quartile over zero in at least two clinical syndromes, otherwise the batch was classified as healthy (score 0). Hair samples were taken at the age of 14 (weaning site) or 36 (fattening site) weeks and analyzed for steroid concentrations using a radioimmunoassay method. Statistical analyses were carried out using SPSS (29.0.1), hair cortisol/DHEA(S) ratio was compared among groups using nonparametric test, the clinical score and age were fixed factors. Age had a significant effect on cortisol/DHEA(S) ratio (90.02 and 63.16 for weaning and fattening respectively, $p < 0.001$). The clinical score didn't significantly affect cortisol/DHEA(S) ratio both at weaning and fattening ages. The hair sample at 14 and 36 weeks of age includes information linked to the intrauterine life, birth and events occurred during the extrauterine life. The higher hair cortisol/DHEA(S) ratio at the age of 14 weeks confirm the physiological trend for the unborn newborn with a high cortisol linked to the perinatal period and DHEA(S) concentrations exerting the antagonist effect on cortisol concentrations [2]. The ratio didn't differ between healthy and clinical conditions, but it showed a sharper decrement for the animals scored clinical compared to healthy ones, highlighting that the clinical score depresses the reactivity of the HPA axis, as previously reported in foals by Lanci et al. [3]. In conclusion, the endocrine asset of pigs was strongly influenced by the age and additional research is needed to draw a relationship between clinical score, allostatic load and resilience.

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77° CONVEGNO SISVET

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Evaluation of the cellular response to osmotic stress in porcine intestinal epithelial cells (IPEC-J2) through transporter regulation

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The gut epithelium is a critical barrier between the luminal environment and the host, playing a pivotal role in nutrient absorption, immune defense, and health of animals. Intestinal epithelial cells face various physiological challenges due to differences in osmolarity across different intestinal segments. Osmolarity measures solute concentration in a solution, vital for water movement and body fluid balance, influencing physiological processes significantly. This balance is crucial for maintaining morphology, cellular functions, and overall health [1]. Numerous stimuli, such as bacterial products, at the intestinal level can trigger hyperosmotic conditions, which influence absorption, potentially exacerbate diarrhea, and impair growth performance in young animals [2]. Hyperosmotic stress primarily induces the upregulation of osmolyte transporters, with variations depending on the solute used. In response to high osmolarity, intestinal cells accumulate osmolytes such as betaine, taurine, and myo-inositol. Taurine regulates cell volume and calcium levels, while myo-inositol partecipates in osmoregulation and mediates cell signalling. Betaine and myo-inositol stabilize proteins, supporting adaptation to hypertonicity and preventing cell death [3].The aim of this study is to evaluate osmolyte transporter modulation during osmolarity changes in intestinal epithelial cells. The intestinal epithelial cell line (IPEC-J2), derived from neonatal piglet jejunal epithelium, were cultured under different osmotic conditions, i.e. sodium chloride (NaCl), mannitol, or sucrose at different osmolarity concentrations (500, and 600 mOsm/L). Morphology, cell viability, nitric oxide production (nitrite detection), and expression of osmolytes transporter-related genes were evaluated to assess the effects of hypertonicity. The hyperosmotic condition (500, and 600 mOsm/L) induced for 24 hours with mannitol or for 72 hours with NaCl, led to a significant reduction ($p < 0.05$) of cell viability, spherical morphology, increase of detachment and nitrite release. On the other hand, IPEC-J2 with sucrose at 500 and 600 mOsm/L after 72 hours showed a positive trend in viability. Notably, the expression of the taurine transporter (TauT) increased significantly ($p < 0.05$) in response to NaCl 500 mOsm/L. Mannitol 500 mOsm/L at 24 hours positively stimulated ($p < 0.05$) sodium- and chloride-dependent betaine transporter (BGT1) expression. Sodium-myo-inositol transporter (SMIT) and BGT1 expression increased ($p < 0.05$) under sucrose 500 and 600 mOsm/L conditions at both times. The results suggest specific adaptation mechanisms for common osmotic stressors in intestinal cells. Hypertonicity caused water efflux, resulting in cell shrinkage, and increased intracellular ion and macromolecule concentrations. Therefore, IPEC-J2 cells adapt to hyperosmotic stress by upregulating membrane transporters for organic osmolytes, crucial for maintaining macromolecular structures.

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77° CONVEGNO SISVET

SOCIETA´ SCIENTIFICA DI RIFERIMENTO **SOFIVET**

TITOLO **EVALUATION OF MAMMARY EXCRETION OF DRUGS FOLLOWING SYSTEMIC ADMINISTRATION IN LACTATING SOWS**

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Testo e Riferimenti bibliografici

Goal of the ConcePTION project is to deepen knowledge about medicine safety during human breastfeeding, due to the limited amount of information concerning the effects of drugs taken by the lactating woman on the child upon passage through the blood-mammary barrier. To establish a risk level for a treatment that is labeled to have an unidentified risk, it takes on average 27 years [1]. Therefore, one of the aims of the project is to create an in vivo model to generate quantitative data. In order to create such model for lactation studies, swine species was chosen due its to similarities in functionality of the mammary gland to humans [2], but also due to ethical sustainability and overall feasibility of the procedures. The 3 chosen test medicines to assess passage in Göttingen Minipigs sows' milk were: metformin, venlafaxine and levocetirizine. Twelve experienced sows were enrolled in the trial (4 for each medicine), supplied by Ellegaard Göttingen Minipigs at 70/75 days of pregnancy. Since arrival, animals were accustomed to the presence of the operator and to be manipulated both in the udder region and ear, respectively for manual milking and blood sampling through a PICC line. Animals were let out of their box every day and were rewarded sweets and fruit juice upon positive interaction with the operator. This training routine allowed animals to maintain a cooperative and docile attitude throughout the entire trial. Four days after farrowing, a PICC line was inserted through an auricular vein upon deep sedation (Tiletamine/Zolazepam, 5 mg/kg IM). From postpartum day 7 and for the following 3 weeks, sows were orally dosed with the chosen medicine once a day with feed. The dosages were differentiated into low dose (during second and third lactation week) and high dose (during fourth lactation week): metformin 500 and 850 mg/die, venlafaxine 75 and 375 mg/die, levocetirizine 15 and 40 mg/die. Sampling was scheduled as follows: SOW DAY (twice a week for 3 weeks, matched milk/ blood samples: before, 1, 3 and 6h after dosing); SOW/PIGLETS DAY (twice a week for 3 weeks, matched milk/blood samples from the sow before and 4h after dosing, plus blood from piglets 25 minutes after sow's sampling). Medicines were always quantifiable in both maternal matrices upon HPLC, with different pharmacokinetics patterns. The levels of medicines into piglets' plasma were low and, for venlafaxine, below the detection. The average milk/plasma ratio (metformin: 1.13; venlafaxine: 2.73; levocetirizine: 0.32) resembles the ones reported for women, suggesting a good translational value of the proposed species. The trial was conducted without any particular problem, except for the venlafaxine test which caused lethargy in sows and gastroenteric problems in piglets. According to our experience the model seems to work well, also considering the 3R concept.

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77° CONVEGNO SISVET

SOCIETA´ SCIENTIFICA DI RIFERIMENTO **SISVET**

TITOLO **INFRARED THERMOGRAPHY AS A NON-INVASIVE DIAGNOSTIC TOOL OF ASSESSING THE STRESS RESPONSE IN ATHLETIC HORSES DURING ROAD TRANSPORT OVER DIFFERENT DISTANCES.**

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Testo e Riferimenti bibliografici

Horses travel frequently during their life activities, and transport is considered as one of the major stress events [1]. Body temperature monitoring is a valuable resource for assessing welfare, physiological state and stress response in mammals. Eye region offers an ideal location. Previous studies suggest that infrared thermography (IRT) of eye temperature could be useful for measuring stress in domestic animals [2]. Given such considerations, the current study aimed to address whether IRT measurements of eye temperature may reflect cortisol release in show jumping horses subjected to two different transport distances. The study was performed in accordance with good veterinary practices, European (2010/63), and national legislations (DL 2014/26). It compares the effect of two journeys (length: 100 and 300 km; duration: 1:15 and 4 hours, speed: 80 and 75 km/h respectively) on eight adult healthy Italian Saddle horses enrolled after the owner consent (age: 8±12 years old, body mass: 450 ± 50 kg). From each animal, blood sampling, rectal (RT) and eye temperature (ET) assessments were performed before (T1), after (T2) and 60 minutes (T3) from the arrival. Cortisol concentrations were assessed by the obtained serum by species-specific commercial enzyme linked immunoassorbent assay kit (ABNOVA). After blood sampling RT was measured using a digital thermometer. Thermographic acquisitions of ET were performed with a thermal infrared camera (FLIR T440) in left and right eye considering three regions of interest: EL1 (medial canthus), EL2 (central cornea) and EL3 (lateral canthus). For each region T_{max}, T_{min} and T_{avg} were automatically obtained. Environmental conditions were recorded inside the truck during both journeys to characterize microclimatic experimental conditions and temperature humidity index (THI) was calculated. Student t-test was applied to verify statistical differences between left and right eye and therefore, mean values of both eyes were used for the analysis. All data were analysed by means of two-way analysis of variance for repeated measures ANOVA (Graph Pad Prism). Statistically significant increase was observed at T2 for $EL1_{max\text{-}min\text{-}avg}$ ($P<0.01$), $EL2_{max\text{-}min\text{-}avg}$($P<0.01$), $EL3_{max\text{-}min\text{-}avg}$ ($P<0.01$) and RT ($P<0.01$) during 100 km and for RT ($p<0.01$) after 300 km. Cortisol concentration didn't show significant changes after both journeys. ET values were positively correlated with cortisol and RT at each time points (T1, T2 and T3) during 100 km journey. During 300 km ET positively correlated with cortisol at T2 and T3 and at T2 with RT. It is evident that adult show jumping horses, used to travelling on a monthly basis, are not particularly stressed by journeys. The 100 km journey caused a significant increase in ET and a slight increase in cortisol, although not significant, suggesting that animal doesn't easily adapt to the new situation in 1 hour. In contrast, maintenance of the studied parameters was observed during the 300 km journey, reflecting the animals' adaptation to transport after 4 hours. This study highlighted the usefulness of IRT as an immediate and non-invasive physiological tool to assess the stress response in athletic horses using an innovative region of interest, allowing practical and fast strategies for monitoring the physiological state of the animal during daily activities.

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77° CONVEGNO SISVET

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ACCUMULATION OF∆2 TUBULIN IN DORSAL ROOT GANGLIA DISPLAYS SOMATOSENSORY NEURON PERTURBATION

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Sensory perturbation, the alteration or disturbance in sensory perception, often arises from nerve damage within the peripheral nervous system (PNS) in cases of peripheral neuropathy. This perturbation can manifest in multiple forms, including pain, numbness, tingling, hypersensitivity (increased sensitivity to stimuli), or loss of sensation (hyposensitivity). Various conditions, such as neurological disorders, chemotherapy drug toxicity, and injuries, can result in degeneration of nerve fibers, leading to disruption of sensory perception (1). Unfortunately, the mechanisms underlying the etiology of most peripheral neuropathies are still poorly understood, creating a burden for the development of neuroprotective therapies (2). We previously observed that ∆2 tubulin (∆2), the only irreversible tubulin posttraslational modification (PTM) and a marker of hyperstable microtubules, accumulates in the PNS of human (sural nerve) and rat (dorsal root ganglia (DRG), and sciatic nerve (SN) after treatment with the chemotherapy drug bortezomib, and this increase is associated with disruption of mitochondrial transport and axonal degeneration in cultured adult DRG neurons (3). We recently tested the effects of targeted in vivo accumulation of ∆2 in DRG and SNs of TTL-floxed mice by ablating the expression of the tubulin re-tyrosinating enzyme tubulin tyrosine ligase (TTL) in the adult mouse. We found that acute loss of TTL induces accumulation of ∆2 in DRG and results in both thermal hyposensitivity and hypersensitivity to osmotic stress. We examined the mechanisms underlying these defects and determined that in addition to disrupting mitochondrial motility, ∆2 accumulation affects mitochondrial bioenergetics and transient receptor potential (TRP) channel activity, underscoring novel roles for excessive ∆2 in damaging sensory function.

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77° CONVEGNO SISVET

SOCIETA´ SCIENTIFICA DI RIFERIMENTO **SOFIVET**

TITOLO **STRESS RESPONSE IN GILTHEAD SEABREAM BROODSTOCK FED NOVEL DIETS**

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Testo e Riferimenti bibliografici

Nutrition is a key aspect influencing fish physiology. This is especially true for carnivorous broodstock, which require diets rich in high-quality proteins, essential amino acids and polyunsaturated fatty acids to maintain reproduction efficiency. In terms of protein sources, vegetable proteins such as soybean meal have been largely tested to substitute fishmeal in farmed fish diets to increase the sustainability of aquaculture. However, soybean meal contains anti-nutritional factors and may lack of essential nutrients, potentially leading to stressful conditions which could affect the reproductive performance. Nowadays, insect meals and algae represent promising alternative to soybean meal, being rich in amino acids and fatty acids, respectively. Nevertheless, the effects of dietary protein sources in carnivorous reproducers such as gilthead seabream have been poorly investigated. Therefore, this study aimed at evaluating the stress response of gilthead seabream broodstock (n=66) fed three experimental diets: a control diet (CTRL) with conventional protein sources, and two novel diets containing four alternative protein meals, i.e. *Hermetia illucens* larvae, duckweed (*Lemna minor*), microalgae (*Nannocloropsis gaditana*) and macroalga (*Alaria esculenta*), with inclusion levels of 5% (L1) and 10% (H1). After the six-month feeding trial, fish were sampled at the Spallanzani Institute's facility, and blood was immediately collected from the caudal vein. Following blood sampling, muscle, fin and scales were also collected. Cortisol, the primary stress-related hormone in fish, was determined in all these matrices using radioimmunoassay to gain a complete picture of both short- and long-term primary stress responses. Moreover, cortisol levels were also measured in the eggs, which are continuously released over the lengthy reproductive period. This measurement aimed to provide insights into the animals' stress status at various points throughout the reproductive cycle, potentially revealing any distinct trends. Both alive and dead eggs were collected monthly from January to May (n=16 per diet, per month). Spectrophotometric analyses were carried out to determine secondary stress response indicators (serum glucose-lactate), as well as oxidative stress products (total protein, advanced oxidation protein products and malondialdehyde in serum and muscle). After assumption checks, data were eventually logarithmic transformed and analysed using ANOVA. Statistical significance was set at $p<0.05$. Neither cortisol levels, nor the glucose-lactate levels, nor the oxidative stress products, differed among fish fed different diets. It is noteworthy that the measured cortisol levels were comparable to the basal levels reported in the same or related fish species. Interestingly, although no differences were due to the egg condition (i.e. alive/dead), both the diet and the month of collection significantly affected the cortisol content of the eggs ($p<0.001$). Specifically, lower levels were found in the eggs collected from the broodstock fed the H1 diet compared to those fed the CTRL and L1 diets. Concerning the month of collection, cortisol levels did not show a constant trend across the different months, but decreased from January to March, which exhibited the lowest cortisol levels, raised in April and fell again in May. Given that fish were maintained under controlled environmental conditions, differences due to seasonal pattern could be excluded, thus the observed variation might be exclusively diet-related. Nonetheless, the measured eggs cortisol levels were much lower than those reported in other studies. In conclusion, this study suggests that the tested novel alternative diets did not elicit a stress response in gilthead seabream broodstock. Nevertheless, further investigation is required to conclusively demonstrate the possible effects on reproduction and welfare.

77° CONVEGNO SISVET

SOCIETA´ SCIENTIFICA DI RIFERIMENTO **SOFIVET**

TITOLO **EFFECT OF DIETARY SUPPLEMENTATION WITH OMEGA 3 ON THE PRODUCTIVE AND REPRODUCTIVE PERFORMANCE OF FEMALE RABBITS**

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Testo e Riferimenti bibliografici

Mortality and culling rates among female rabbits are extremely elevated, primarily due to intensive reproductive rhythms that induce a negative energy balance and result in reduced fertility. Recent studies are therefore focusing on enhancing both productive and reproductive performance as well as animal welfare by incorporating various nutraceuticals into the diet [1]. This study investigates the combined effects of extruded linseed and algae *Padina pavonica* extract as dietary sources of omega-3 on female rabbits' productive and reproductive performance. Thirty-six nulliparous New Zealand White female rabbits, aged 4 months, were individually housed in conventional cages under controlled environmental conditions. The rabbits were randomly divided into three experimental groups (n=12) and fed different diets: commercial feed (CNT group), commercial feed integrated with 5% extruded linseed (L5% group), and commercial feed integrated with 5% extruded linseed in combination with 0.2% algae *Padina pavonica* extract (L5%PP group). The rabbits were monitored from artificial insemination until weaning of the rabbit kits, and several productive and reproductive parameters were evaluated, including feed intake, body weight, receptivity, fertility, litter size at birth and weaning, litter weight at birth and weaning, perinatal and pre-weaning mortality, as well as milk yield. There were no significant differences in body weight and feed intake among groups, suggesting that both linseed and algae did not negatively affect the diets' palatability. Concerning the rabbit's sexual receptivity, both L5% and L5%PP groups showed a higher percentage of does with red vulvas (75%) compared to the CNT group (58.8%), but statistical analysis revealed only a trend towards significance (p=0.086). In terms of fertility, L5% and L5%PP groups exhibited higher pregnancy rates (83.3%) compared to the CNT group (66.7%), although statistical analysis could not find significant differences among groups (p=0.447). Moreover, no significant differences were observed in litter size and weight among groups, neither at birth nor weaning. However, both supplemented groups had significantly lower perinatal (P<0.001) and pre-weaning (P<0.05) mortality. Dietary omega-3 supplementation during pregnancy is proposed to enhance early neuronal development and regulate neurochemical aspects related to stress response, growth, and cognitive functions, crucial for newborns' vitality. Newborn mammals can also benefit from omega-3 supplementation through milk consumption, as the fatty acid composition of milk typically reflects the composition of the mother's diet [2]. Although milk composition was not assessed in our study, the rabbits' milk yield increased until day 18 *post-partum* (P<0.001) without differences among groups. Overall, dietary omega-3 supplementation with extruded linseed and *Padina pavonica* algae extract appears to be a promising strategy for improving the productive and reproductive performance of female rabbits, although further investigations are needed to elucidate the underlying cellular mechanism involved.

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77° CONVEGNO SISVET

SOCIETA´ SCIENTIFICA DI RIFERIMENTO **SISVET**

TITOLO **HAIR AND BLOOD TRACE ELEMENTS (CADMIUM, ZINC, CHROME, LEAD, IRON AND COPPER) BIOMONITORING IN ATHLETIC HORSE: POTENTIAL ROLE OF HEMATOLOGICAL PARAMETERS AS BIOMARKERS.**

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Testo e Riferimenti bibliografici

In the context of biomonitoring, it is important to study animals in an environmental background that could potentially be polluted and to assess all effects on certain haematological parameters as blood biomarkers [1]. Blood and hair are important biological matrices used to assess the bioaccumulation of trace elements in domestic animals, particularly in athletic horses [2-3]. This research aims to study the potential bioaccumulation of certain trace elements (cadmium- Cd, zinc- Zn, chrome- Cr, lead- Pb, iron- Fe, and copper- Cu) in different biological matrices as blood, serum, and hair (tail and mane) in athletic horses stabled near the industrialized area of Milazzo (Messina, Sicily). On this basis, we wanted to understand the relationships between haematological parameters (RBC, WBC, Hb, Hct, MCV, MCH, MCHC, PLT) and the concentrations of the studied trace elements in the different substrates for potential use as blood biomarkers in athletic horses. Blood, serum, mane, and tail samples from 20 healthy Italian Saddle horses, frequently trained, aged between 10 and 15 years old and with a body weight between 435 and 500 kg were obtained with the owner's consent to determine Cd, Zn, Cr, Pb, and Fe concentrations. Trace elements were determined on all biological samples using a Thermo Scientific iCAP-Q ICP-MS spectrometer. On the EDTA blood samples taken in duplicate, haematological parameters (RBC, WBC, Hb, Hct, MCV, MCH, MCHC, PLT) were determined by means of an automated haematology analyzer (HeCo Vet C; SEAC, Florence, Italy). Descriptive statistical analysis and the Pearson correlation test ($p< 0.05$) were performed to evaluate the relationship among the concentrations of Cd, Zn, Cr, Pb, Fe, and Cu observed in blood, serum, mane, and tail and their relationship with haematological parameters. Mineral concentrations were also evaluated in water, hay and concentrates. Statistical analysis showed a significant difference for Cd ($p< 0.0001$), Zn ($p< 0.0001$), Cr ($p< 0.0001$), Pb ($p< 0.0001$), Fe ($p< 0.0001$), and Cu ($p< 0.0001$) concentrations among the analyzed substrates. Our results revealed a statistically higher concentration of Zn, Cr, Pb, Fe, and Cu in the blood than other substrates and a higher concentration of Cd in the tail.

A positive correlation was found between blood and serum Cr ($p<0.0001$) and Zn ($p<0.01$) concentrations, between mane and tail Zn ($p<0.001$) concentrations and between blood and mane Pb ($p<0.01$) concentrations, whereas a negative correlation was observed between blood and tail Cr ($p<0.01$) concentrations. The trace elements analysed showed both positive and negative correlations among biological substrates and direct and indirect haematological parameters. It is evident from the analysis of the results obtained that there is a close relationship between the bioaccumulation of certain trace elements studied in the various equine biological substrates and haematological parameters, which represent useful biomarkers in horses during sporting activity.

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77° CONVEGNO SISVET

SOCIETA´ SCIENTIFICA DI RIFERIMENTO **SOFIVET**

TITOLO **ATTACHMENT BEHAVIOURS AND STYLES PREDICT ACUTE AND CHRONIC PHYSIOLOGICAL STRESS LEVELS IN DOGS**

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Testo e Riferimenti bibliografici

In human psychology, children´s attachment behaviour patterns may be linked to short- and long-term stress-related physiological changes. Although dogs show similar attachment features towards their caregivers as those observed in human infants, the relationship with acute and chronic stress has been scarcely investigated [1,2]. The aim of this study was to assess whether dog attachment styles and behaviours during the Strange Situation Procedure (SSP) would affect indicators of both acute and chronic physiological stress.
One-hundred dog-owner dyads participated in the SSP. Before the test (T0), a sample of saliva was collected from all dogs. During the test, dogs were classified according to their attachment style towards the owner as secure, anxious, avoidant or disorganized. Additionally, they were scored on a 1 to 5 unidirectional scale for six attachment behaviour dimensions, namely Proximity/contact seeking, Contact maintenance, Resistance, Avoidance, Separation distress, and Distance interactions [3]. After the SSP (T1), another sample of saliva and dog hair were collected to measure cortisol concentrations and physiological measurements were taken to assess sympathetic activation (heart rate, blood pressure, rectal temperature, respiratory rate). During this procedure dogs were also scored for fearfulness, aggression and lack of compliance. Cortisol concentrations were analysed with ELISA kits.
Stepwise regression models were built for each physiological parameter measured as dependent variable, whereas all attachment behavioural dimensions and styles were included as predictors. Bonferroni-Holm adjustment was applied when appropriate.
As for the indicators of sympathetic activation, heart rate was more likely to be lower for avoidant ($p=0.001$) and secure dogs ($p=0.002$) compared to anxious dogs, as well as for dogs who tried to maintain contact with the owner during the test ($p=0.01$). Temperature was more likely to be higher for anxious dogs compared to secure dogs ($p=0.03$) and for dogs who showed aggression ($p=0.02$) and lack of compliance ($p=0.002$) during the measurement procedures. No significant effect was found for blood pressure and respiratory rate.
Furthermore, salivary cortisol at T0 was more likely to be higher in dogs who obtained higher scores for Avoidance towards the owner during the SSP ($p=0.02$), whereas at T1 it was more likely to be higher in anxious dogs compared to both secure ($p<0.001$) and avoidant dogs ($p=0.04$). No significant predictors were identified for salivary cortisol concentrations.
Finally, hair cortisol concentrations were more likely to be lower in dogs who attempted to maintain contact with the owner during the SSP ($p<0.001$).
This study provides evidence that the quality of dog attachment towards the owner may affect the dog´s physiological response to stressful events. For the first time, a link between dog attachment behavioural patterns towards the caregiver and chronic stress has been identified, and a link with acute stress has been confirmed.

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SOIPA

77° CONVEGNO SISVET

Stato: INVIATO - ID: 13083

Dirofilaria immitis serodiagnosis and diffusion of Aedes albobictus in our country: which risk for Italian cats?

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Dirofilaria immitis, the agent of canine and feline heartworm disease (HWD), is a widespread mosquito-borne helminth. The true prevalence of HW infection in cats is likely underestimated due to the difficulty in establishing a definitive diagnosis. Aedes albopictus, a recognized competent vector for D. immitis, is currently considered the most invasive mosquito species worldwide and Italy presents the highest abundance in Europe. The aims of the present study are: (i) evaluating the current seroprevalence of D. immitis in Italian cats and (II) to estimate the potential future risk of feline HWD associated with the presence of Ae. Albopictus through the Ecological Niche Model (ENM). Sera of 812 Italian cats from 13 regions were analyzed for D. immitis antibodies with the protocol described by [1]. The ENM was created using occurrence points of A. albopictus in Italy, obtained from Global Biodiversity Information Facility from January 1990 to April 2023. Bioclimatic data, including 19 bioclimatic variables, related to temperature and precipitation and were downloaded from the online software worldClim.orgThe average prevalence of D. immitis antibodies was 12%. Seropositivity was significantly associated with age (< 6 years) while there was no association with geographical area or sex. The risk map showed that the highest risk of infection was found in northern inland areas and along coastal areas, while the lowest risk was identified at higher altitudes. The ENM correctly classified most of the areas where D. immitis seropositive cats were found, with 80.4% occurring in high and very high-risk areas. Results of the present study suggest that cats in Italy are exposed to D. immitis infection. In this context it appears clear how routine prevention should be part of the general healthcare protocols in cats. Moreover, the resulting risk maps indicate areas with a suitable habitat for Ae. albopictus may put cats at risk of exposure to D. immitis. [1] Morchón R et al. Specific IgG antibody response against antigens of Dirofilaria immitis and its Wolbachia endosymbiont bacterium in cats with natural and experimental infections. Vet Parasitol, 125:313, 2004. [2] Genchi C et al. Is heartworm disease really spreading in Europe? Vet Parasitol, 133:137, 2005. [3] Genchi C et al. Climate and Dirofilaria infection in Europe. Vet Parasitol, 163:286, 2009.

77° CONVEGNO SISVET

Stato: INVIATO - ID: 13295

Occurrence of co-infections with Ehrlichia canis and Anaplasma phagocytophilum in clinically healthy leishmaniotic dogs: emerging findings from Southern Italy

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Canine leishmaniosis (CanL) is a widespread vector-borne disease caused by Leishmania infantum, affecting dogs worldwide [1]. In historically endemic countries for CanL, such as Italy, an overlapping transmission of L. infantum and other vector-borne pathogens (VBPs), including Anaplasma phagocytophilum and Ehrlichia canis has been increasingly reported. Moreover, dogs with clinical leishmaniosis seem to exhibit higher rates of co-infections and more pronounced clinicopathological abnormalities [2]. However, data on the occurrence of co-infections in clinically healthy L. infantum seropositive dogs and the comparison with L. infantum seronegative animals are lacking. In this scenario, this study aims to evaluate the occurrence of co-infections with E. canis and A. phagocytophilum in clinically healthy L. infantum seropositive and seronegative dogs from Southern Italy. Medical records of dogs, of any age, sex, and breed which were referred to the Medical Clinic Unit of the Department of Veterinary Medicine (Valenzano, Italy) and clinically evaluated in recently published clinico-parasitological trials [3] or still ongoing (data unpublished) were retrospectively collected. Dogs were considered eligible for inclusion in this study if they were clinically healthy (i.e., physical examination and laboratory findings unremarkable), tested for L. infantum, E. canis, and A. phagocytophilum by indirect immunofluorescent antibody test (IFAT), and residing in Italy. Records of 154 animals (i.e., n=77 female and n=77 male; 7.51 ± 3.65 years) met all the criteria and were enrolled in this study. Out of 154 animals, 135 (87.7%) and 19 (12.3%) were shelter and hunting dogs, respectively.Out of the 154 dogs, 90 (58.4%) were seropositive for L. infantum by IFAT. Among them, 4 (4.4%) dogs were seropositive for A. phagocytophilum, and 2 (2.2%) dogs were seropositive for E. canis and A. phagocytophilum. None of the L. infantum seropositive dogs tested seropositive for E. canis alone. Out of the 154 dogs, 64 (41.6%) were L. infantum seronegative with one (1.6%) seropositive for A. phagocytophilum, and one (1.6%) seropositive for E. canis and A. phagocytophilum.No statistically significant differences were found between the L. infantum seropositive and seronegative groups in the proportion of A. phagocytophilum seropositive ($X^2=0.9889$, $p=0.32$) and E. canis and A. phagocytophilum seropositive($X^2=0.0852$, $p=0.77$) animals.This study reports a low percentage of co-infections with A. phagocytophilum and E. canis, and no co-infection with E. canis alone in either seropositive or seronegative dogs for L. infantum from Southern Italy. The low occurrence of co-infections in the analyzed dog population may be related to the climatic conditions, which are known to strongly influence the presence of ticks and other hematophagous arthropods in the environment and consequently the circulation of VBPs. Moreover, our findings may be attributed to a reduced circulation of A. phagocytophilum and E. canis probably due to increased prevention measures and treatment for tick-borne pathogens in the canine population.

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77° CONVEGNO SISVET

Stato: INVIATO - ID: 13458

Development of a multiplex real-time PCR for the detection of Dirofilaria immitis, D. repens, Ehrlichia canis and Anaplasma spp. in dogs

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Canine Vector-borne diseases (CVBDs) pose a diagnostic challenge, due to a wide range of clinical signs, from unspecific, up to severe health implications or death, particularly when there is a coinfection with different pathogens, which can complicate the clinical pattern [1]. The aim of this study was to develop a multiplex Real-time PCR (Rt-PCR) for the identification of different CVBDs caused by Dirofilaria immitis, Dirofilaria repens, Ehrlichia canis and Anaplasma spp. This approach could be very useful to obtain a rapid and reliable diagnosis of the abovementioned pathogens, allowing a fast and effective treatment strategy.Initially, different couple of primers and hybridization probes were designed, based on the most used target sequences: i) the mitochondrial cytochrome c oxidase 1 (COX1) and ii) the internal transcribed spacers (ITS-1 and ITS-2) of ribosomal RNA (rRNA) for detection of filarial nematodes; iii) 16S rDNA and iv) groEL gene for the identification of E. canis and Anaplasma spp. DNA. Twenty DNA samples extracted from blood of experimentally infected dogs with D. repens [2], 15 DNA samples extracted from blood of dogs naturally infected with D. immitis and specific reference strains from the American Type Culture Collection for E. canis and A. phagocytophilum were used as positive controls. For each pathogen, primers and probes concentration, as well as the annealing temperature were optimized, using a gradient Rt-PCR thermocycler CFX96. The limit of detection for each pathogen was also evaluated. Moreover, repeatability and reproducibility were assessed using three replicates of each sample for each run by estimating the variation of results within PCR amplification (intra-assay) and between two PCR amplifications (inter-assay), respectively.The best results for multiplex Rt-PCR amplification were obtained with primers amplifying ITS-1+ for D. immitis, ITS-2 for D. repens and 16S for E. canis and Anaplasma spp. Rt-PCR mix was prepared in a final volume of 25 μL, containing 1X Bio-Rad Universal Master Mix, 1 μM of each primer and 0.5 μM of probe for D. immitis and D. repens, 10 pmol of each primer for E. canis and Anaplasma spp., 3.75 pmol of each probe for E. canis and A. phagocytophilum, 6.0 pmol of A. platys probe and 2 μL of extracted DNA. Each amplification was performed with the following thermal conditions: 95 °C for 10 min followed by 35 cycles at 95 °C for 30 s, 55.6 °C for 30 sec. The multiplex Rt-PCR confirmed high repeatability between three replicates in the same run (coefficient of variation, CV%= 3.0%) and high reproducibility between two runs (CV%= 4.0%). All the samples were correctly identified by the multiplex Rt-PCR (100% specificity). The limits of detection were: 1 microfilaria/μl for D. immitis and D. repens, 3.6 pg/μL for E. canis and 0.5 ng/ μL for Anaplasma spp. A validation phase of these results is required and is currently being carried out on blood samples from symptomatic dogs with suspected dirofilariosis, ehrlichiosis and anaplasmosis to confirm the preliminary findings and validate this a rapid, specific and sensitive molecular technique.

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77° CONVEGNO SISVET

Stato: INVIATO - ID: 13485

Therapeutic advances in Giardia infections in non-human primates

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Therapeutic advances in Giardia infections in non-human primates Michele Capasso1, Lavinia Ciuca1, Isabel Guadano Procesi2, Federica Berrilli2, Laura Rinaldi1Dept. of Veterinary Medicine and Animal Production, University of Naples Federico II, Naples - Italy AbstractGiardia duodenalis is a ubiquitous enteric flagellated protozoan of global importance that infects a wide range of hosts, i.e., >40 animal species, such as humans (1). Non-human primates (NHP) are kept under human care in zoos, research laboratories, rescue centers and could play an important role as reservoirs of zoonotic Giardia infections. Though Giardia is a common parasite in NHP, a limited number of field studies have been conducted on the efficacy of antiparasitic treatments in NHP. Therefore, the aim of this study was to evaluate the synergistic effect of fenbendazole and metronidazole for the treatment of G. duodenalis infection in different species of NHP housed in a zoological garden of southern Italy. Moreover, the study also aimed to better define the circulation of G. duodenalis zoonotic assemblages in NHP and the potential occurrence of zoonotic transmission between the staff (zookeepers, veterinarians) from the zoo and NHP. Briefly, six species belonging to four families (Lemuridae, Cercopithecidae, Atelidae, Hylobatidae) of NHP (N=23 animals) and housed in six cages were identified as Giardia-positive, and divided in two groups. Group F was treated with fenbendazole (50 mg/kg, orally, every 24 hours for 5 consecutive days) and Group M was treated with metronidazole (25 mg/kg, orally, twice a day for 5 consecutive days). After five days from the first round of therapy, all the animals were retreated by inverting the drugs in each group. At each sampling day (SDs -3-24) the faecal samples were tested for the presence of Giardia cysts using the FLOTAC technique (2). Moreover, multiple faecal tests for the antigen-detection of Giardia were performed at each sampling day only on samples that resulted positive for Giardia cysts with FLOTAC. The results showed the synergistic effects of fenbendazole and metronidazole (98–100%) over the combination of the two drugs (52–90%) against the infection by Giardia in NHP. Only two positive samples were successful sequenced showing 100% of identity with Assemblage B (Accession number: MF095053), sub-assemblage BIV. In addition, all the zookeepers and veterinarians from the zoo included in the study, resulted negative for Giardia cysts. Overall, the study emphasizes the need for regular monitoring and control of Giardia infections in NHP housed in zoological gardens. Köster et al. Intestinal protists in captive non-human primates and their handlers in six European zoological gardens. Molecular evidence of zoonotic transmission. Front Vet Sci. 8:819887, 2022. Cringoli et al. FLOTAC new multivalent techniques for qualitative and quantitative copromicroscopic diagnosis of parasites in animals and humans. Nat Protoc.5:503–15, 2010.

77° CONVEGNO SISVET

Stato: INVIATO - ID: 13502

Anthelmintic efficacy of borage (Borago officinalis) and wild mallow (Malva silvestris) aqueous macerates to control gastrointestinal nematode infection in sheep

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Gastrointestinal nematodes (GINs) are ubiquitous in grazing small ruminants and cause significant costs due to production losses. Moreover, anthelmintic resistance (AR) is now widespread throughout Europe and poses a major threat to the sustainability of modern small ruminant livestock farming [1]. For this reason, the exclusive use of commercial anthelmintics for the treatment of GIN infections in ruminants is less sustainable due to AR, as well as the problem of drug residues in animal products and the environment. Therefore, an integrated and complementary therapeutic approach is needed, including the search for alternatives to synthetic anthelmintic drugs [2]. The aim of this study was to evaluate the possibility of using the aqueous macerates of borage (Borago officinalis) and wild mallow (Malva silvestris) to control GIN infections in sheep. The borage and wild mallow plants present in seminatural pastures of southern Italy were sampled. After drying, the two plants were subjected to aqueous extraction processes using the conventional maceration technique in order to extract different bioactive compounds. The in vivo trial was conducted in a farm of southern Italy (Campania region), where the prevalence of GINs was high. Sheep with natural-mixed infection and different worm burdens were used. For the trial, sheep were divided into four homogeneous groups by age, body weight and grazing season (n = 12 animals/group): G_Bor: 0.5 liter of borage macerate (2.5 g of extract) in a single administration; G_Mal: 0.5 liter of wild mallow macerate (2.5 g of extract) in a single administration; G_ALB: 3.8 mg/kg of albendazole (positive control); G_CNT: 0.5 liter of water (negative control). Individual faecal samples were collected rectally before treatment (D0) and 7, 14 and 21 days after treatment (D7, D14 and D21) and analysed using the Mini-FLOTAC technique [3]. The coprocultures were performed for each group in order to identify the GIN genera.The faecal egg count reduction (FECR) was calculated for each group using the formula FECR = 100 × (1 # [T2/C2]). The results showed the infection of different GIN genera in the farm examined: Trichostrongylus (26%), Teladorsagia (37%), Haemonchus (28%) and Chabertia (9%). The FECRT showed a mean reduction of GIN eggs in the G_Bor and G_Mal groups of 50.2% and 64.4% at Day 7, 47.7% and 48.5% at Day 14, 24.5% and 9.9% at Day 21, respectively. The FECR in the G_ALB group was 89.8 %.Due to their complex chemical compositions, numerous bioactive ingredients, and natural origin, herbal formulations represent a potentially valuable alternative for the control of GINs in sheep. In this context, the results of the present study showed that both the aqueous macerates of borage and wild mallow are promising candidates.

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77° CONVEGNO SISVET

Stato: INVIATO - ID: 13509

Validation of cLSH-Abby Imhotep, a new rapid immunochromatographic test for Canine Leishmaniosis diagnosis

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Canine Leishmaniosis (CanL) is a vector-borne zoonotic disease caused by *Leishmania infantum* and vectored by phlebotomine sand flies. Infected and/or diseased dogs serve as one of the main reservoirs of infection in Mediterranean countries. Diagnosis of CanL is still a challenge due to the variety of clinical signs and the high percentage of asymptomatic dogs; therefore availability of sensitive and reliable diagnostic tools is of paramount importance to achieve a definitive diagnosis. The gold standard for the diagnosis of CanL is the detection of anti-*Leishmania* antibodies using serological methods (i.e., immunofluorescent assay, IFA, and enzyme-linked immunosorbent assay, ELISA). Commercial rapid detection assays for anti-*Leishmania* antibodies in dogs have been reported to show lower sensitivity and specificity when compared to conventional serological methods, although these commercial tests are very attractive for the practitioner as screening tests for their simple and rapid use. Recently, a new tool, the cLSH-Abby Imhotep (DongGuan) Medical Industry Investment Co, LTD), based on immunofluorescence chromatography technology using microspheres wrapped with Europium (Eu) lanthanide as a marker, has been launched in the market for the diagnosis of CanL.
The diagnostic performance of the LSH-Abby Imhotep (DongGuan) and another immunochromatographic test (ICT), the *Leishmania* IgG/IgM Rapid Test (Cassette, Citest Diagnostic imminc.) were evaluated using as reference a commercial indirect ELISA test (ID Screen® Leishmaniasis Indirect Test, VET- Innovate ID Diagnostics).
A total of 61 sera of privately owned adult dogs (34 seropositive, 22 seronegative, and 5 doubts) diagnosed by a commercial indirect ELISA test for the detection of anti-*Leishmania* antibodies were used. The samples were submitted to the ICTs following the manufacturers' instructions. Statistical analysis was performed to evaluate the diagnostic performance of each ICT in comparison with the ELISA and Cohen's kappa coefficient was calculated for the accuracy of the tests.
Both ICTs scored 97.06% for sensitivity while specificity was 81.81% and 90.90% for *Leishmania* IgG/IgM Rapid Test and cLSH-Abby Imhotep, respectively. Cohen's kappa coefficient underlined a perfect agreement between cLSH-Abby Imhotep and ELISA results (i.e., Cohen's *k*: 0.81), while a substantial agreement between *Leishmania* IgG/IgM and ELISA results was detected (Cohen's *k*: 0.73).
Rapid tests are a valuable tool for the diagnosis of CanL, due to their simplicity, low cost, and practical results. The screening of dogs in *Leishmania* endemic areas is a fundamental act of public health surveillance policy, which can be implemented through the use of rapid tests coupled with a thorough clinical evaluation before decision-making. The tests evaluated in this study showed good performances, and, according to the results, the cLSH-Abby Imhotep seems a reliable diagnostic tool that can be used in clinical and epidemiological investigations.

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Ferroglio et al. Evaluation of a rapid device for serological diagnosis of Leishmania infantum infection in dogs as an alternative to immunofluorescence assay and Western blotting. Clin Vaccine Immunol, 20 (5): 657-659, 2013.

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77° CONVEGNO SISVET

Stato: INVIATO - ID: 13514

WILD UNGULATE UNCONTROLLED GROWTH IN SICILY: A REGIONAL PROJECT FOR THE EARLY DETECTION, SURVEILLANCE AND PREVENTION OF WILDLIFE-RELATED ZOONOSES

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Wild ungulates play a crucial role in the transmission and maintenance of several zoonotic pathogens. In addition, wild boars, feral pigs and fallow deer are regarded as the most important hosts for several tick species and tick-borne pathogens some of which are zoonotic concerns. In the last decades, a demographic growth of the populations of wild ungulates has been observed in Sicily. This increase in number poses eco-pathological issues and may represent a threat for both human and animal health. Therefore, considering the relevance of wild ungulates in the epidemiology of some infectious and parasitic agents of zoonotic concern surveillance activities in these species are strongly advocated. The research project Wild Ungulate Uncontrolled Growth in Sicily, funded by the Ministry of Health (GR-2021-12373930), aims to investigate the potential health risk posed by the increase in the number of wild ungulate populations in Sicily. Tick-borne pathogens (TBPs), and food-borne agents in areas where ticks, wild, domestic animals, and humans live in sympatry will be investigated. A risk analysis with a set of parameters to evaluate the potential hot spots in Sicily for the spread of TBPs associated with wild ungulates was performed in the first phase of the project. Risk factors included climatic-ecological conditions, wild ungulate population dynamics (uncontrolled growth of fallow deer and/or wild boar population, culling plans), tick abundance, human activities (presence of urban centers/small towns, recreational areas, grazing areas) were assessed and analyzed and transferred onto a map using a Geographic Information Systems (GIS). A risk ranking was elaborated, and five different potential hot-spot areas were identified, namely: S1 (Madonie Alte), S2 (Madonie Basse), S3 (Ficuzza/Corleone), S4 (Nebrodi), and S5 (Peloritani). All the study sites are characterized by the presence of natural areas, grazing areas for livestock, and the presence of a large number of wild ungulates. In particular, the S1, S2, and S3 are characterized by a large increase in wild boar and fallow deer populations, while in S4 and S5 only wild boar are present. In the second phase of the project, a monthly tick sampling from the environment and the molecular detection and identification of pathogens in the collected ticks will be performed. Moreover, blood, feces, and tissue samples from hunted animals and/or from animals undergoing selective control will be performed in the same sites. The results of this project will be useful to address future mitigation measures in the highest-risk areas and the dissemination of the obtained results will increase the awareness of people and workers daily at risk of pathogen transmission.

77° CONVEGNO SISVET

Stato: INVIATO - ID: 13537

Balantioides coli: an emerging zoonotic parasite in fattening pigs raised in northern Italy

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Balantioides coli is a ciliated protozoan with a direct life cycle and is mainly transmitted via the faecal-oral route, colonizing the caecum, colon and rectum of several hosts, as pigs, primates, humans, birds and rodents. It is considered a neglected zoonotic pathogen, included among the emerging foodborne parasites that should be placed on a priority list for the development of standardized control guidelines [1]. Pigs represent the main reservoirs for human infection; indeed, most cases are reported in people living in close contact with domestic pigs, wild boars and pig manure [2]. Little is known about the genetics of B. coli and the only available markers can be used for phylogenetic studies allowing the identification of different genetic variants of the parasite [2].
Considering the lack of current data on B. coli circulation, a survey was planned to investigate its prevalence and genetic diversity in pigs raised in northern Italy.
Overall, 440 faecal samples were taken from 22 fattening pig farms located in northern Italy, including the regions of Lombardy, Emilia-Romagna and Piedmont. In each farm, 20 faecal samples were randomly collected from the rectal ampulla of different animals, which were fatteners aging 9 months old and weighing 170 kg (OPBA_90_2023). Samples were analyzed by both the sedimentation and the FLOTAC® dual technique, using FS2 (sodium chloride, NaCl) and FS7 (zinc sulphate, ZnSO4) flotation solutions. Moreover, the Cohen's kappa coefficient was performed to evaluate the agreement between the copromicroscopic methods. Then, a conventional PCR targeting the complete ITS1–5.8S rRNA-ITS2 region and the last 117 bp (3' end) of the SSU-rRNA sequence was performed on a positive sample from each farm and PCR amplicons showing the expected size were purified and sequenced. The sequences were compared with those available in the GenBank database using the BLASTn algorithm and when more than one sequence was detected in the chromatograms, the PCR products were cloned. At the farm level, all the sampled farms were positive and a total of 422 out of 440 samples were positive by the sedimentation technique (95.9%), while 377 samples out of 440 were positive by the FLOTAC technique with zinc sulphate solution (85.7%) and 39 were positive by the FLOTAC technique with sodium chloride solution (8.9%). The Cohen's kappa coefficient revealed a moderate concordance between the sedimentation and the zinc-based FLOTAC technique (0.47) while the concordance was slight between the sedimentation and the salt-based FLOTAC technique (0.15), confirming that this latter method is not suitable for the detection of B. coli cysts in pig faeces. Of the 22 samples sent for sequencing, 19 had genotype B, 2 had genotype A and 1 showed mixed sequences, comprising both genotype A and B. Following cloning, the sample revealed the specific sequence variants A0 and B1.
This study demonstrated a high prevalence of B. coli in fattening pigs underlining their central role as reservoirs for humans and the importance of conducting in-depth studies to define its public health significance.

[1] Bouwknegt et al. Prioritisation of food-borne parasites in Europe, 2016. Euro Surveill, 23:1-11, 2018.
[2] Ponce-Gordo et al. Balantioides coli. Res Vet Sci, 135:424-431, 2021.

77° CONVEGNO SISVET

Stato: INVIATO - ID: 13723

Ostertagia ostertagi and Fasciola hepatica antibodies in bulk tank milk in relation to productive parameters in dairy cattle farms in Italy

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Among the available diagnostic techniques, antibody detection in bulk tank milk represents a useful tool to screen the presence of parasitic agents in dairy herds. Ostertagia ostertagi is one of the most important gastrointestinal nematodes for cattle worldwide. Fasciolosis, caused by the liver fluke Fasciola hepatica, can have a major economic impact on livestock industry. Both parasitic diseases mostly occur in subclinical forms but may lead to production losses in affected herds.

Therefore, the study aimed to evaluate the spread of O. ostertagi and F. hepatica and their impact on herd milk production parameters in dairy cattle in Italy.

Bulk tank milk (BTM) samples from 350 dairy herds of the largest dairy production area in Italy (Lombardy) were analyzed by indirect ELISA for the detection of O. ostertagi and F. hepatica antibodies (SVANOVIR, Svanova). BTM samples were classified according to ODR values [1,2]. Data on 5 qualitative and quantitative milk production parameters were collected; generalized linear models (GLMs) were developed.

The overall mean ODR for O. ostertagi revealed a value of 0.59. 107 herds resulted positive (ODR>0.6) with a prevalence of 30.6%. Besides, 138 farms showed ODR between 0.3 and 0.6 considered as a “grey zone” including animals within the pre-patency or weeks after treatment, when antibody titers are decreased [3]. A higher prevalence was detected in the provinces of Mantova (P=62%), Bergamo and Brescia (P=50%) and Pavia (P=28%). Lower prevalence values between 6 and 10% were evidenced in Cremona, Lodi and Milano. An association was evidenced between the ODR values and the productive parameters: in particular, in herds with ODR>0.5, both daily milk production (24.8 vs 24.1) (p>0.05) and mature equivalent milk yield (9388.9 vs 10229.3) (p=0.001) were reduced. Somatic cell count was higher in positive herds (253554 vs 238289) (p=0.05). Fat (3.85 vs 3.81) and protein (3.40 vs 3.38) content in milk were similar in positive and negative herds.

In 24 herds the infection with liver fluke was evidenced (P=6.9%). Positive farms were located in the provinces of Milano, Mantova, Bergamo, Pavia and Cremona with prevalence values between 4 and 18%. An effect of F. hepatica on productive parameters was demonstrated: daily milk production (20.7 vs 24.8) (p=0.026) and mature equivalent milk yield (8895.6 vs 9992.6) (p=0.046) were lower in positive versus negative farms. Somatic cell count was slightly higher in positive herds (253307 vs 243204) (p>0.05). Fat and protein content in milk were similar in positive and negative herds (3.84 vs 3.82 and 3.46 vs 3.38, respectively).

This study provides an assessment of the exposure to O. ostertagi and F. hepatica evidencing the impact of these parasites on herd performances in Italian dairy herds considering both quantitative and qualitative milk parameters. The screening for antibodies, assessing the infection level, is an instrument to determine the need for anthelmintic control in the herds.

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[2] Charlier et al., Prev Vet Med, 78:57-66, 2007.
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77° CONVEGNO SISVET

Stato: INVIATO - ID: 13847

Not just Cyathostomine: characterization of the intestinal strongyle population in stabled horses in northern Italy, with a focus on the presence of Strongylus vulgaris

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The routine use of anthelmintic drugs to horses has significantly decreased the prevalence of intestinal strongyles and the incidence of large strongyle-associated disease [1]. Nonetheless, this practice has also fostered the emergence of resistance to a few available anthelmintic drugs, especially in Cyathostominae populations. Nowadays, an approach based on selective or strategic treatments is being promoted to mitigate the further spread of drug resistance. It is therefore important to evaluate the reappearance of highly pathogenic species. The present study aims to assess the parasitological status of stabled horses in northern Italy and investigate the presence of Strongylus species using both morphological and molecular diagnostic techniques.
Fecal egg count of 440 horses from 25 stables was performed (FLOTAC Dual-Technique); third-stage larvae (L3) obtained obtained from individual coprocultures were i) morphologically identified and ii) subjected to Strongylus spp. real-time PCRs [2], followed by sequencing for species confirmation. Statistical analysis, using Generalized Mixed Models (GLMM), was carried out to evaluate individual and management risk factors influencing the spread of S. vulgaris.
Overall, 226 horses tested positive for strongyle infection (P=51.36%, mean UPG±s.d.=135.98±418.037). The morphological analyses performed on 4699 L3 revealed Cyathostominae Type A and Type C as the most widespread (mean%: 85 and 9.3, respectively), with a variability in richness among animals and stables. Concerning migratory strongyles, morphological and molecular analyses revealed S. vulgaris in 4 and 19 animals, respectively, with an overall of 20 positive horses (4.5%) from 12 different stables; no other species of the Strongylus genus were detected. The age of S. vulgaris positive animals was between 3 and 21 years (mean±s.d.=9.05±5.115), with 11 horses showing less than 200 UPG. Interestingly, three horses had been only recently introduced into the stables. Statistical analysis highlighted the young age of the animals (B±s.e. =0.069±0.032; p-value=0.035) and the possibility of access to the paddock (B ±s.e.=-2.206±0.592; p-value=0.0001) as predictive variables for the presence of S. vulgaris.
The results obtained from the study highlight how S. vulgaris, a parasite now considered relatively rare, is still circulating in the equine population in Italy. Considering its presence in horses with low strongyle UPG values, the combination of morphological and molecular techniques, preferably on individual samples, should be suggested as part of the routine coprological diagnosis.
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INVADERS OF THE INVADER: PARASITES AND HYPERPARASITES OF THE BLUE CRAB *CALLINECTES SAPIDUS* FROM NORTHERN ADRIATIC SEA

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The anthropogenic-mediated translocation of plant and animal species into geographic regions outside their native range is considered one of the most distinctive ecological features of the Anthropocene era.
An increasingly globalized world subject to environmental changes has led to a growing spread of alien species, some of which are considered invasive due to their marked predatory attitude towards indigenous species, food competition issues and/or their possible role as reservoirs/vectors of transmissible pathogens potentially harmful to native populations.
The blue crab (*Callinectes sapidus*), reported for the first time in the Mediterranean waters in the 1930s, has recently gained attention due to its considerable spread favoured by climate change; it has become almost ubiquitous in national coastal waters, where it is currently among the invasive alien species with the greatest impact on biodiversity and shellfish production.
In the Mediterranean, data on transmissible pathogens potentially carried by this species are scarce; to address this lack of information, we carried out a preliminary parasitological investigation in *C. sapidus* collected from different coastal areas of Emilia Romagna and Veneto, aimed at characterizing parasitic agents of interest to animal and public health, and to food safety.
The investigation was mainly focused on *Hematodinium* spp., a protozoan capable of causing heavy mortality in native crustacean populations, and on the presence of potentially zoonotic digenean metacercariae.
Overall, 68 specimens of *C. sapidus* were sampled from selected coastal areas of Emilia Romagna and Veneto, namely Punta Marina (RA), Marina Romea (RA), Codigoro (FE), Rosolina (RO), Scardovari (RO), Cesenatico (FC). All collected specimens were subjected to biometric measurements and to parasitological and histopathological investigations: haemolymph smears were stained with Hemacolor® for cytological examination; the gills and, after compression, the muscles were subjected to microscopic examination. Portions of different organs were also preserved in 10% buffered formalin for histological analysis.
Cytological examination of smears allowed to observe cellular elements attributable to blood stages of *Hematodinium* spp, in conjunction with a drastic decrease in the presence of haemocytes, in analogy with the descriptions available in the literature [1]; histological examination of the gills, highlighted the presence of some patterns attributable to infections by the parasite. Metacercariae of digenean trematodes were found in the muscle of 7 crabs, particularly in the claws musculature. The metacercariae were all viable and mobile, and morphologically attributable to the Microphallidae family, of which several species - all without zoonotic potential - have already been described in *C. sapidus* [1].
In some individuals, cyst-like formations containing spores of microsporidia were found in the muscle and identified through molecular analysis as *Unikaryon panopei*, a species already recently described in the USA [2] but never reported in the Mediterranean. Furthermore, some of the metacercariae found were also hyperparasitized by microsporidia not yet identified.
Further analyses are still underway, nevertheless preliminary results highlight the need to carry out large-scale investigations, focused on both parasites and other transmissible pathogens, in order to have a more complete picture of the health risks linked to the spread of the blue crab in our coastal environments.

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[2] Sokolova et al. Two new species of *Unikaryon* (Microsporidia) hyperparasitic in microphallid metacercariae (Digenea) from Florida intertidal crabs. Journal of Invertebrate Pathology, 182:107582, 2021.

77° CONVEGNO SISVET

Stato: INVIATO - ID: 13873

How to control Fasciola hepatica distomatosis in dairy sheep in Italy?

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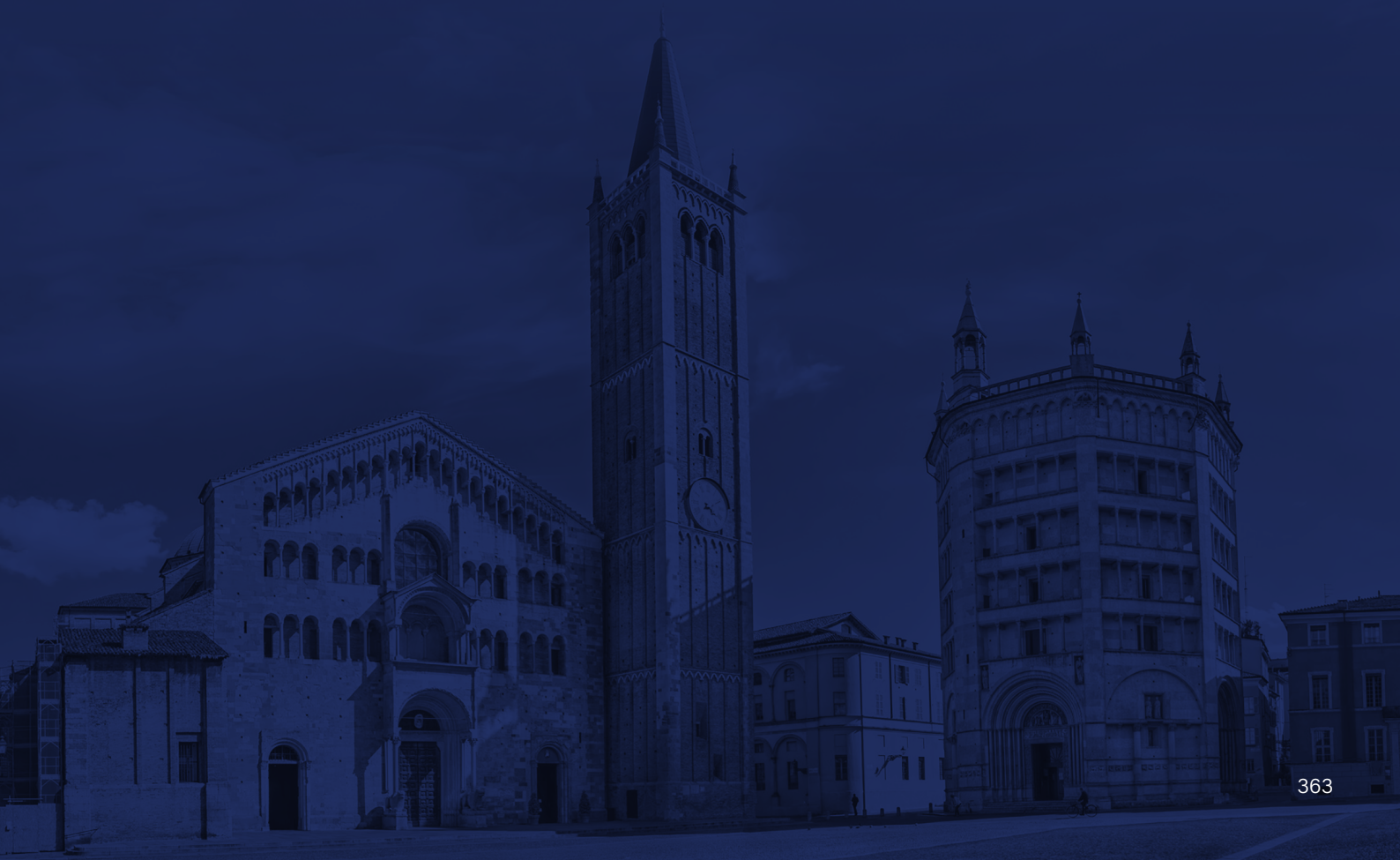
The aim of this work is to analize what prophylactic and terapeutic protocols applied in Italy for the control of fasciolosis by Fasciola hepatica in sheep.

SESSIONE POSTER

Di seguito vengono riportati i programmi
e i relativi contributi pervenuti



AIPVet				
ID	COD. AFFIS.	CORRESPONDING AUTHOR	TITOLO	AUTORI
13085	1	Alessia Tognoloni	Proteomic insights into tenocyte response: IL-1β alters the molecular and metabolic networks essential for tendon integrity and function	A. Tognoloni, M. Peffers, Y. Ashraf Kharaz , M. Seccaroni, G. Cerrotti, L. Urbanelli, C. Emiliani, A. Di Meo , E. Chiaradia
13126	2	Giovanni Martino	Mesothelial reactivity with multifocal mineralization in a dog	G. Martino, M. E. Gelain, B. Sacchetto, T. Banzato, S. Burti, S. Ferro
13183	3	Caterina Romanello	Study of PDGFR-β expression in normal and pathological canine placental tissues.	C. Romanello, S. Dell'Aere, D. Groppetti, F. Riva , G. B. M. Bianchi, E. Giussani, A. Inglesi, A. Del Carro, P. Roccabianca
13204	4	Sofia Tomasoni	Histopathological and immunohistochemical characterization of a new murine experimental model for the study of Neurofibromatosis type 1 (NF1)	S. Tomasoni , R. Verin , P. Finotti, F. Ciscato , I. Masgras, A. Rasola, F. Scantamburlo , A. Mazzaro , S. Negro , M. Pirazzini
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AIPVET

Proteomic insights into tenocyte response: IL-1β alters the molecular and metabolic networks essential for tendon integrity and function.

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Recent studies have linked interleukin-1beta (IL-1β) and chronic inflammation to the development and healing of tendon disorders [1]. The elevated levels of this cytokine after tendon injury, seem to drive abnormal ECM remodelling and tendon fibrosis [2]. Despite its recognized impact, the underlying molecular mechanisms of IL-1β are not well understood. Recently, the influence of IL-1β on gene expression profiles in equine tenocytes has been highlighted [3]. However, no proteomic investigations have been performed so far.

The present *in vitro* study investigates the response of equine tenocytes to IL-1β exposure using a proteomic approach, in order to elucidate the molecular mechanisms underlying the effects of this cytokine in the pathogenesis of tendon disorders.

Equine tenocytes, from superficial digital flexor tendon (SDFT), were exposed to different concentration of IL-1β for 24, 48 and 72 hours, and cell viability and gelatinase activity of matrix metalloproteases, such as MMP2 and MMP9, were analysed by MTT assay and gelatin zymography, respectively. The proteome of tenocytes treated with 10 ng/mL of IL-1β for 48 hours, compared to untreated cells, was analyzed using quantitative label-free proteomic analysis. Bioinformatic tools such as STRING and Cytoscape were used in Protein-Protein Interaction analysis and functional analysis respectively, aiding in the identification and interpretation of the complex protein networks and biological pathways affected by IL-1β treatment.

Tenocytes demonstrated resistance against all IL-1β concentrations when exposed for 24 hours. However, a decline (approximately 13%) in cell viability was observed when the exposure to IL-1β (10 ng/mL) was 48 hours. Similarly, MMP9 and MMP2 gelatinase activities measured in the cell culture medium, remained unchanged after 24 hours to IL-1β exposure with a significant increase after both 48 and 72 hours. Proteomic and bioinformatic analysis evidenced that IL-1β modified the abundance of a large numbers of protein species, most of which are involved in molecular ad metabolic pathways already linked to tendinopathy, confirming the role of these cytokine in tendon disorders. In particular, changed levels of proteins involved in ECM organization and degradation, cytoskeleton organization, cell adhesion, metabolic pathways, hypoxia response, oxidative stress ferroptosis, apoptosis, were highlighted.

This study clarifies the role of IL-1β in the alteration of tenocyte homeostasis, highlighting some molecular processes underlying the etiopathogenesis of tendon disorders. The cellular pathways in which the deregulated proteins are involved could represent potential targets for novel therapeutic approaches. Moreover, considering the SDFT as a valid biological and functional model for the human Achilles tendon, these findings could have significant translational value for human medicine from a One Health perspective.

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77° CONVEGNO SISVET

Stato: INVIATO - ID: 13126

Mesothelial reactivity with multifocal mineralization in a dog

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Mesothelial hyperplasia is a benign reactive condition that may result from acute or chronic insults to mesothelial surfaces in response to infectious, toxic, inflammatory, or neoplastic stimuli. Differentiating a mesothelial reactive proliferation from a mesothelioma can be difficult as reactive cells may exhibit, as well, papillary growth, cellular atypia, mitotic figures, and necrosis [1]. Some studies in human and veterinary medicine have shown that in malignant mesothelioma neoplastic cells can also have an osteogenic potential [2,3]. We describe a 4 year-old male Lagorai Shepherd dog evaluated for progressively worsening of abdominal and right scrotal enlargement. Abdominal ultrasound revealed severe abdominal and scrotal effusion characterized by an anechoic corpuscular fluid. The right hepatic lobe showed an irregular profile and an inhomogeneous echostructure along with some small areas of surface mineralization. Computed tomography confirmed severe ascites with diffuse reactivity of the parietal and visceral peritoneum, along with multifocal peritoneal nodules, and several areas of peritoneal mineralization on the spleen, diaphragm, stomach, abdominal wall. Blood count revealed mild neutrophilia and eosinophilia. The clinical chemistry results showed a mild reduction in total calcium, mild hypoproteinemia, hypoglobulinemia, increased A/G ratio, and mild hypocholesterolemia. The peritoneal nodular lesions were surgically sampled for cytological and histological examination. Cytology described mesothelial cells occasionally organized in papillary groups, with mild anisocytosis and anisokaryosis features, and rare mitosis. Histology confirmed the cytological findings and revealed the presence of a multifocal moderate chronic inflammation associated with small areas of mineralization. The mineralized material appeared to involve collagen fibers and the lumen of some vessels. Final diagnostic hypothesis was mild to moderate mesothelial reactivity with mild atypia and mild multifocal peritonitis with mineralization. The differential diagnosis was represented by a mesothelioma. Therapy was set with 1mg/kg of prednisolone for 7 days, progressively decreased for the following 22 days. Clear reduction in abdominal and scrotal effusion was noted after the first week of treatment. The patient had normal clinical parameters during the entire observation period. Three months after the end of the therapy, a clinical check-up, including an ultrasound examination, showed no effusion in the abdominal cavity and mild effusion of the tunica vaginalis of the right testicle. Few millimetric calcifications were still present, one over the right lobe of the liver, and several on the surface of both testicles. Based on the clinical improvement and the evolution of the pathological lesions at first follow-up, the patient was considered to be affected by mesothelial reactivity with unusual peritoneal mineralizations rather than a neoplastic condition. The cause has not been yet determined and further follow up is needed to confirm the diagnostic hypothesis.

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77° CONVEGNO SISVET

Stato: INVIATO - ID: 13183

Study of PDGFR-β expression in normal and pathological canine placental tissues.

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Platelet-derived growth factor (PDGF) receptor-β (PDGFR-β) belongs to the class III tyrosine kinases (TKRs). Coordinated signalling through PDGFR-β and its ligands PDGFs activates pathways that regulates vasculogenesis and proliferation of extra villous trophoblast, allowing for normal development of the placenta in humans, consequently granting the normal embryo development [1].The role of PDGF and PDGFR-β has been similarly demonstrated for mice placenta [2]. This study aims to characterize PDGFR-β immunohistochemical expression in 10 normal and 10 pathological canine placental samples collected in pregnant bitches undergoing caesarean sections. Most frequent lesions included necrosis, intervillous haemorrhage, oedema, and mineralization in 10/10 pathological placental samples.PDGFR-β in normal and pathological placental tissues was intensely expressed by amnios, glandular chambers and marginal hematoma and was moderately expressed by fibroblasts in the stroma. PDGFR-β was also expressed by maternal and foetal vascular endothelium and sincytiotrophoblast, with higher intensity in pathological placentas compared to the normal ones.PDGFR-β was not expressed by the cytotrophoblast, in necrotic areas and in the allantoid connective of both normal and pathological placenta. Increased PDGFR-β may represent a response to placental lesion eliciting hypoxia and stimulating vasculogenesis via PDGF pathways.

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77° CONVEGNO SISVET

Stato: INVIATO - ID: 13204

Histopathological and immunohistochemical characterization of a new murine experimental model for the study of Neurofibromatosis type 1 (NF1)

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Neurofibromatosis type 1 (NF1) is an autosomal dominant genetic disorder characterized by loss-of-function mutations in the NF1 tumor suppressor gene in humans and occasionally reported in other species. NF1 patients are predisposed to the development of malignant tumors arising from Schwann cells (1). Improvement in molecular biology, neuroimaging, and the development of new animal models contributed to elucidate the pathogenesis of NF1 and to test therapeutic approaches. To this aim we developed and characterized a new murine model of NF1-related malignant tumors with interesting results in terms of reduced timing of onset and reproducibility of lesions if compared to existing models. Specifically, tamoxifen chow was administered to NF1flox/flox; p53flox/flox; PLP-Cre+ mice for NF1 and p53 gene specific ablation in Schwann cells. After nerve damage, sciatic nerve was monitored through ultrasound inspection performing 3D reconstruction. To evaluate the development of neoplasia along the damaged sciatic nerve and to follow the growth we established four different groups (n= 6 per group) and mice were humanely sacrificed at 15, 30, 45- and 60-days post-surgery (dps). Each group included 3 controls NF1flox/flox; p53flox/flox; PLP-Cre- (Cre-) and 3 NF1flox/flox; p53flox/flox; PLP-Cre+ (Cre+) mice. All mice underwent necropsy and the main organs were sampled and submitted for histopathological and immunohistochemical (IHC) investigations. Interestingly, the lesion was detectable in Cre- and Cre+ mice at 15 days, but at 30-45-60 days nerve enlargement was not present in Cre- mice. A growing trend was noticed in Cre+ mice at 30, 45 and 60dps in the damaged sciatic nerve whereas the contralateral one was unremarkable.
Histopathology (H&E) of the sciatic nerves showed proliferation of Schwann cells and perineurial cells at 15dps in both Cre- and Cre + groups and interpreted as a regenerative attempt after trauma. Similar changes, with lower severity were observed at 30dps in Cre- mice with complete regeneration of the nerve at 60dps. As expected, the Cre+ groups at 30, 45 and 60dps showed a constant growth of the sciatic lesion in time with haphazardly proliferating Schwann cells showing atypical features morphologically consistent with nerve sheath tumors (NSTs). IHC for S-100 confirmed the diagnosis of NSTs and IBA-1 showed the presence of numerous tumor-associated-macrophages (TAMs). In order to identify the subset (M1 and M2) of TAMs specific IHC for iNOS, Arginase-1 (ARG1) and Glutamine synthetase (GS) was also performed.
In conclusion this newly proposed experimental model for NF1 is able to develop and reproduce NSTs that mirror a subset of tumors associated to human NF1 in a reduced timeframe (30dps) if compared to existing animal models. Furthermore, this murine NST model recapitulates tumor heterogeneity of human NF1 associated tumors as indicated by the high macrophage infiltration rate (2). As a perspective, this model can be of interest to study new pathogenetical features and to identify novel therapeutical approaches.

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77° CONVEGNO SISVET

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First report of megacolon in a sea turtle: a case report in Caretta caretta

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The term megacolon refers to a colon of increased both diameter and length in apparently absence of organic disease. Usually clinical signs could include constipation, anorexia, and weight loss. Although this pathologic condition is widely diffuse in humans and it has been also reported in dogs and cats, the pathophysiology is still unknown and symptom management is sometimes hard [1]. Here, we report for the first time a case of megacolon in a loggerhead sea turtle. A Caretta caretta was recovered from stranding on Western Mediterranean coasts (Messina, Sicily) in August 2023 and admitted at the National Reference Centre for the Wellness, Monitoring and Diagnostic of Sea Turtles Diseases, in Palermo. Biometric measures (CCL) and weight were recorded. Clinical examination and blood collection were performed. The subject (35 cm CCL, 5 kg) was in poor body condition and the absence of the right back fin was evidenced; on the carapace was found the presence of ectoparasites (Lepas) and algae. A severe anaemia and neutrophilia were observed. During the period of hospitalization in the water tank of the rescue centre, the subject showed anorexia and constipation. Radiographic exam was performed, showing the presence of air in the gastrointestinal tract. Supportive and medical therapies were set up. Defecation of a low quantity of faeces was observed only two weeks later. A total of 0,54g of dry mass of different type of plastics was found in faeces. The subject suddenly died in September 2023 and necropsy was performed. Considerable decreased liver volume, granulomatous and capsulated liver lesion, adhesion of the liver to the right lung, abnormal distension of colon with thickened wall containing massive necrotic material mixed to mucous fluids, were observed as gross lesions. Samples were then collected for microbiological and histological assays. Bacterial isolation was performed on selective and differential agar media, and the identification was carried out with the biochemical API method; for mycetes analysis, samples of liver and intestine were plated on Sabouraud agar. Visual inspection of heart, liver, spleen, urinary bladder and intestine and flotation of faeces were performed for parasitological assays. A strain of Yersinia enterocolitica was isolated from liver. Histologically, liver granulomas appeared with acellular central area composed by eosinophilic material and surrounded by a fibrous capsule. The origin of megacolon could vary between species and may results from long-standing mechanical or functional bowel obstruction as well as from obstructive lesions and from neurological abnormalities [2]. Gastrointestinal obstruction by foreign bodies (e.g. hooks, fishline, plastics) in sea turtle is the most common cause of stranding and often lead to death [3]. In these case report, even if liver granulomas and megacolon could suggest the presence of foreign bodies, it was found only a little quantity of plastics and, consequently and unfortunately, the cause of the origin of megacolon remains unknown.

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A case of tonsillar epidermoid cyst in a cat: endoscopic, cytologic and histologic findings

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The present work reports the first case of epidermoid cyst located in the tonsil of a cat. A female European shorthair cat, 2,5 years old, underwent endoscopy of the upper respiratory tract for inspiratory noise present since the first months of life but worsening over time. The subject showed no further symptoms, and the hematologic and biochemical findings were unremarkable. At endoscopy a cystic mass, with fluid content, of globular shape, of about 1.5 cm in diameter was seen in the right tonsillar crypt. A fine needle aspiration was performed to collect the cystic fluid content for cytological examination; the lesion was then opened with the removal of a small superficial tissue flap subjected to histological examination. Direct smears of the fluid were prepared to submit to cytopathology. On a bluish granular background with numerous cholesterol crystals, the preparations showed good cellularity. A mixed population was observed with a predominance of squamous cells showing variably keratinized cytoplasm, without features of atypia; moreover, sparse foamy macrophages were seen sometimes showing phagocytosis of bluish material (possible hemosiderin), occasionally showing bi- or multi-nucleation (multinucleated giant cells); few small lymphocytes and rare eosinophils were also seen. Cytology was consistent with a cystic lesion of squamous epithelial origin associated with mixed macrophagic inflammation. On histological examination of the superficial flap removed to perform the opening of the lesion, the sample was representative of a portion of mucosal tissue with the presence, on the deep side, of a segment of stratified squamous epithelium with superficial aspects of keratinization, composed of keratinocytes without morphological features of atypia. Around this segment of squamous epithelium, compatible with a portion of the cystic wall, there was a dense leukocytic infiltrate composed of lymphocytes and plasma cells, accompanied by less abundant segmented neutrophils. The morphologic picture was compatible with the sampling of an intramucosal cystic structure lined with normotypic squamous epithelium: possible epidermoid cyst. Following the fenestration procedure performed endoscopically, there was an immediate disappearance of the respiratory noise, with no recurrence of symptoms in the follow-up period (3 months). Epidermoid cysts (termed dermoid if they also include cutaneous appendages) are benign lesions linked to the congenital development of epithelial components of ectodermal origin in anomalous locations or their implantation by traumatic or surgical events. These lesions have been described in various species and can also be found in oral and pharyngeal locations. Few reports of oral/pharyngeal lesions in the feline species are present; the first detailed report of a dermoid cyst located in the nasopharinx was recently published, while one of the previous studies reported a cyst in the soft palate, but histological details regarding the appearance and nature of the cystic wall were lacking. To date, there are no reports of epidermoid cysts in cats in the tonsillar site (reported instead, for example, in humans), hence the interest in the case presented here.

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Mammary gland, Skin, and Soft Tissue Tumours Rates in Pet Cats: Findings of the Feline Tumours Collected from 2002 to 2022

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Abstract

Cancer is a leading cause of death in cats, and the rate of such disease has been increasing over the recent years. Feline oncology represents an important area of study not only for the health and wellbeing of cats, but also for human health, as many cats' cancers share similarities with human counterparts [1]. Therefore, epidemiological studies on feline oncology can provide insights into environmental and genetic factors that contribute to cancer in cats, which may ultimately inform improvements in human cancer care. To gain initial insights into the epidemiology of feline neoplasms, a descriptive study was undertaken using a dataset documenting cases of feline cancers collected from the Liguria region (north-west Italy) between 2002 and 2022. Then, the cancer cases were cross-referenced with the metal pollution data of the municipalities of the Liguria region. The information on heavy metal pollution derives from the monitoring activities conducted on wild boars. Wild boars, being exposed to Cadmium pollutant, accumulate cadmium in their liver and kidneys, serving as environmental proxies, i.e. *biomonitors*.

The database includes tumour location, morphological codes of the ICD-O-3, feline's breed, sex, neuter status, date of birth, date of diagnosis, national territorial unit code of the town of owners' residence and Cadmium concentration (obtained by *biomonitors)*. The dataset involves a population of 4399 cats including 3195 females (1425 neutered) and 1204 males (750 neutered). The tumour location was obtained by grouping the topographical codes into groups according to Grüntzig et al. [2]. To identify potential risk factors for the tumours occurrence, a GLM (Generalized Linear Model) was performed considering metal concentrations (i.e., Cadmium), sex, age class, neuter status as covariates and tumours prevalence as the outcome variable.

The results indicate that mammary gland tumours are the most represented in the female population, while skin cancers are more prevalent in males. Furthermore, the estimated models suggest that non-neutered female cats have a significantly higher probability of developing mammary gland tumour compared to the neutered females (OR=2.19, 95%IC:1.18-4.09). Conversely, neuter status appears to be a risk factor for soft tissue and skin tumours (OR=0.63, 95%IC:0.46-0.86; OR=0.91, 95%IC:0.64-1.30, respectively). Additionally, the data indicate an increased probability of cancer develop in polluted area (ORs from 3.63 to 40.85, 95% IC: 1.68-50.25), consistent with findings in human literature [3]. Overall, the data suggest that the feline population could serve as a valuable animal model for human cancers studies.

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77° CONVEGNO SISVET

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IS THERE ANYBODY OUT THERE? PRELIMINARY ASSESSMENT OF IMMUNE CELL MARKERS ON CANINE SOFT TISSUE SARCOMAS

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Canine soft tissue sarcomas (cSTS) represent a heterogeneous group of mesenchymal neoplasms accounting for 18% of canine cutaneous tumors. STS are considered immunologically "cold" tumors. Data regarding human STS (hSTS) indicate that tumor-associated macrophages (TAMs) are the most abundant cell type in the tumor immune microenvironment (TIME) of these neoplasms. Studies report that hSTS characterized by high infiltration of B and T lymphocytes, and the formation of tertiary lymphoid structures (TLSs) display a more favorable prognosis. Literature regarding the TIME of cSTS is extremely limited, and there are no studies examining its cellular populations. The present study aimed to perform a preliminary TIME characterization of cSTS using immunohistochemistry (IHC) for different immune cell populations. Thirty FFPE cSTS (15 perivascular wall tumors, 4 peripheral nerve sheath tumors, 4 liposarcomas, 4 fibrosarcomas and 3 undifferentiated sarcomas) were reviewed, graded, and analyzed using IHC to identify MAC387+, CD3+, Foxp3+, CD20+, and MUM-1+ cells. MAC387+ cells showed a widespread intratumoral distribution, while tumor-infiltrating lymphocytes (TILs) were primarily observed near tumor blood vessels. cSTS displayed low immunogenicity, with approximately half of the cases demonstrating a lack of immune cell infiltration. Conversely, a small subset of cases was characterized by high infiltration of immune cells and distinguished by the presence of dense lymphocytic aggregates with a follicle-like structure interpreted as TLSs. No significant differences were found in the TIME composition among cSTS of different histotypes or grade and no correlations were observed with the mitotic index. Further studies would benefit from a panel of antibodies targeting M1/M2 TAMs and cytotoxic T lymphocytes. Moreover, a larger cohort of clinically documented cases is needed to determine the impact of the TIME on treatment response and patient outcomes in cSTS.

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77° CONVEGNO SISVET

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Clinical-pathological features in 18 cases of granulosa-theca cell tumour of the equine ovary

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Granulosa -theca cell tumor (GTCT) in mare is the most common ovarian tumor. Clinical diagnosis of the tumor is made by transrectal palpation, ultrasonographic examination, endocrine profile, and sexual behavior [1] and the definitive diagnosis is made by histological examination. The current histological classification [2] reports two patterns: follicular (microfollicular and macrofollicular) and solid (insular, trabecular, tubular and diffuse); the tubular pattern is characterized by a “Sertoli-like” arrangement. Moreover, Call Exner bodies, theca cells and luteinized granulosa cells can be observed [2]. In this study, 18 cases of GTCT were included, the median age of the mares was 12 years with a range from 3 to 25 years. The breeds were: 6 Arabian Thoroughbred, 5 Italian Saddle, 2 Friesian, 1 Spanish Thoroughbred, 1 Spanish Arabian, 1 Thoroughbred, 1 Quarter Horse and 1 heavy draft mare. Mares presented infertility problems, in 18/18; stallion-like behaviour characterized by mounting other mares and aggressive behaviour, in 10/18. The other 6/18 presented anestrous and 2/18 abnormal estrous behavior. All showed unilateral enlargement of the ovary and a hypotrophic contralateral ovary. Ultrasound examination allowed to firstly investigate the internal architecture of the mass. Seventeen mares had unilateral ovariectomy performed by standing laparoscopic approach, while one mare with an ovary larger than 25 cm, underwent midline ventral celiotomy. In 7 cases the right ovary was affected and in the remaining 11, the left. Grossly, the neoplastic ovary had an ovoid or spherical shape, with a smooth surface, delimited by a fibrous capsule and on the cut surface, it was cystic (1 case), multicystic (11 cases) and solid (6 cases). The cystic structures contained serous or serohaematic material, delimited by solid connective septa of different thicknesses and of a greyish-white or yellowish colour. GTCTs were classified histologically according to most prevalent pattern. Histological patterns were follicular (macrofollicular, microfollicular or macro-microfollicular) in 8/18, solid in 3/18 (prevalently insular, tubular and diffuse), mixed in 7/18 (mainly macro or microfollicular and insular, tubular or trabecular). Cell population was characterized by neoplastic granulosa cells in all cases and by luteinized cells in 16/18 cases. In addition, 7/18 cases presented "Sertoli-like" elements and 17/18 cases a fibro-thecal
component. Heterologous bone elements were present in two cases, one with predominantly solid Sertoli-like pattern, and another with mixed pattern. Areas of dystrophic calcification were present in 2/18 cases (prevalently solid pattern). Call Exner bodies were present in only 2/18 cases. Mitotic activity was assessed, as low in 16/18 and moderate in 2/18 (prevalently solid pattern). The findings of this case series mostly agree with reports in literature [2,3]. A combination of macroscopic and microscopic patterns seems to characterize equine GTCTs, and no further morphological classification is probably possible. Despite the size, presence of calcification and moderate mitotic activity, equine GTCT typically remains a benign and unilateral tumour. No correlation between abnormal behaviours and presence of luteal, theca or Sertoli-like cells was found.

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Morphological and immunohistochemistry modifications in progressive post-mortem changes of mice central nervous system

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The estimation of post-mortem interval (PMI) is a daily challenge in forensic medicine and no single reliable method exists for an accurate estimation, even in human forensic medicine. Despite the brain is often a neglected organ in forensic pathology, its localization within the cranium preserves it from the action of external factors and scavenger animals, making it a hypothetical source of information even long after death. Nowadays the usefulness of forensic neuropathology has been largely highlighted [1], but very few studies focused on the study of central nervous system (CNS) autolytic changes, mainly restricted to some specific CNS areas, cellular populations, or selected temperatures [2-3]. In the study performed herein, temporal sequences of post-mortem CNS changes in brain kept at different temperature were investigated in different areas of C57BL/6J mice brains. In detail, fixation of mouse brains kept at different storage temperature (4°C, 22°C, and 37°C) was delayed for four timepoints (24, 120, 168, 336 hours; T1-T4). The brain of control animals was formalin-fixed straight after euthanasia. Histology (hematoxylin-eosin, luxol fast blue, Nissl's staining method) and immunohistochemistry (IHC) were carried out to investigate how post-mortem may affect morphology and identification of neuronal and glial cell epitopes, using markers such as NeuN, SMI-32, 2F11, Olig2, GFAP. A semiquantitative score ranging from 0 (absent) to 3 (severe) was used to described autolytic changes both in the grey (GM) and white matter (WM). Immunoreactivity was evaluated using both a qualitative score from 0 (negative) to 3 (strong) and a quantitative analysis for NeuN immunolabelled neurons. Statistical analysis was performed using Krustal-Wallis test, followed by Dunn's Multiple Comparison test using GraphPad Prisma 7. Results showed that autolytic changes started earlier in brains at 22°C and 37°C comparing to those at 4°C, and in GM comparing to WM, with cerebellum and hippocampus showing the earliest post-mortem changes. A complete loss of nuclear stainability with Nissl's staining was observed at T2 and T3 in samples at 22°C and 37°C. The cellular antigens were differently affected by autolysis process overtime: NeuN and Olig2 immunoreactivity were gradually lost at nuclear site and diffused to the cytoplasm; GFAP and SMI-32 showed an increase in immunolabelling, while 2F11 immunoreactivity decreased, until being no more detected at T4 and T2 in brains kept at 22°C and 37°C, respectively. Reduction of NeuN-positive cells was statistically significant between the control group and samples at 37°C ($p<0.01$) and 22°C ($p<0.05$) at all time points. This study suggests that CNS morphological analysis and immunohistochemical investigation could be satisfyingly applied to forensic cases providing useful data for the estimation of PMI.

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77° CONVEGNO SISVET

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Methodological protocol for the histological, histochemical and immunohistochemical evaluation of swine liver decellularized 3D-scaffolds loaded with pancreatic BxPC3 tumor cells and magnetic nanoparticles

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For many years, 2D in vitro cell cultures and in vivo animal models were the gold standard in cancer research; however, these models frequently do not fully recapitulate the complexity of the tumor microenvironment (TME). Recently, significant efforts have been made to develop novel in vitro models to replicate the complex interplay between tumor and TME, leading to the development of 3D in vitro models of decellularized extracellular matrix (dECM). [1] Pancreatic cancer has poor prognosis, and since a complete surgical remove is often not possible, treatment with radiotherapy (RT) is usually performed. Alternative anti-tumor approaches include Hadrontherapy (HT) and Magnetic Fluid Hyperthermia (MFH), by using magnetite nanoparticles (MNPs) injected into the tumor, to generate heat locally by an external alternating magnetic field. 3D in vitro models of dECM could represent a valuable model to test novel therapeutic approaches against various types of tumors, including pancreatic cancer. [2,3]
This study aimed at developing a methodological protocol for the histological, histochemical and immunohistochemical evaluation of swine liver decellularized 3D-scaffolds loaded with human pancreatic BxPC3 tumor cells receiving different treatments (i.e. HT and MFH).
Scaffolds were loaded with BxPC3 cells and treated with HT and/or MFH. MNPs were added to cells either prior to or after loading them into the scaffolds. After treatment, scaffolds were formalin-fixed, paraffin-embedded, and 4 μm sections were stained with Hematoxylin-Eosin (H&E) and Perl's Iron staining (visualization of Fe3+). Immunohistochemistry for Ki67 (cellular proliferation), Cleaved Caspase-3 (apoptosis), and γH2AX (DNA damage) was additionally performed.
Histological evaluation with standard H&E-staining allowed the assessment of scaffold colonization by loaded cells and identification of cell viability/cell death. The presence of MNPs was highlighted by Perl's iron staining, revealing a greater amount of particles when added after the loading of the cells on the scaffold. Immunohistochemistry for Ki67 allowed the quantification of proliferating cells, and γH2AX detected variable amounts of cells with DNA damage. CC3 identified only few apoptotic cells, indicating that the not viable cells visible in HE-stained sections were not apoptotic but rather necrotic.
The proposed protocol proved to be useful in evaluating swine liver 3D-scaffolds loaded with BxPC3 human pancreatic cells and MNP. This protocol can be also applied for the evaluation of other types of 3D-scaffolds.

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Hepatobiliary toxicity and distribution of 10 nm silver nanoparticles after single intravenous administration in mice

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Abstract
Silver nanoparticles (AgNPs) are an important category of nanomaterials characterized by their dimensions typically ranging from 1 to 100 nm. These small sizes result in a high surface area to volume ratio, which in turn leads to a spectrum of distinctive chemical, physical and biological properties not shared by their bulk material counterparts [1]. The widespread application of AgNPs in multiple fields, such as medicine and industry, raises concerns about their potential environmental impact and toxicity. Toxicological studies are thus necessary to foster knowledge on the toxic effects of AgNPs. Some studies in animal models report AgNPs toxic action on different organs (lungs, liver, kidneys, spleen, brain and parotid glands), either after single or repeated administration, and following different routes of exposure [2].

In view of the need to improve the understanding of the AgNPs-related toxicity, this work aimed at investigating the impact of 10nm AgNPs on *in vivo* hepatobiliary toxicity assessing the AgNPs' tissue distribution.

Male CD-1(ICR) mice of 4-5 weeks were purchased from Charles River (Calco, Italy) and randomly divided in 2 groups (n=3). Animals were intravenously treated with vehicle (sterile water) or 10 mg/kg bw of 10 nm AgNPs purchased from NanoComposix (San Diego, USA). 24 hours after treatment, liver and gallbladder were fixed in 10% NBF, paraffin-embedded, and 4 µm sections were stained with Hematoxylin&Eosin (H&E). To analyze the tissue distribution and cellular localization of silver, autometallography (AMG) and immunofluorescence were performed. For immunofluorescence, the following primary antibodies were used: anti-Iba1 (macrophages), anti-Arginase1 (hepatocytes), anti-CD31 (vascular endothelial cells), and anti-LYVE-1 (lymphatic and sinusoidal endothelial cells). Images were acquired using the TCS SP8 confocal microscope (Leica microsystems) with a 63x/1.4 oil immersion objective exciting and acquiring the emission of the Alexa 555 or Alexa488 directly conjugated primary antibodies, while nuclei were visualized by DAPI staining. Silver aggregates were imaged in reflection mode using the 561 or 488 nm laser lines. A Z-stack of few microns was acquired for each field of view.

In mice treated with 10 nm AgNPs hepatobiliary lesions, including severe diffuse midzonal hepatocellular necrosis and hemorrhage, and mild to severe diffuse mural and intraluminal hemorrhage of the gall bladder were observed, while no lesions were present in control mice treated with vehicle. In 10 nm AgNPs treated mice, silver aggregates were mainly detected in Kupffer cells, but also within hepatocytes, and endothelial cells.

Our study revealed that i.v. administration of a single dose of 10 mg/kg of 10 nm AgNPs induced severe hepatobiliary toxicity, associated with presence of silver aggregates within different cell types (Kupffer cells, hepatocytes, endothelial cells), indicating the ability of nanoparticles to penetrate within cells of different origin. The hepatobiliary toxicity of these small AgNPs is likely related to their massive elimination in the bile [3].

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77° CONVEGNO SISVET

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Exploring novel immunohistochemical markers of Doxorubicin-induced cardiotoxicity in mice

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Doxorubicin (DOX) is the most used anthracycline drug for the chemotherapeutic treatment of many types of cancers, including solid and non-solid malignancies. However, dose-dependent cardiotoxicity progressively leading to lethal myocardial damage is reported in association with its usage [1]. DOX cardiotoxic molecular mechanisms are complex, multifactorial, and considered to be related to the reduction of ATP levels, increased ROS production, mitochondrial calcium and iron overload, DNA damage, and cell death [2]. The heart is the most metabolically active organ in the body, with a greater mitochondrial content but a comparatively lower amount of antioxidant enzymes (e.g. catalase and glutathione peroxidase) compared to other organs. For these reasons, mitochondrial and oxidative damage are considered contributors to DOX-induced cardiotoxicity. In the mouse model, DOX-induced cardiac histopathological findings are found only at cumulative high doses, whereas at lower doses Transmission Electron Microscope (TEM) is the most suitable method to detect ultrastructural mitochondrial damage, hallmark of DOX-induced cardiotoxicity [3].

This study aimed at identifying and quantifying immunohistochemical markers of DOX-induced cardiotoxicity in a mouse model with proven ultrastructural mitochondrial damage but without evident histopathological changes.

Six 5-week-old females outbred athymic nude (Foxn1nu) mice were subcutaneously injected with patient derived xenograft of triple negative breast cancer HBCx-17. When tumors reached about 100 mm^3 of volume, animals were randomly divided in control and DOX-treated groups (n=3). Mice received six injections of either DOX (2.5 mg/kg) or saline every 2-3 days for 21 days. On the 3rd day after the last administration, mice were sacrificed, and hearts were sampled for TEM, and histology. For TEM, hearts were fixed in 2.5% glutaraldehyde, embedded in epoxy resin, and ultrathin sections were evaluated for mitochondrial damage. The area of mitochondria (at least 100/sample) and the percentage of mitochondrial damaged area were measured using ImageJ software. For histology, 4 μm sections were obtained from FFPE samples and stained with Hematoxylin-Eosin. For immunohistochemistry, the following primary antibodies were used: Iba-1 (macrophages), Cleaved-Caspase-3 (apoptosis), GPX4 (oxidative stress), and γH2AX (DNA damage). QuPath software v0.3.2 was used to quantify the number of positive cells (γH2AX, CC3) and the percentage of positive area (Iba-1, GPX4) per mm^2. Statistical analysis was performed with GraphPad Prism v10.

At TEM, DOX treatment induced significant ultrastructural damage to cardiomyocytes: the percentage of the area occupied by the mitochondrial matrix increased, a depletion of cristae and an increase in the damaged area were found. No relevant histological findings were observed in the heart. At immunohistochemistry, GPX4 was increased in DOX-treated mice as compared to control mice, whereas no differences were found for the other markers.
The results of this study highlight the utility of GPX4, a marker of oxidative stress, as immunohistochemical marker of DOX-induced cardiotoxicity, especially in absence of histologically detectable changes.

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77° CONVEGNO SISVET

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Mycoplasma bovis pneumonia in cattle: the role of co-infections in lesion modulation

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The Bovine Respiratory Disease Complex (BRDC) is a major cause of production losses in cattle farming, with significant implications for animal health and welfare. The combination of environmental and management stressors impairs pulmonary defences, leading to pneumonia with polymicrobial etiology [1]. Mycoplasma bovis is a major bacterial agent of BRDC and is recognized as a primary cause of pneumonia and other diseases in calves and adult cattle [2]. However, how bacterial co-infections impact on lesion development in naturally occurring M. bovis pneumonia is still not fully elucidated. This study aimed to describe histopathological findings typical of M. bovis lung infection and to investigate the role of concurrent infections on lesion modulation. Lung samples collected at necropsy from calves and adult cattle were subjected to a standardized diagnostic protocol including gross and histological examination, and bacteriological, virological and molecular analyses. Giemsa and Gram histochemical stains, along with M. bovis immunohistochemistry (IHC), were also performed to identify bacteria and M. bovis antigen in lung tissues. Overall, 35 cases were collected and divided into the following categories: pneumonia associated with M. bovis infection (15), pneumonia negative for M. bovis (20) and non-pneumonic cases (10). Histologically, pneumonia associated with M. bovis infection were classified according to the following morphological diagnosis: necrosuppurative bronchopneumonia (NSB), necrotizing pneumonia (NP), and fibrinous pleuropneumonia (FPP). In 14 out of 15 cases (93%) three types of necrotic lesions (endobronchial, bronchocentric, and parenchymal) were observed. Endobronchial necrosis was characterized by hypereosinophilic material filling the airway lumen and diffuse M. bovis IHC positivity. Bronchocentric necrotic foci were characterized by their circular shape, regular margins, and loss of bronchiolar epithelium. Smaller lesions showed diffuse M. bovis IHC positivity while, in larger ones, this was peripherally distributed. Finally, necrotic areas with irregular shape were defined as parenchymal necrosis and were characterized by peripheral M. bovis IHC positivity. M. bovis antigen was also detected in cases of suppurative bronchiolitis and bronchitis. Regarding co-infections, 9 out of 15 cases (60%) tested positive for other microbial species by bacteriological, virological and/or molecular analyses, with P. multocida and T. pyogenes being the most frequently isolated bacteria. M. bovis was not visualised by either Giemsa or Gram staining in IHC-positive lesions. Histochemical evaluation revealed bacteria other than M. bovis within necrotic lesions in 11 out of 15 cases (73%). Overall, 55% of cases with co-infections were categorized as severe NP with large areas of parenchymal necrosis, 27% as FPP, and 18% as NSB. All cases with only M. bovis lung infection were categorized as NSB. In conclusion, necrotic lesions are strongly indicative of M. bovis lung infection. Acute stages show suppurative bronchiolitis and endobronchial necrosis. Lysis of the bronchiolar wall then allows the lesion to expand centrifugally, resulting in bronchocentric and parenchymal necrosis. M. bovis lung infection is often associated with other respiratory pathogens. Although identifying the exact role of each microbial species in lung lesion development is challenging, obtained results suggest that concurrent bacterial infections may exacerbate the necrotizing process primarily caused by M. bovis.

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77° CONVEGNO SISVET

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Gross pathology and etiopathogenetic findings in loggerhead sea turtles (Caretta caretta)

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Loggerhead sea turtle (Caretta caretta) is the most abundant turtle species in the Mediterranean Sea and the only one that currently reproduces there on a stable basis [1]. Although this sea basin provides the ideal habitat for the entire life cycle, the populations are in constant decline and therefore Caretta caretta is classified as a “vulnerable species” by the IUCN. In this study we analyzed 12 cases of stranded Loggerhead Sea turtles that were submitted over the years to the School of Biosciences and Veterinary Medicine of the University of Camerino, for post-mortem examination. The main causes of death were categorized according to an anatomic-pathological classification into inflammatory, chronic/degenerative, systemic/vascular, and others. They were then classified based on the affected systems and for each system we described the main post-mortem findings including histopathological lesions. The main cause of death was inflammation linked to bacterial involvement, followed by chronic/degenerative causes. The hepato-biliary system was overall the most involved, although systemic conditions were also very represented. The gastrointestinal system was instead the most affected by inflammatory conditions. All the turtles examined over the years were heavily parasitized by epibiont barnacle (relatable to Chelonibia testudinaria). Although barnacles are considered non-pathogenic for turtles and their number is associated with the state of reactivity and general health of the host, a strong burden is linked to the Debilitated Turtle Syndrome (DTS) [2]. Nevertheless, it is not yet clear whether the debilitation of turtles causes colonization or the opposite. A thorough microscopical examination, by means of optical and transmission electronic microscopy (TEM), also allowed the evaluation of barnacles’ lesions on the carapace and the possible association of bacterial colonization of the skin. In some cases, TEM also highlighted structures compatible with Herpesvirus virions in the hepatocytes. These observations may be of help to better clarify the combined role of Herpesvirus spp. and barnacles in the pathogenesis of DTS.

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77° CONVEGNO SISVET

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Are smudged erythrocytes only induced by lipids?

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Smudged erythrocytes (SE) are erythrocytes that lyse during blood film preparation or during blood collection of lipemic samples [1]. Circulating lipids, and especially triglycerides, if increased compared to their normal values, increase the permeability and the osmotic fragility of erythrocytes, that in turn may appear smudged or resemble ghost cells [2,3]. In our routine practice, SE may be found also in blood samples that do not appear grossly lipemic or, conversely, may not be present in all the samples that have a higher lipid concentration based on biochemical testing. To assess the reliability of this anecdotal finding, we evaluated the presence of SE in blood smears from samples with known concentration of cholesterol and triglycerides. This retrospective study was done on 290 blood smears prepared from anticoagulated canine blood samples routinely submitted to our diagnostic laboratory and collected from patients of the Veterinary Teaching Hospital as a part of clinical visits. Since no samples were collected specifically for this study, a formal approval from the ethical committee was not required (decision n° 2 2016 of the ethical committee of the University of Milan). May Grünwald-Giemsa stained blood smears from dogs that performed both hematology and clinical chemistry (including the measurement of cholesterol and triglycerides) were retrieved from our archive and microscopically examined to assess the presence of SE. The prevalence of samples with SE in cases with or without macroscopically evident lipemia or with or without increased concentration of cholesterol and/or triglycerides was then calculated and statistically compared. SE were found in all the samples with visually lipemic serum (47/47, 100.0%), but also in 115/243 samples (47.3%) that did not have macroscopic lipemia. However, the difference between these proportion was statistically significant (P<0.001). No significant differences (P=0.341) were found in the proportion of samples with SE between cases with normal cholesterol and triglycerides (54/90, 60.0%) and cases with hypercholesterolemia and/or hypertriglyceridemia (108/200, 54.0%).The proportion of cases with SE was similar in samples with hypertriglyceridemia alone (46/75, 61.3%) in samples with normal triglyceride and cholesterol (54/90, 60.0%) and in samples with hypertriglyceridemia and hypercholesterolemia (52/97, 53.6%) but notably lower (10/28, 35.7%) in samples with hypercholesterolemia alone, even though a significant difference among groups was not found (P=0.096). Similarly, no significant differences were found between the proportion of cases with SE in samples with (62/125, 49.6%) or without (100/165, 60.0%, P=0.062) hypercholesterolemia, irrespective of the presence in both groups of hypertriglyceridemia, or between the proportion of cases with SE in samples with (98/172, 57.0%) and without (64/118, 54.2%, P=0.644) hypertriglyceridemia, irrespective of the presence in both groups of hypercholesterolemia.This study confirms that SE may be found in samples with macroscopic lipemia and in many, but not all, samples with hypercholesterolemia and/or hypertriglyceridemia, suggesting that the effect of lipid on RBC membranes may be dose dependent or influenced by factors other than the simple presence of increased cholesterol and triglycerides. This last hypothesis is supported also by the detection of SE in about half of the samples with normal triglycerides and/or cholesterol. Therefore, further studies are needed to determine which factors other than lipemia and hypertriglyceridemia or hypercholesterolemia may induce smudging and lysis of RBCs.

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Viral infections in free-living cats, the role of passive surveillance. Preliminary data.

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The spread of viral agents in free-living cats (free-roaming and colony cats) is not completely known in Italy, there are only sporadic patchy studies. Several host and population-related factors contribute to pathogen prevalence and their pathogenicity, including transmission route, frequency and type of feline contact within and out of the group and other variables such as age, sex and immune status. Furthermore, viral co-infections are also observed.Feline Panleukopenia (FP) is a highly contagious viral disease of cats, caused by Feline Parvovirus (FPV). Most of infections are subclinical in adult cat, while kittens are affected more severely. Infection rate is high in unvaccinated cats. Infections occurs through contacts with feces and secretions. FPV is highly stable in the environment. Feline Coronavirus (FCoV) is a common feline pathogen that can cause mild diarrhea in kittens and systemic or individual organs disease in adult cats (Feline Infectious Peritonitis, FIP). It has been hypothesized that the FIP virus is a mutation of the enteric FCoV. Viral transmission occurs mainly via the fecal-oral route. Feline Immunodeficency Virus (FIV) and Feline Leukemia Virus (FeLV) are Retroviruses which can cause persistent infections in cats, resulting in severe immunosuppression. FIV is mainly transmitted by aggressive interactions among cats while FeLV is transmitted by saliva during grooming or sharing food and water bowls. Viral infections affect both domestic and stray cats and the contact between them may facilitate virus transmission. Viral co-infections have been observed in cats and these can lead to more severe symptoms respect to the single pathogen.Aim of the study was to investigate the health status and viral circulation in free-living cats belonging to AULSS 6 Euganea of Padua (Veneto region) to assess viral prevalence and improve health management in cat colonies. 150 cats were collected dead or in precarious health conditions in the territory of AULSS 6 Euganea and delivered to the Public Veterinary Service, between January 2023 and March 2024. Necropsy examinations were carried out at IZSVe laboratories on deceased or euthanized cats. The animals were grouped according to their presumed age: i. Young: ≤5 month's old, immature, absence of permanent dentition (n.17); ii. Adults 6 months-8 years old: definitive dentition and sexual maturity (n.73); iii. Advanced age: >8 years old, based on dental wear and physical conditions (n.60). Based on the suspected diagnosis, PCR tests for FPV, FCoV, FIV and FeLV was performed on pool spleen and mesenteric lymph nodes and, when present, organs with lesions.Among tested animals, 13.3%, 13.0%, 18.4% and 21.6% were positive for FPV, FCoV, FIV and FeLV, respectively. FPV and FCoV were detected mainly in adult cats, whereas FIV and FeLV in elderly cats. Less positivity of viral infections were detected in young cats, probably due to the low animal number, none of them tested positive for FIV.Co-infections were detected in 25.0% of positive cats. This study highlight a viral positivity about in 35.0% of sampled animals. Differences in positivity of viral infections were observed among the three age groups. Interestingly, the percentage of co-infections in our cases is higher than that reported in literature (1.2)

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Hospital-Acquired Infections, don't forget pets: surveillance in Veterinary Facilities- Preliminary data

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In humans, the Hospital-Acquired Infections (HAIs), usually associated with antibiotic-resistant bacteria, including Methicillin-Resistant Coagulase-Positive Staphylococci (MRCoPS), Extended Spectrum Beta Lactamase (ESBL)-producing Enterobacteria (i.e. E.coli, K.pneumoniae), Vancomycin-Resistant Enterococci (VRE), Pseudomonas aeruginosa, and other pathogenic microorganisms (i.e. Salmonella spp.), are widely described. Otherwise, limited data are available in the veterinary field. The aim of this study, was to investigate the bacteriological contamination of selected small animal veterinary facilities (VFs), particularly for the presence of potentially pathogenic bacteria for animals, human operators and owners. 15 VFs of North-eastern Italy were involved in this study which lasted from February 2022 to February 2024. A questionnaire was filled out for each facility, including information on cleaning procedures, healthcare personnel, number of operating rooms, average number of patients per week, number and type of surgical procedures performed per week, number of postoperative infections, etc. Samples were collected from several defined critical points of waiting rooms, visiting room, hospitalization areas, anterooms and operating rooms, using sponges and dip-slides, in different seasons and times of the day. Bacteriological examination included: i. specific selective/enrichment media to detect the presence of ESBL-producing Enterobacteria and Pseudomonas aeruginosa (McConkey Agar + cefotaxime), MRCoPS (Chromagar MRSA II), VRE (Cromid VRE), Salmonella spp, (Modified semi-solid Rappaport-Vassiliadis and Xylose Lysine Deoxycholate Agar); ii. determination of the total aerobic mesophilic count and the count of coagulase positive staphylococci; iii. detection of dermatophytes and yeasts by placing 3 plates with selective medium (Mycobiotic Agar) on the floor of each room for at least one hour. Moreover, MRCoPS were confirmed for the presence of mecA genes by PCR. A disk diffusion test was performed for phenotypic confirmation of ESBL-producing strains. A total of 5514 samples were examined. The proportion of MRCoPS, VRE and Pseudomonas aeruginosa was around 1% of the samples; ESBL was detected in 3.3% of samples; Salmonella spp was found in 3 VFs. Only one clinic resulted positive for dermatophytes (Nannizzia gypsea), while Malassezia pachydermatis was detected in different rooms and at different sampling times in 6 facilities. The highest counts of aerobic mesophilic bacteria were found in hospitalization and visiting rooms, while counts of coagulase-positive staphylococci were under 4 ufc/ml in most facilities. As expected, the mesophilic count, which included saprophytic bacteria, was higher in the hospitalization areas and in the visiting rooms, characterised by a greater influx of animals and/or humans, and in some critical points such as sinks, door handles and hospital cages. Even though the prevalence of antibiotic-resistant bacteria were low, some of the isolated bacteria might be pathologically relevant. Our findings underline the importance of regular active surveillance of pathogens in small animal clinics, as a pivotal step in managing hospital's infection-control program, keeping in consideration the unique pathogen risks, facilities, animal populations and personnel characteristics.

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CHALLENGING DIAGNOSTIC CASE: ENTERITIS AND MENINGOENCEPHALITIS OF UNKNOWN AETIOLOGY IN 2 FEMALE BUFFALOES FROM A FARM IN NORTHERN ITALY

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Post mortem investigation is often difficult in livestock animals, primarily because of the obvious impediments of transporting large animals to the nearest Veterinary Department, and secondarily for the financial implications of such process. However, pursuing a thorough *post mortem* investigation in livestock animals has important implications for the health of the entire livestock, and for public health in general. Here we report an interesting case of *post mortem* diagnostic investigations in 2 young (6-month-old) female buffaloes coming from the same farm in Northern Italy. The two animals were referred to the Anatomical Pathology Service of the Veterinary Teaching Hospital of the University of Milan (Lodi, Italy), the first in December 2020 and the second in February 2024. Both buffaloes were found dead in the farm with previous neurological signs. At necropsy, an acute and diffuse necrohemorrhagic enteritis was found. At histology, severe necrosis of the small intestine was found, along with hemorrhages and fusion of villi. Because of the reported neurological signs, brain, cerebellum, and brainstem were also harvested, despite not having gross alterations. In both cases, severe fibrino-necrotizing meningoencephalitis was found at histology, and it was characterized by severe perivascular cuffing of macrophages and neutrophils, necrosis of the vascular endothelium with thrombi, and neuroparenchymal microabscesses. Mild mixed periportal hepatitis was also found in the second case. Our differentials for the enteritis included *Listeria monocytogenes*,[1] *Escherichia coli*, *Salmonella* spp., *Clostridium perfringes* type A,[3] Malignant catharral fever, Bovine viral diarrhea, and Infectious bovine rhinotracheitis. The differentials for the meningoencephalitis included *Histophilus somni*, *Listeria monocytogenes*,[1] *Salmonella* spp., and *Escherichia coli*. An association between enteritis and meningoencephalitis was supposed. To narrow down the list of differentials, additional stainings on FFPE samples of small intestine, brain, and brainstem were performed. These included Gram stain, which was negative, and immunohistochemistry for *Listeria monocytogenes* and *Escherichia coli* on the first and second case, respectively, which were both negative. Bacteriology was also performed on the second case and led to isolation and typing of *Psychrobacter phenylpyruvicus* from the brain. Various strains of this bacterium have been noted to cause infant meningitis and general bacteremia,[2] however its pathological role in animals is not clear. Here we reported a dual case of enteritis and meningoencephalitis in 2 female buffaloes from the same farm, that represented a diagnostic challenge and a still unresolved case. Further analysis will be needed to help clarify the case.

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Immunohistochemical Characterization of Mononuclear Phagocyte System Cells in Cattle

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The Mononuclear phagocyte system (MPS) consists of a network of circulating monocytes and tissue-based macrophages and dendritic cells serving distinct, yet interconnected roles in embryonic development, tissue homeostasis, immune surveillance, antigen presentation and pathogen clearance.[1] Phenotypical studies on bovine MPS populations have mainly focused on pathological conditions and rely on a combination of monocytes isolation, flow cytometry, immunofluorescence and immunohistochemistry, in the latter cases often with a limited number of antibodies tested. [1,2,3] To the authors' knowledge, immunohistochemical investigation with a broad panel of markers in both physiological and pathological conditions is lacking. The aim of the study was, therefore, to apply a panel of antibodies to investigate their applicability on bovine tissues and to characterize different subsets of MPS cells in bovine healthy and selected pathological samples. Iba1, CD206, MARCO, HO-1, Lysozyme, Arginase 1, iNOS, MHC-II, Galectin-3 and CD86 were applied to sections of healthy bovine ileum and to a subset of representative pathological conditions comprising a case of granulomatous lymphadenitis (nodal tuberculosis), a case of granulomatous ileitis (ileal paratuberculosis) and a case of chronic fibrino-suppurative pleuro-pneumonia with multiple alveolar multinucleated macrophages. Among the applied markers, Iba1, MHC-II and Galectin-3 were the most effective, with strong positive signal in absence of background. CD206, HO1, Arginase 1, CD86 and Lysozyme effectively identified MPS cells in the tested samples albeit with variable background signal while INOS and MARCO resulted negative in all examined samples. In healthy tissues the combination of immunohistochemical positivity, cellular morphology, and cellular localization allowed us to classify different subsets of intestinal MPS populating both the lamina propria and the mucosa-associated lymphoid tissue of Peyer's patches. The same characterization was also applicable to pathological cases where markers proved useful in detecting different subsets of inflammatory MPS cells. Remarkably, alveolar multinucleated cells in the pneumonia case were prominently Iba1, galectin-3 and MHC-II positive while in the tuberculosis case they were Iba1, galectin-3 and MHC-II negative, possibly suggesting a distinct ontogenesis or different functional roles.

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77° CONVEGNO SISVET

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Fifty Shades of Brown: A Histochemical Exploration of Tissue Pigments

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Pigmentation refers to the pathophysiological process in which abnormal deposits of exogenous or endogenous substances accumulate in tissues, leading to alteration in color and occasionally to tissue damage and inflammation.1The characterization of pigmentations in tissues is fundamental in both diagnostic and experimental histopathology and requires the application of histochemical stains capable of highlighting specific molecules based on their chemical properties.2 Melanosis, anthracosis, hemosiderosis, lipofuscinosis, and pigmentation from bile and hemoglobin/myoglobin represent some of the most common pigmentations observed in domestic and laboratory animals.3An adult Friesian cow, a beagle dog, and a Fenretinide-treated apoE-knockout (EKO) female mouse with coarsely granular brown pigment in renal cortical tubular epithelium, an adult dog with brown to amphophilic pigment in small intestine myofibers and an old dog with neuronal cytoplasmic golden-brown pigment were included in the study. Cases were stained with hematoxylin and eosin (HE) and with a panel of histochemical stains including Giemsa, Perls Prussian blue, Ziehl-Neelsen stains and Periodic acid-Schiff reaction. Both an unstained blank section and an HE-stained sections were, additionally, analyzed under UV light to assess pigment autofluorescence.The applied stains proved useful in discriminating the nature of pigments, identifying them as hemosiderin in the murine kidney, a consequence of treatment-related hemolysis, as ceroid-lipofuscins in the canine intestine possibly related to dietary or genetic factors and as lipofuscins in canine neurons, a product of the lipid metabolism in aged animals' nervous system. However, it was not possibly to fully elucidate the nature of the pigment observed in the cortical tubular epithelium of bovine and canine kidneys, highlighting the need for a broader panel of histochemical stains or for more advanced techniques such as electron microscopy or histology-guided mass spectrometry profiling.

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FATAL INTOXICATION BY INGESTION OF *TAXUS BACCATA* IN TWO DONKEYS: PRELIMINARY CASE REPORT RESULTS

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Taxus baccata, also known as European yew, is an evergreen tree native to Europe, primarily grown as an ornamental plant. Plant toxicity has been known since ancient times, and all parts of the plant are toxic, apart from the aril, the red pulp of the berries. [1] Yews possess a variety of toxic compounds, in particular the taxine alkaloids, which are the main responsible for *T. baccata* toxicity. [2]
The aim of this study is twofold: to document a fatal poisoning case involving donkeys (*Equus asinus*) and to highlight the dangers of European yew ingestion for both animals and humans, and consequently to emphasize the need for reliable methods to detect these toxic compounds also in veterinary medicine.
Two donkeys coming from the same farm in mainland Venice, north-eastern Italy, were found dead on December 20th, 2023. As reported by the owners during the anamnesis, there were thin leaves and sticks compatible with *Taxus baccata* inside the paddock, and no additional symptoms were documented apart from sudden death. The animals were accepted at the Dept. of Comparative Biomedicine and Food Science (BCA) laboratories the following day, and immediate complete necropsy was performed. A standardized sampling was performed for histology, and suspecting *T. baccata* poisoning, also for toxicology for both animals, including stomach content, feces, liver, and kidney. Further forensic botanic analysis was performed at the Dept. of Earth, Environmental and Life Sciences (DISTAV) laboratories of University of Genoa, while the toxicological examination, consisting of high-performance liquid chromatography-mass spectrometry (HPLC-MS) was performed at the Dept. of Chemical, Biological, Pharmaceutical and Environmental Sciences (CHIBIOFARAM) laboratories, University of Messina.
During the post-mortem examination dark green needle-like leaves, similar to *T. baccata,* were identified in the stomach of one animal; the main pathological findings observed in both animals were severe pulmonary edema and hemorrhages, thoracic and abdominal effusion, atonic dilatation of the right ventricle, and liver degeneration; even if aspecific, these are the most common lesions associated with *Taxus* poisoning in herbivores [3]. The forensic botanical and toxicological examinations are to this day still ongoing.
Although only two donkeys were examined, this case suggests that yew toxicity can be dangerous for farm animals, and a standardized procedure for taxine identification must be developed to effectively identify unexplained deaths, focusing also on wild animals.

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77° CONVEGNO SISVET

Stato: INVIATO - ID: 13738

Histological characterization of lesions induced by perfused and non-perfused radiofrequency thermal ablation needles in liver on a swine in vivo model

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RFA (Radiofrequency thermal ablation) is a technique currently applied in human oncology, based on tissue coagulative necrosis induced by means of several type of needles. The aim of this study was to characterize lesions induced by perfused and non-perfused RFA needles in liver on a swine in vivo model. The in vivo trial (aut. n. 885/2016-PR, 22/09/2016) involved eight 4-months-old Landrance x Large White sows, weighing about 45 kg. The animals underwent median celiotomy, from xiphoid cartilage to a point 5 cm caudal to the umbilicus, under general anaesthesia. The RFA procedure was carried out, under ultrasound guidance, inserting the electrode in one single liver lobe. Different RFA conditions were explored (six replicas for each), using 18G internally cooled needles (RF Medical Co. Ltd., Seoul, Korea), either perfused (P) or not (NP), selecting a fixed time of delivery of thermal energy (60 seconds). Four different saline solutions were used with P needle: saline 0.9% (P 0.9%), hypertonic saline 3% (P 3%), 7% (P 7%) and 10% (P 10%). The animals were euthanized at the end of RFA procedures, still under general anaesthesia, by an intravenous overdose of thiopental sodium. Samples from each RFA area were formalin fixed, routinely processed and the histological sections were H.E. stained and analysed to determine the total number of lobules, to investigate microscopical alterations and to characterize the type and amount of cell infiltrate. A semiquantitative score was attributed to the alterations observed in each examined lobule in the lesions and in the surrounding areas. A total score was calculated multiplying the mean score for the total number of lobules counted in the specific zone.Kruskal-Wallis test (followed by Dunn's post-test) was applied to compare the patterns of the lesions induced by the different treatments and to evaluate the differences in areas closer and more distant from the insertion site. P-value less than 0.05 was considered significant.Thermoablated areas showed histologically the presence of three different concentric zones: a central area involving coagulation necrosis (zone 1), an intermediate haemorrhagic area (zone 2), and a surrounding zone (zone 3), characterized by the presence of both cell injury and healthy tissue. Lymphocytes were the prevalent type of infiltrate detected in each examined section. The number of lobules involved in zone 1 was significantly higher in P 7%, compared to NP ($p<0.05$), whereas no significant differences were detected in the two more peripheral areas of the lesion among the treatments. No differences were observed comparing the scores obtained in the same areas by different treatments. The scores decreased along with the distance from the insertion site, and in the more distant areas they were negative, confirming the safety and effectiveness of RFA treatments.Moreover, the total score showed a significant reduction of lesions in zone 3, compared to zone 1 or 2, for each tested condition.The analyses confirm and support previous results obtained by morphometric investigations, showing that P 7% could represent the most promising choice to be further investigated, in order to obtain larger and more reproducible ablation effects.

77° CONVEGNO SISVET

Stato: INVIATO - ID: 13843

EXPRESSION OF INTEGRIN aV IN FELINE INJECTION SITE SARCOMA, PRELIMINARY INVESTIGATIONS

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Feline injection-site sarcoma (FISS) is a common feline tumor characterized by asymmetric and infiltrative growth and a high local recurrence rate. Its etiopathogenesis is related to the proliferation of fibroblasts and myofibroblasts in areas of chronic inflammation, particularly at sites of injection or trauma.
Treatment of FISS is primarily surgical, and complete excision with neoplastic cell-free margins is paramount to reduce recurrence. Recently, the intraoperative use of αvβ3 integrin has been introduced to mark the extent of neoplasm and guide the surgeon's hand in excision. The rationale of this method assumes that integrin is expressed on the surface of tumor cells and in the vascular endothelium, with higher intensity in areas of neo-angiogenesis. Integrin-αv could be a promising marker of tumor extent allowing for neoplasm-free margins from the first surgery1. However, the definitive assessment of margin status relies on histopathologic analysis. This is considered the gold standard for defining the presence of tumor cells in excision margins, but is not free of pitfalls, especially in distinguishing between neoplastic myo/fibroblasts and reactive fibrous connective tissue.
The present study aimed to investigate the immunohistochemical expression of integrin-αv in FISS to see if it could distinguish reactive tissue from tumor tissue, specifically in the histopathological analysis of excision margins.
Ten FISS (7 fibrosarcomas, 3 pleomorphic sarcomas) in as many cats were selected from the archives of Veterinary Pathology. Specimens were processed for histopathologic examination using the en-face/3D margin analysis technique. One subcutaneous injection-site granuloma and one osteosarcoma were also included. All sections were stained with HE and immunostained (ABC-method) using a polyclonal anti-integrin-αv antibody (AB1930, Merck)2. In all samples, vascular endothelium was diffusely positive for integrin-αv (internal positive control).
In all tumors, neoplastic cells were positive for integrin-αv: widespread, moderate to intense cytoplasmic positivity was observed in spindle cells in all tumors, intense and distinct membrane positivity was detected in multinucleated giant cells (MGCs) and round mononuclear cells in pleomorphic-sarcomas. MNGCs in osteosarcoma had moderate intracytoplasmic positive reaction, and epithelioid macrophages in the granuloma were negative. Integrin-αv was also variably positive (from weakly to intensely) in fibroblasts within excision margins, while the immunolabeling signal was generally fainter, or absent in mature fibrocytes. Inflammatory cells (lymphocytes, plasma cells), in FISS, excision margins, and granuloma, were consistently negative.
In conclusion, integrin-αv immunostaining was not significantly different between presumably reactive and frankly neoplastic fibroblasts, therefore they cannot be reliably distinguished, either because they are already genetically mutated or because integrin does not specifically bind neoplastic cells. This finding does not invalidate the surgical utility of integrin-av, which is likely related to the integrin's ability to bind to and mark neo-angiogenesis. The distinct membrane localization of integrin-αv in neoplastic round mononuclear and MNGCs of pleomorphic sarcomas is an interesting and novel finding of the present study, suggesting that integrin-av may play an important role in the oncogenesis of pleomorphic sarcoma variant of FISS. Integrin-αv expression has been positively related to metastasis to lymph nodes and a higher tumor stage in several tumors. In the authors' opinion, the role of integrin-αv in feline pleomorphic sarcoma deserves further study.
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AISMEVEM

From sanitary dogs to rescue dogs: the history of an irreplaceable collaboration.

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The collaboration between men and dogs date back to the ancient times and the use of dogs in the art of war is very old too. The Greeks and the Romans used them as messengers for important news among the enemy lines and they were also used for patrols and outposts. In the Middle Ages Henry VII of England sent Charles I four thousand dogs which took part in the war against the king of France. It was quickly clear that dogs, due to their ability in detecting human scent from living or dead people, possessed a natural skill in finding missing persons, but they also had an instinct to help and protect them. The military organizations throughout the ages identified these traits and shaped canine behaviour so that dogs could help them in their activities on and off the battlefields. One of the first times in which the sanitary dogs were employed was during the Russian-Japanese war in the early 20th century. During the First World War, all the fighting Nations employed dogs in battlefields. In Italy several thousands of dogs were used to supply ammunition, food and water and also for patrolling, exploring and for safety services. They were also used to search wounded soldiers on the battlefield [1]. Probably, the German troops displayed the best organization of sanitary dogs – *Sanitätshund* – and approximately six thousand dogs (mainly German Shepherds, Dobermanns, Airedales) were trained and employed. In 1893, in Germany the Association of sanitary dogs – *Deutscher Verein für Sanitätshunde* – was created, with the aim of convincing the military to use dogs as “ambulance dogs” [2]. During the wartime everywhere, among the people, the use of sanitary dogs generated great appreciation. Many reports appeared in newspapers and illustrated postcards were produced in order to collect funds. Indubitably the military organization played a fundamental role in developing the service of sanitary dogs. Progressively the skills acquired in wartime were employed in the training of rescue dogs. Dogs and handlers are still a unique and unsurpassed search team [3].
The goal of this poster, by the use of old pictures and propagandist postcards, is to emphasize the use of dogs as sanitary helpers and the “evolution” of new roles in the Armies and the use of rescue dogs in disasters and calamities.

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AIVI

77° CONVEGNO SISVET

Stato: INVIATO - ID: 13005

STANDARDIZING THE ANALYTICAL DETERMINATION OF SULPHITING AGENTS IN FOOD: THE ITALIAN TECHNICAL SPECIFICATION UNI/TS 11868:2022

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Sulfites (or “sulphiting agents”) are a class of food additives identified by the codes from E220 to E228. In Europe, their addition is authorized in food with several restrictions, as defined in the Regulation No. 1333/2008/EC. The attention of the scientific community has been focused on sulfites due to cytotoxic and mutagenic effects caused by high intake of this class of food additives. Moreover, these compounds may cause intolerance and asthma in sensitive individuals and, through the reaction with the disulfide bonds, they may alter protein metabolisms, other than degrade thiamine, folic acid, nicotinamide and pyridoxal. The addition of sulfites in fresh meat preparations, also called “sulphuring treatment”, is a widespread practice due to its simplicity and cheapness. Given the significant health effects of sulfites, described above, this practice is not permitted by the actual legislation, so, the laboratory in charge of food inspections need analytical methods able to identify and quantify this compound in meats as accurate and selective as possible. The rapidity and cheapness should be another important characteristic of these methods, since the processing of large amounts of samples is often required. This type of determination is usually carried out by using the titrimetric (Monier-Williams) or the enzymatic method. However, it is well known that these approaches may result as low reproducible, selective and accurate, due to low levels to detect, rapid sulfite oxidation to sulfate in aqueous medium and presence of matrix interfering compounds.In this study, a novel analytical method, based on ion chromatography with conductivity detection, is proposed as new standard procedure for this type of determination. This protocol, already validated and published by the IZS Puglia and Basilicata [1], was submitted to a standardization procedure by the “Chemical Analysis of Meats” Working Group of the Italian Standardization Body (UNI). The analytical procedure is composed of sample extraction (4g) using 40 mL of a solution composed of NaOH and fructose, in a horizontal shaker for 30 min. After centrifugation for 5 min at 1200 rpm at room temperature, the extract (#2 mL) is filtered on paper (Whatman No. 40), and then on 0.22 µm filter prior to chromatographic analysis. The chromatographic determinations were performed using a ICS-6000 (Thermo Fisher Scientific) ion chromatography system equipped with an anion self-regenerating suppressor and a conductivity detector. The chromatographic separations were performed using an IonPac AS9-HC® column eluted by a gradient (1.0 mL/min) of 2 solutions: 8 mM Na2CO3 and 2.3 mM NaOH (A) and 24 mM Na2CO3 (B). Three laboratories were involved in this inter-laboratory validation. Fresh meat, meat products and shrimp samples were analysed both before and after fortification at different levels of sulfites. The suitability of the following parameters was ascertained: method linearity (r2 > 0.999) in the measurement range 8.0 – 160.0 mg kg-1; selectivity; accuracy, as recovery% and precision, equal to 97.7% and 5.9%, respectively, in compliance with the requirements of European Decision 808/2021/EC.
ANOVA one-way test confirmed the homoscedasticity of data obtained by 3 laboratories ($p < 0.05$). The method was also submitted to Proficiency Test round, obtaining a z-score value of 0.3, confirming method suitability for the determinations of sulfites in fresh meat, meat products and shrimp samples [2].

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77° CONVEGNO SISVET

Stato: INVIATO - ID: 13333

Exploring kidney and muscle metabolome for Antibiotic-Free pig authentication purposes

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Beside the implementation of EU regulatory framework in the field of the monitoring of antibiotic residues, the introduction of voluntary certification schemes for Antibiotic-Free (ABF) labelling may pose a risk to the integrity of pork chain. Metabolomics has been used to tackle specific authenticity issues and has shown promising results in the context of food fraud [1]. In the current study, an untargeted metabolomic approach based upon Nuclear Magnetic Resonance spectroscopy (1D 1H NMR) in combination with chemometrics techniques was employed to investigate changes in metabolome due to antibiotics exposure in relevant pig matrices for authentication purposes. The findings related to the metabolic fingerprinting of pig liver have already been considered in a previous presentation. Kidney and muscle tissue were further investigated to gather more information and get a broader and complete picture.
A total of 103 heavy pigs, reared in different farms of Northern Italy and slaughtered at the same commercial abattoir, were randomly selected and divided in two groups according to the exposure to antibiotics administration during their entire lifecycle: ABF group and Antibiotic Treated (ABT) group. Kidney and diaphragm samples were extracted following the readaptation of Bligh & Dyer protocols [2], a biphasic extraction procedure to separately collect lipophilic and hydrophilic extracts. 1D 1H NMR spectra of both extracts were recorded on a NMR spectrometer operating at magnetic field of 600.17 MHz (JEOL ECZ 600, JEOL Ltd., Tokyo, Japan). To examine the structure of samples under investigation, an unsupervised Principal Component Analysis (PCA) was conducted on the spectral data separately on lipidomic and metabolomic dataset obtained by the lipophilic and hydrophilic extracts, respectively. Briefly, the two-dimensional PCA score plot for the polar extract highlighted a good grouping trend in muscle samples and a discrete performance in the case of kidney. On the contrary, the PCA score plot for the non-polar extracts of both matrices showed the two groups strongly overlapped each other and no clusterization among samples of the same group was evident. The metabolomic dataset (polar extract) of kidney and diaphragm were furtherly submitted to a supervised Orthogonal Partial Least Squared-Discriminant Analysis (OPLS-DA). The score plots provided ABF samples perfectly separated from the ABT samples in the bi-dimensional space. The most discriminant NMR spectra signals were annotated and the regulation of the obtained metabolites between the two phenotypes – ABF vs. ABT – were highlighted.
In the light of these findings, further investigation may be encouraged since the research of biomarker molecules-proving meat authenticity is pivotal for assessing the integrity of the food chain and introducing new tools to monitor antibiotic usage by integrating in a One Health approach aspects related to authenticity and public health issues.
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77° CONVEGNO SISVET

Stato: INVIATO - ID: 13462

Effects of the permanence of microplastics in sea-water on capture and ingestion by mussels

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This study aims to evaluate how the permanence of microplastics in marine water can influence their likelihood of being captured and ingested by mussels. A total of 96 fresh specimens of Mytilus galloprovincialis were sampled from a local market in Messina (southern Italy) and subjected to experimental contamination with a known number and type of microplastics inside glass containers filled with seawater. In detail, two groups of containers were separately prepared with filtered (8 μm pore-size filters) and unfiltered seawater, and both spiked with blue virgin polyethylene (PE, 1.050 g/cm3) microplastics ranging in size from 200 μm to 500 μm. The experimental design foresaw to individually place a first group of mussels inside each container immediately after particle addiction while a second group of mussels were placed inside containers after 5 days. This last time frame was set up to simulate real environmental conditions since it is well-known that, during the permanence of microplastics in water, organic materials can adhere to their surface [1]. A total of 50 PE particles was added in each spiked sample while unspiked samples were used as control. The experimental contaminations were conducted inside an incubator at 15 °C. After 4 and 8 hours of exposure, mussels were removed and the water of each container was filtered through 8 μm pore-size filters. Particles present on the filter surface, which represented those not captured by the mussels, were observed and enumerated under a stereomicroscope. No significant differences were detected in the number of particles captured by mussels placed in the containers immediately after particle addition in any of the different samples tested. In contrast, as regards mussels placed inside the containers after 5 days, a greater number of microplastics was captured by samples placed in unfiltered seawater (20 % and 20.67 % of the spiked particles after 4 and 8 h, respectively) rather than in filtered seawater (1% and 3% of the spiked particles after 4 and 8 h, respectively). The hours of exposure did not affect the mussels' ability to capture particles. We could speculate that the permanence of microplastics in water favors the adhesion of organic material on their surface from the surrounding unfiltered seawater misleading mussels that mistake them for food. In this regard, there are several reports of how organic biofilm formation on microplastic surfaces during their permanence in water, not only influences their environmental distribution due to physic-chemical changes (e.g. density and hydrophobicity variations) but also prone to their ingestion by biota [2,3]. Nowadays, most of the experimental contamination studies have used virgin microplastics that, based on our results, could not reflect real environmental conditions. Therefore, the present investigation highlights how future studies should consider the use of weathering particles in the experimental setup thus simulating the real conditions of exposure.

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77° CONVEGNO SISVET

Stato: INVIATO - ID: 13540

Alternative Protein Sources: Exploring Consumer Perceptions and Food Trends

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The food security and sustainability debate has spurred the quest for new protein sources, including insects, snails, algae, and plant-based high-protein preparations. Scientific research is focusing on these alternatives to meet global needs in a responsible and efficient manner. Thus, the delicate balance between safety, quality and sustainability of new protein sources was investigated, tracing a path from established food traditions to the most recent innovative solutions.
A survey was created to study the availability, perception, and buying habits of meat alternatives like insects, snails, seaweed, and plant-based proteins. The questionnaire was divided into three parts: one about demographics, one about personal eating habits and one about purchasing patterns and food preferences. It was distributed during gastronomic events in Piedmont during summer 2023 and via a link on the website www.izsplv.it. Subsequently, statistical analyses were conducted using regression and non-parametric methods.
The final sample consisted of 627 individuals from 128 municipalities in Piedmont, with a majority of women and an even distribution across age groups, except for the over-70s. The majority had higher education and full-time employment, mainly in the medical services, public administration, education, and training sectors. 22.7% of the respondents reported suffering from diet-related disorders, such as hypertriglyceridemia and blood pressure and blood sugar problems. 32.7% of the participants stated that they consume alternative protein products to meat, with differences related to age, gender, and type of diet. Women and younger people were more likely to consume such products, especially following a flexitarian, semi-vegetarian or vegetarian diet. 71.1% of non-consumers would be willing to try vegetable-based products, but only 36.3% were interested in insect- or snail-based products. 59.2% of the interviewees showed interest in all alternative protein sources, with a smaller percentage expressing indifference or disgust. Factors influencing the willingness of respondents to try such products were age and gender, with a decrease in interest in the older groups and a greater likelihood of interest from the male gender than from the female gender.
Qualitative characteristics, such as appearance and taste, were often reported as limitations for the consumption of these products. A minority (12.4%) perceive alternative proteins as less controlled or healthy than traditional ones.
Transitioning to insect- and plant-based diets, reducing meat intake, is crucial for promoting a more sustainable diet. Despite the majority identifying as omnivores, there's a growing interest in flexitarianism. The analysis of food preferences showed that respondents consume more plant-based foods than animal-based foods. Women, young people, and those following a flexitarian/vegetarian diet are more likely to consume alternative protein sources, especially of plant origin. While some concerns relate to taste, quality and origin of products, social acceptance, and consumption habits also play an important role. Social acceptance and consumption habits play an important role, while some consumption concerns relate to taste, quality, and origin. However, concerns about safety, health and environmental impact persist. In conclusion, the survey highlighted diverse consumer views on new protein sources, emphasizing the necessity for further detailed studies.
Project funded by the Ministry of Health, IZS PLV 04/22 RC; CUP J19I22001130001
Onwezen, Marleen C., et al. "A systematic review on consumer acceptance of alternative proteins: Pulses, algae, insects, plant-based meat alternatives, and cultured meat." Appetite 159 (2021): 105058.
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Preliminary investigation of metal concentration in mussels (*Mytilus galloprovincialis) from* Butrinti Lagoon and Shengjin (Albania)

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Heavy metals (HMs) are significant marine pollutants. Bivalve molluscs, particularly filter-feeding organisms, possess high metal absorption capacities due to their feeding mechanism. HMs accumulation in aquatic environments poses health risks. The European Commission (EC) has set Maximum Levels (MLs) for Cd (1 mg/kg) and Pb (1.5 mg/kg) in bivalve molluscs, and for Hg (0.5 mg/kg) in seafood under Regulation (EC) No. 915/2023. On the other hand, mussels are recognized as nutritious dietary sources rich in omega-3 fatty acids and proteins, contributing to human health. In this respect, the study aimed to conduct a preliminary monitoring of only three elements, namely: Cd, Cu, and Zn in *Mytilus galloprovincialis* from two sites along the Albanian coast: Butrinti Lagoon and Shengjin. From June to September 2023, 111 mussel samples were collected. Analysis was done at the Element Analysis Laboratory of Istanbul University using Inductively Coupled Plasma Optical Emission Spectroscopy (ICP-OES). Results were reported in µg/g. Cu and Zn showed relatively high concentrations (Cu=Butrinti: 21.72 µg/g ± 9.75, Shengjin: 19.77 µg/g ±13.04; Zn= Butrinti: 234.28 µg/g ± 140.99, Shengjin: 360.05 µg/g ±239.10). However, mussel consumers' exposure to Cu and Zn remained below the Provisional Maximum Tolerable Daily Intake (PMTDI) levels set by JECFA [1] at 0.5 and 1 mg/kg body weight per day respectively. All samples exceeded the maximum Cd limit of 1 mg/kg. An average serving of mussels can expose a 60 kg adult to weekly cadmium levels surpassing the Provisional Tolerable Weekly Intakes (PTWI). Even though this study offers only partial results, it highlights a critical scenario concerning Cd. Thus, systematic monitoring programs are necessary to oversee aquatic pollution and seafood quality in Albania. Authors are screening for all elements to provide comprehensive results for thorough risk analysis.

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77° CONVEGNO SISVET

Stato: INVIATO - ID: 13600

EXPLORING THE RESISTOME IN TWO EUROPEAN LARGE-SCALE SWINE SLAUGHTERHOUSES USING METAGENOMICS

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Antimicrobial resistance genes (ARGs) pose significant challenges in animal food production environments, such as large-scale swine slaughterhouses[1]. This study aimed to assess the occurrence and distribution of ARGs and their associated microbial communities in two slaughterhouses, SH1 and SH2 located in Italy and Spain, respectively. In total, thirty-two environmental samples were collected, evenly divided between the clean and dirty areas of the processing plant. This comprised four samples each from dirty surfaces, dirty drains, clean surfaces, and clean drains in both swine slaughterhouses. Up to five swabs were pooled per sample, aiming to provide a comprehensive representation of the entire slaughtering environment, from the entrance of the animals to the end of the line before the pre-sectioning area. Sampling was performed over four days, with one batch collected per week (on the same day of the week) over a one-month timeframe and conducted at the same time each day. The DNA of each pooled sample was extracted using the Power Soil Pro Kit (Qiagen®). All DNA samples were analysed using assembled reads generated from Illumina NovaSeq whole metagenome sequencing technology. The findings, related to both abattoirs, revealed that the dirty areas, comprising both surfaces and drains, exhibited the highest concentration and diversity of ARGs, with approximately 70% of ARGs-contigs detected in them, while approximately 30% were found in samples from clean zones. Predominant ARGs identified in both slaughterhouses belonged to the Tetracycline(25% in both SH1 and SH2), Aminoglycoside(22%in both SH1 and SH2), and Beta-lactam(17%SH1 versus 11%SH2) families, followed by Macrolide in SH1 and multi-drug Macrolide-Lincosamide-Streptogramin B resistance in SH2, with 10%. The top ARGs-carrying bacterial genera included Acinetobacter, Aeromonas, for both abattoirs, followed by Streptococcus and for SH1, and Clostridium and Enterococcus for SH2. Analysis on mobile genetic elements(MGEs) showed the presence of plasmidic DNA carrying ARGs in both abattoirs. The highest percentages were found for Aminoglycoside ARGs, with 10,7% in SH1 versus 8,2% in SH2, Tetracycline ARGs, with 7,5% in SH1 versus 6,6% in SH2, and Folate pathway antagonist ARGs, with 3,5% in both SH1 and SH2. Other plasmid associated ARG families represented less than 2%, such as Amphenicol and Lincosamide and other multi-drug resistance genes. Notably, one of the lowest percentages was detected for Beta-lactam plasmid-associated ARGs(around 0,5% for both SHs). Overall, a minor occurrence of Lateral Gene Transfer events(LGT events) was detected(<0,05%).This study underscores the importance to characterize and evaluate the occurrence of antimicrobial resistance genes within animal food production environments, with emphasis on the potential dissemination of ARGs across different areas within swine slaughterhouses[1]. Additionally, analysing ARGs in these environments serves as a valuable method to comprehend the true extent of resistance to clinically significant antimicrobials, relevant to both human and veterinary medicine. Finally, such insights are necessary to continuously define effective strategies aimed at mitigating the spread of antimicrobial resistance to humans through the food chain and/or through direct contact during occupational exposure[2].

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Marine biotoxins in seafood from Southern Mediterranean: results of monitoring plan of DSP, PSP and ASP in 2022-2023.

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In the last ten years, climate changes and raising sea temperatures caused an increase in the severity, frequency and geographical range of harmful algal blooms, that can produce marine biotoxins. These toxins belong to different classes of compounds, and can accumulate in high concentrations in filter-feeding organisms, especially bivalve molluscs, as well as in plankton feeding species of fish and crustaceans. Marine biotoxins can provoke mortalities in fish and contaminate seafood, causing foodborne intoxication in humans. The symptoms include vomiting, diarrhoea, dizziness or, in extreme cases, respiratory disease because of toxic aerosols. Monitoring programmes are carried out by health authorities to control possible contamination, above all in mussel farming. These control programmes are aimed to prevent both people intoxication and to save economic losses.

In this work, we present the results of monitoring plans of some groups of marine biotoxins during 2022 and 2023 in seafood from the coasts of Southern Mediterranean, collected in the farming sites of Italian Southern Regions, that is Campania, Apulia and Sicily. The local health Authorities

Domoic acid (ASP, causing Amnesic Shellfish Poisoning), the PSP group, that is saxitoxin and other 12 derivatives (causing Paralytic Shellfish Poisoning), the DSP lipophilic group, including okadaic acid and yessotoxins, azaspiracids, pectenotoxins and dinophysistoxins (causing Diarrhoeic Shellfish Poisoning) were monitored. We employed a panel of confirmatory chromatographic methods. PSP toxins were analysed using liquid chromatography with fluorescence detection (HPLC-FLD) by the Lawrence method; ASP was tested by a HPLC-DAD method, while the DSP toxins were tested by high resolution mass spectrometry coupled to liquid chromatography (UHPLC-HRMS). All the methods were accredited and their analytical performance monitored by external proficiency test.

In the years 2022-2023 we tested 2162 samples of mussels, clams, sea urchins. The samples were from Campania (61% in 2022 and 51% in 2023), Apulia (31% in 2022, 36% in 2023) and Sicily (7% in 2022, 11% in 2023). The local Competent Authorities did not request the same analyses for all samples, depending on their own risk evaluation.

The contamination rates were variable. The DSP toxins were the most abundant, and were determined in 70 samples out of 643 (10.9%), while PSP toxins were found out only in 19 samples out of 1062 (1.8%). ASP was never detected in 2022-2023.

We observed seafood contamination depends on the Region of provenience. In samples from Campania only DSP were determined. DSP and PSP were found out in seafood from Apulia. In samples from Sicily only the PSP were determined. The major contamination rate was observed in samples from the Southern Adriatic Sea, both in 2022 and 2023.

The biotoxins most frequently detected are okadaic acid, yessotoxin and homoyessotoxin, among the DSP group, and saxitoxin, among the PSP. The biotoxins GTX2,3 (PSP) and DTX2 (DSP) were also detected in a lower extent. Some considerations about the levels of contamination measured, as well as not compliant samples, are also reported.

This study shows that marine biotoxins are a possible concern for consumers in Southern Italy. The DSP biotoxins were the most abundant, but along the coasts of Apulia also the presence of PSP is noteworthy. The monitoring activity allows to control the contamination of mussel farming in our regions and protect consumers in the case of algal bloom outbreaks.

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77° CONVEGNO SISVET

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Environmental surveillance of antimicrobial resistance: an integrated approach

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The complex dynamics of antimicrobial resistance (AMR) call for integrated surveillance plans involving humans, animals and the environment. There are indeed multiple possible pathways by which AMR can spread, and it is genarally challenging to monitor bacterial transmission, especially in natural environments. There is therfore a compelling need to develop environmental surveillance programs including all the involved drivers, in order to define each contribution and evaluate AMR circulation within natural ecosystems.

We carried out a pilot study aimed at developing an environmental surveillance protocol including samples of human wastewater, livestock manure, and surface water using extended-spectrum β-lactamase (ESBL/AmpC) - and carbapenemase-producing *Escherichia coli* as pathogen-model. Sampling was carried out from April to June 2023 in a wastewater plant in Northern Italy and in farms and along a stream at 3 km, 6 km and 9 km from the plant. A total of 24 samples of human wastewater (two per week), 18 of livestock manure (once a month in 3 swine and 3 bovine farms, one of each per sampling point) and 39 of surface water (3 per week at the 3 sampling points) were collected. All the samples were screened for resistant *E. coli* by phenotypical and molecular methods.

Overall, 28.9% and 31.6% of all the samples tested positive for ESBL/AmpC- and carbapenemase-producing *E. coli*, respectively. In particular, 31.3% of wastewater and 30.8% of surface water samples tested positive for ESBL/AmpC strains, while prevalence in swine and bovine manure samples was 44.4% and 11.1%, respectively. Prevalence of carbapenemase-producing *E. coli* was instead 56.3% and 17.9% in wastewater and surface water, while 44.4% of swine samples and 11.1% of bovine samples were positive. Humans and swine appear the two major contributors to the spread of resistant *E. coli* in the environment, and the high prevalence of ESBL/AmpC *E. coli* detected in surface water raises indeed concerns as this is supposed to be an antimicrobial-free matrix.

Interestingly, 4 carbapenemase-producing *E. coli* were resistant to all the classes of β-lactams tested by MIC (cephalosporins, carbapenems, penicillins): particularly, two of them were isolated during a single week in May, in a human wastewater sample and in a surface water sample at the sampling point closest to the treatment plant (3 km downstream). The other two were isolated during the same week in June, one from swine manure from the farm closest to the plant and the other in bovine manure sampled in the farm located 6 km away from the plant. This pattern is suggestive of a single strain of human origin spreading through the environment, and WGS analyses are currently underway to assess whether this is the case.

Implementing One Health surveillance is pivotal to monitor AMR spread in natural ecosystems and requires a holistic approach that goes beyond considering humans, livestock and the environment as separate entities. Although this is a pilot study with a limited sample size, our results show that such an integrated approach can offer insight into AMR circulation and be a valuable tool for large-scale surveillance of AMR.

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Evaluation of olive mill wastewater extracts on Listeria monocytogenes planktonic cells and biofilm

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Listeria monocytogenes is a foodborne pathogen that is widely distributed in the environment, still causing the highest lethality rate, according to the latest EFSA report on foodborne diseases [1]. It can easily penetrate food processing facilities and contaminate food, posing a public health risk. Furthermore, L. monocytogenes biofilm-forming ability helps this microorganism persist under harsh conditions in a variety of habitats, resisting routine cleaning/disinfection plan of food industry. Food business operators (FBOs) are in charge of controlling L. monocytogenes in the food processing environment, especially in facilities that produce ready-to-eat food [2].In recent years, fascinating natural extracts have been identified as potential tools that FBOs can use to reduce the risk of L. monocytogenes in foods and food production facilities. In regard of this, olive mill wastewater is the principal by-product of the olive oil business, originating mostly from the treatment and pressing of olives in mills. It is high in minerals and phytochemicals, which have a variety of biological qualities. Since natural antibacterial compounds are more foodgrade than chemical sanitizers and are also preferred by consumers, studies on the applicability of such compounds as novel sanitizing agents obtained from by-products have received much attention in recent years [3].The current research focuses on the antimicrobial ability of olive mill wastewater extracts (OMWE) to control L. monocytogenes even when organized in biofilm form. In the present study, four strains of L. monocytogenes were tested, two isolated from processing environment and two from food matrices, previously characterized for biofilm production. L. monocytogenes ATCC 13932 was tested as a quality control. According to the protocol of Mic (CLSI) with some modification and standardized protocol for anti-biofilm activity (Mbec Assay), each strain was tested.On Mic test, the extract was evaluated at concentrations of 500 mg/ml, 250 mg/ml, 125 mg/ml, 62,5 mg/ml, 31,25 mg/ml. On Mbec test, concentrations of 1 g/ml, 500 mg/ml, 250 mg/ml, 125 mg/ml and 62,5 mg/ml were used.Preliminary data show that environmental isolates of L. monocytogenes are sensitive at concentrations half as high (125 mg/ml) as those isolated from food matrices (250 mg/ml). Concerning the eradicating capacity of the biofilm by the OMWE, a concentration double with respect to the Mic is necessary to achieve the Mbec. The ability of OMWE to inhibit biofilm formation by L. monocytogenes and the time required to eradicate biofilm need further investigation.

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AMV

Investigating Prolonged Excessive Weight in Pigs as a Potential Model for Knee Osteoarthritis

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Osteoarthritis (OA) is a chronic debilitating disease that causes pain and immense discomfort to the affected individual. The etiology of OA is multifactorial and the pathogenesis is not completely understood, although overweight is a clear risk factor for the development of OA. The pathology is characterized by degenerative loss of articular cartilage, narrowing of the joint space, synovial inflammation, and bone remodeling in humans, dogs, and horses. Preclinical models of osteoarthritis in which damage occurs spontaneously may better reflect the onset and development of osteoarthritis. This work aimed to evaluate the sow at the end of its career as a model of spontaneous osteoarthritis associated with obesity, examining the integrity of the articular cartilage of the bone of the knee (femoral condyle and tibial plateau) and the meniscus in the medial side which is the one more subjected to pathological changes. For this work, sows weighing 250 kg (4-6 years mean age) and pigs weighing approximately 110 kg (7-8 months average age) were examined. The study was carried out through sampling at the slaughterhouse. The cartilage integrity of the femorotibial joint was determined by magnetic resonance imaging (MRI) scoring systems, macroscopic chondropathy scoring, and histological proteoglycan staining (Safranin O). The production of leptin, visfatin, and IL-6 was also evaluated on synovial fluid by ELISA test to assess joint inflammation. The scoring analyses carried out on the macroscopic aspect of the femoral condyles, tibial plateaus, and menisci (Photographic Chondropathy Score [1]: femoral condyles $P<0.05$; tibial plateau $P<0.05$; PAULI scoring meniscus $P<0.05$ [2]) as well as the scoring performed on MRI (Outerbridge score[3], $P<0.05$) demonstrated that the animals belonging to the 250kg group presented statistically higher values compared to the 110kg group, highlighting pathological aspects affecting the sows. The analysis of the synovial fluid revealed that leptin, IL6, and visfatin levels showed no significant differences ($P>0.05$, all comparisons). However, a common trend can be detected, with higher levels of each target in the 250 kg groups. Finally, histological analyses performed on safranin O staining showed similar results to those obtained by macroscopic scoring; femoral and tibial condyles presented severe fibrillation of the cartilage as well as hypocellularity and proteoglycan loss. Similarly, the meniscus showed severe fibrillation, unorganized collagen fibers, hypocellularity, and slight matrix staining. In conclusion, it has been observed that sows at the end of their career develop spontaneous joint pathologies due to long-term excessive weight.

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THE MENISCUS VASCULARIZATION: ENDOSTATIN AND VASCULAR ENDOTHELIAL GROWTH FACTOR BALANCE FOR TISSUE ENGINEERING PURPOSES

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The neonatal meniscus is fully vascularized but, with advancing age, the inner zone gradually becomes fully avascular due to a balance modification between angiogenic and anti-angiogenic factors [1]. Moreover, this change in vascularity coincides with cell differentiation from fibroblast-like to chondrogenic-like phenotype. In particular, as the reduction of vascularization is strongly connected with meniscus maturation [2], it is important to determine a correlation between vascular changes and the modulation of cell phenotype during meniscus development [3]. These two processes could be regulated by common molecules already known as vascular modulators but have not yet been associated with the signaling involved in the regulation of cell phenotype. Although several studies have been performed to clarify the cellular and structural heterogeneity of the meniscus, there are still some open questions regarding its structure and its insufficient repair and regenerating capability. New insights about the pathways involved in vascular and phenotypical changes will guide to the generation of better meniscal substitutes and possibly clarify the physiological development of the meniscus itself.
Therefore, this work aimed to characterize the expression of factors involved in the angiogenesis of porcine meniscus at birth. The menisci were removed from the knee joints of newborn piglets, which died under the weight of their mother, so that no animals were sacrificed for research purposes. Then, samples were evaluated through their molecular and morpho-functional characteristics, with a particular focus on vascular factors i.e. the angiogenic factor Vascular Endothelial Growth Factor (VEGF) and the anti-angiogenic marker Endostatin (ENDO). Morpho-functional analyses showed that endothelial cells were barely marked at the nuclear level by ENDO, and that the signal in the extracellular matrix was nearly negative. On the opposite, the endothelial cells were positively marked for VEGF, and the signal was distributed homogeneously throughout the tissue, which is coherent with the diffuse vascularization. Moreover, gene expression revealed that endostatin had a lower expression than VEGF. In conclusion, newborn piglets' menisci show that the fibroblast-like phenotype of the cells is characterized by a high VEGF and a low ENDO expression, which reflects the vascularization of the meniscus at this age. An extensive knowledge of the morphology of the neonatal meniscus is mandatory to be able to develop an engineered tissue. Funding: PSR2022_DIP_035_AIDOS

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Establishing an in vivo analysis to explore the feeding behavior of Nothobranchius Furzeri

Aniello Vitale, Daniela Giaquinto, Viviana Giuliano, Maria Chiara Di Meo, Ettore Varricchio, Livia D'angelo.

Nothobranchius Furzeri, also known as "African turquoise killifish", has become the popular model organism for aging and neuroscience research. The main feature resides in its very short lifespan compared to the other vertebrates.
Recently this model has been used for designing behavioral tests, particularly addressed to assess behavior-associated changes upon aging. An automated set up to evaluate the feeding intake over lifespan has been proposed [2] and in parallel a number of papers have clearly demonstrated that this species can be considered a selective feeder displaying preference for live food [3].
Therefore, we have designed a behavioural study to assess food-related behavioural changes over aging. To this aim, we tested 15 male experimental subjects, subdivided into 3 groups (n=5/group) according to the age (8, 24 and 52weeks old), in an experimental tank and exposed to two different types of food, namely: bloodworms (as live food) and SDS-400 (as dry food). The experimental tank, made of transparent plexiglass, was rectangular and divisible into 3 areas, a central area where the animal was initially positioned, and two lateral areas, each holding the different type of feed (live and dry). We mainy valuated exploratory behaviour and food preference.
Our results display that levels of immobility increased from young to old animals, and conversely the exploratory behaviour decreased over aging, according to [1], and thus confirming the usefulness of our *de novo* designed experimental tank for a behavioral analysis. As for the food preference test, the preliminary results highlight the clear preference for the bloodworms in all animals, independently of the age, confirming what is indicated in the literature. However, despite the clear preference towards live food, this does not limit the possibility of carrying out future studies aimed at increasing the palatability of dry food. Determining an optimal palatable dry diet help to solve the problem of nutritional variability linked to live food, ensuring higher standardization.

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Comparative analysis of the primary motor cortex in pigs and wild boars: possible effects of domestication.

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The primary motor cortex (M1) and the cerebellum are the main structures involved in the control of motor activity. Recently, we compared some aspects of the cerebellum of wild boars with that of pigs to evaluate possible effects of domestication [1], since domestic pigs (*Sus scrofa domesticus*) are descended from Eurasian wild boars (*Sus scrofa*). Domestication seems to alter the morphology and physiology of the central nervous system. Macro-anatomical data suggest that the brains of domesticated species are smaller compared to those of their wild ancestors [2]. In this context, we compared the cytoarchitecture of the M1 in pigs with that of wild boars, focusing on parvalbumin (PV)-expressing interneurons as they play an important role in modulating the excitatory activity of pyramidal neurons, and thus tuning motor information.

The M1 (left and right) was identified and sampled from the brains of 6 adult pigs and 6 adult wild boars. The pigs were slaughtered at a local abattoir, and the wild boars were donated postmortem by local hunters during routine hunting activities. No animals were killed for the purpose of the study. Two blocks of tissue, one centimeter apart, were sampled from the left and right M1, and routinely processed for paraffin embedding. Five-µm-thick tissue sections were stained for histological and immunohistochemical analysis to assess the following parameters: M1 thickness (M1T, µm), density of PV-immunoreactive (ir) neurons (DPVN, cells/mm^2), total density of neurons (DN, cells/mm^2), and the ratio between DPVN and DN. Measurements were performed on four sections of each side per animal. To determine significant differences in M1T, DPVN, DN and DPVN/DN between pigs and wild boars, considering the left/right hemisphere, statistical analyses were performed using the Mann-Whitney test, with significance set at $p < 0.05$.

Nissl-stained coronal sections of the M1 in both pigs and wild boars displayed a laminar organization consisting of five neuronal layers with a nearly absent layer 4 and a layer 5 characterized by large pyramidal cells.

The immunohistochemical localization of the PV-ir interneurons did not reveal a distinct difference in laminar organization within the M1 between the two groups. PV-ir cells were primarily distributed in layers 2 and 6 in both pigs and wild boars. Statistical analyses of the morphological parameters showed only left-right asymmetry in pigs, with the left cortex displaying a higher value ($p<0.0001$) of M1T. When comparing pigs with wild boars, values of both M1T ($p<0.0001$) and DPVN/DN ($p=0.018$) were significantly higher in wild boars than in pigs.

The data presented here suggest that the cytoarchitecture of pig M1 may have been altered during the domestication process. In this regard, of particular interest are the findings regarding the decrease in density of both PV-ir interneurons and Purkinje cells, previously reported in the cerebellum [1], as they are consistent with a reduced neural processing of the pig primary motor cortex.

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77° CONVEGNO SISVET

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Examination of the microtubular cytoskeleton, in papain-dissociated canine primary neurons from fetal brain sampled in compliance with the principles of the 3Rs

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In basic research, the identification of the most suitable experimental model has characterized the past decades [1]. In fact, in the field of research on neurodegenerative diseases, the full limitation of the mouse model has been highlighted. Very short average lifespan, and lack of spontaneous onset of some lesions typical of neurodegenerative diseases, have caused researchers' attention to turn to new experimental models closer to humans. Among these, the canine model has attracted much attention lately. As a companion animal, it often shares the same environment as humans, the same stressors, and the same food. In addition, during aging, dog may develop neuropathological and clinical signs superimposed on that shown by humans, making it as a model for the study of human brain aging [2]. Alongside the search for experimental models closer to humans, the need to reduce the use of experimental animals has become increasingly present in recent years, looking for experimental alternatives in line with the principles of the 3Rs (Reduction, Refinement and Replacement). The approach of the present work was twofold: first, to describe the procedure of obtaining primary canine neurons from occasionally sampled fetal brains; second, considering the importance of microtubular network during neuronal morphogenesis [3] by immunofluorescence, we examined the presence and the cellular distribution of tubulin post-translational modifications as tyrosinated and acetylated α- tubulin (markers of dynamic and stable microtubule respectively), and the pattern of two microtubular associated proteins, i.e. CLIP-170 and Kinesin-1. Through collaboration with our veterinary teaching hospital, as part of the agreement with the Public Health Offices, to ensure the spay of stray bitches, pregnant uteruses were collected at different gestation ages (especially 25 and 45 gestation days). The brain tissues were dissociated through an enzymatic papain system, cultured, and the development, morphology and the number of neuronal cells were assessed. We noticed that he dissociation of cells by the papain method, allows a mild enzymatic action thus obtaining many viable cells. Moreover, explants from early gestation days fetuses (25 days) yielded the higher number of neurons in comparison with those obtained from older fetuses (45days). Regarding the arrangement of the microtubular network, there was a clear immunoreactivity for both tyrosinated and acetylated α-tubulin. The data on motor proteins, showed that CLIP-170 was more immunoreactive than kinesin-1, and there was high overlap between tyrosinated α-tubulin and CLIP-170. Our findings brought to light some novel information regarding the function of the microtubular network in the early stages of the development of canine neurons. Furthermore, the protocol set up for obtaining primary neuronal cultures from random sampling and exploiting biological material destined for destruction, highlighted the usefulness of this alternative experimental model, which may bypass the shortage of available commercial canine neural cell lines as well as the requirement of experimental canines while respecting the 3Rs principles.
This work was supported by “Fondazione di Sardegna–Gadau 2017”.
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Effect of melatonin supplementation during in vitro maturation on developmental competence and tubulin organization of ovine oocytes during non-breeding season.

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Melatonin is a key factor involved in the control of seasonality of reproduction and is related to the quality of the oocyte in seasonal [1] and not seasonal species such as in pig [2].
In seasonal ruminants, melatonin plasma concentration and oocyte quality are higher during the reproductive season (September to December). The aim of the study was to determine the role of this key molecule controlling seasonal effect on oocyte quality using ovine as animal model. To discriminate between the direct effect of melatonin on cumulus oocyte complexes (COCs) from the effects mediated by the environment, the COCs were recovered during the non-reproductive season and matured in vitro by adding melatonin to the maturation medium.
COCs recovered from ovaries of ewes slaughtered during the non-breeding season (April to June) were in vitro matured adding melatonin to maturation medium at a final concentration of 10^{-7}M (MEL group). COCs in vitro matured in standard conditions (without melatonin supplementation) were used as controls (CTR). Following 24 hours of in vitro maturation, oocytes from the two experimental groups were fertilized and cultured to the blastocyst stage to determine developmental competence. Furthermore, tubulin organization and chromosomal configuration were assessed in CTR and MEL MII oocytes by indirect immunofluorescence and confocal laser scanning microscopy (CLSM). The results of embryo production, as percentages of fertilization and first division and number of blastocysts were significantly higher in oocytes supplemented with melatonin compared to CTR group ($\chi 2$ test $P<0.01$ for fertilization and first division; $P<0.05$ for blastocyst output). α-total tubulin assessment showed a higher immunoreactivity in MEL oocytes compared to CTR (ANOVA one-way $P<0.05$). Furthermore, oocytes with normal spindle and chromosome organization decreased in control group compared to MEL ones (60.5% versus 79.6%, respectively; $\chi 2$ test $P<0.05$). Our results indicate that melatonin play a central role in the acquisition of the developmental competence of ovine oocytes, influencing microtubular organization and spindle morphology factors involved in oocyte quality [3].
This work was supported by “Fondazione di Sardegna–annualità 2017” and “DM 737/2021 risorse 2021-2022 Finanziato dall’Unione Europea- NextGenerationEU”.
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77° CONVEGNO SISVET

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Validation of a rat embryonic stem cell model at pre- and post-implantation stage to reduce in vivo developmental toxicity testing

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The 2023 report on animal usage for scientific purposes in the European Union and Norway in 2020 highlights the extensive use of animals, particularly in regulatory and industrial chemical testing. Notably, prenatal developmental toxicity studies require a large number of pregnant rats per tested compound, raising ethical and economic concerns and emphasizing the need for alternative in vitro methods in line with the 3R principle (Reduction, Replacement, Refinement). The European Centre for the Validation of Alternative Methods (ECVAM) has validated alternatives such as rat whole embryo culture (WEC) and mouse embryonic stem cells (mESC) for predicting developmental toxicity. However, these systems suffer of several limitations including lineage specificity, low predictive cell lines, and poor translatability compared to in vivo studies.

This study explores the use of a rat embryonic stem cell (RESC) in vitro model to mimic pre- and post-implantation embryonic development stages. RESCs were derived from E4.5 Sprague-Dawley rat blastocysts stage as previously described [1], and cultured as single cells (SC). RESC-SC were maintained in SCML medium without mitogens (no LIF, no bFGF), seeded on 0.1% gelatin solution (EmbryoMax, Merck-Millipore Burlington, MA, USA). The applicability of this cellular platforms for developmental toxicology screening has been tested on the environmental pollutant 2,2',6,6'-tetrabromobisphenol A (TBBPA). TBBPA is classified as "thyroid-disrupting chemical", inhibiting the binding of T3 to nuclear receptors, and RESCs cells are T3-sensitive, through the crosstalk with retinoic acid (RA) [2].
In the pre-implant model, RESC cells are treated with vehicle, maintaining pluripotency, while RA treatment induces neuroectodermal differentiation in the post-implant model. Using morphological and molecular techniques, the study analyzes proliferation, differentiation, and gene expression in both models.

RA treatment significantly downregulates pluripotency genes and markers for endoderm and mesoderm in the post-implant model (FOXA2, SOX17, DKK, KLF4, OCT4, SOX2, NANOG), confirming neuroectodermal differentiation. Next, the study evaluated the acute toxicity of TBBPA using MTT viability assay and nuclear morphology by high-content screening (HCS) analysis. TBBPA toxic dose hindered cell proliferation and reduced nestin-positive cells percentage.Further analysis involved transcriptomic profiling using PCR arrays to assess gene expression related to differentiation and cell death pathways. TBBPA exposure in the post-implant model perturbed gene expression associated with differentiation, e.g. up-regulated EMT-related genes (MSNL, G6PC), primitive streak inducing gene (FOXA1), and early germinal layers inducers (neurectodermal, NEUROD1; hindbrain, GBX2; embryonic mesoderm, SMTN); while down-regulated of pluripotency and preimplantation-associated genes (SOX17).
A rescue experiment was also conducted using a TR antagonist to block TBBPA's action on neuroectodermal differentiation. Results showed that TR antagonist pre-treatment inhibits TBBPA-induced neuroectodermal differentiation, suggesting TBBPA's action is mediated by thyroid hormone receptors.

Overall, the study demonstrates the utility of the RESC-based model for evaluating the embryotoxic potential of TBBPA and highlights its potential for screening other endocrine disruptors.

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77° CONVEGNO SISVET

Stato: INVIATO - ID: 13560

Neurodevelopmental and neurobehavioral effects of in utero and pre-weaning exposure to short-chain per- and polyfluoroalkyl substances (PFAS) in rats

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Humans and animals are daily exposed to environmental pollutants that can alter organism homeostasis. Among the pollutants, per- and polyfluoroalkyl substances (PFAS) are of particular concern due to their widespread use in industrial processes. Exposure and bioaccumulation of long-chain PFAS has been associated to adverse health effects as possibly mediated metabolic and endocrine dysfunction. Due to this adverse effects, long-chain PFAS has been recently replaced by short-chain PFAS characterized by lower bioaccumulation. However, very few data are available on harmful effects of short-chain PFAS. The purpose of this research is to evaluate the impact of prenatal exposure to two short-chain PFAS: PFBA (perfluorobutanoic acid) and GenX (ammonium 2,3,3,3-tetrafluoro-2-(heptafluoropropoxy)-propanoate) on neurodevelopment and behavior in CD Sprague Dawley rats (Authorization 206/2022-PR). We selected to expose animals during gestation and lactation because of the well-known effect of metabolic and endocrine dysfunction on neurodevelopment.

Pregnant mothers were exposed via PFBA- and GenX-contaminated feed at two concentrations (0.5 and 5 mg/kg/p.c./day) for about 100 days, covering the prenatal and pre-weaning periods. Control group was fed standard diet for laboratory rodents. A total of 100 pups of both sexes (to assess potential sex-specific effects) were tested with a battery of neonatal motor tests to evaluate neurological development from post-natal day (PND) 7 until PND 21. In adulthood, animals were tested for complex behaviors (motor coordination, spontaneous locomotion, learning and memory) by rotarod and Morris Water Maze (MWM).

Maternal exposure to PFAS-contaminated diet did not lead to significant changes in neurodevelopment respect to control group (CTRL). In adulthood, we observed behavioural alterations in rat exposed to PFBA and GenX. In particular, both males and females exposed to high-dose of PFBA and GenX showed impairment in MWM probe trail, which were more severe in female compared to male rats (one-wayANOVA: malePFBA vs maleCTRL, $p= 0.0018$; femalePFBA vs femaleCTRL, $p= 0.002$; maleGenX vs maleCTRL, $p=0.0035$; femaleGenX vs femaleCTRL, $p<0.0001$). Moreover, female rats only, displayed significant alteration at low-dose PFAS exposure (one-wayANOVA: femalePFBA vs femaleCTRL, $p= 0.0058$; femaleGenX vs femaleCTRL, $p<0.0001$). No alteration in locomotion and sensory-motor alteration were observed.

These findings indicated that prenatal + postnatal exposure also to low/very low short-chain PFAS doses alters learning and memory abilities in adult rats, possibly acting directly or indirectly on the development of related brain pathways.

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77° CONVEGNO SISVET

Stato: INVIATO - ID: 13565

ALZHEIMER'S-LIKE CLINICAL PHENOTYPE IS ACCELERATED IN YOUNG TG2576 MICE BY EXPERIMENTAL COLITIS

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Systemic inflammation and neuroinflammation exert a significant impact on the progression of sporadic Alzheimer's disease (AD), as demonstrated by epidemiological and preclinical studies, with growing evidence suggesting a higher incidence of AD among individuals with inflammatory bowel disease. In order to explore if peripheral inflammatory diseases take part in AD worsening in "at risk" subjects, we investigated whether colitis induced by dextran sulfate sodium (DSS) in young presymptomatic/preplaque mice exacerbates or accelerates age-related cognitive decline in the Tg2576 mouse model of AD. The experimental design (aut. 742/2020-PR) consisted of four distinct groups: vehicle-treated Tg2576 mice (Tg2576 control), DSS-treated Tg2576 mice (Tg2576 DSS), vehicle-treated non-transgenic littermates (WT control), and DSS-treated non-transgenic littermates (WT DSS). Baseline assessments of learning and memory were conducted using Morris water maze when mice were 2.5 months old, prior to DSS administration. Subsequent evaluations were performed at 4 and 5 months of age, with mice sacrificed immediately after the last test at 5.5 months. Blood samples were collected at baseline, during colitis, and at sacrifice while brain tissues and gut specimens were harvested for morphological and molecular analyses. Four supplementary cohorts were employed for cytofluorimetric analysis of microglial cells and qRT-PCR neuroinflammation array in the hippocampus during the acute phase of DSS-induced colitis.Our findings revealed that DSS-induced colitis in young Tg2576 mice precipitates the onset of cognitive impairment, as observed in the Morris water maze test, in absence of amyloid pathology. To elucidate potential mechanisms underlying the hastened cognitive decline, we examined systemic inflammation, neuroinflammation markers and changes in gut microbiota composition. We observed a shift in the Firmicutes/Bacteroidetes ratio in Tg2576 DSS compared to C57BL6 DSS mice, mirroring alterations observed in elderly Tg2576 mice. Significant differences in inflammation and neuroinflammation-related parameters were evident between Tg2576 and WT mice as early as 3 months of age, preceding plaque deposition. It's noteworthy that these differences evolved rapidly (between 3 and 5.5 months of age) in Tg2576 mice treated with DSS compared to untreated Tg2576 and WT littermates. Specifically, following DSS colitis induction, WT and Tg2576 mice displayed contrasting patterns in the expression levels of inflammation-associated astrocyte genes in hippocampus. While no alterations in microglial characteristics were observed in the hippocampus among the experimental groups, reduced GFAP immunoreactivity was noted in Tg2576 compared to WT mice. Overall, astrocyte analysis suggests an atrophic "loss-of-function" phenotype, further exacerbated by DSS treatment, accompanied by decreased GFAP mRNA expression levels. In summary, our results suggest that unidentified peripheral mediators triggered by DSS colitis, accentuate a "loss-of-function" phenotype of astrocytes in young Tg2576 mice, possibly compromising the hippocampal synaptic function.

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77° CONVEGNO SISVET

Stato: INVIATO - ID: 13726

SKELETAL DEVELOPMENT OF RAINBOW TROUT FRY (Oncorhynchus mykiss) REARED IN A RECIRCULATING AQUACULTURE SYSTEM WITH A TiO2-BASED PHOTO-ELECTROCATALYSIS FILTERING TECHNIQUE – PRELIMINARY RESULTS

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In recirculating aquaculture systems (RAS) fish can be subjected to increased levels of ammonia, nitrates, and nitrites, which can cause toxic effects and impaired health and growth [1]. Skeletal anomalies are frequent in farmed fish, and their occurrence depends on the species, developmental stage, and rearing method [2]. In salmonids, these have been described as associated with fast-growing rearing conditions [3], causing a change in swimming and feeding performance, with negative consequences on the growth rate, economic value, and welfare status. This study is part of a major project, the Fish-PhotoCAT (PRIMA2019), and aimed at evaluating the impact of a PEC system on the growth and skeletal development of rainbow trout fry.
Five grams fry were reared at 5 kg/m3, for 21 days in 500 L tanks. All the tanks were equipped with the standard water filtration set-up: in three tanks this constituted the only filtration system (CTR) and in the other three tanks, a PEC system was installed (T). Water parameters were monitored and at the end of the trial, fish were weighed, measured for total length, scored for dorsal and caudal fins, and sampled for skeletal analyses with alcian blue-alizarin whole-mount double staining. Skeletal structures were studied to assess the osteogenesis in the vertebral column and fins. Authorization code: OPBA_20_2020.
No significant differences were found regarding NH3 and nitrite concentrations between the experimental groups. The mean concentration of nitrates, however, was significantly higher in the CTR (122.211 mg/L vs. 108.510 mg/L; $p<0.001$), likely due to the parallel ammonia oxidation to molecular nitrogen performed by the PEC. All groups exhibited similar weight, length, and fin lengths. Classification of skeletal structures as dermal or endochondral bone was based upon their affinity for the histological stains revealing that the origin of the skeletal elements studied did not differ from that seen in other teleosts. The fry double staining revealed that individuals from both groups were similarly ossified revealing that no vertebral abnormalities were detected nor spine deformities. No differences were found in the head length versus in the groups. Analysis of the number of vertebrae revealed that the number varied between 59 and 64 and the mean was not significantly modified by the PEC. Caudal and dorsal fin scores showed no differences between treatments. No difference was found between the experimental groups regarding the other fins organization.The results of this study indicate that both experimental groups presented a similar skeletal development, but further studies are necessary to deepen the different aspects of the use of the PEC.

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77° CONVEGNO SISVET

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Gastric and duodenal visfatin expression in piglets fed with different polyphenolic supplements

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Visfatin is an adipokine with pleiotropic effects, one being the mediation/attenuation of inflammation. In pigs, visfatin transcript is expressed in the stomach at relatively low levels [1], and seems not to be involved in swine fattening [2], but information is lacking on its role in the GI tract. Our aim is to preliminary explore the expression and localization of this molecule in the stomach and duodenum of piglets fed with and without different levels of polyphenolic additions, well-known antioxidants and immunomodulants.
Nine piglet litters, local Sardinian breed, were divided into three groups. Starting from the 14th day of life, a commercial dry creep feed (ctr group), or the commercial creep feed added with a polyphenolic extract dosed at 120 ppm (s1 group) or dosed at 240 ppm (s2 group) was offered to suckling piglets. The extract was obtained from olive mill wastewater, and the predominant polyphenols were tyrosol, hydroxytyrosol, and verbascoside. The animals were regularly slaughtered right after 14 days of experimental creep feed administration. Samples from the glandular stomach and cranial portion of the duodenum were collected from 13 selected piglets (crt: 5; s1: 4; s2: 4). FFPE sections underwent to immunohistochemical reaction, and were visualized using a primary rabbit polyclonal antibody against Visfatin (1:100; ab233294, Abcam, UK) and DAB as chromogen. Digital-image analysis using QuPath software was performed on the tunica mucosa of the latter whole-scanned slides, comprising positive cell detection and classification of “epithelial” vs. “extra-epithelial” cells training a machine learning based model (random forest). A multivariate approach (PCA) was used to evaluate trends in morphometrical data of classified positive and negative epithelial cells. To characterise Visfatin-secreting cells, a double-label immunofluorescence was performed to co-localize the adipokine with Serotonin. Moreover, relative gene expression of visfatin via RT-qPCR was carried out in both the glandular stomach and duodenum.
Visfatin-positive cells have been detected in 5 out of 13 individuals, in both glandular stomach and duodenum, without dependence between experimental groups (χ^2: 0.536, p: 0.764). The immunostaining was typically cytoplasmic and highlighted cells located at the basal portion of the gastric and duodenal glands. The morphology of those cells was consistent with neuroendocrine cells, and confirmed by co-localization of Visfatin and Serotonin. No significant difference (Mann-Whitney test, $p > 0.05$) was detected between negative and positive epithelial cell percentages and H-scores within IHC positive individuals of the same tissue from different groups. Similarly, there was no statistical difference in epithelial/extra-epithelial composition and cell positivity between the glandular stomach and duodenum (Student T-test, $p > 0.05$). PCA did not highlight particular trends in the morphology of positive and negative epithelial cells. Transcript levels of visfatin were statistically different between groups in both the glandular stomach and duodenum (one-way ANOVA, $p < 0.05$), and increased with the dose of polyphenolic extract.
A pro-inflammatory challenge could likely give better clues on the role of visfatin in the GI tract, given its attenuator effect on mucosal inflammation. Factors that lead to its variation between individuals and supplements need further investigation.

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CHARACTERIZATION OF ADIPOSE-DERIVED STEM CELLS (ASCs) FROM SUBCUTANEOUS AND INTRAMUSCULAR FAT: FIRST STEP IN REALIZING 3-D PLANT STRUCTURES TO PRODUCE ADIPOSE TISSUE FOR CELL-BASED MEAT

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With the global population steadily increasing, the development of sustainable and efficient food production methods has become increasingly crucial. Cultured meat represents a promising solution to the environmental, ethical, and health challenges associated with conventional meat production. To date, alternatives to traditional meat, including both plant-based and cultured options, have primarily focused on replicating the muscle component of meat. However, adipose tissue, found within intramuscular fat, also plays a crucial role in determining meat's sensory, nutritional, and technological attributes and its derived products. Adipose cells require a 3D substrate to adhere, proliferate, and develop mature tissue [1]. In our project (PRIN 2022: Food for future: 3D plant-derived structures to produce adipose tissue as innovative food ingredient for cultured meat) we will evaluate the feasibility of creating 3D structures using plants, such as cabbage leaves, which possess specific morphologies featuring cavities capable of facilitating adipose cell growth and differentiation. In this abstract, we discuss the first step of our project: the isolation and the characterization of two distinct Adipose-derived stem cells (ASCs) sources: subcutaneous SC and intramuscular IM fat, both obtained from pigs (120 Kg). ASCs were isolated from adipose tissue collected at the slaughterhouse and characterized by following the criteria dictated by the International Society for Cellular Therapy [2], which consist of verifying i) adherence to plastic and proliferation, ii) specific surface gene expression (positivity for MSC markers and negativity for hematopoietic markers), iii) multipotent differentiation potential. Samples of SC and IM fat tissue were also collected, and formalin fixed to perform histometrical analysis (Adipocytes area and perimeters, and percentage of stromal fraction).

At passage P3, the colony-forming unit assay was performed to assess MSCs' proliferative potential. Preliminary data suggest no differences between IM and SC. MSCs were also screened for positive MSC markers (≥95%) and MHC-II expression by flow cytometry (FACS). The statistical analysis revealed no significant differences between SC and IM-derived MSCs markers expression. The chondrogenic, osteogenic, and adipogenic differentiation potential was assessed and tested by specific histochemical stainings (Alcian blue, Alizarin Red, and Oil Red O staining, respectively). In both SC and IM, positive staining was observed thus confirming successful differentiation. The analysis carried out has established that, similarly to SC, IM is endowed with multipotentiality.

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ANIV

77° CONVEGNO SISVET

Stato: INVIATO - ID: 13095

First detection of Leptospira ST198 related to L. interrogans serogroup Australis in red foxes (Vulpes vulpes) in Emilia Romagna region (Italy)

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Leptospirosis is a worldwide zoonosis affecting numerous wild and domestic mammalian species, sustained by pathogenic and highly motile spirochete bacteria of the genus Leptospira. Leptospires are maintained in nature by several subclinical wild and domestic reservoir hosts that serve as source of exposure for wildlife, livestock, companion animals and humans. The most important reservoir host for several Leptospira serovars is represented by rodents, but Leptospira spp. infection has been documented in accidental hosts as domestic dogs and wild animals such as red foxes (Vulpes vulpes) [1]. In this study, kidney samples from red foxes culled or found dead in the provinces of Bologna, Modena and Ferrara (Emilia Romagna region, Italy), from January 2022 to March 2023 were screened for pathogenic Leptospira spp. DNA using a SYBR Green real-time PCR assay. The identified leptospires were genotyped using a multi-locus sequence typing (MLST) approach, adopting a scheme based on seven housekeeping genes [2], and a protocol developed for direct application on DNA extracted from biological samples. Of the 126 red foxes included in the study, two tested positives for Leptospira spp. DNA, and the identified bacteria were genotyped by MLST analysis as Leptospira sequence type (ST) 198, related to L. interrogans serogroup Australis serovar Australis. To date, Leptospira ST198 in Italy has been detected exclusively in dogs, hedgehogs [1], and in a horse [3] and, to the best of the author's knowledge, this represents the first report of this variant in red foxes. This finding suggests that Leptospira ST198 is widespread in the Italian territory, involving different animal species as maintenance or accidental hosts, and confirms that the red fox may be a reliable sentinel for epidemiological monitoring. Further studies are necessary to understand the role of red fox in the maintenance of this pathogen not only in the wild but also in urban and peri-urban environments.

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Evaluation of the humoral response in water buffalo after two doses of an inactivated phase I Coxiella burnetii vaccine

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Ruminants, both domestic and wild, are the main reservoirs of Q fever, a zoonosis that causes reproductive disorders in animals and acute (self-limiting flu-like syndromes) and chronic forms (characterized by atypical pneumonia, hepatitis, and endocarditis) in humans [1]. The infection is widespread worldwide, as evidenced by the persistent outbreaks reported in both humans and domestic ruminants, whose control and management are currently based on vaccination.
The only currently licensed vaccine for the prevention and control of Q fever in ruminants is Coxevac® (Coxevac, Ceva Santé Animale, France), a phase I vaccine whose administration significantly reduces the risk of abortion, shedding, and therefore the spread of Coxiella in livestock [2]. Although buffalo are intensively farmed in several countries and represent a reservoir for Q fever, no evidence has been described regarding the efficacy of vaccination in this species [3]. This work aimed to evaluate the humoral response (using appropriate phase-specific ELISAs) and the effects on the abortion rate in buffalo through a field study. A total of 35 water buffalo (15 seropositive and 20 seronegative) were vaccinated with 2 doses of a commercial vaccine (Coxevac®) and tested for the presence of antibodies targeting phases I and II at different timepoints (t0 = time of the first vaccination; t1 = 3 weeks from the first vaccination and moment of the second vaccination; t2 = 3 weeks later from the second vaccination). The response was concentrated towards phase II at both timepoints, with high standard deviations indicating a wide variability in response to the vaccine. A significant increase in seroreactivity was found between t0 and t1 and, even if reduced, between t1 and t2. Seroconversion did not significantly depend on age or natural infection status. Although seropositive animals (naturally pre-infected) had slightly higher reactivity than negative animals at t1 and t2, no statistically significant differences were observed (for both phase I and phase II). Similarly, animals older than 20 months had greater seroreactivity at t1 than younger animals (for both phase I and phase II), although not statistically significant. After completion of the vaccination cycle, the herd study observed a reduced rate of abortion and placenta retention. The effects of vaccination on abortion rates were reported monthly. In the month of June, a total of 19 placenta retentions and 3 abortions were reported in the herd study, diagnosed via specific real-time PCR for Coxiella burnetii (Ct 12.6, 20.2, and 20.4). In July, the month in which the first vaccination occurred, retentions were reduced to 17 and a single abortion occurred (Ct 24.2). In the following five months (all animals had completed the vaccination cycle), only five placenta retentions and one abortion occurred (negative for Coxiella in real-time PCR). These preliminary data appeared to support vaccination in buffalo (even in seropositive animals), although further studies are needed to better define the dynamics concerning seroconversion in this species.

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77° CONVEGNO SISVET

SOCIETA´ SCIENTIFICA DI RIFERIMENTO | ANIV

TITOLO | CANINE DISTEMPER VIRUS IN SARDINIA (ITALY): DETECTION AND PHYLOGENETIC ANALYSIS IN FOXES.

Autori | Elisabetta Coradduzza[1]*, Fiori Mariangela Stefania[1], Luca Ferretti[2], Alice Ledda[3], Gian Simone Chessa[1], Angela Maria Rocchigiani[1], Lostia Giada[1], Renata Rossi[1], Antonio Pintore[1], Marcella Cherchi[1], Daniele Denurra[1], Flavia Pudda[4], Marco Muzzeddu[4], Angelo Ruiu[1], Paolo Briguglio[5], Davide Pintus[1], Ciriaco Ligios[1], Giantonella Puggioni[1]

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Canine distemper virus (CDV) is the etiological agent of a highly prevalent viral infectious disease of carnivores and poses a serious threat to the conservation of the affected species worldwide. This work aimed to characterize phylogenetically distemper strains infecting foxes, circulating in Sardinia (Italy) from 2014 to the present. With this objective, we sequenced the H gene of all canine distemper virus strains isolated from infected foxes that were part of our dataset, since this viral gene is also the most frequently used gene for CDV phylogenetic classification. A total of 42 specimens were examined. Subjects recovered alive manifested ataxia, sensory depression, muco purulent conjunctivitis, and died within 2 to 48 hours. Brain, lung, conjunctival mucus, and blood in EDTA were subjected to biomolecular investigation to diagnose the presence of the virus by RT-PCR amplification. We performed Maximum Likelihood phylogenetic reconstruction from Sardinian and international sequences using IQ-TREE. We also performed Bayesian reconstruction of the dated phylogeny of the Sardinian sequences using BEAST. Molecular distances for nucleotide and amino acid sequences were computed using R ape package with default parameters. The reconstruction of the global CDV phylogeny shows that all our Sardinian sequences from foxes belong to a single clade. The only other sequence in the same clade is from the dog sample sequenced in this work that lies in the middle of this clade, suggesting a transmission involving a host jump from foxes to dogs. This possibility is also confirmed by examining the amino acid sequence of our strains, in particular assessing the receptor-binding site 549 in the SLAM-binding region of the haemagglutinin, which McCarthy already hypothesized in 2007 to be a strong candidate in determining host cell tropism. The mutation Y549H is associated with independent incidents of the spread of CDV to non-dog hosts, proving that amino acid substitution at this site is involved in this phenomenon. Our analysis also shows that Sardinian and Italian sequences represent a sister clade of the published central European sequences classified as belonging to the Europe-Wildlife lineage. Note that these sister clades are very different from sequences belonging to the Europe-Wildlife lineage from Germany and Austria: the nucleotide divergence is 5.0% and 5.2% for Sardinian and Italian sequences respectively, and the amino acid divergence is 5.5% and 5.3%. This divergence is comparable to the divergence between different CDV lineages. Most likely, Sardinian sequences belong to a novel “Sardinia-Wildlife” lineage. In conclusion, this study shows the dynamics of CDV infections within the fox populations in Sardinia, enriching our knowledge of the strains responsible for natural CDV infection in this species and that of surveillance of their spread in the territory.

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77° CONVEGNO SISVET

SOCIETA´ SCIENTIFICA DI RIFERIMENTO **ANIV**

TITOLO **R0 ESTIMATION FOR THE SPREAD OF THE MAIN BLUETONGUE VIRUS EPIDEMICS IN SARDINIA, ITALY.**

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Testo e Riferimenti bibliografici

Over the last 20 years, Italy has experienced multiple incursions of different serotypes of Bluetongue virus (BTV), the causative agent of bluetongue (BT), a major disease of ruminants. Since 2000, when the first BT outbreak was notified in Sardinia (Italy), the region has been the most affected area of the Mediterranean basin. BT has a great impact on the livestock industry, mainly due to the direct consequences of the viral infection and the ban of animal trade from infected areas. The main strategies to face BT are based on vaccination and the control of vectors within the farms. The spread of the disease is very sensitive to climate conditions, although management practices mitigate disease transmission. The use of sentinel animals represents a useful system for a rapid alert of viral circulation in the first epidemic phases. However, the re-emergence of already present serotypes and the potential arrival of new ones, make epidemic phenomena still unpredictable.
This work aimed to estimate BTV's doubling time and the R0 from the data obtained during the main BT Sardinian epidemics, for each serotype involved. Furthermore, this study aimed to improve the position of the sentinel on the territory. The estimated parameters allow for the quantification and comparison of the spread of the infectious disease in terms of season and epidemic starting area, the prediction of its speed, and the evaluation of the surveillance effort currently in place. Combined techniques were applied to the 13,603 BTV outbreaks notified in Sardinia from 2012 to 2023 to estimate the R0 from the doubling time (td), assuming that the number of secondary cases increases exponentially and that BTV infectious period (T) was of 12-15 days. Three main different starting areas were identified: southwest, northeast, and southeast. Most of the time, the epidemic season starts in August and ends in December. In 2012-2013, the BTV-1 epidemic started in the southwest area recording about 7,000 cases with a slow speed (R0 = 1.73; 95% CI = 1.54-2.86). The most dangerous serotype in terms of the number of cases, doubling time, R0, and duration of the epidemic trend was the BTV-4. It plagued the susceptible population in 2017 (2,500 cases) and 2021 (3,300 cases), starting from the southeast area, and was characterized by a very short doubling time (td = 3.12 respectively), and a higher R0 value (3.66, 95% CI = 2.82-5.71). In 2023 a BTV-8 transmission occurred later (October), with a rapid spread (R0 = 1.97; 95% CI = 1.64-5.63) from the southwest area, and contemporarily with the BTV-4 in the northeast. The epidemic lasted much shorter (about two months) probably due to the climatic starting period, and the two serotypes remained distinct in the two areas, probably thanks to the BTV-4 vaccination strategy. Although the new EC regulations focus the disease control activities to guarantee trade, clinical studies and control of epidemic events must be guaranteed by scientific community. These parameter estimation could represent a fundamental tool for monitoring the surveillance system's sensitivity.

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77° CONVEGNO SISVET

SOCIETA´ SCIENTIFICA DI RIFERIMENTO **ANIV**

Potential in vitro antiviral activity of fungal metabolites Sphaeropsidin A and B against bovine coronavirus

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Pimarane diterpenes are a representative group of secondary metabolites frequently produced by phytopathogenic fungi including several species belonging to the genus Diplodia. Sphaeropsidin A (SphA) and its analogue sphaeropsidin B (SphB) are well-known members of this class of natural products and, due to their effective antimicrobial, insecticidal, herbicidal, and anticancer activities, SphA and SphB are promising natural substances to be used in agriculture as well as in medicine [1]. Nonetheless, to date no targeted studies have been carried out to evaluate its antiviral properties. Considering that the scientific community's interest in the antiviral properties of natural compounds has significantly increased after the emergence of severe acute respiratory syndrome coronavirus (SARS-CoV-2), new studies are evaluating the use of natural drugs for the treatment of SARS-CoV-2 infection. From this perspective, bovine coronavirus (BCoV), a betacoronavirus responsible for neonatal diarrhea, winter disease and bovine respiratory syndrome in ruminants [2,3], could represent a good model for screening new anti-coronavirus molecules. In this study, the antiviral effects of SphA and SphB were evaluated on Madin Darby Bovine Kidney (MDBK) cell cultures infected with BCoV by bioscreen, immunofluorescence staining and virus yield analyses. After BCoV infection, non-toxic concentrations of SphA and SphB significantly increased cell viability. These results occurred in association with a reduction in features due to morphological cell death. In addition, the presence of both SphA and SphB resulted in a significant reduction of virus yield and spike S protein expression. Our preliminary results suggest that nontoxic concentrations of both fungal secondary metabolites have potential antiviral activity against BCoV. Moreover, animal model for screening potential antivirals agents, avoid handling extremely dangerous viruses, like SARS-CoVs and/or Middle East Respiratory Syndrome (MERS)- CoV.

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Modulation of the gut microbiota and metabolic potential of honey bees with integrated feeding

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Apis mellifera is the world's main pollinating insect and plays a crucial role in maintaining the biodiversity of the global ecosystem. The integrity of the honeybees' gut microbiome is essential to prevent diseases that can afflict them [1]. In this context, probiotics are increasingly used to support the gut microbial balance and, consequently, the health of these insects.
In this study, a multistrain probiotic formulation was administered as diet supplement to the subspecies *A. mellifera ligustica* worker honeybees. The effect on intestinal metabolism was evaluated by comparison with a control group of bees not receiving the supplementation. Hive A received 50% glucose syrup; hive B received 50% glucose syrup supplemented with probiotics Honeybeeotic^{TM}, 2 x 10^{11} ufc/ml. The latter was formulated from 7 bacterial strains isolated from a particular honeybee population found in the Roti Abbey area (Matelica, Marche Region, Italy). All nutritional support was administered *ad-libitum*, daily for one month.
Genomic data on gut microbial community were obtained by next-generation sequencing (NGS, Shotgun technique). Pools of 4 intestinal segments (crop, midgut, ileum and rectum) of 20 honeybees from hives A and B, at time T0 (start of work) and T1 (end of treatment) were analysed. Phenol oxidase activity was assessed on hemolymph samples [2] belonging to 20 honeybees taken from each hive at time T1. Results were statistically described, and alpha- and beta-biodiversity were evaluated.
Data obtained show that dietary supplementation with the probiotic mixture is safe and well-tolerated. Also, it modulates the composition of the gut microbiota in healthy honeybees. Following probiotic consumption, the components of the 'core' microbiota were retained, with both positive and negative variations. Some components, such as Lactobacilli, *Snodgrassella* and *Bifidobacterium*, were enriched at the crop level, indicating a greater diversity of OTUs composing the microbiota after treatment. Likewise, the decrease of some microorganisms is also positive (*Frischella perrara*, *Parasaccharibacter apium*), as it reflects the induction of improved insect fitness by the probiotic.
Several genes were found to be more abundant in the probiotic group, like KEGG genes involved in amino acid metabolism and carbohydrate metabolism, as well as BCAA transport genes, suggesting an effective nutritional supplement to the host.
Phenol oxidase activity decreased significantly in the hive B (1.75 ± 0.19 U/mg), with respect to the hive A (3.62 ± 0.44 U/mg, $p<0.005$), indicative of the improved state of well-being of the honeybees, which do not require the activation of mechanisms involved in immune defence.
In conclusion, the use of this probiotic appears to totally improve the health of honeybees, allowing the intestinal microbiota to acquire a balanced condition with an increase in the metabolic activities involved in digestion. Consequently, it can be understood how the increasing use of probiotics in beekeeping is a means of support for healthier and more resistant colonies, able to cope with biotic and abiotic factors potentially harmful to this precious insect.

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77° CONVEGNO SISVET

Stato: INVIATO - ID: 13405

Development of a molecular assay for the detection of Borrelia spp. in animal samples

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Vector Borne Diseases (VBDs) are an emerging health problem for both humans and animals due to an increase in their spread and prevalence, closely related to climate change and loss of biodiversity [1]. Many VBDs constitute zoonoses as the pathogen habitually resides in animal hosts that act as reservoirs and sources of infection for humans. Of particular importance are tick-borne pathogens (TBPs); it is estimated that in Northern Italy up to 40% of ticks are positive for one or more aetiological agents [2-3]. This, associated with the increase in outdoor activities and close cohabitation with dogs and cats, plays a primary epidemiological role in the transmission of some TBPs to humans such as Borrelia burgdorferi sensu lato, a genospecies complex causing Lyme Disease (LD). The diagnosis of this disease is mainly serological, while molecular assays, often less sensitive, allow to genetic characterise the identified bacteria. Few studies have been carried out on the molecular prevalence and genetic characterisation of the different Borrelia species in animals. Our study aimed to develop a molecular assay for the detection of Borrelia spp. DNA in animal samples. For this purpose, a SYBR Green Real-Time PCR (qPCR), designed on 16S rRNA gene of Borrelia spp. and capable of identifying a broad spectrum of Borrelia species belonging to both the Relapsing fever group and the LD group was developed. The qPCR reactions were performed using the PowerUp SYBR Green Master Mix (Thermo Fisher Scientific, Life Technologies, Carlsbad, CA, USA) in the QuantStudio 3 Real-Time PCR System (Thermo Fisher Scientific, Life Technologies, Carlsbad, CA, USA). Bacterial DNA copy number determination was carried out by absolute quantification using the standard curve method. Serial 10-fold dilutions of a plasmid (pCR4 plasmid, TOPO TA Cloning Kit, Life Technologies, USA) containing one copy of the target sequence were used as external standards for the construction of the assay standard curve. Sensitivity was evaluated determining the limit of detection (LOD) of the assay. Specificity was established testing several genospecies of Borrelia burgdorferi sensu lato as positive controls (B. afzelii, B. garinii, B. lusitaniae, B. valaisiana and B. burgdorferi sensu stricto). The qPCR developed showed a LOD of 10 copies/μL and amplified all the positive controls. Therefore, the assay showed excellent sensitivity and specificity for Borrelia spp. Spleen samples of wild animals from Emilia Romagna region are currently being analysed to validate the developed assay on biological specimens and to assess the molecular prevalence of these bacterial species. This research was supported by EU funding within the NextGenerationEU-MUR PNRR Extended Partnership initiative on Emerging Infectious Diseases (Project no. PE00000007, INF-ACT).

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77° CONVEGNO SISVET

Stato: INVIATO - ID: 13533

Molecular Characterization and Phylogenetic Analysis of Hepatitis E virus (HEV) circulating in Sardinia (Italy) pig farms

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Hepatitis E Virus (HEV) is a significant public health concern globally, primarily transmitted through the fecal-oral route, that can lead to acute hepatitis in humans. While traditionally associated with waterborne transmission in developing countries, HEV has garnered attention in developed nations due to zoonotic transmission, particularly from pigs. The etiological agent is a small RNA virus, characterized by a high heterogeneity, and classified into 8 genotypes, 4 of which cause most of human cases. HEV-1 and HEV-2 only infect humans and circulate in low-income countries; HEV-3 and HEV-4 are zoonotic. A previous study conducted in Sardinia, Italy, evidenced an high HEV seroprevalence (42.3%) in pigs, shedding light on the potential risks associated with pig farming. In order to characterize the viral strains circulating in Sardinia, a total of 128 fecal specimens (pool) were collected from the seropositive farms and screened for the presence of HEV by qualitative real-time RT-PCR. Seventeen samples resulted positive for HEV, for 11 of which it was possible to obtain the amplification of partial ORF1 and/or ORF2 genome regions. PCR-products were purified and subjected to Sanger sequencing. The sequences were edited using the free Aliview software and aligned to all Italian and international strains with a nucleotide identity greater than 92%, retrieved from NCBI GenBank. Maximum Likelihood (ML) trees for both genome regions were produced with IQTREE2 software using the model suggested by the software's ModelTest. Statistical support for specific clades was obtained via 1000 bootstrap replicates. The Sardinian strains were classified as genotype HEV-3. The phylogenetic analysis showed that seven strains clustered in the subtype 3f, and two strains in the subtype 3e. Two identical sequences clustered with strains belonging to a novel subtype not classified yet. This is the first study that molecularly characterize the HEV strains circulating in swine population in Sardinia.

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77° CONVEGNO SISVET

Stato: INVIATO - ID: 13622

Equine viral hepatitis: serological evidence for circulation of Equine Hepacivirus and Equine Parvovirus-hepatits in Piedmont, Italy

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Viral hepatitis has recently assumed greater relevance in equine clinical practice since a variety of new viruses have been discovered, such as the Equine Hepacivirus (EqHV) and the Equine Parvovirus-hepatitis (EqPV-H)[1]. These viruses are worldwide distributed and associated with mild to severe liver pathology. Affected horses may be asymptomatic, with transient subclinical infection and occasional elevation of liver enzymes, or present with overt and relevant clinical signs, such as icterus, colic episodes, and hepatic encephalopathy. Iatrogenic transmission through inoculation of biological products of equine origin is the major source of virus spread, although the infection can also occur among in-contact horses.

EqPV-H is a single-stranded DNA virus whose genome contains two large ORFs, encoding non-structural and structural proteins. The antigenic protein VP1 is used as a target for virus detection in serum and liver samples by PCR techniques, but also for serological tests like the Luciferase Immunoprecipitation System (LIPS) assay[2]. EqHV, the closest known relative of human Hepatitis C Virus, is a single-stranded positive-sense RNA virus presenting a large ORF encoding structural and non-structural proteins, including the NS3 protein, which is commonly used as target molecule in antibody detection methods. Although the circulation of the two viruses has been demonstrated worldwide, epidemiological information on their distribution in Italy is still limited. A recent epidemiological investigation highlighted a prevalence of EqHV between 4,7% (Southern Italy) and 6,25% (Northern Italy)[3].

In general, the diagnosis of these two viruses mainly relies on the molecular detection of pathogens while a lack of serological assays is the main challenge for their serological investigations.

In this context, the study aimed to develop an in-house ELISA test for the detection of antibodies against the two viruses and to explore their serological prevalence in Piedmont (Italy).

The recombinant NS3 and VP1 antigens were produced via mammalian expression strategy, using a transient expression system based on high density, suspension-adapted, HEK cells. Performances of the prototype ELISA were evaluated with reference sera provided by the Department of Veterinary Medicine of Bari. Field serum samples from different Piedmontese stables (mares and foals from 5 breeding farms, animals presented to the Veterinary Teaching Hospital of Turin, adults from the hippodrome that collects race-horses from different parts of Italy) were then tested for the presence of antibodies against the two viruses.

Test validation allowed us to set the method cut-off at 40% of the positive control. Out of 150 field samples tested, EqPV-H specific antibodies were detected in up to 10.91% of horses (95% CI, 5.08-16.74%), while a prevalence of 5.45% (95% CI, 1.21-9.70%) was detected for EqHV. No animals with co-infection were reported.

Our results are consistent with those reported in Italy and other European countries in previous studies.

In conclusion, we detected the presence of antibodies against the two viruses and confirmed their circulation in Piedmont horses. Further investigations are needed to confirm our data and to deeply explore the epidemiology as well as the antibody response against these two viruses.

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77° CONVEGNO SISVET

Stato: INVIATO - ID: 13629

A five-years retrospective epidemiological study of feline immunodeficiency virus (FIV), feline leukaemia virus (FeLV), and feline coronavirus (FCoV) infection in cats from Sicily, southern Italy

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Feline leukaemia virus (FeLV), feline immunodeficiency virus (FIV), and feline coronavirus (FCoV) still threatening feline health, causing significant morbidity and mortality. These viral infections are worldwide documented in the feline population, still nowadays generating concerns on the available therapeutic options and management strategies of positive cats. To date, well documented rapid tests have been successfully used to directly or indirectly confirm FIV, FeLV or FIP infection, supporting decision making to control these diseases and prevent virus transmission, particularly for shelter or colonies management. Prevalence in the Italian feline population still deserve to be in-depth determined and, particularly, in Sicily is almost unknown. To obtain baseline data, a five-years retrospective study on FeLV, FIV and FCoV prevalence in cats from Sicily was performed.
For this purpose, the results of commercial tests performed on a total of 260 sera from household, stray or colony cats collected in 2019-2023 were evaluated. Samples were collected by public and private veterinary practitioners and submitted to the Istituto Zooprofilattico della Sicilia "A. Mirri" (IZSSi) for diagnostic purposes. The presence of FeLV antigen and antibodies to FIV using the commercial rapid enzyme-linked immunosorbent assay (ELISA) kit SNAP Combo Plus FeLV/FIV (IDEXX, USA) [1]. FCoV antibody testing were performed using the ELISA assay ImmunoComb Feline Coronavirus (FCoV) [FIP] Antibody Test Kit (Biogal Galed Laboratories, Israel) [2]. Information on the province of collection for each cat was obtained from the Informative System of Laboratories (SILAB) database of the IZSSi.
Samples considered in this study were collected from 7 of the 9 Sicilian provinces. A total of 4 (1.6 %), 41 (16.9%) and 107 (66%) cats tested positive to FeLV antigen and to FIV or FIP antibody detection, respectively. Considering cats tested for all three viruses (n=142), n=90 tested positive only for one virus (FIV or FCoV), n=10 for two viruses (FIV and FCoV), while only two cats tested positive for all three viruses. Variations of positivity rates (FIV: 5.3-38.5%, mean 19.3%; FeLV: 0-3.7%, mean 1.8%; FCoV: 53.8-83%, mean 64.7%) were observed between each year of collection. Even with variations based on year and province of collection, the highest total positivity rates were observed in Caltanissetta (88% of tested cats), Catania (79%), and Agrigento (50%) provinces.
Epidemiological data, particularly from underestimated territorial areas as Sicily, are necessary to optimize strategies for the prevention and control of viral infection. This study defined baseline data of the positivity rates of FIV, FeLV, and FCoV in Sicily, awaking on their diffusion and suggesting future necessary research focusing potential associated risk factors, necessary for an optimal protection and management of cats, particularly in critical or overcrowded environments.

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77° CONVEGNO SISVET

Stato: INVIATO - ID: 13691

Study on the prevalence of extended-spectrum beta-lactamase-producing bacteria in the feces of llamas and alpacas from northern Italy.

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South American camelids (SACs), particularly llama (Lama glama) and alpaca (Vicugna pacos), are spreading in Europe too, especially in Italy. While traditionally were used for fiber and land management, they're increasingly kept for animal therapy, trekking, or even as pets (2). However, despite close contact with humans and to other animal species, research on potential disease transmission from SACs remains limited (1). The aim of this study was to evaluate the distribution of extended-spectrum beta-lactamase (ESBL)-producing gram-negative bacteria in SACs.Nine farms from different regions of northern Italy (Lombardy, Piedmont, and Veneto) have been enrolled. The farms were selected based on the owners' availability and the presence of at least one SACs on the farm. After identification, a clinical examination was performed, and species, breed, sex, age, and any antimicrobial treatment performed were recorded. Fecal samplings were collected by transrectal stimulation, stored in sterile vials at 5 °C and processed within 24 hours.Fresh feces (0,1 g) were enriched in 5 mL of Müeller Hinton broth and incubated at 37°C for 24 h. All the feces were cultured on CHROMagar™ ESBL agar plates and MacConkey agar and incubated at 37°C for 24 h. Using the direct transfer method, the species identification was accomplished via matrix-assisted laser desorption-ionization mass spectrometry (MALDI-TOF MS; Bruker Daltonics, Fremont, CA). All the isolates recovered from CHROMagar™ ESBL agar plates were subjected to ESBL phenotyping assessment using the double-disc synergy test (DDST). Furthermore, to assess carbapenemase production, a disc diffusion test was performed according to EUCAST guidelines.A total of 125 SACs (19 llamas and 106 alpacas), ranging in age from 5 months to ten years old were included. Of these, 23 (18.4%) tested positive for ESBL-producing bacteria. The most frequently identified bacteria were Pseudomonas spp. (n=20) and Escherichia coli (n=3).The prevalence of ESBL-producing bacteria (18.4%) detected in this study is comparable to other European studies (1). From the data obtained, SACs appear to have a low distribution of ESBL-producing bacteria, but these are present throughout the studied area. This suggests a minimal risk of transmission of these types of antibiotic-resistant bacteria between animals, humans and the environment.

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ARNA

77° CONVEGNO SISVET

Stato: INVIATO - ID: 13081

POLYETHISM AND SEASON AFFECT HAEMOLYMPH PROTEIN PROFILE IN HONEYBEES (APIS MELLIFERA)

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The decline of honeybee (Apis mellifera) populations has negative consequences not only for agriculture and beekeeping, but also for ecosystems. There is an urgent need to understand the factors contributing to this decline and to establish objective criteria for assessing the health of hives. In human and veterinary medicine, serum proteins serve as valuable biomarkers to assess the health and nutritional status of organisms. Although the unique proteome of honeybee haemolymph provides valuable biomarkers of hive health, it remains underexplored from a clinical perspective. Some of these proteins reflect essential metabolic processes such as lipid metabolism (apolipophorins) and iron homeostasis (transferrin), while others play key roles in nutrition, development, immune response, and longevity (vitellogenin and hexamerins). However, these proteins can vary with age, role in the hive, and season. This study investigates variations in haemolymph proteins in newly emerged, nurse and forager bees in three different seasons.Samples of haemolymph were collected in 2023 from honeybees in an apiary located in the province of Bologna, surrounded by wild flora and vineyards.
Samples were collected in May, July and November. Three different categories of workers were collected: newly emerged, nurses and foragers. Total protein concentration was measured using the Bradford method, and then haemolymph proteins were separated and quantified by 1D sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE) as reported by Isani et al [1].
Total haemolymph proteins have consistently shown a positive association with the quantity and quality of dietary protein intake and have also been correlated with nutritional status. In this study, the concentration of total proteins was consistent with those reported in the literature [1, 2] and the highest concentration was measured in November in nurse bees (63 ±0.2 μg/μL). The electrophoretic profiles of haemolymph proteins found in this study are also consistent with those reported in the literature [1]. Interesting differences were found between newly emerged, nurse and forager bees. Nurse bees had the highest vitellogenin concentration compared to the other two sub-castes, with the peak vitellogenin concentration observed in November (12.11 ± 4.01 μg/μL), when the lack of brood determines the increase of this protein. In contrast, newly emerged bees had a higher concentration of apolipophorin, a multifunctional high molecular mass protein involved in the transport of lipids in the haemolymph. The vitellogenin/apolipophorin ratio in nurse bees favoured vitellogenin in May and November, whereas the two were equal in July. Conversely, the ratio in newly emerged bees is consistently in favour of apolipophorin. In conclusion, these are the first data on the concentration of a panel of haemolymph proteins in different phases of the life cycle and in different seasons in Italian honeybees. These data can be considered as a starting point to establish in the future reference values useful in clinical practice to evaluate the health and nutritional status of bees.
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77° CONVEGNO SISVET

Stato: INVIATO - ID: 13084

Biochemical characterization of a by-product from Arthrospira platensis extraction and its possible use as feed supplement

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Due to their high nutritional value, microalgae are added to animal feeds [1]; however, production costs are still too high to be competitive with other sources, such as soybean. The present research analyses microalgae by-products as a source of proteins with high biological value and low environmental impact for their use as feed supplements in a circular economy approach. The research focuses on the characterisation of Spirugrass®, a by-product resulting from the extraction of phycocyanin from the biomass of the cyanobacterium Arthrospira platensis. The research was carried out in two phases: 1) the characterisation of Spirugrass® obtained from AlgoSource (France), with particular emphasis on iron content and iron-binding proteins; and 2) the effect of high-pressure pasteurisation at 6000 bar for 5 minutes (HPP) on Spirugrass® proteins.
Soluble protein extraction was performed using dried biomass of A. platensis and different batches of Spirugrass®. Supernatants were fractionated by size exclusion chromatography (SEC) and phycocyanin was detected by reading the absorbance at 620 nm. Gel electrophoresis (SDS-PAGE) was used to assess protein quality and integrity and phycocyanin content; samples were loaded onto precast polyacrylamide gels (4-12%) and ferogram analysis was performed using ImageLab 5.2.1 software. Iron content was determined by atomic absorption spectrometry (AAS) after sample digestion, while supernatants or chromatographic fractions were analysed directly.
SDS-PAGE of the supernatants obtained from the extraction of soluble proteins from A. platensis and Spirugrass® was performed to verify the quality and integrity of the proteomes. In all samples, including Spirugrass®, the most abundant bands were at 18 and 17 kDa. These bands contain C-phycocyanin (C-PC) [2]. Intense protein degradation was not observed in Spirugrass® samples; however, the electrophoretic profile was characterised by lower levels of phycocyanin and lower molecular weight bands, suggesting possible partial fragmentation. The lower amount of phycocyanin in Spirugrass® than in the total biomass of A. platensis was confirmed by SEC: the protein appeared in two peaks of different molecular mass, the first at >75 kDa and the second at 35-40 kDa, suggesting the presence of different aggregation states. For Spirugrass®, SDS-PAGE analysis also revealed batch-to-batch variability. In terms of iron content, Spirugrass® samples contained a mean value of 261 ± 15 μg/g dry weight, representing 70% of the total iron present in the intact biomass. After SEC, the iron peaks overlapped with those of phycocyanin, confirming that this protein can still bind iron in Spirugrass®. HPP treatment revealed slight differences in the SDS-PAGE protein profile; however, a decrease in band intensity was detected, suggesting that HPP may cause mild protein degradation. These preliminary data suggest a possible use of Spirugrass® in animal feed, due to the high protein and iron content.
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77° CONVEGNO SISVET

Stato: INVIATO - ID: 13222

Exploring the Relationship Between Laser and Sniffer Techniques for Methane Detection in Dairy Cows During Robotic Milking: Preliminary Findings

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Methane, a potent greenhouse gas, significantly contributes to environmental concerns due to its short half-life, making it useful to control methane emissions to reduce the carbon footprint of dairy farming [1]. Addressing this issue, researchers have explored various techniques for methane detection, including laser methane detectors (LMM) and sniffer methods (SNF) [2]. However, presently, there are no published equations correlating the application of these techniques in the Automatic Milking System (AMS). This study aimed to evaluate the correlations between methane emissions measured with SNF (Moologger, Tecnosens, Italy) and LMM (Laser Methane Mini, Cowcron, UK) in dairy cows milked using an AMS (Merlin2, Fullwood, UK). Eight Italian Holstein dairy cows were enrolled in the trial, with an average lactation number of 2.38 ± 0.58, and an average Days in Milk (DIM) of 69 ± 15, milked at fixed times (06:00, 14:00, and 22:00) for four days. Methane emissions were simultaneously measured using both the LMM and SNF techniques. During milking in the AMS, while the cows were consuming supplementary concentrate, the LMM was directed at the cow's muzzle, and the SNF aspirated air from the AMS manger. The LMM recorded a total of 98.29 ± 59.96 ppm*m, while the SNF recorded 2.14 ± 1.18 mcg/s. Pearson correlation analysis was employed to study the relationship between these two measurement methods. The results revealed a 41% coefficient of determination (Rsq) and a 64% correlation (R) (RSQME = 44.27; $P < 0.01$) between the two techniques. While this preliminary finding is promising, it remains relatively weak. To enhance the results, two approaches could be implemented: increasing the number of animals and/or sampling at additional time points, and evaluating the effectiveness of the cow's muzzle placement in the AMS feeders. In fact, we are aware that when the cows finish consuming the supplemental concentrate in the AMS feeder, usually they start moving their heads in other directions, hence getting further from the SNF aspirator. As a result, methane stops being detected by the SNF, but the LMM's operator continues to follow the cow's muzzle. These strategies will be adopted in subsequent stages of the experiment to achieve a better outcome. In conclusion, the obtained results suggest a certain degree of correlation between the LMM and SNF techniques. However, further implementation and refinement are necessary in the subsequent phases of experimentation.[1] Lanzoni et al., Animal, 17(5):100794, 2023; [2] Sorg et al., Comput. Electron. Agric., 153:285-294, 2018.

77° CONVEGNO SISVET

Stato: INVIATO - ID: 13321

The use of milk amyloid A as a biomarker for the udder health of dairy cows

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Milk amyloid A (MAA), produced by mammary gland epithelial cells [1], increases significantly during intramammary infections before serum levels rise [2]. This precedes a rise in milk somatic cell count [2], indicating the potential role of MAA as a mastitis biomarker [3]. The aim of this study was to quantify the MAA content in bovine milk and to assess its variability related to milk quality, animal [days in milk (DIM), parity], and environmental (herd, season) factors. A total of 636 individual bovine milk samples were collected during the evening milking in 54 herds located in the northern Italy. The concentration of MAA was assessed with a commercially available enzyme-linked immunosorbent assay kit and data analyzed through a mixed linear model that included the random effect of herd and the fixed effects of season, days in milk, parity and somatic cell count. The average MAA concentration in milk was 1.78 μg/ml with a standard deviation of 1.73 μg/ml. The high variation was ascribed to the high presence of cows with MAA content in milk close to zero. The statistical analysis showed that MAA was higher in older cows (≥4 parities) and during the first weeks of lactation (within 120 days in milk). A positive association was observed between MAA and somatic cell count in milk, indicating that the higher MAA release into milk is directly related to immune system activation in the mammary gland. This increase also seems to precede that of somatic cells in milk, highlighting the potential role of MAA as a biomarker of cow health, particularly in relation to mastitis. Our results have the potential to enhance the efficacy of diagnostic protocols and traceability mechanisms at the herd level. This improvement aims to mitigate economic losses associated with mastitis, minimize antimicrobial utilization, and reduce the risk of antimicrobial resistance development.

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77° CONVEGNO SISVET

SOCIETA´ SCIENTIFICA DI RIFERIMENTO **ARNA**

TITOLO **CHEMICAL CHARACTERIZATION AND FUTURE PERSPECTIVE ON THE BLUE CRABS (CALLINECTENES SAPIDUS RATHBUN, 1896)**

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Testo e Riferimenti bibliografici

The blue Atlantic crabs (Callinectes sapidus Rathbun, 1896) is a decapod crustacean of the *Portunidae* family, native to a vast stretch of the western Atlantic seaboard from Maine to the Río de la Plata. Blue crabs are common in the Gulf of Mexico and western Atlantic Ocean where they are the target of several large recreational and commercial fisheries. They serve as prey for many organisms (fish, rays, and larger invertebrates) and are also opportunistic omnivores that feed on plants, animals, detritus, and carcasses when available[1]. In the Mediterranean the species was accidentally introduced in Greece in 1948 and since then its abundance has been gradually increased posing a threat to native fisheries, and the diver [2].
Both fresh and marine crabs provide abundant nutrients essential for human health. Due to their distinct flavor and delightful taste, crab meat and innovative crab-derived processed products enjoy significant popularity, leading to a consistent rise in demand both domestically and globally. [3].
A total of 10 blue crab samples from the Adriatic Sea and 10 blue crab samples from the Mediterranean Sea were chemically characterized to detect differences; Protein, ash and fatty acid analyzes were carried out to evaluate edibility and nutritional quality.
The aim of this work is to analyze samples from different areas to evaluate their chemical differences and at the same time evaluate the edibility of the product and its nutritional characteristics to be able to introduce it more widely onto our tables.
It is of fundamental importance to comprehensively evaluate the chemical footprint of the blue crab, in order to consider its possible exploitation as a source of bioactive substances, thus contributing to sustainability and the circular economy.

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77° CONVEGNO SISVET

Stato: INVIATO - ID: 13376

INSECTFISH. THE USE OF INSECT MEAL IN THE FISH SECTOR: CREATING VALUE FROM FARM TO FORK

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The aquaculture industry has grown significantly in recent years. However, it faces a major challenge in maintaining sustainability. Fish meal and fish oil, which are widely used in aquaculture diets for their high crude protein content, essential amino acids, and omega-3 content, are becoming increasingly scarce and expensive due to the shortage of marine resources. Therefore, the feed industry is looking for cost-effective and nutritionally suitable alternative ingredients that won't affect fish growth performance or quality. The InsectFish project aims to enhance aquaculture sustainability by replacing fish meal with insect meal. The project takes a multidisciplinary approach and has specific actions planned out based on the two Research Units part. It will provide and validate necessary tools to fine-tune fish farming using insect meals and promote the final products for targeted users, including retailers, food service operators, and consumers, according to geographical location, eating behaviours, and acceptance level. The project's focus is investigating the characteristics of gilthead seabream (Sparus aurata) fed with insect-based feed, from a nutritional, quality, and sensory point of view. The sensory perception of consumers might be affected by the intrinsic characteristics of the insect-fed fish, as well as by the information provided regarding the sustainable value and aquatic production that used insect meal. These factors may influence not only consumers' sensory perception, but also preference, and willingness to pay (WTP). The final goals of the InsectFish project are: i. to refine the production process of farmed fish fed on insect meals; ii. to compare fillet traits in terms of nutritional values and quality; iii. to understand stakeholders' perception regarding the use of insects in aquaculture production; iv. to understand whether food service experts and consumers can recognize sensory differences between insect-fed fish and insect non-fed fish; v. to explore the attitude, preferences, and willingness-to-pay of Italian consumers towards farmed fish-fed insects. This knowledge will be useful for all stakeholders, including producers and distributors across the food chain. Effective communication tools will be developed to enable consumers to make informed and conscious choices, as well as enhance the insect industry and market. The InsectFish project was funded under the Next Generation EU call from the Italian Ministry of University and Research (MUR) PRIN 2022 PNRR (Progetti di Rilevante Interesse Nazionale 2022 Piano Nazionale di Ripresa e Resilienza).

77° CONVEGNO SISVET

Stato: INVIATO - ID: 13519

Application of a cell assay to evaluate the effect of grape by-products on the modulation of the immune response in chicken immune cells

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Agro-industrial by-products are produced in abundant quantities annually and present sustainable and cost-effective opportunities to substitute conventional feed resources. Grape by-products (GP) derive from the production of wine and related beverages and are particularly noted for their rich content of bioactive compound, including polyphenolic substances. Previous research has already highlighted the antioxidant and antimicrobial properties of GP when used as feed supplements [1]. This study aims to investigate the immunomodulatory effects of various GP forms on chicken peripheral blood mononuclear cells (PBMC) using an in vitro immune cell assay. The research project started with the processing of the original GP (oGP) via in vitro digestion using the INFOGEST protocol, yielding digested GP (ivdGP). Additionally, a polyphenol-enriched extract was prepared from oGP using a water:ethanol mix (extGP). The phenolic content was determined in extGP and ivdGP through the colorimetric Folin-Ciocalteu method, as described by Serra et al [2]. The PBMC were isolated from blood samples of eight chickens, following the optimized protocol by Larsberg et al [3]. The cells underwent treatment with oGP, ivdGP, extGP, and concanavalin A as a positive control across two experimental setups. Initially, the PBMC proliferation was tracked bi-hourly over a span of 60 hours using the Incucyte® live cell imaging system. In a second setup, the PBMCs were harvested after 24 hours for immunophenotyping via flow cytometry, following staining with a specific antibody mix. Two experiments were carried out in independent replicates. The phenolic content of the extGP sample was 4193 ± 382 mg/100 g tannic acid equivalents (TAE), while it dropped to 1559 ± 275 mg/100 g TAE in ivdGP, as expected after in vitro digestion. The preliminary results of the monitoring of PBMC proliferation revealed no significant variation in proliferation after all treatments over 60 hours. However, flow cytometry analysis demonstrated that extGP treatment significantly increased the proportion of activated CD4+CD25+ and CD8+CD25+ T cells ($P<0.01$) compared to control, thus suggesting a specific T cell stimulation by extGP. Instead, the extGP treatment did not significantly affect B cell activation. In summary, a non-quantitative but qualitative change in PBMC was observed following extGP treatment, as extGP did not alter PBMC proliferation compared to all other treatments but it was the only treatment among all tested GP forms to enhance CD4 and CD8 T cell activation, indicating its potential as an immunomodulatory agent in chicken immune response. Nonetheless, additional research is necessary to confirm the utility of GP as a bioactive feed supplement for enhancing poultry health and nutrition.

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77° CONVEGNO SISVET

Stato: INVIATO - ID: 13527

Influence of protein production process on petfood sustainability: comparison of the environmental impact of a hydrolysed and a processed animal protein of the same species

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A careful selection of ingredients, in particular protein sources, is one of the factors that can influence petfood sustainability. The environmental impact of a petfood or a raw material can be estimated using Life Cycle Assessment (LCA), a process which analyses all the input and output of the production process [1]. The aim of this study was to compare the environmental footprint of two animal proteins of the same species (chicken), processed with a different production technology.

A chicken meal (CM, processed animal protein) and a hydrolysed chicken protein (HCP) produced by the same company, starting from the same chicken raw materials (i.e. category 3 chicken by-products) were evaluated. CM (crude protein 65%, digestible protein 77%) was obtained with the rendering process and HAP (crude protein 20%, digestible protein 96%) with enzymatic hydrolysis.
CM and HCP were compared by assessing their environmental impact per unit of 1 kg. Additionally, to provide comprehensive information for nutritional purposes, the environmental impacts were also adjusted according to the content of digestible protein.

LCA calculation is developed with respect to cradle-to-gate and gate-to-gate system boundaries. The methodology used is based on the international standards ISO 14040 and ISO 14044[2]. The data used for meal and hydrolysate production are primary data derived from accounting for the input and output flows of the producer. The data used for the upstream phase (which includes animals rearing and slaughtering and by-products transportation) are derived from major international databases. The characterisation factors and impact categories calculated are those found in EN 15804[3] as per EPD (Environmental Product Declaration) certification.
The results show that for 1 kg of CM production 1,2 kg CO2 eq are released, while for 1 kg of HCP only 0,22 kg CO2 eq are released, i.e. 17% of CM. To reach the same amount of digestible protein, 2,61 kg of HCP are necessary to substitute 1 kg of CM, therefore, LCA of HCP adjusted for protein content is 0,57 kg CO2 eq, i.e. 44% than CM's. The higher environmental impact of CM results mainly from the higher consumption of electrical and thermal energy.

These preliminary data suggest a lower environmental impact of the production process of hydrolysed animal proteins if compared to the one of processed animal proteins. These data could be useful to build a reliable state of the art regarding LCA of petfood protein sources and thus to improve environmental performances of petfood producers and to promote truly sustainable products.

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77° CONVEGNO SISVET

Stato: INVIATO - ID: 13553

Analysis of the impact of nutritional supplementation on canine gut microbiota through an in vitro colonic fermentation model

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Modern science states that improving gut health is essential to guarantee dogs an optimal quality of life [1]. The Simulator of the Canine Intestinal Microbial Ecosystem (SCIME) was recently developed and validated to offer a proper platform to analyze the effects of nutritional interventions on the canine gut microbiota [2]. The setup of a typical reactor representing the gastrointestinal tract of the adult human (SHIME®) has already been described since 1993 [3]. Given the high similarity between the human and dog intestinal conditions, this general reactor was adapted to simulate the canine intestinal ecosystem. The present study aims to evaluate the impact of a feed product on the composition and metabolic activity of healthy canine donors' luminal and mucosal gut microbiota. The nutritional supplementation is Microbiotal (NBF Lanes, Milan, Italy), a complementary feed that includes in its composition dried oligofructose, microencapsulated tributyrate, inulin, and inactivated Lactobacillus reuteri.
The experiments to unravel the microbial modulation by Microbiotal were conducted using the SCIME platform, which consists of three temperature-controlled reactors representing the different parts of the canine gastrointestinal tract. The properties of Microbiotal were evaluated using the microbiota of a healthy ±20 kg canine donor. After inoculation of the colon reactors with the fecal sample, the experiment followed three steps: a stabilization period that allowed the microbial community to differentiate (up to three weeks.); a control period (about three weeks) during which an analysis of the sample determined the baseline microbial community composition and activity; a treatment period of two weeks where the SCIME was operated with a diet supplemented with Microbiotal on top of the normal composition. Dietary supplementation effects were evaluated at the level of the composition (using the 16S-targeted Illumina sequencing) and metabolic activity (through the continuous monitoring of the acid/base consumption, the concentration of short-chain fatty acids - SCFA -, lactate, ammonium, and branched SCFA) of the luminal and mucosal gut microbiota.
The supplementation of the Microbiotal product enhanced acidification in both the proximal and distal colon, which was linked with significant stimulation of acetate production in the proximal colon ($p<0.05$). Concerning markers for proteolytic fermentation, a trend towards increased ammonium and branched SCFA levels was observed in the distal colon. Repeated administration of Microbiotal significantly enriched different bacterial groups involved in primary substrate degradation (Bifidobacterium, Limosilactobacillus, Prevotella, Bacteroides, and Enterococcus) in the proximal colon, indicating a boost of saccharolytic fermentation within the canine microbial community.
Acetate is one of the critical metabolites formed during primary substrate fermentation that a wide range of gut microbes can produce. Its significant increase in the proximal colon confirms the Microbiotal beneficial effect. This work highlights how a feed containing prebiotics and bacterial fermentation products can diversify the bacterial flora and enhance the modulatory effects on the gut microbiota.

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77° CONVEGNO SISVET

Stato: INVIATO - ID: 13555

Modulation of the canine gut microbiota with prebiotic, probiotic, and postbiotic supplementation as assessed in the Mucosal Simulator of the Canine Intestinal Microbial Ecosystem (M-SCIME)

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The Simulator of the Canine Intestinal Microbial Ecosystem (SCIME) was recently developed and validated to provide a proper platform to analyze the effects of nutritional interventions on the canine gut microbiota [1,2]. Previous experiments tested the benefits of Microbiotal (NBF Lanes, Italy), a feed consisting of prebiotics and bacterial fermentation products (unpublished data). The present study aims to evaluate the impact of Lactobacillus reuteri probiotic supplementation [3] (NBF1, NBF Lanes, Italy), alone or in combination with Microbiotal, on the composition and metabolic activity of healthy canine donors' luminal and mucosal gut microbiota.
The experiments to unravel the microbial modulation by L. reuteri with or without Microbiotal were conducted using the SCIME platform. The properties of the test products were evaluated using the microbiota of a healthy ±20 kg canine donor. After inoculation of the colon reactors with the fecal sample, the experiment followed three steps: a stabilization period; a control period; a treatment period where the SCIME was utilized with a regular diet supplemented with L. reuteri or with L. reuteri and Microbiotal. Both the effects of the dietary supplementations were evaluated at the level of the composition (using the 16S-targeted Illumina sequencing) and metabolic activity (through the continuous monitoring of the acid/base consumption, the concentration of short-chain fatty acids - SCFA -, lactate, ammonium, and branched SCFA) of the luminal and mucosal gut microbiota.
The treatment with L. reuteri stimulated lactate production, alone or in combination with Microbiotal. In contrast with the results previously observed with Microbiotal treatment alone, probiotic supplementation reduced acetate and propionate levels compared to controls. Concerning markers for proteolytic fermentation, a trend towards increased ammonium and branched SCFA levels was observed in the distal colon following L. reuteri treatment, both in the presence or absence of Microbiotal. The Limosilactobacillus genus was strongly enriched following supplementation with the probiotic, independently of co-supplementation with Microbiotal, especially in the proximal colon.Furthermore, treatment with L. reuteri significantly enriched the Pseudomonas, Stenotrophomonas, and Faecalibacterium genera in the proximal colon compared to the control. Interestingly, next to the stimulation of Limosilactobacillus, significant enrichment of Bifidobacterium was observed in the distal colon upon treatment with the combinatory supplementation.
The SCIME experiment displayed that L. reuteri supplementation promoted significant changes in microbial community fermentation patterns. Following probiotic treatment, the RNA gene profiling highlighted the activation of cross-feeding interactions in the canine microbiome. Moreover, significant metabolic differences were observed between the proximal and distal colon regions. In the proximal colon, a significant metabolic shift was due to treatment with pure L. reuteri and in co-supplementation with Microbiotal. In the distal colon, a significant impact was revealed upon supplementation with the probiotic engrafted with Microbiotal. This work points out the ability of a feed containing probiotics to improve the modulatory effects on the canine gut microbiota, especially when coauthored with prebiotics and postbiotics products.

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Effect of Dietary Supplementation of Functional Seaweeds in F4+ Escherichia coli Challenged Piglets

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Despite progress in reducing antimicrobial use in the veterinary field, it is crucial to find alternatives to preserve effectiveness and limit antimicrobial resistance. The role of Escherichia coli strains as indicators of resistant bacteria has recently been highlighted [1]. In pig farming, pathogenic strains of E. coli are the main cause of gastrointestinal disorders and antibiotic use [2]. The European Union encourages the exploration of alternatives to antimicrobials in livestock, including the use of functional ingredients. In this field, algae represent a possible innovation in animal nutrition that aligns with livestock sustainability principles and provide a high content of functional molecules [3]. The aim of this study was to evaluate an innovative dietary combination of Ascophyllum nodosum and Lithothamnium calcareum, on growth, duodenum gene expression, jejunum morphology, and serum oxidative status in F4+ E. coli challenged piglets. Forty-eight weaned pigs (28±2 days) were divided into two groups (n=24 pigs/group). The control (CTRL) group was fed a commercial diet, while the seaweeds (ALGA) group was fed commercial diet supplemented with a mixture of A. nodosum and L. calcareum (1.5% and 0.5%, respectively) from day 0 to 27 (ethical authorization n°884/2021-PR). After 13 days, half of the animals were orally challenged with a single dose of 108 CFU of F4+ E. coli, resulting in two infected groups (CTRL+ and ALGA+, n=12 pigs/group). The seaweeds were characterized for functional properties by assessing the antioxidant activity through ABTS assay, F4+ E. coli growth inhibition ability by the microdilution method and metabolomic profile by Q-TOF MS/MS analysis. Growth performance was evaluated by measuring the individual body weight weekly. After 27 days, blood samples were individually collected to assess serum oxidative status and antioxidant barrier assessments using colorimetric tests. At the end of the trial, six animals per group were slaughtered, and duodenum and jejunum sections were sampled for gene expression analysis via qRT-PCR and histological evaluation. The results showed a higher body weight in ALGA+ group compared to CTRL+ after 7 days post-challenge ($p < 0.0001$). The seaweeds-supplemented animals revealed a higher antioxidant barrier in blood serum compared to pigs on a commercial diet at 27 days ($p < 0.05$). The morphology of jejunum showed lower villus height and width in CTRL+ compared to ALGAE+ ($p < 0.05$). Gene expression profile of duodenum showed a tendency towards increased mRNA transcription of transforming growth factor beta in CTRL+ compared to ALGA+ after 27 days ($p < 0.09$). In conclusion, the algae mixture demonstrated a protective effect against intestinal damage caused by F4+ E. coli; this proposes the supplementation of functional seaweeds as an interesting strategy to enhance gut health and reduce antibiotic treatments in weaned piglets.

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77° CONVEGNO SISVET

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Increasing docosahexaenoic acid (DHA) content in cow milk: dietary supplementation and rate of transfer

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The beneficial effect of long-chain omega-3 fatty acids (FA), as DHA (cis-4,7,10,13,16,19-Docosahexaenoic acid), on human health is now well known, but reaching an adequate intake by consumers can be challenging. To enrich DHA content in milk and dairy products could be a solution. With the aim to increase DHA content in milk, 2 homogeneous groups of 20 mid-lactating dairy cows each were supplemented with 300 g/d of an algae extract containing 12.4% of rumen protected DHA microencapsulated in a matrix of saturated fats (TRT group) or with 300 g/d of matrix fats only (59% palmitic, 39% stearic; CTR). Supplements were top dressed on TMR for 21 days. Milk yield, composition, and FA profile were measured on 0, 14, 21, and 31 days from the beginning of supplementation. During the supplementation period, milk yield, protein, lactose, somatic cell count, and clotting properties were similar between groups, but milk fat content decreased in TRT (3.04 vs 3.96%, P<0.01). Feeding DHA modified the composition of milk fat, decreasing the proportions of short-chain FA (C6, C8, C10, C12, C14), and causing little but significant changes in long-chain FA proportions. Among those, the greatest variations were observed in trans FA (C18:1t 6.17 vs 1.33%, P<0.05; and C18:2t – CLA, 1.92 vs 0.44%, P<0.05) and DHA, which level was 11 times higher in TRT than in CTR (0.21 vs 0.02% P<0.01). The average total output of DHA in milk was 8.4 times higher in TRT (0.24 g/d in CTR and 1.99 g/d in TRT), with a rate of transfer from diet to milk of 5.4%. The transfer was low, but DHA was likely partially accumulated in the cow blood lipids, since 10 days after treatment withdrawal the DHA output in milk was still 1.21 g/head/d (5 times higher than CTR). The supplementation with DHA increased its content in milk fat, but without reaching levels that allow legal recognition as with 100 mL of milk the intake would be 6.4 mg. Moreover the reasons for its low transfer rate, as well as more effective methods to protect unsaturated FA from rumen biohydrogenation need to be investigated further.

RNIV

ID: 13343

IP-10 as a potential biomarker for detection of *Mycobacterium bovis* infection in water buffalo: preliminary results

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Bovine tuberculosis (bTB) is a worldwide zoonosis that affects many species of domestic and wild animals. *Mycobacterium bovis* (*M. bovis*) is the main cause of infection in *bovidae*, including water buffalo (Bubalus bubalis). The interferon-gamma (IFN-γ) release assay (IGRA) is widely used in the diagnosis of *M. bovis* infection in cattle, by detecting IFN-γ released by previously sensitized T-cells *in vitro*, and we have recently shown the utility of this test in water buffalo [1]. In literature it is suggested that other cytokines, such as the interferon gamma-inducible protein 10 (IP-10), could be diagnostic biomarkers for *M. bovis* infection in cattle or wildlife [2, 3]. In this study, we evaluated the potential use of IP-10 in the diagnosis of *M. bovis* infection in cattle and water buffalo.

Animals used in this preliminary study were analysed within the context of the Official Italian National bTB eradication program in the Campania region (Southern Italy). A total of 10 Italian Mediterranean buffaloes and 10 cattle were enrolled. Animals were divided in four groups: infected buffaloes (N = 5), uninfected buffaloes (N = 5), infected cattle (N = 5), uninfected cattle (N = 5). Naturally infected animals were selected from herds with confirmed bTB outbreaks, with positivity at IGRA and bTB lesions at the slaughterhouse. Uninfected animals were selected from Officially Tuberculosis-Free herds, and tested negative at SIT or IGRA performed in the last 6 years [1]. Heparinized blood samples of each animal were dispensed in aliquots of 1 ml and stimulated with Phosphate-buffered saline (PBS, Nil Control Antigen), 10 µg of antigen bovine PPDB, Pokeweed Mitogen (PWM, final concentration 1 µg/ml, control of lymphocyte viability), respectively. Plasma was collected 16-24 h post-stimulation and stored at -80°C until analysed. Levels of IFN-γ and IP-10 were measured using Bovine Cytokine/Chemokine Magnetic Bead Panel Multiplex assay. Cytokine's data were graphically and statistically analyzed with GraphPad Prism 10.01, using an un-paired T-test or the non-parametric Mann-Whitney test.

As expected, all the animals released both IFN-γ and IP-10 in response to PWM, and *M. bovis*-infected cattle and buffaloes released IFN-γ in response to PPDB stimulation, with statistically significant differences between PPDB-treated samples and PBS (p value < 0.05). Regarding IP-10, we observed that *M. bovis*-infected cattle and buffaloes released IP-10 in response to PPDB stimulation, with statistically significant differences between PPDB-treated samples and PBS (p value < 0.05). On the contrary, no differences were observed in the IP-10 levels of PPDB-stimulated and PBS samples (p value > 0.05 in both bovine and water buffalo) in uninfected animals.

Overall, these preliminary data suggested that IP-10 can be a biomarker for detection of *M. bovis* infection in water buffalo as in cattle. Therefore, we are planning to carry out further analysis, on a larger number of naïve and *M. bovis* infected animals, in particular in water buffalo, where improving bTB diagnostic performance is a major issue.

This research was funded by the Italian Ministry for Health (Ricerca Corrente IZSME 14/2022).

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77° CONVEGNO SISVET

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Omic evaluation of resilience and adaptation to the combination of heat and exercise stressors in horse athletes.

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Endurance race, low intensity-long duration exercise, is one of the most challenging disciplines for horses, due to the long distances (up to 160 km divided in several phases) at an average speed of 20 km/h or more. Endurance horses are susceptible to metabolic imbalance due to dehydration, acid balance and electrolyte abnormalities, substrate depletion and heat accumulation, which can result in life-threatening conditions [1]. For this, equine endurance competitions are governed by the National and International Equestrian Federations (FEI) rules, which safeguard and ensure the animal welfare. Although endurance horses are subjected to specific training that induces the physiological adaptations, prolonged exercise linked to sub-optimal temperature can lead to negative effects on health and welfare such as myopathies, colic, laminitis, diaphragmatic flutter, cardiac arrhythmias and massive rhabdomyolysis [2]. Moreover, strenuous exercise under hot, or hot and humid conditions, increases the onset of heat stress due to the the accumulation of body heat that exceeds dissipation capabilities [3]. Incremental field standardized exercise tests (fSETs) are valuable systems for horse training and fitness evaluation [4].
This research aimed to address molecular features of resilience and adaptation to a combination of heat and exercise-induced stresses in horses through a next generation sequencing approach.
Six Arabian horses, stabled and homogeneously trained at the Italia Endurance Stable & Academy (Perugia, Italy), were monitored during fSETs in summer and winter, collecting blood samples before and after each fSET. Total RNA was extracted from serum and sequenced with Illumina® technology. Lactatemia and hematocrit levels significantly increased in summer vs. winter. From NGS data, a set of differentially expressed small RNAs were obtained and a protein-protein interaction network for targets of micro RNAs (miRNAs) was built. On this, a gene ontology (GO) enrichment analysis was carried out highlighting, in summer vs. winter, enriched terms related to: innate immune response, protein kinase activity and DNA methylation (for targets of up-regulated miRNAs); cellular response to cytokine stimulus, NF-kappa B signaling and signal transduction (for targets of down-regulated miRNAs). These preliminary results should be considered in outdoor sports, in animals as in humans, since the horse athlete is an optimal model to study exercise-related physical disorders sharing, with human athletes, poor performance syndromes, overtraining and potentially lethal consequences if the physical effort is associated with thermal stress.

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Abstract ID 13848

Diagnosis of brucellosis: comparison of three serological tests

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Brucellosis is an important zoonosis, still widespread in some Italian territories. Current legislation provides, among the indirect diagnostic tests, buffered Brucella antigen agglutination tests (BBAT), complement fixation test (CFT), and indirect ELISA (I-ELISA) [1].

The combination of BBAT and CFT tests, despite having shown poor specificity in differentiating infected animals from reactive false-positive animals [2], still remains the main approach used for the diagnosis of brucellosis in domestic species. Indeed, EU Regulation 689/2020 also includes the I-ELISA test among the possible diagnostic tests for brucellosis.

The aim of this study is to compare the three different serological tests for the in vivo diagnosis of brucellosis in three domestic species, particularly affected by this infectious disease: buffalo, sheep and goat.

In the context of Italian National Brucellosis eradication programs, in herds with brucellosis outbreaks, blood samples of the present experiment were taken from 190 buffaloes, 200 sheep and 175 goats.

Serum samples were used for the BBAT and CFT tests according to WOAH Terrestrial Manual [1], and the I-ELISA test was performed according to the manufacturer's instructions (ID Screen® Brucellosis Serum Indirect Multi-Species, Innovative Diagnostics, Grables, France).

Statistical analysis was carried out with the R software version 4.3.2. (R Foundation for Statistical Computing). The agreement between the three diagnostic tests was estimated using Cohen's Kappa index. A kappa value of 1 indicates perfect agreement and a value of 0 indicates no agreement beyond chance.

In buffaloes high level of agreement was found among all serological tests, the kappa value ranged from 0.89 to 0.98. In particular, kappa index was 0.98 between I-ELISA and BBAT, and 0.9 between I-ELISA and CFT, while between BBAT and CFT the kappa agreement was 0.89.

In sheep, a substantial concordance was found between I-ELISA and BBAT (Kappa 0.65) and CFT (Kappa 0.67), while a perfect concordance between BBAT and CFT was found with a kappa agreement of 0.99.

In goats, the agreement between I-ELISA and BBAT was slightly lower with a kappa value of 0.83 while kappa index between I-ELISA and CFT was 0.82, and between BBAT and CFT was 0.92.

Our preliminary results, although requiring confirmation of test performance through post-mortem investigations, allow us to hypothesize a possible use of the I-ELISA test in the investigated species since the test in cattle has already shown excellent performance [3].

The use of an additional diagnostic test could help to improve diagnostic accuracy and contribute to the eradication of brucellosis in herds and regions where the disease is still present.

This research was funded by the Italian Ministry for Health (Ricerca Corrente IZSM 11/2021).

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Welfare assessment: correspondence analysis of welfare score and haematological and biochemical profiles of dairy sheep

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This work aimed to investigate the association between animal welfare (AW), assessed with the protocol developed and validated by the Italian National Centre of Reference for Animal Welfare (ClassyFarm System), and haematological and biochemical profiles in dairy sheep. This study was approved and funded by the Italian Ministry of Health (Prot. No. 12786 of 12/05/2023).
AW was assessed in 15 dairy sheep farms in the Sardinia region using the ClassyFarm self-assessment protocol. On each farm, blood samples were taken from the same sheep on which the Animal Based Measures (AMBs) were assessed. Blood count, hepatorenal profile, serum protein electrophoresis and determination of C-reactive protein were performed on 297 samples. The results of the analyzed parameters were compared with i) the reference ranges for the species, to verify the animals' health status; and ii) with the farm AW score, to verify their association with the animals' detected welfare status. The laboratory results of the animals housed on the farms with the best and worst AW scores were also compared.
Statistical analysis was carried out using STATA BE software (v17.0). Data were summarized using median and IQR; the comparison with the reference ranges was carried out considering the 5th and 95th percentiles of the sample distribution. A correlation matrix was used to evaluate the association between variables (Spearman's correlation coefficient). Student's t-test and Wilcoxon test were performed according to variable distribution. A significance value of $p<0.05$ was chosen.
All 15 farms included in the study had a higher than sufficient AW score (median 87; IQR 84-93). Similarly, all the analyzed laboratory profiles were within the normal ranges, with few exceptions: ALP, AST and BUN showed higher variability and tended to higher values, whilst platelet count tended to lower levels.
Correlation analysis revealed a moderate negative correlation ($r = -0.30$ - -0.45, $p<0.05$) between the ClassyFarm AW score and some laboratory parameters (e.g., MCH, total bilirubin, phosphorus), suggesting a correspondence between the health and welfare status of the animals, in line with what has been demonstrated in dairy cows [1].
The animals housed on the farm with the worst AW score (74) showed significantly higher levels of calcium, phosphorus, GGT, RBC, HCT, WBC, number of neutrophils, lymphocytes, and basophils, compared with the animals housed on the farm with the best AW score (95) ($p<0.001$). Our results highlighted how semi-extensive farming is quite respectful of dairy sheep welfare. The haematological and biochemical profiles were aligned with the ClassyFarm AW scores. Therefore, the use of a validated AW protocol in combination with the identification of well-known laboratory parameters can be a fundamental tool for veterinarians to detect stress conditions early.

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SICLIMVET

77° CONVEGNO SISVET

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Employing Fluorescent light therapy to hasten wound healing in cat: a comparative analysis of clinical and microbiological outcomes.

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Employing Fluorescent light therapy to hasten wound healing in cat: a comparative analysis of clinical and microbiological outcomes.
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For decades, veterinary medicine has shown extensive research and clinical focus on wound management. A novel approach to improving skin wound healing involves the application of a fluorescent light energy (FLE) system, consisting of a blue light-emitting diode (LED) device and a topical photoconverter gel. When illuminated by the LED device, this system emits low-energy light in the form of fluorescence, capable of penetrating the skin and stimulating healing. Extensive exploration and application of the FLE system has yielded promising results for improving various canine dermatological conditions and promoting skin wound healing [1-3]. However, there is limited knowledge regarding the acceleration of natural skin regeneration by FLE therapy in cats. The objective of this study is to determine the effect of FLE (Phovia, Vétoquinol Italia S.r.l.) on the healing of wounds and different dermatological conditions in a feline population. Additionally, we aim to evaluate the possible impact of FLE on the microbial load of cutaneous lesions through skin swabs taken before and after treatment. Eleven cats attending the Veterinary University Teaching Hospital of the Department of Veterinary Medicine and Animal Productions for cutaneous lesions, but in general good health, were enrolled. The cats received three FLE therapeutic sessions over the course of three consecutive weeks. During each session, the wounds received two consecutive 2-minute FLE applications (back-to-back protocol), and microbiological sampling through skin swabs was carried out. No antimicrobial treatments were performed in the previous 6 weeks and during the entire trial. FLE led to complete clinical resolution in all enrolled cats, except one cat because she was licking a lesion, and the owner declined the use of an Elizabethan collar. No adverse events were reported. Microbiological tests demonstrated a negative outcome in five cases after the third application with FLE, whereas three demonstrated a decrease in bacterial count, and three of them a slight increase in bacterial count. As demonstrated by the data presented in this study, alternative therapeutic approaches like FLE evaluated herein emerge as a beneficial strategy for the management of feline wounds. This alternative approach aims to reduce the duration of treatment regimens and decrease the reliance on medication use, particularly antibiotics that contribute to the growing issue of antimicrobial resistance.
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77° CONVEGNO SISVET

Stato: INVIATO - ID: 13064

Fluorescent light energy applications in veterinary medicine

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The use of therapeutic light and lasers has experienced an intense spread in the past few decades representing new appealing management options for canine dermatological disorders which account for the most commonly presented complaints in daily practice. Low-level laser (light) therapy (LLLT), or photobiomodulation (PBM) are synonyms of the same practice that uses photons at different wavelengths and at non-thermal irradiance to influence biological activity. A novel approach to PBM is through application of a fluorescence light energy (FLE) system consisting of blue light which activates topical photoconverter hydrogel containing specialized chromophores (molecules able to be excited by certain wavelengths) that generate fluorescence. FLE uniquely uses chromophores that are not absorbed by the tissues, nor produce heat: instead, they are only employed to produce and deliver the fluorescent light energy to the tissue.1 Moreover, FLE is free from those side effects and risks related to the use of lasers. FLE exhibited its aptitudes in veterinary dermatology: being able to reduce inflammation and control bacterial overgrowth, it has the potential to be considered as an option in different dermatological conditions, possibly replacing some topical treatments and improving owner compliance. In fact, FLE may be an effective sole treatment for canine superficial pyoderma, eliminating the need for, or decreasing the length of time of administering topical products and/or systemic antibiotics and supporting antimicrobial stewardship programs. In addition, it has the potential to accelerate time to clinical resolution for canine interdigital furunculosis and deep pyoderma in comparison with systemic antibiotic treatment.2 Interestingly, FLE can be used to cure multidrug resistant skin infection without administering neither topical nor systemic antibiotic.3 The technologies behind light-based therapies are advancing rapidly, and in many cases, their utility in the clinical setting is still being actively explored. It is realistic to hypothesize that this technology can be optimized not only for dermatological applications, and its value in clinical care will continue to grow. Marchegiani A, Spaterna A, Cerquetella M. Current Applications and Future Perspectives of Fluorescence Light Energy Biomodulation in Veterinary Medicine. Vet Sci. 2021 Jan 25;8(2):20. doi: 10.3390/vetsci8020020. PMID: 33504091; PMCID: PMC7912178. Marchegiani A, Spaterna A, Cerquetella M, Tambella AM, Fruganti A, Paterson S. Fluorescence biomodulation in the management of canine interdigital pyoderma cases: a prospective, single-blinded, randomized and controlled clinical study. Vet Dermatol. 2019 Oct;30(5):371-e109. doi: 10.1111/vde.12785. Epub 2019 Aug 13. PMID: 31407840. Marchegiani A, Fruganti A, Bazzano M, Cerquetella M, Dini F, Spaterna A. Fluorescent Light Energy in the Management of Multi Drug Resistant Canine Pyoderma: A Prospective Exploratory Study. Pathogens. 2022 Oct 18;11(10):1197. doi: 10.3390/pathogens11101197. PMID: 36297254; PMCID: PMC9608719.

77° CONVEGNO SISVET

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Transcutaneous abdominal ultrasonography in healthy donkeys

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Transcutaneous abdominal ultrasonography is a fast and non-invasive tool that gives immediate information about the location, volume and pattern of abdominal viscera, intestinal motility and wall thickness. In horses, ultrasound is an important component of the diagnostic workup for patients presenting clinical signs of acute abdomen and the knowledge of normal ultrasound anatomy is fundamental to understand pathologic changes [1,2].Aim of this study was to document which abdominal viscera could be identified using a transcutaneous ultrasonography technique under field condition in donkeys; to determine where the different abdominal structures could be easily displayed and to describe any variation that occurred between different donkeys.Ten clinically healthy adult donkeys (Equus asinus), age 14±8 yo, weight 217±90 kg, were included in this study. The wall thickness of the oesophagus, stomach, duodenum, left colon, right colon and cecum were assessed. Moreover, images of the renal cortex and medulla, liver and spleen were obtained.On the left side, the kidney could be visualized in the paralumbar fossa for all the donkeys with the length measuring 9.34±0.82 cm, the renal cortex 0.86±0.28 cm and the medulla 1.63±0.64 cm. The spleen was identified from the 11th to the 16th ICS (intercostal space) in all the donkeys; some cases extending more cranially up to the 8th ICS and/or caudally up to the paralumbar fossa. The stomach wall measured 0.66±0.15 cm. The left colon was easily visualized in the 16th ICS, but in few cases, it could be displayed more cranially (15th) or caudally (up to the paralumbar fossa). The wall thickness was 0.44±0.13 cm.On the right hemiabdomen, the kidney was identified in the 17th ICS except one donkey that showed it in the 16th ICS. The organ length was 9.87±0.94 cm, with a cortex of 0.83±0.24 cm and a medulla of 1.53±0.20 cm. Cranially to the right kidney, the duodenum was visualized in the 16th ICS for all the cases and it could be followed for at least two ICS; occasionally it could be followed cranially up to the 12th ICS. The wall thickness was 0.35±0.1 cm. The liver was displayed from the 12th to the 15th ICS in all the donkeys; in some cases, it extended cranially till the 9th and/or caudally up to the 16th ICS. The right colon wall could be measured in the 14th or 15th ICS and it was 0.32±0.06 cm. The caecum wall measured 0.28±0.08 cm and could be easily found in the right paralumbar fossa and occasionally in the 17th and 16th ICS.The oesophagus was examined just caudal to the pharynx in the upper neck on the left side, dorsal to the trachea. The diameter ranged between 1.01 cm and 2.46 cm (mean 1.58±0.56) and the wall measured 0.36±0.08 cm.This is the first preliminary study describing how to perform a complete transcutaneous ultrasonography in donkeys, providing references for acoustic windows. The ultrasonographic findings are similar to those of horse, excepting for the parenchymatous organs which are smaller and positioned more caudally in the abdomen.Good knowledge of these standard and reference values represents a step in the early diagnosis in case of acute abdomen symptoms and other abdominal diseases.[1] Ibrahim et al., Reference values and repeatability of transabdominal ultrasonographic gastrointestinal tract thickness and motility in healthy donkeys (equus asinus). J Equine Vet Sci, 92, p.103153, 2020[2] Le Jeune et al., Ultrasound of the equine acute abdomen. Veterinary Clinics: Equine Practice, 30(2), pp.353-381, 2014

77° CONVEGNO SISVET

SOCIETA´ SCIENTIFICA DI RIFERIMENTO **SICLIMVET**

TITOLO **A Comprehensive Case Study on Laser Therapy and Hydrotherapy Integration Post-Femoral Head and Neck Resection**

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Testo e Riferimenti bibliografici

The aim of this study was to assess the therapeutic effects of super pulsed laser therapy on a canine patient (a 7-year-old male Setter) who had recently undergone surgery (bilateral femoral head and forehead resection). The muscles were subjected to several ballistic projectiles that could potentially lead to myositis with exercise or overheating with laser therapy. However, no such effects were observed, even though the physiotherapy treatment approach involved the use of super pulsed laser therapy along with controlled, low-impact exercises using a water treadmill. Patient arrived in the rehabilitation sector, with pelvic extension pain, gluteal muscle atrophy, quadriceps and femoral biceps, contracture of the iliopsoas muscle, pain in the lumbar thoracic spine, retraction of the joint capsule. Difficulty sitting and getting up. Goniometry of left coxofemoral joint pretreatment flexion 48º extension 158º. Goniometry of the right coxofemoral joint pre: flexion 47º extension 157º perimetry right thigh 28 cm left thigh 29cm The treatment plan consisted of 20 physiotherapy sessions using the laser for pain management and osteoarthritis, along with aquatic treadmill sessions for muscular reinforcement, balance, proprioception exercises, therapeutic exercises, and core strengthening. Rehabilitation sessions, were conducted twice a week over a two-month treatment period, reducing to once a week for the final two weeks, totaling two months and two weeks of treatment. Laser Treatment: To control pain in the thoracolumbar region, a 1000 Hz frequency was applied for 5 minutes in direct contact, moving the emitter slowly, over the thoracolumbar region to release endorphins. For controlling chronic pain/inflammation at the surgery site, a 50 Hz frequency was used around the joint where the femoral head would be, in direct contact, scanning for 10 minutes. To address contracture of the pectineus muscle a frequency of 1000-3000 Hz was used in static mode, placing the laser in direct contact with the muscle for 5 minutes. Water Treadmill Treatment: The initial sessions (first two weeks) involved water at hip joint level. Subsequent sessions (8 sessions) focused on the middle third of the femur .The remaining sessions were conducted in knee-deep water for 10-15 minutes each. After the treatment, we obtained a report of improvement from the owner, who states that he is no longer reluctant to walk, sit or stand up. And in the measurements: Post treatment flexion of left coxofemoral joint 50º extension 160º Goniometry of the right coxofemoral joint post treatment flexion 50º extension 159º Post treatment Right thigh perimetry 30 cm; left thigh 31 cm. We therefore conclude that with hydrotherapy and laser therapy we can help with the recovery of muscle contractures, joint extension, and support strength, with minimal risk, as the super pulsed laser does not cause burns or abrasions on the patient's skin and there is no need to shave it. Bibliography:[1] Minto B.W. et al., 2012. Clinical evaluation of acetabular denervation in dogs with coxofemoral dysplasia treated at the FMVZ Veterinary Hospital - Botucatu - SP. Veterinary and Animal Sciences. 19(1):91-8.[2] Mueller M. et al., 2007. Effects ects of radial shock wave therapy on limb function of dogs with osteoarthritis of the hip. Veterinary record. 160(1): 762–765. [3] Off W, Matis U. Arthroplasty for excision of the coxofemoral joint in dogs and cats. Results of clinical, radiographic and gait analysis of the Department of Surgery, Faculty of Veterinary Medicine, Ludwig-Maximilians-University of Munich, Germany. 1997. Vet Comp Orthop Traumatol. 2010;23(5):297-305. PMID: 20945541

77° CONVEGNO SISVET

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The use of photobiomodulation for the resolution of antibiotic resistant osteomyelitis: a case report

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The use of photo biomodulation for the resolution of antibiotic resistant osteomyelitis: a case report Miriam Caramico, Stefania De Dominicis, Laura Silenzi, Michele Rasola, Francesca Salvati, Roberto Tamburro Osteomyelitis is an inflammatory process accompanied by bone destruction usually caused by bacteria, mycobacteria or fungi. They can infect bones by spreading through the bloodstream or, more often, by spreading from nearby infected tissue or a contaminated open wound. The infection can be limited to a single portion of the bone or can involve several regions, such as marrow, cortex, periosteum, and the surrounding soft tissueOsteomyelitis can be classified into:Acute osteomyelitis, when symptoms last less than 1 month.Chronic osteomyelitis when symptoms last more than 1 month. If during the acute phase the infection is not eradicated, due to resistance of the bacteria to antibiotics, the infectious process becomes chronic and this leads to anatomical-pathological changes to the affected bone; non-vital areas are created in the most infected bone areas, called "seizures", which represent the body's attempt to confine the infection by avoiding its spread through the formation of a bone envelope. In the long run, chronic osteomyelitis leads the bone to important structural changes with the presence of sclerosis and deformities, which antibiotics are unlikely to reach, thus explaining the chronic nature of the process and the difficulty of eradicating the infection.All these factors make recovery from this pathology difficult, with considerable disability for the patients, up to the possible amputation. This study aims to demonstrate the control/cure of antibiotic non-responsive osteomyelitis using photo biomodulation (super pulsed laser) and avoid a amputation. The therapy was applied in a single case, which will be reported subsequently. Animal adopted from a municipal kennel (still as a puppy) with angular bone deformity of the femur, tibia and fibula, bilaterally.The deformity, being worse in the left posterior limb, caused him to drag the limb, with lateral rotation of the tarsus and causing a severe abrasion. This wound was being treated with antibiotic therapy and they were bandaging it with antibiotic and anti-inflammatory ointments.Male dog, 27 kg, not neutered, mixed breed 3 yearsA protocol using Activet PRO was developed in order to control and resolve the infection and inflammation promoted by osteomyelitis.1 to day 90 of treatment, statically with red, blue and infrared lights, 1000 hz Between the treatments we also did the treatment for inflammation, tissue local inflammation 50 hz statically for 3 minutes at the site of the injury, for a month three times a week From the 30th to the 120th day we switched to the 5000 Hz Inhibitory configuration, , statically with red, blue and infrared lights, but only once a week.After treatment with the super-pulsed laser, a cure for osteomyelitis was evidenced through a new culture and antibiogram examination.We believe that this could be the beginning of the development of a protocol for the use of super pulsed laser in deep infections.Because it is just a case report, we do not have an N to report how effective the treatment is for the control and cure of multidrug resistant osteomyelitis. Bibliography:[1]Ikpeme IA, Ngim NE, Ikpeme AA. Diagnosis and treatment of pyogenic bone infections. Afr Health Sci. 2010;10(1):82–8. [2]Waldvogel FA Medoff G Swartz MN Osteomyelitis: a review of clinical features, therapeutic considerations and unusual aspects: 3. osteomyelitis associated with vascular insufficiency.N Engl J Med. 1970; 282: 316-322[3] Uskokovic V. Nanostructured platforms for the sustained and local delivery of antibiotics in the treatment of osteomyelitis. Crit Rev Ther Drug Carrier Syst. 2015;32(1):1-59. doi: 10.1615/critrevtherdrugcarriersyst.2014010920. PMID: 25746204; PMCID: PMC4406243

77° CONVEGNO SISVET

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DISSEMINATED ASPERGILLOSIS ASSOCIATED WITH SEVERE NONREGENERATIVE ANEMIA IN A LAGOTTO ROMAGNOLO DOG

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Aspergillosis in dogs is a fairly common infectious disease in its localized sinonasal form; disseminated aspergillosis is rarely described and it is characterized by non-specific clinical signs. Aspergillus terreus and A. deflectus are commonly involved in systemic infections whereas A. fumigatus, A. niger, and others less frequently [1]. The aim of this report is to describe a rare case of disseminated aspergillosis in a dog, highlighting the most relevant clinical signs, the correlation between the infection, the systemic inflammation and the severe anemia, and the crucial role of the cytological examination in the diagnosis of fungal infection.
The dog, a 6.5 years old, intact male, Lagotto Romagnolo dog was referred to the Veterinary Teaching Hospital of the University of Perugia (PG-VTH) for severe anemia. The onset of the clinical history dated back more than 8 months earlier when the dog was presented to the referring veterinarian with head tilt and cervical and lumbosacral pain. Few months previously, a nasal grass awn foreign body was removed from the nasal cavities. Serologic tests (IFAT) for Toxoplasma gondii, Neospora caninum, Rickettsia spp., Ehrlichia spp. and Leishmania infantum were performed with negative results. Therapy with meloxicam and enrofloxacin was started with good clinical improvement, but the dog subsequently developed a progressively worsening anemia. When referred to the PG-VTH, physical examination revealed a body condition score of 3/9 with diffuse muscle hypotonia, weakness, lumbosacral pain and pale mucous membranes. A mild leukocytosis and severe nonregenerative anemia were observed on complete blood count and blood smear evaluation. The biochemical analysis revealed mild azotemia, increased concentration of aspartate aminotransferase, alanine aminotransferase, alkaline phosphatase and gamma-glutamyl transferase, and mild hypoalbuminemia. An increase in C-reactive protein level (92 mg/L, RR: 0-10.7 mg/L) was also found. At abdominal ultrasonographic evaluation, splenomegaly and two complex renal cysts were found, which showed heterogeneous hypoechogenic content and were interpreted as septic emboli because at the basis of chronic renal infarctions. A computed tomography scan also revealed the presence of an intracranial lesion, generalized discospondylitis, left ventral nasal concha atrophy and retropharyngeal and retrosternal lymphadenopathy. Renal cysts were sampled by fine needle aspiration. Cytology revealed the presence of fungal hyphae and conidia, and Aspergillus fumigatus was identified at the colture. Based on these findings, a systemic mycosis was suspected. The dog was hospitalized and a blood transfusion was performed. Concurrently, antifungal therapy with itraconazole at 10 mg/kg q24h OS was started along with anti-inflammatory and supportive therapy. The dog was discharged after few days and at subsequent follow-ups, the anemia gradually became regenerative showing substantial improvement. >5 months after diagnosis, the dog is still alive, under antifungal therapy and in clinical remission.
This case of systemic aspergillosis is probably the result of fungal dissemination driven by the penetration of a nasal grass awn foreign body. Disseminated aspergillosis is reported to be characterized by non-specific clinical signs, which is the reason why diagnosis may be challenging and the number of cases underestimated; in this report, cytological examination, a low-cost minimally invasive procedure, was critical for diagnosis. Another interesting aspect of this report was the relationship between the severe nonregenerative anemia and the inflammatory status, and how both regressed with the antifungal therapy.

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77° CONVEGNO SISVET

Stato: INVIATO - ID: 13499

Genotypic frequencies of PNPLA1-related Ichthyosis in an Italian population of Golden Retrievers

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Ichthyoses are a group of hereditary and mainly congenital diseases characterized by dry and scaly skin due to a defective formation of the stratum corneum. In humans, more than 60 genes related to ichthyoses have been identified. In dogs, several breed-specific forms of ichthyosis are recognized; however, only six have been associated with their underlying genetic defect1. The most common canine ichthyosis is an autosomal recessive form that affects Golden Retrievers (OMIA 001588-9615). It is caused by a homozygous insertion-deletion variant in the gene encoding patatin-like phospholipase domain-containing protein 1 (PNPLA1) on CFA12 (mutation c.1445_1447delinsTACTACTA). The defective protein causes malformation of the intercellular stratum corneum lipid layer and abnormal desquamation, inducing a non-epidermolytic form of the disease called ichthyosis type 12. A commercial DNA test for the PNPLA1 variant is available to detect wild-type, heterozygous and homozygous dogs. The aim of this study was to evaluate genotypic frequencies of the PNPLA1 gene variant in an Italian population of Golden Retrievers, to provide a prevalence of the mutation for this breed in the country. This is a retrospective observational study conducted between 2017 and 2023. DNA tests for PNPLA1 mutation detection conducted on Golden Retrievers from all over Italy during this period have been included in the study. Each subject was privately-owned. Blood sampling was operated by authorized veterinarians. DNA tests were conducted in the Vetogene Laboratory, which is one of the official reference laboratories of the Ente Nazionale Cinofilia Italiana (ENCI). No ethical approval was required because data were collected from clinical practice and their use was previously agreed with the owner through written consent. DNA extraction was carried out, and samples were analyzed through real-time PCR. Descriptive statistics were generated, and statistical differences were declared at a p value < 0.05. A total of 183 Golden Retrievers were included, 64.4% (n. 118) of which females, and 35.6% (n. 65) males. The population age ranged from 6 months to 8 years (median: 2 years); it was not significantly different between males and females ($p = 0,665$). In 31.2% of the dogs, the test result was clear (n = 57), while 38.8% of the dogs were heterozygous carriers (n = 71) and 30.0% were genotypically affected (n = 55). Among females, genotypic frequencies were as follows: 25.4% (n. 30) wild type, 43.2% (n. 51) heterozygous carriers, and 31.4% (n. 37) affected. Among males, 41.5% (n. 27) were wild type for the mutation, 30.8% (n. 20) heterozygous carriers, and 27.7% (n. 18) affected. Percentages were significantly different basing on sex ($p < 0,001$); however, the discrepancy between the number of females and males could have affected the results. Although the number of dogs is limited, the present study provides new insights about genotypic frequencies of ichthyosis type 1 for Golden Retrievers in Italy by widening the current knowledge of its prevalence in the country3. Since the numbers of heterozygous carriers and affected subjects are very high, sensitivity of breeders towards DNA testing must increase to orient selected reproduction towards healthier breeding strategies.

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77° CONVEGNO SISVET

Stato: INVIATO - ID: 13501

von Willebrand's disease in Dobermann dogs in Italy

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At the end of 2023, 773 genetic diseases in dogs, 418 of whom with simple Mendelian transmission, were identified. Nevertheless, every year new diseases are discovered, due to the increase of the breed consanguinity for incorrect mating. The von Willebrand's disease (vWD) is the most common inherited coagulation disease in dogs. It is an autosomal recessive monogenic disease, causing high morbidity and low mortality, occurring due to a defect or deficiency of von Willebrand factor (vWF). The vWF is a large plasmatic multimeric glycoprotein that allows normal adhesion and aggregation of platelets, resulting in proper blood clotting. Three different forms of vWD exist, depending on the qualitative or quantitative abnormalities of plasmatic vWF multimers. In Dobermanns, the mutation is caused by a nucleotide base frameshift (c.7437G>A, NM_001002932,1) in the exon vWF 43, which allows the formation of a truncated protein of 119 amino acids. The disease causes haemorrhages of different severity, depending on the type, going from mild to severe. Type I vWD (vWD1), the mildest form, is the most diffused in Dobermann. This study aimed to analyze the diffusion of the vWF mutated gene for the vWD1 in Italian Dobermanns through a PCR evaluation performed on extracted DNA from K3EDTA whole blood or GenoTube® buccal swabs. DNA extraction, amplification, and purification were performed. Between 2019 and 2023, 159 genetic tests were performed in Vetogene (official ENCI – Ente Italiano della Cinofilia - laboratory). The demographic analyses showed that 57% of tested Dobermanns were below 18 months, 33% were 2 years old and 10% were older than 3 years. The mutated allele occurred in the tested population with an allelic frequency of 0.06. In the examined sample, the genotype mut/mut was not identified, but only healthy subjects (wt/wt) (88%) and carriers (wt/mut) (12%). These data are not representative of the entire population of Italian Dobermanns because only 5% of registered dogs were tested during the enrolment period. Although the analyzed data come from only one of the main Italian veterinary genetic laboratories, it can be assumed that the total percentage of the tested dogs does not reach even half of the registered dogs. This can be related to owners' and breeders' lack of knowledge of the genetic tests to monitor the inherited diseases of their animals. To improve this situation, the owner should be aware of the existence of this genetic disease. Based on the results obtained in this study, it would be possible to introduce, through specific training courses for responsible owners, the genetic foundations of hereditary diseases and educate them on proper breeding. Selected breeding and genetic testing must be proposed as a guarantee of animal health for future offspring. Mating between healthy subjects and carriers should only be carried out if the gene pool of the breed is very small and integration of the greatest number of different subjects is necessary to increase the genetic variability, avoiding mating between affected dogs, or between affected and carriers.

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77° CONVEGNO SISVET

Stato: INVIATO - ID: 13579

Serum D-Lactate concentrations in dogs with Inflammatory Bowel Disease

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The D-enantiomer of lactic acid (D-lactate) is normally produced from exogenous sources such as bacterial fermentation in the gastrointestinal tract in mammals. In humans, increased D-lactate concentrations are related to gastrointestinal diseases including short bowel syndrome and malabsorptive syndrome. Similarly, increased D-lactate concentrations were described in calves affected by diarrhea and in cats with gastrointestinal diseases. Idiopathic inflammatory bowel disease (IBD) in dogs is defined as a syndrome in which dogs show chronic (duration > 3 weeks) gastrointestinal signs characterized by mucosal inflammation without overt evidence of an etiologic agent or causative factor. Recent studies have identified alterations in various bacterial groups within the intestinal microbiome between dogs with chronic enteropathies and healthy dogs. The purpose of the present study was to measure serum D-lactate concentrations in dogs with IBD. Our hypothesis was that dogs with IBD have increased serum D-lactate concentrations, as a consequence of dysbiosis, compared to healthy dogs. We retrospectively reviewed data from the database of Veterinary Teaching Hospital (VTH) of Perugia University and dogs affected by IBD with serum sample stored at -80°C were considered eligible for inclusion. To obtain a group of clinically healthy dogs (Control group), animals of VTH blood donor program were reviewed and dogs with serum stored at -80°C were included. A total of 18 dogs with IBD and 10 healthy dogs were included in the study. The IBD group was divided into three subcategories based on the severity of the disease. For these reasons a scoring index for IBD activity was created based on Canine Chronic Enteropathy Activity Index (CCECAI), World Small Animal Veterinary Association (WSAVA) histopathological grading, and level of serum albumin (Alb). This activity index had a total score of 9 (mild < 3, moderate between 3 and 6 and severe > 6). For each class (CCECAI, WSAVA histopathological grading, and Alb level) was attributed a score from 0 to 3. Dysbiosis index (DI) was available only for 1 dog and was 4.5, with a reduction of C. hiranonis and an increase of E. coli. Determination of serum D-lactate concentrations (μM) was performed using a commercially available colorimetric assay kit (D-Lactate Colorimetric Assay Kit, BioVision Inc, California). Mean D-lactate concentrations of IBD group were 223.59 μM/ml (range 66.53 – 386.56 μM/ml). Mean D-lactate concentrations of the Control group were 209.77 μM/ml (range 139.77 – 300.50 μM/ml). Our results showed no significant difference ($p > 0.05$) in the serum levels of D-lactate between dogs with various degrees of IBD and healthy dogs. However, the wide variability of D-lactate concentrations in IBD dogs encourages further studies on this topic to understand potential factors able to influence serum D-lactate levels in dogs affected by IBD.

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77° CONVEGNO SISVET

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Hematological and biochemical reference intervals for the endangered Persano and Salernitano horses.

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The Persano horse (Ph) and Salernitano horse (Sh) are Italian horse breeds considered endangered (1) and knowledge of the physiologic parameters is useful to support conservation strategies. The aim of the study was to determine reference intervals (RIs) for hematological and serum chemistry parameters in healthy Ph and Sh and to compare them with other horse breeds. After a complete clinical exam, a total of 23 clinically healthy Ph (11 females, 7 males and 5 geldings) aged between 3 and 21 years old (mean: 9.4 ± 5.9) and 36 Sh (24 females, 8 males and 4 geldings) aged between 4 and 21 years (mean: 9.6 ± 5.5) were enrolled in this study with the owner consent. Blood samples were collected from the jugular vein on the morning. The hematological parameters analyzed were red blood cell count; hematocrit; hemoglobin concentration; mean corpuscular volume; mean corpuscular hemoglobin; hemoglobin concentration distribution width; RBC distribution width; total white blood cell; leukocyte subpopulations (neutrophils, basophils, eosinophils, lymphocytes, and monocytes) as percentage and as absolute count (n# cell × 103 /μL). Platelets and their indices were also analyzed including platelet count and mean platelet volume. All hematological analyses were performed with an automatic cell counter equipped with software dedicated for veterinary blood analysis (ADVIA 2120, Siemens Healthcare Diagnostic, Germany). Biochemical parameters, including total protein, albumin, creatine kinase, aspartate amino transferase, alkaline phosphatase, γ-glutamyl transferase, creatinine, urea nitrogen, glucose, triglyceride, total cholesterol, sodium, potassium, chloride, magnesium, calcium, phosphorus and total bilirubin levels, and serum protein electrophoresis (Albumin, α1, α2, β1, β2 and γ-globulins fractions) were performed on an automated biochemistry analyzer (Clinical Chemistry Analyzer AU 680, Beckman Coulter, USA) and on Hydrasys 2 scan focusing (Sebia, France), respectively. The statistical analysis was carried out using the software GraphPad Prism V.6.01. The RIs were established according to the norms of the American Society of Veterinary Clinical Pathology. From each variable, extreme outliers were excluded from the calculation of RIs. The normality was checked by the D'Agostino Pearson's test. According to distribution, the reference intervals were given as mean ± SD or as median and 2.5th and 97.5th percentiles. A comparison between parameters obtained from the breeds was performed using the unpaired t-test or the Mann-Whitney test. A p value <0.05 was considered significant. Hematological parameters in Ph and Sh were closer to limits stated for other warm-blooded horse breeds (2). On biochemical profile, the upper limits of creatine kinase, total bilirubin, total protein, aspartate aminotransferase and total cholesterol were slightly lower while the lower limit of albumin was slightly higher compared other breeds (3). The slight differences may be attributed to differences in environments, feed, and the physiological features of the two breeds. On serum protein electrophoresis, only mildly higher albumin levels were found compared with previously published data. No differences were found in blood parameters between Ph and Sh. The Ph is genetically very close to the Sh (1) confirming the results obtained in our study. Hematological evaluation is an important step for health assessment in equine medicine, playing an important role in the strategies aimed at the welfare and conservation of endangered breeds. (1) Domestic FAO. Animal Diversity Information System (2023) Available online at: http://www.fao.org/dad-is /(Last update August 29, 2023). (2) Satué et al. Age- and Sex-Related Modifications of Hematology in Spanish Purebred Horse, Journal of Equine Veterinary Science, 93:1-6, 2020. (3) Sample et al. Hematologic and biochemical reference intervals for adult Friesian horses from North America, Veterinary Clinical Pathology, 44:194-199, 2015.

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Preliminary data on endoscopic evaluation of the cloaca in Dracaena daudin

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The aim of this work is to describe, for the first time, the cloacal anatomy, the possibility of early diagnosis of abnormalities, pathological findings, and immediate determination of sex in Dracaena daudin specimens. Cloacal endoscopy was the diagnostic technique used by us, as has recently been done for other species of lizards. The study was conducted on two subadult specimens (one male and one female) maintained in captivity from a private breeding facility. A rigid endoscope with a diameter of 4 mm, 0°, and a length of 8.5 cm (Olympus Medical, Japan) was used. The animals were positioned in dorsal recumbency without the use of anesthetics while the endoscope was inserted into the cloaca in a caudal-cranial direction. To improve visibility, a warm sterile saline solution supplemented with lidocaine was infused into the cloaca. These procedures allowed direct observation of all cloacal portions, the proctodeum, urodeum, and coprodeum, as well as visualization of the oviduct ostia present in the urodeum of females, which allows them to be distinguished from males. Female Dracaena also have a pair of exclusively ureteral papillae, arranged more caudally. In males, the anatomy is different because the urodeum is blind-ended and the pair of papillae includes both the openings of the ureters and deferent ducts (urogenital papilla) [1;2].In this work, cloacoscopy is proposed as an observational tool for the study of comparative anatomy, sex determination, and opens new horizons as a diagnostic technique for obstructive pathologies of the cloaca frequently observed in captivity, such as dystocia, fecalomas, and foreign bodies that could be removed or aspirated immediately after their identification. The study of the organs associated with the cloaca, their comparison with species of lizards whose anatomy is better known, and techniques for sperm collection and artificial insemination will be the subject of future work [3].

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SICV

77° CONVEGNO SISVET

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Unilateral nephrectomy in three horses with positive athletic outcome

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Unilateral nephrectomy is a rare surgical procedure in horses, indicated for neoplasia, hydronephrosis, abscessation, pyelonephritis, nephrolithiasis, ureterolithiasis, nematodiasis, idiopathic renal hematuria and ectopic ureter[1].
Different surgical techniques are described: transcostal and transthoracic approaches in lateral recumbency, standing laparoscopic nephrectomy, standing hand-assisted laparoscopic transperitoneal (LTP) nephrectomy, ventral midline celiotomy[1,2].
Nephrectomy is considered a safe surgery if the controlateral kidney is compensating, although few reports underline the importance of serial monitoring of renal function and the outcome on athletic activity[2,3].
The aim of the study is to describe the surgical approach and the athletic outcome in three horses presented for monolateral nephrectomy.
Case-1 (11-year-old) had a history of inappetence and increased serum creatinine, correlated to the presence of a right ureterolith with secondary degenerative changes in the ipsilateral kidney; *case-2* (16-year-old) and *3* (12-year-old) had a neoformation at the right and left kidney, respectively, with a previous long-term history of weight loss and polyuria without hematological alterations.
Horse-1 underwent a standing LTP right nephrectomy.
In *horse-2*, a right flank standing laparoscopy was planned to collect biopsy samples, followed by a nephrectomy under general anesthesia in lateral recumbency by a trans-costal approach.
Horse-3 underwent a standing LTP left nephrectomy; after complete isolation of the kidney, due to its dimensions and a notable catecholaminergic response, general anesthesia was required for removal through a ventral laparotomy.
No significant complications occurred in the postoperative period.
A severe, chronic hydronephrosis was diagnosed in *case-1*, while a papillary renal carcinoma and a papillary renal adenocarcinoma resulted in *case-2* and *case-3*, respectively.
A positive outcome was recorded in all horses. Long-term follow up is present for *horses-1* and *2* (27 months and 20 months, respectively): *case-1* persisted with mild increase in serum creatinine (mean 2.8 mg/dl, reference ranges 0.9-2) without other hematologic alterations nor clinical signs and it is competing at high-level show jumping; *case-2* has not shown hematologic alterations and returned to full show jumping activity. *Case-3* has a shorter 8-month follow-up but it is back to jumping activity without hematologic alterations.
In this case series, standing LTP was performed in two horses, entirely in *case-1* and partially in *case-3*, where isolation of the kidney by laparoscopy was followed by its removal by ventral midline laparotomy under general anesthesia. In *case-2*, renal biopsies by standing laparoscopy allowed the surgeon to plan nephrectomy in lateral recumbency through a trans-costal approach, given the large dimension of the neoplasia. Therefore, three different cases requiring nephrectomy have been approached with different surgical techniques, highlighting the importance of considering each case alone to identify the best approach.
Even if monolateral nephrectomy has been performed with a good prognosis for normal life[1,2,3], there is poor evidence in literature about the return to athletic activity of horses[2].
In this case series, 3 horses affected by unilateral renal disease underwent nephrectomy with complete resolution of the initial complaint and a good prognosis on athletic activity after short- and long-term follow-up. No clinical signs of renal insufficiency have been reported; the elevation in serum creatinine in *case-1* could be explained by the complete loss of activity of the right kidney, which probably overloaded the controlateral before surgery, while the neoplasia of *case-2* and *3* coexisted within a partially functioning kidney with an adequate compensatory response, explaining the absence of blood alteration.

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77° CONVEGNO SISVET

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Mesh-Enhanced Sutures: A Successful Approach for Treating complete Achilles Tendon Rupture in Dogs

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Abstract: The common calcaneal tendon (Achilles tendon) is the strongest and largest tendon and is one of the commonly affected by traumatic rupture in dogs. Different suture techniques are used to repair the tendon ruptures. This case report describes an original suture, utilizing a polypropylene mesh augmentation combined with a double 3-loop pulley pattern, to manage a complete and chronic rupture of the right Achilles tendon in a 6-year-old, male, mixed-breed dog. The dog was referred to the Hospital of the Department of Veterinary Medicine of Perugia for a plantigrade stance in the right hind limb secondary to a traumatic fall two weeks prior. The initial assessment included a comprehensive orthopedic, radiographic and ultrasound examinations. Following an extensive debridement involving about ¼ of the length of the tendon, the structure was reconstructed using a combined technique with a double 3-loop pulley pattern and a polypropylene mesh. An external fixator was applied to support the tibiotarsal joint for 16 weeks, with biweekly adjustments to gradually reduce extension and enhance tendon strength. Appropriate anti-inflammatory and antibiotic treatments were prescribed for one month postoperatively. Biweekly ultrasonographic follow-ups up to eight months postoperative confirmed complete recovery, with the patient demonstrating weight-bearing and no signs of lameness or swelling. This case highlights the effectiveness of mesh-enhanced sutures in successfully treating a dog with traumatic, chronic, and complete tendon damage.Keywords: Common calcaneal tendon, Achilles tendon, Polypropylene mesh, Tendon repair, 3-Loop pulley suture.

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THE LONG TERM USE OF ENFLICOXIB IN DOGS WITH OSTEOARTHRITIS: CLINICAL SAFETY AND EFFICACY

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Abstract

Osteoarthritis (OA) is a pathology characterized by progressive destruction of various components of synovial joints. The OA is generally associated with pain and inflammation and therefore lameness, which are capable to decrease the quality of dog life. Unfortunately, there is no treatment for solving OA, but it is possible to slow down its progression through a correct therapeutic approach which could relieve pain and improve the quality of life of the dog and, consequently, of the owner.

The objective of the present study was to evaluate the efficacy and safety of enflicoxib for the treatment of naturally occurring canine OA.

Fourteen dogs were treated for 13 weeks with enflicoxib (Daxocox®, Ecuphar NV, Italy) administered once a week at 4 mg/kg, with an initial loading dose of 8 mg/kg. From day 0 to day 90 efficacy was assessed by the veterinarian by using clinical pain and lameness scores, and by the owners using the Canine Brief Pain Inventory.

For the veterinary assessment: percentages of CSS responders on D7, D14, D28, D56 and D90 were 29%, 50%, 57%, 71% and 57%, respectively. CBPI percentage of responders increased progressively reaching values of 93% except for day 90 when it settled down to 79.

The analysis of the CSS total scores compared to the basal values showed high significance ($P<0.01$) at all time points. Comparisons between CBPI components (PIS and PSS) basal scores and those recorded during the weekly CBPI assessments also showed high significance ($p<0,01$) at all time points. Lameness using the 5 grades NRS was classified at D0 as 5 in 7% of dogs, as 4 in 21%, as 3 in 43%, and as 2 in 21%. On D90 no dogs showed 5 or 4 grade lameness. 14% of cases were recorded as grade 3, 43% as grade 2 and 21% as grade 1. 21% of dogs were free from lameness. The statistical analysis showed significance ($p<0,01$) in the difference between the degree of lameness recorded on D0 and that registered on D7, D14, D28, D56 and D90 veterinary assessments. At day 0 and 90 a complete blood count and a biochemistry profile were performed in all treated animals. Significance was found in the difference between blood urea and Creat values obtained at the beginning and at the end of the study ($p<0,05$). Taking as reference the ranges of minimum and maximum values suggested by the laboratory machine used for blood tests, at the end of the study 11 patients (79%) showed a significant increase in urea values compared to the basal. Among these, only 4 dogs (29%) exceeded the indicated threshold limit.

From the first weeks of treatment, a meaningful improvement in the clinical and owner scores was noticed. In conclusion, long term weekly administration of enflicoxib at the proposed dosage, resulted in great benefit for the quality of life of the dog affected by OA.

Key words: Osteoarthritis, enflicoxib, COX-2, NSAID, dog.

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77° CONVEGNO SISVET

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Medetomidine and Butorphanol, followed by Isoflurane and local Lidocaine, for surgical anesthesia in Dwarf Hamsters (Phodopus sungorus)

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Dwarf hamsters (Phodopus sungorus) are popular pets, and veterinary attention for their medical conditions is increasing, often necessitating surgical interventions [1]. Despite their relatively short lifespan, ensuring the possibility of surgical procedures is essential for the welfare of these animals [1,2]. Unfortunately, studies on hamster anesthesia are scarce and often limited to laboratory and research contexts, and anesthetic and surgical procedures are often not recommended due to the high mortality rate of this species. Therefore, there is a need for more data regarding alternative and safe anaesthetic protocols for pet dwarf hamsters [1]. This study aims to describe the effects of medetomidine and butorphanol as premedication before inducing anesthesia with isoflurane, supplemented with local anesthesia using lidocaine, in hamsters undergoing surgical excision of subcutaneous masses.
The study enrolled 15 dwarf hamsters with a mean weight of 46 ± 13 g. Subjects received subcutaneous (SC) administration of medetomidine and butorphanol (MB: 0.2 and 0.5 mg/kg, respectively). Sedation levels were assessed after five minutes based on a previously established scoring system utilized in canine studies employing a similar protocol [3]. Anesthesia was subsequently induced using 5% isoflurane in an induction chamber; maintenance of anesthesia was achieved with 1% isoflurane in pure oxygen via a handcrafted face mask following the loss of righting reflex. Prior to surgery, fur clipping was performed, and 2 mg/kg lidocaine was locally administered around the mass, with lidocaine diluted in sterile saline to obtain a 0.2 ml volume for injection. Heart rate (HR), respiratory rate (RR), and peripheral oxygen saturation (SpO2) were monitored at five-minute intervals. After the surgical procedure, atipamezole (1 mg/kg, SC) was administered, and the reappearance times of palpebral and pedal reflexes, spontaneous movements, and the resumption of the righting reflex (RRR) were recorded. Post-operative care included the administration of 1 mg/kg meloxicam. Mean sedation score was 7 ± 3, while mean surgical duration was 27 ± 12 minutes, with atipamezole administered 40 ± 16 minutes after MB administration. Mean HR, RR, and SpO2 were 231 ± 45 bpm, 41 ± 7 breaths/min, and 97 ± 3%, respectively. All subjects maintained spontaneous ventilation throughout the procedure, and no intraoperative responses to surgical stimuli were observed. Reappearance times for palpebral and pedal reflexes were 1.6 ± 0.5 and 2.1 ± 0.8 minutes, respectively, while movements and RRR were recorded at 3.6 ± 1.4 and 6.3 ± 2.2 minutes, respectively. All recoveries were smooth, devoid of complications, and no perioperative mortality was recorded. All the subjects returned to their usual behaviors once discharged.
Medetomidine and butorphanol have additive effects when used in combination to produce sedation in a premedication protocol before surgical procedure. Notably, both agents possess analgesic properties, with butorphanol being often favored over other opioids in rodents due to its low cardiorespiratory adverse effects [2,3]. The dosages employed, coupled with the adjunct use of lidocaine, allowed the utilization of low percentages of isoflurane, resulting in expedited recovery without perioperative complications or mortalities in the context of this challenging species, the dwarf hamster.

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77° CONVEGNO SISVET

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Retroperitoneoscopic adrenalectomy in four dogs using SILS device

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Adrenalectomy is traditionally viewed as one of the more challenging interventions in soft tissue surgery no matter what surgical approach is used. Approaches used in the past include a ventral celiotomy, a paracostal approach and more recently transperitoneal laparoscopic resection. Jeong, et al. proved in 2016 the feasibility of pneumoretroperitoneum in dogs. One year later, the same corean group published this article of single port retroperitoneal adrenalectomy in healthy Beagle dogs. The aim of this study is to describe the left retroperitoneoscopic adrenalectomy in four dogs. Four female intact dogs were enorolled for this study. Left adrenalectomy was performed based on the presence of hypercortisolism in all cases. These cases were diagnosed on citology after fine needle aspiration (FNA) carcinomas. CT whole body was done for all dogs. No adrenal gland vascular invasion was seen.Dogs were positioned in lateral recumbency with lumbar elevation using a cushion and secured with self-adhesive tape. The dorsal and lateral aspect of the hemithorax and hemiabdomen was clipped from the level of the 11th thoracic vertebra to the level of the seventh lumbar vertebra for aseptic surgery. To access the retroperitoneal space, needed to transect the skin, fascia, and separate muscular layers. A SILS port (Covidien, New Haven, Connecticut) was placed by using a stay suture for retraction of the skin and muscles.After placing three 5-mm cannulas through the SILS port for triangulation of the laparoscopic instruments, the retroperitoneal space was investigated by using a 5-mm, 0° telescope (Karl Storz, Tuttlingen, Germany) When the retroperitoneal space was visualized, pneumoretroperitoneum was induced by using an insufflator (Karl Storz) at a pressure of 5 mmHg. An ultrasonic scalpel (Harmonic™ or Sonicision™) was used to accomplish the vascular dissection and sealing. Other's devices, as advanced bipolar sealers, were also used.Dissection started with the surgical plane between the fat pad and the lateral aspect of the kidney to gain access to the adrenal gland Retroperitoneoscopic examination of the kidney and its pedicle, adrenal gland, vena cava and the indemnity of the peritoneum was done.Absence of liver macrometastases and macroscopic vascular invasion into the caudal vena cava was confirmed in all dogs.The distal segment of the phrenicoabdominal vein was identifed, sealed, and transectedExposure and dissection of the adrenal glands were performed from caudal to cranial (close to far from the camera) After careful dissection, the remaining glandular tissue was progressively entirely removed along with the SILS port to prevent abdominal wall contamination.The retroperitoneal space was inspected for hemorrhage, and the adrenalectomy site was locally rinsed with small volumes of warmed lactated Ringer solution and concurrent use of close suction to avoid abdominal contamination with neoplastic cells. All remaining gas must be aspirated to avoid postsurgical pain. After the total gas elimination, the incision is closed.Excised adrenal tissue was submitted for pathologic examination. No major or minor complication was seen.

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Collivignarelli F. et al. Two - port laparoscopic adrenalctomy in the dogs Animals (Basel) 25;12(21):2917, 2022

LOCAL ANAESTHETIC EFFICACY PROVIDED BY LIDOCAINE OR LIDOCAINE-TRAMADOL IN DOGS UNDERGOING MAXILLARY FOURTH PREMOLAR EXTRACTION.

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Complicated tooth fracture in dogs is a common condition that often requires extraction of the affected tooth. Local anaesthetic drugs are considered the safest and the most effective agents to inhibit oral pain. This study wants to compare the analgesic effect of maxillary nerve block induced by lidocaine or lidocaine-tramadol on perioperative pain in dogs undergoing maxillary fourth premolar surgical extraction (Approval No. 088/2022). Sixteen mixed-breed dogs presented to the Veterinary Teaching Hospital of University of Messina for upper fourth premolar complicated fracture, were enrolled in a randomized, prospective, blinded clinical trial. All dogs were premedicated with a combination of dexmedetomidine 1 µg/kg and methadone 0.3 mg/kg administered IV. Anaesthesia was induced with IV propofol and maintained with isoflurane in 100% oxygen. ECG, invasive SAP mmHg, DAP mmHg and MAP mmHg, HR/min, RR/min, SpO_2, T °C, E_tCO_2 mmHg and E_tIso % nwere monitored every 10 minutes. The dogs were randomly assigned to one of two groups, with eight animals in each group: the Lidocaine Group (lidocaine alone, Group L) and the Lidocaine-Tramadol Group (lidocaine + tramadol, Group LT). An assistant prepared the drugs and performed the intraoral maxillary nerve block with the principal investigator and the surgeon blinded about the drugs used. In Group L, the maxillary nerve was blocked by infiltrating of 2 mg/kg 2% lidocaine, while in Group T, the block was performed by infiltrating 2 mg/kg of lidocaine and 2 mg/kg of tramadol. In order to obtain the same volume between groups, a saline solution was added. After 3 minutes from the injection, the surgery was started. Rescue analgesia was provided with the administration of 2 µg/kg fentanyl IV and E_tIso was increased by 0.1-0.2% if animals had signs of inadequate anaesthesia.

The principal investigator performed postoperative pain score assessments once the dogs were able to standing (T0) and at 1, 2, 4, 6 h and 12 h (T1, T2, T4, T6, and T12) using the Italian version of the Glasgow Composite Pain Scale-Short Form (ICMPS-SF). All data were assessed for normality distribution by Shapiro–Wilk test and presented as the mean ± SD. The Analysis

of Variance and Bonferroni post hoc test or t-test, was used to compare all cardinal variables between the groups. The frequencies of dogs requiring an additive dose of sedative were analyzed with Fisher's exact tests. The ICMPS-SF scores were analyzed between the two groups using the Mann-Whitney test. Differences between the two groups were considered statistically significant for p-value <0.05.

HR, RR, SAP, DAP, MAP, SpO2, E_tCO2, and E_tIso showed no statistically significant differences between groups ($p>0.05$) suggesting that both drugs provided effective pain control during the surgery, while the ICMPS-SF evaluation highlighted a statistically significant different between group L vs group LT regarding animals that required analgesia at the post-operative time point (t0 4.750 +/- 0.462 vs 3.250 +/- 0.707) $p<0.01$; T1 (4,750 +/- 0.462 vs 3 +/- 0.534) $p<0.001$; t2 (5.375 +/- 0.5175 vs 3.625 +/- 1.061) $p<0.01$; t4 (5.5 +/- 0.577 vs 3.750 +/- 1.389) $p<0.05$; t6 (6 +/-0.001 vs 2.857 +/- 1.464), suggesting that tramadol added to lidocaine to perform maxillary block extend the analgesia longer than lidocaine alone.

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77° CONVEGNO SISVET

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The use of antibiotics in small-animal surgery rooms: an experience from the University Veterinary Teaching Hospital of Naples

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The escalating prevalence of bacterial infections worldwide has elevated antibiotic resistance to a matter of global significance. Preoperative antibiotic prophylaxis is administering antibiotics before performing surgery to help decrease the risk of postoperative infections. Importantly, the guidelines recommend that antibiotics should be used to prevent infections only before and during surgery, a crucial measure in stopping the spread of antibiotic resistance [1].This retrospective study examined the administration of antimicrobials in small animals receiving surgery at the University Veterinary Hospital of Naples (Italy), where monitoring studies on antimicrobial prescription in medical clinic unit were already performed [2,3].A total of 121 animals (precisely n. 102 dogs, n. 14 cats and n. 5 exotic animals) underwent surgical procedures under general anaesthesia. These surgeries were performed from September 2023 to January 2024. The objective of this study is to map the current evidence on surgical antibiotic prophylaxis administration at the University Veterinary Hospital of Naples. The study was conducted taking into account the two different hospital surgical rooms, one used for the orthopedic and neurosurgical procedures (room1) and the other one for soft tissues surgery (room 2). During the studied period, 78 and 43 surgical interventions were performed in room 1 and room 2, respectively. The age of the patients ranged from 3 months to 18 years with 53 females and 68 males. Antibiotics were administered before the surgery in 11. 6% of patients with amoxicillin being the most used, while 79.3% of patients received antibiotics in the intraoperative phase with ampicillin (37.5%) and cephazolin (32.2%) as the most administered. Furthermore, 87. 6% of patients continued the antibiotic therapy after the surgery, with amoxicillin-clavulanate as the most prescribed antibiotic (50.4%). The efficacy of amoxicillin-clavulanate as antibiotic prophylaxis in surgery has been already assessed in many clinical studies for its broad spectrum of actions against Grm-positive and Gram-negative bacteria as well as anaerobic pathogens that have a relevant role in postoperative infections. However, the use of this antibiotic should be monitored, since a growing rate of resistance has been recorded both in human and veterinary medicine.

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77° CONVEGNO SISVET

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Computer-Aided Design Planning and 3D-printed Patient-specific Guide to Address an Oblique Plane Antebrachial Deformity in a Dog

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Abstract
Computer-aided design (CAD) and patient-specific 3D-printed guides have been demonstrated to improve the accuracy of antebrachial deformity correction in dogs (1,2). This report describes a successful application of CAD and a patient-specific 3D-printed guide to correct a complex antebrachial deformity in a 6-month-old Maltese dog presented with left forelimb lameness. Radiographs of the affected limb showed elbow incongruity, a short ulna, and a long curve radius. A multiplanar antebrachial deformity was diagnosed based on CT scans. Employing CAD (Grasshopper algorithmic modeling tool integrated into Rhinoceros software), the center of rotation of angulation was identified in the distal radius. An oblique plane deformity was diagnosed, characterized by 21° valgus, 30°excessive procurvatum, and 42° external torsion. Using CAD, the surgery was stimulated virtually, and an osteotomy guide with high-profile features was custom-designed for the radius. Intra-operative models and surgical guides were 3D-printed in polylactic acid. The surgery involved a bi-oblique ulnar osteotomy and an oblique-plane closing-wedge ostectomy of the radius. The guide was perfectly positioned and stabilized with K-wires to the bone. After the ostectomy, a mini-series Fixin plate was placed dorsally on the radius. Postoperative radiographs showed resolution of deformity and elbow incongruity. Radiographical union of the osteotomy site was observed 60 days post-op. After seven months, radiographs showed a decrease in radial bone density underneath the plate, indicative of stress protection ascribed to the plate's rigidity. Staged plate dynamization was performed at 7 and 9 months, followed by complete plate removal at 11 months. This case report highlights the benefits of using CAD planning and patient-specific 3D guides in an antebrachial corrective osteotomy; as well as the importance of long-term follow-ups for early diagnosis and management of stress protection. Future research should focus on objective assessments of CAD and 3D technologies in corrective procedures, comparing them to free-hand surgeries (2,3).

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77° CONVEGNO SISVET

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A case of a South American tapir (Tapirus terrestris) with dental problems

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Tapirs frequently experience dental and oral issues that can significantly impact their overall wellbeing, health, and comfort [1]. Treatment of these problems often requires sedation, with a critical focus on ensuring safe airway management, particularly due to the potential challenges associated with intubation in this species [2].
At the Cappeller Zoological Park (Cartigliano, Italy), a captive 22-year-old male tapir (Tapirus terrestris, 190 kg) presented with a history of progressive weight loss and alteration in food chewing behavior. Over the previous 3 months, distinct changes were observed, including prolonged chewing duration and significant food spillage from the mouth during mastication, especially when compared to his housemate. Medetomidine (0.005 mg/kg), ketamine (1 mg/kg), and butorphanol (0.1 mg/kg) were administered intramuscularly to perform physical examination, blood sampling, accurate oral cavity evaluation, and dental x-ray. Subsequently, an intravenous catheter was inserted into the femoral vein. During diagnostic procedures, the animal showed regurgitation, and orotracheal intubation was performed immediately. Intravenous Ringer's lactate was administered and propofol was titrated to maintain an appropriate anesthesia depth. Supplemental oxygen was provided throughout the procedure. Physiological parameters (pulse rate, oxygen saturation, respiratory rate, arterial blood pressure) were continuously monitored.
Physiological parameters were stable throughout the procedures. Complete blood work was unremarkable. Dental x-ray and oral inspection revealed irregular tooth consumption. Sharp dental surfaces were rasped using motorized equipment and water-dip cooling. The procedure lasted 100 minutes, and the animal recovered smoothly, successfully standing at first attempt within 15 minutes of the end of propofol administration. The tapir regained normal chewing behavior within 2 days, and showed weight gain and improved physical condition within 3 weeks.
This clinical case emphasizes the importance of monitoring chewing behavior in tapirs to promptly detect dental problems. Additionally, it underscores the need for safe airway management during anesthesia for the treatment of these problems, to prevent respiratory issues such as aspiration pneumonia.

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L-TRF IN AVIAN SPECIES: PROTOCOL STANDARDIZATION, MACROSCOPIC AND HISTOLOGIC EVALUATIONS

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In the last decade, regenerative medicine and platelet concentrates have garnered increased interest within the medical and surgical domains, particularly for their roles as wound sealants and haemostatic agents in the regeneration of surgical wounds. In veterinary medicine, Leukocytes-Platelet Rich Fibrin production protocol has already been described for dogs and cows [1][2], but to the authors' best knowledge, a centrifugation protocol has not yet been standardized for Leukocytes-Thrombocyte Rich Fibrin (L-TRF) membrane in Gallus gallus Domesticus. The aim of this prospective study was to start from the canine L-PRF protocol described in dogs to standardize a valid protocol in Gallus gallus Domesticus and evaluate the L-TRF membranes obtained macroscopically and histologically. This study is preliminary to the evaluation of the use of L-TRF membranes in wound with loss of substance in avian species.Twenty subjects of Gallus gallus Domesticus species kept as PETs were included in the study approved by the Ethics Committee (prot. No. PG/2024/0041725 of 08/04/2024). Exclusion criteria were defined as follows: subjects with a body weight less than 1.2 kg, concomitant or prior respiratory diseases characterized by symptoms, clinically suspected and/or instrumentally diagnosed infectious diseases, and subjects exhibiting behaviour incompatible with the required procedures. Three millilitres of blood were drawn from the left wing vein of each subject: one 1-ml aliquot was designed for routine hematologic testing (complete blood count-CBC), while the remaining 2 ml were used for the L-TRF production. A centrifugation protocol was employed, comprising the following steps: acceleration, centrifugation, and deceleration. A dedicated device was then used to obtain L-TRF membranes. The weight and size of each membrane were registered with a goldsmith digital scale and electronic caliper, collected in a sterile Eppendorf, and preserved in 10% neutral buffered formalin for histologic analysis. All the animals showed a CBC in normal range. 8 clots and 7 L-TRF membranes were obtained. The mean (±SD) length and width were 1.35 ± 0.63 cm and 0.75 ± 0.29 cm, respectively. The mean (±SD) weigh was 0.47 ± 0.17g. Histologic analysis of the L-TRF membranes showed an overall well-defined histoarchitecture consisting of a first layer composed of erythrocytes, followed by a layer of leukocytes, erythrocytes and thrombocytes, and finally a layer of an amorphous, eosinophilic material (plasma) intermingled with a fibrillar substance. In conclusion, is it possible to state that the proposed protocol is valid for production of L-TRF in Gallus gallus Domesticus.

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DORSAL RECUMBENCY LAPAROSCOPIC OVARIOHYSTERECTOMY IN A DONKEY WITH CHRONIC PYOMETRA

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A 6-years-old 135 kg jenny was presented to University of Bologna Veterinary Teaching Hospital for weakened urinary stream and pollakiuria developed following a dystocia episode occurred two months earlier. A purulent and malodorous vulvar discharge was reported as a sporadic symptom. At a visual exam the vaginal vestibule appeared stenotic, a colposcopy revealed irregular vaginal walls and cervix, the urethral orifice was partially covered by hyperplastic cicatricial tissue, causing urovagina. A transrectal ultrasound showed uterine collection from which Proteus mirabilis was isolated. A McKinnon technique urethroplasty was performed to correct the urination defect and the donkey was treated with trimethoprim-sulfadiazine 5+25 mg/kg orally for 13 days according with the antibiogram. Additionally, cloprostenol 1,3 mcg/kg intramuscularly (IM) was administered once a day for three consecutive days and an intrauterine lavage was performed with lactate ringer and gentamicin.
Since these procedures had proven ineffective in resolving uterine collection, a laparoscopic ovariohysterectomy was scheduled. Despite being described in literature, the standing procedure was excluded due to the size of the animal, so a modified hand-assisted dorsal recumbency surgery was chosen. After fasting for 24 hours, the patient was sedated with detomidine 20 mcg/kg and morphine 0,1 mg/kg intravenously (IV) and caudal epidural anaesthesia was performed with morphine 0,1 mg/kg. Romifidine 80 mcg/kg was used to reach optimal sedation, general anaesthesia was induced with tiletamine/zolazepam 1 mg/kg and maintained with Isoflurane 1%; positive pressure ventilation was used. The patient was placed in dorsal recumbency and the ventral abdomen was prepared for aseptic surgery. After placing the table in a 13° Trendelenburg position, a 1.5-cm incision was made through the skin and linea alba over the umbilicus through which a 10-mm laparoscopic sleeve with sharp pyramidal trocar was passed into the abdomen. The sharp trocar was removed and replaced by a laparoscope, capnoperitoneum was obtained with 10-15 mm Hg and maintained using an automatic insufflator. Two 5-mm instrument portals were made: one on the midline, approximately 10 cm caudal to the laparoscope, and one at the same level on the left side, spaced 10 cm apart. As urinary catheterization was not possible due to recent urethroplasty, the bladder was emptied using a 20G spinal needle and an aspirator under laparoscopic guidance. Laparoscopic claw forceps and Enseal© Trio 3mm curved jaw were used to transect mesovarium and mesometrium.
Once the uterus and ovaries were freed from other structures, a 6 cm caudal median celiotomy was performed. Ovaries and uterus were exteriorized with laparoscopic guided hand assistance and drawn caudally; the table was set back to horizontal position. Two enterostats were placed close to the cervix and transection of the entire uterus was performed between the two. The uterus was sutured with a double continuous inverting suture of the serous layer and the stump replaced inside the abdomen. A three layers closure was performed for the four abdominal incisions.
Post-operative care included enrofloxacin 2.4 mg/kg IV sid, flunixin meglumine 1.1 mg/kg IV bid and sucralfate 4 g orally bid for five days. No complications were reported and at 3-months follow-up the donkey is in general good health.
The case is the first described dorsal recumbency laparoscopic ovariohysterectomy in an equine patient with a pathological reproductive tract.

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77° CONVEGNO SISVET

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Experimental model of glaucoma filtering surgery in rabbit: preliminary results.

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Glaucoma is an optic neuropathy characterized by progressive degeneration of retinal ganglion cells, and increasing intraocular pressure (IOP) represents the main risk factor[1].
Glaucoma filtering surgery (GFS) is performed when topical drugs or laser treatment fail to lower IOP. GFS lowers IOP by diverting the humor aqueous to a subconjunctival bleb thus creating a new drainage pathway. However, the success of GFS is mainly limited by subconjunctival fibrosis; consequently, antimetabolites are used off-label in clinical practice to inhibit fibrotic response[1].
An experimental GFS model (Ministerial Authorization no. 461/2022-PR July 2022) was developed in rabbits to evaluate hereafter new formulations for post-operative treatment, with higher safety profiles and enhanced therapeutic efficacy compared to antimetabolites.
The current experimental GFS model was derived from the work of Cordeiro et al. 2 and was designed to produce a subconjunctival filtering bleb in albino New Zealand White rabbits.
Two experimental sets were carried out on 12 rabbits (6 each). All surgeries were performed on the right eye with the aid of an operating microscope and under general and topical anesthesia. Briefly, the conjunctiva was detached from the scleral plane for 5 mm along the limbus and 8 mm posteriorly by using blunt-tipped Westcott scissors and conjunctival forceps. Then, a half-thickness scleral tunnel was fashioned with a sharp 23G angle blade through which a 22G cannula needle was inserted up to the anterior chamber. Finally, the needle was removed, and the cannula was cut, leaving 1 mm exposed. The conjunctiva was sutured watertight to the limbus to obtain the bleb.
Challenges were encountered with the positioning of the cannula in the initial set; as a result, in the subsequent set, the cannula was consistently placed between the dorsal rectus muscle and the nasal fornix, and then sutured to the sclera3. In both experimental sets, bleb evaluation was performed at 1, 3, 5, 7, 10, 14, 21 and 28 days after the surgery, using the "Indiana Bleb Grading System" method to describe height, extension, vascularization, and bleb leakage with the Seidel test.
Results of the first set showed that 83% (5/6) of the rabbits experienced cannula dislocation during the follow-up whereas a functioning bleb was maintained up to timepoint 7.
The second set's results showed that 17% (1/6) of the rabbits experienced cannula dislocation at day 21, and a functioning bleb was maintained up for all rabbits to time point 14.
Based on these results, all the steps of the experimental surgical model were defined, highlighting critical issues. Therefore, some solutions have been developed to prevent them, such as standardizing the position and fixation of the cannula to the sclera. Overall, the development of this model allows the testing of future innovative drugs to improve the success and safety of glaucoma surgery.
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77° CONVEGNO SISVET

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COMPARISON BETWEEN TWO DIFFERENT SUBCUTANEUS SEDATION PROTOCOLS IN RABBITS: ALFAXALONE-BUPRENORPHINE-DEXMEDETOMIDINE vs KETAMINE-BUPRENORPHINE-DEXMEDETOMIDINE

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The study aimed to evaluate the effectiveness of subcutaneous (SC) sedation in rabbits using alfaxalone or ketamine in combination with buprenorphine and dexmedetomidine. It was a randomized, cross-over, prospective study approved by the Italian Health Ministry with a 4-week washout period.Twelve 12-month-old New Zealand white rabbits (Oryctolagus cuniculus) weighing 3.5 ± 0.33 kg underwent experimental orthopedic surgery after being deemed healthy based on clinical examinations. Two SC premedication protocols were used: Ketamine 15 mg kg-1 or Alfaxalone 5 mg kg-1 associated with Dexmedetomidine 100 μg kg-1 and Buprenorphine 30 μg kg-1 (KDB group and ADB group). The drugs were injected subcutaneously in the intrascapular area using a 2.5 mL syringe and a 23 G needle. After injection, the rabbits were left undisturbed in their cage and scores were recorded by the same operator. The sedation score (SS) was assessed ten minutes after the subcutaneous injection and every five minutes for a total of twenty minutes (T10, T15, T20) using a simple descriptive scale [1]. The time from subcutaneous injection to sternal recumbency, immobilization, and no response to stimulation (recumbency time, RT) was also recorded. Afterward, the animals were removed from their cages and placed in a sternal recumbent position for preoxygenation. The ears were then clipped, disinfected, and a 22 G intravenous catheter was inserted into the marginal vein. Following tracheal intubation (using a blind technique), the rabbits were connected to a non-rebreathing Bain system and administered an oxygen/isoflurane mixture to maintain anesthesia. Propofol was administered as needed to facilitate intubation, and the number of cases that required it was recorded. Heart rate (HR), respiratory rate (RR), oxygen saturation (SpO2) in air, non-invasive systolic arterial pressure (SAP), mean arterial pressure (MAP), and diastolic arterial pressure (DAP) were continuously monitored and recorded 5 minutes after intubation.All data were analyzed using MedCalc 12.7.0.0 software. The normal distribution of the data was confirmed by the Shapiro-Wilk test. Mean, standard deviation and 95% confidence intervals (CI) were calculated for all data. Student t test was used to evaluate SS, RT, HR, RR, SAP, MAP, DAP, and SpO2 between groups and times. A p-value < 0.05 was considered statistically significant. SS at T10 and T15 was higher ($p = 0.0004$) in the KDB group (11.58 ± 2.27; 12.08 ± 2.27) compared to the ADB group (1.19 ± 2.46; 6.54 ± 3.90). The RT was higher ($p < 0.001$) in the ADB group (21.75 ± 3.51) compared to the KDB group (9.75 ± 5.95). In the KDB group, 25% of the rabbits received propofol versus 75% in the ADB group ($p = 0.04$). SAP was lower and HR and SpO2 were higher in the KDB group (79.58 ± 15.73; 206.33 ± 18.99; 98.54 ± 2.46) compared to the ADB group (96.41 ± 14.52; 149.5 ± 22.32; 93 ± 5.98). In conclusion, the two protocols proved to be safe for sedation of rabbits. KDB provides a deeper sedation with a faster onset compared to ADB. Furthermore, the KDB protocol seems to have a better oxygenation profile but a worse hemodynamic condition compared to ADB.

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77° CONVEGNO SISVET

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Pneumocephalus and pneumorrachis after transoral transphenoidal hypophysectomy in a dog

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Pneumocephalus and pneumorrhachis consist of intracranial and intraspinal trapped air and are recognized after neurosurgery in humans. In veterinary literature, pneumocephalus and pneumorrhachis are rarely described and their association has been reported in a single case report after transfrontal craniotomy. The aim of this report is to describe a case of pneumocephalus and pneumorrachis after transoral hyphophysectomy in a dog.An 8-year-old male Belgian Malinois was referred for pituitary-dependent hyperadrenocorticism. The pituitary macroadenoma (pituitary-brain ratio 0.5) was treated by transsphenoidal hypophysectomy, without any intraoperative complications. Seven days after surgery and after discharge, the dog presented with acute progressive painful ambulatory tetraparesis, consistent with C1-C5 neurolocalization. Differential diagnoses were infective/inflammatory (i.e., meningitis) or degenerative/traumatic (i.e., intervertebral disc disease causing compressive myelopathy).Inflammatory markers were unremarkable at blood works. The Magnetic Resonance Imaging (MRI) detected free air in the third and lateral ventricles and in the epidural space at C1-C2 and C5-C6, causing severe compressive myelopathy. The diagnosis was of pneumocephalus and pneumorrhachis. Rest and pain management was set. Neurological signs improved within 72 hours. Four weeks later, pneumocephalus and pneumorrhachis disappeared on control MRI, analgesia was progressively tapered, and the dog completely recovered. Pneumocephalus represents an expected finding following intracranial surgery, especially in human medicine: it is generally asymptomatic and spontaneously resolves within one month from surgery. The air is stored inside the cranial cavity during surgery due to a valve effect, assuming an anti-declining position. Less commonly, tension pneumocephalus occurs, i.e. when the air inside the cranial cavity causes an increase in intracranial pressure leading to neurological deterioration. A decompressive surgery should be considered as an option in these cases. Pneumorrhachis rarely occurs after intracranial surgery, despite an existing communication between cerebral and spinal subarachnoid spaces, possibly causing spinal compression. In the case herein described a progressive air accumulation in the cervical subarachnoidal space might have been caused by a possible communication between the rhinopharynx and the ventricular system due to failure of sphenoid bone closure by bone wax, or air migration from the cerebral ventricles, entered during surgery. In this case pneumocephalus and pneumorrachis completely resolved by conservative treatment and pain management within 4 weeks from surgery, as already reported in human medicine. Pneumocephalus should be considered as postoperative consequence of transsphenoidal hypophysectomy; pneumorrhachis may occur as a rare complication.

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77° CONVEGNO SISVET

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EFFICACY OF LYMPHATIC DRAINAGE KINESIOLOGY TAPING IN THE TREATMENT OF STASIS EDEMA IN HORSES

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Expansion and compression of lymphatic vessels, and the deformation of the tissues in which they are embedded, can explain formation and transport of lymph. (1) Stasis edema is a condition caused by stagnation of lymphatic fluid in the subcutaneous tissue of the distal limb, caused by incorrect lymph circulation.(2) Use of kinesio taping began with treatments for musculoskeletal disorders and has progressed to circulatory and lymphatic systems disorders. Its mechanism of action is based on microconvolutions due to elastic recoil of the tape, which promote vessel pumping. (3) The aim of this case report is to describe a low-cost and non-invasive method to treat stasis edema in the distal limbs of horses.
Two horses training in showjumping activities participated in the study protocol with owner consent. All measurements were performed using a Gulick II tape measure. (2) The measurements were carried out at T0, before applying the tape, and at T1 one week after application.
Case one: Italian saddle horse, gelding, 20 years old. The patient showed localized swelling of both hind limbs, after resolution of left hind suspensory ligament desmopahy. The T0 measurement of the edema at the level of the fetlock joint was 30 cm on the left and 30.5 cm on the right. A rehabilitation protocol was implemented, followed by an application of taping with lymphatic drainage effect in association with free daily movement. On the 7th day after application, the tape was removed and a new measurement was carried out: T1 fetlock joint measurements of the left and right hind limbs were 28.5 cm and 29 cm, respectively, demonstrating a 1.5 cm decrease in the circumferences of these joints.
Case two: Dutch horse, gelding, 8 years old. Anamnesis revealed a traumatic superficial wound to the right hind fetlock without compromise of tendon and ligament structures, with an evident and non-malleable swelling from the tarsal joint downwards. The measurements at T0 were taken at the level of the fetlock joint (33.5 cm), at the middle of the metatarsal bone (30.5 cm) and at the level of the hock joint (47.5 cm). A rehabilitation protocol was implemented, and application of taping with lymphatic drainage effect from the proximal part of the hock up to the ventral part of the fetlock. On the 7th day after application, the tape was removed and a new measurement was carried out: in T1 fetlock joint, metatarsal bone and hock joint measurements were 30.5 cm, 27 cm and 44 cm respectively, demonstrating a decrease of approximately 3 cm in the joints' circumferences.
The results demonstrate a reduction in swelling in the distal joints of the hind limbs, after the application of kinesio tape using drainage technique. This is an easy to apply and inexpensive technique. Further studies needed to confirm the effects of kinesio tape in animals with stasis edema of the forelimbs and hindlimbs.

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77° CONVEGNO SISVET

Stato: INVIATO - ID: 13694

Ultrasonographic and elastosonographic changes of patellar ligament in dogs affected by cranial cruciate ligament disease.

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The aim of this study is to evaluate the changes in thickness and elasticity of the patellar ligament in dogs with cranial cruciate ligament disease. The hypothesis is that the patellar ligament may show increased thickening and stiffness with increasing days from onset to diagnosis instead of trauma. Thirty-three dogs with unilateral naturally occurring cranial cruciate ligament disease were enrolled. The subjects were divided in three groups: based on the time elapsed from the onset of lameness to diagnosis: Group 1 (1–15 days), Group 2 (16–60 days) and Group 3 (over 60 days).All patients underwent to ultrasonographic and elastosonographic examination of patellar ligament.The dogs were positioned in lateral recumbency with the affected stifle up and in maximal manual passive flexion. A patellar ligament was considered normal when the fibrillar echotexture was homogeneous and parallel, and slightly broadened at origin. Ligaments that exhibited ultrasonographic evidence of pathologies, such as disrupted patterns, increased cross-sectional diameter or internal mineralization, were considered abnormal.The ligament thickness was calculated on the longitudinal ultrasound images in its exact half.The elastosonographic images were obtained by applying light rhythmic pressure with the probe and only longitudinal sections were acquired.The elastosonographic images were characterized by a colour translucent map superimposed on the B-mode images.Each colour indicated the relative elasticity of the different structures compared with the mean elasticity of the entire area: blue (mostly hard), green (intermediate) and red (soft).The acquired data were compared between groups (1,2 and 3) and, additionally, the group of dogs with cranial cruciate ligament disease was compared to a group of dogs with healthy stifles that was already studied [1].Statistical significance was set at p-value < 0.05. Thirty-three patellar ligaments from 33 dogs affected by unilateral cranial cruciate ligament rupture were evaluated. Eleven dogs belonged to Group 1, eight dogs to Group 2 and fourteen dogs to Group 3. All ligaments showed alteration in echo structure. The cross-sectional diameter of patellar ligament was 2.4 ± 0.55 mm in the group 1; 2.1 ± 0.35 mm in the group 2; 2.6 ± 0.41 mm in the group 3. There were statistically significant differences ($p < 0.05$) in the comparison between groups 2 and 3; and between group 1 and 3 which mean a progressive thickening of patellar ligament with increasing time between the onset of the lameness and the diagnosis.There were not significant differences in elasticity between groups although the patellar ligament tended to become harder and less soft with increasing days after rupture. After comparison with a study group of the patellar ligament of healthy dogs, it was found that the patellar ligament of dogs with cranial cruciate ligament rupture is harder and less soft than that of dogs with healthy stifle.The ultrasonographic and elastosonographic findings highlighted a progressive thickening and a tendency to lose elasticity of patellar ligament related to time increases between the onset of cranial cruciate ligament rupture and diagnosis and treatment.

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77° CONVEGNO SISVET

Stato: INVIATO - ID: 13709

Positive outcome in a 5-year-old dog with IV grade medial patellar luxation treated with De Angelis modified technique: a case report

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Patellar luxation (PL) is a common cause of lameness in dogs, primarily affecting small breeds, where medial luxation (MPL) represents the most prevalent form [1]. The PL is clinically classified into four grades, with grade IV being the most severe, characterized by a permanent luxation. Appropriate surgical treatment for grade IV MPL relies on the aetiology, age, clinical presentation and imaging findings. To the best of our knowledge, the use of the De Angelis modified technique [2] as a surgical treatment of grade IV MPL has not been reported in adult dogs. This case report describes the successful application of this technique in a 5-year-old female Chihuahua diagnosed with grade IV bilateral MPL. The dog was referred due to progressive worsening of ambulation over the last three years, resulting in a complete reluctance to move or walk. At the time of presentation, the dog was unable to stand and walk on both pelvic limbs and had the tendency to transfer the weight onto the thoracic limbs. Clinical examination confirmed the presence of bilateral grade IV MPL, and both tibiae appeared to be severely internally rotated. The cranial tibial thrust and cranial draw tests were negative, and the neurological examination was unremarkable. Survey radiographs and computed tomography revealed exaggerated internal rotation of the tibia of approximately 45° in the limb axis with concomitant agenesis of the trochlear groove, bilaterally. The tibial tuberosity did not appear to be excessively medially positioned and axial deviation of the tibial anatomic axis (leading to proximal tibia vara or valga) was not detected. A femoral V-sulcoplasty, medial and lateral arthrotomy and a modified De Angelis technique were therefore performed to allow the patella and quadriceps mechanism to be sagittally aligned. Surgery was initially performed on the right stifle; post-operative radiographs confirmed satisfactory reduction of the patella within the femoral groove and correct limb alignment. In the follow-up, rest in cage for two weeks and a progressive increase of activity was requested. The same procedure was performed two months later on the contralateral stifle. A follow-up orthopaedic examination was performed 3 and 4 months after surgery and the client was extremely satisfied with the outcome. On the left, it was no longer possible to luxate the patella whilst, on the right side, a mild degree of laxity (MPL grade I) was still present although it did not appear to be clinically significant. This case report describes the successful surgical management of grade IV medial patellar luxation characterized by exaggerated internal rotation of the tibia in the absence of concurrent osseous deformities. Nevertheless, although the application of a modified De Angelis technique combined with trochleoplasty appeared efficacious in addressing such a severe degree of patellar luxation, meticulous patient selection is strongly recommended.

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77° CONVEGNO SISVET

Stato: INVIATO - ID: 13834

Squamous cell carcinoma of the third eyelid in cattle (BOSCC): benefits of early surgery.

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Bovine squamous cell carcinoma (BOSCC) is one of the most common eye cancers. The predisposing causes have not been fully clarified, but the onset of the neoplastic process is multifactorial: Long-term exposure to UV rays and lack of skin pigmentation play a fundamental role. In fact, breeds with depigmentation of the eye, such as Pezzata Rossa (PRI) and Frisona Italiana (FI), are most impacted when bred extensively and have access to pasture[1-2].This study was carried out in the province of Ragusa (Sicily), an area of high livestock density. In the first time, an epidemiological investigation was carried out to assess the frequency of neoplastic processes at the eye region.Together, whole herds were monitored within 12 farms where one or more animals with ocular lesions were detected or reported; this screening was carried out to highlight preneoplastic lesions (e.g. papillomas) in clinically healthy animals, to undergo preventive surgical excision. 1244 animals were clinically examined, of which 24 adult bovine aged between 3 and 13 were included in the study for lesions in the ocular region: in detail, 13 subjects equal to 54,17 % PRI, 5 subjects equal to 20,83% FI, 1 subject equal to 4.17% Charolaise (CHR), while 5 subjects equal to 20.83% crossbreeds (CRS). Neoplastic lesions of different extent and severity were subjected to a unique protocol that includedEOG and EOP of the ocular region. In 12 of the 24 animals (8 PRI, 3 FI, 1 CRS) were found neoformations limited to the third eyelid, on which it was possible to adopt an early surgical treatment of excisional type.The administration of a loco-regional anaesthesia was carried out to obtain the blockage of the auricular-palpebral nerve and further multiple inoculation at the peribulbar level.The results showed the lack of preneoplastic lesions in the third eyelid but of already cancerous lesions, although small. These were macroscopically ascribable to rounded nodules, pinkish and with a smooth surface, the size of a few millimetres, or to small cauliflower growths of up to 1-2 cm. To determine the nature and degree of tumour, all neoformations were examined histologically and stage (5 grade I, 2 grade II, 5 grade III). On all animals undergoing surgical treatment, three follow-ups were carried out at 30, 60 and 90 during which there were no recurrences. Early surgical treatment avoided further development and invasion of nearby structures, improving animal welfare and reducing economic losses. In agreement with some authors, it is possible to highlight a predisposition of the PRI breed (54,17%) and a higher prevalence of neoplasms localized at the third eyelid (50%) [3]. It is therefore clear and essential to implement prevention techniques, educating and sensitising farmers to recognise such injuries in a timely manner.

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TOPICAL AUTOLOGOUS PLATELET-RICH PLASMA IN MANAGEMENT OF PERIANAL FISTULAS IN 5 GERMAN SHEPHERD DOGS

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Platelet-rich plasma (PRP), thanks to its ability to speed up the healing process, is used in the treatment of many diseases in which tissue regeneration is required. Canine perianal fistulas disease (PAF) is a painful and chronic disease of the perianal tissues that affects predominantly German shepherd dogs. Immunosuppressive medications are currently considered the cornerstone of treatment for PAF, with therapy often lasting for extended periods and sometimes yielding unsatisfactory results.

The aim was to describe if adjuvant topical injection of autologous PRP as a rescue therapy in the treatment of PAF could ameliorate the healing of the lesions.

Five female German Shepherds aged between 5 and 11 years were brought to our facility for PAF. All of them had previously been treated for extended periods with immunosuppressive doses of cyclosporine and/or prednisone without achieving complete wound healing. All cases were considered affected by severe PAF: 3 cases had at least 2 large sinuses and several draining tracts (depth range 15-50 mm); 2 cases exhibited marked thickening of perianal tissue and disseminated draining tracts extending 360° (depth range 3-35 mm). For suspicious cases, biopsy confirmed the absence of neoplastic lesions. They underwent three weekly sessions (day 0, day 7 and day 14) of autologous PRP injection directly into perianal fistulas. PRP, obtained after two centrifugations of collected whole blood from dogs, was resuspended at final concentration of 8×10^5 PLT/µL and immediately injected into fistulas. For fistulas less than 25 mm deep, 0.25 ml was injected, while for deeper fistulas, 0.5 ml was administered. From day 0, the diet of all dogs was restricted to a novel-protein (fish) diet and the therapy was supplemented with topical tacrolimus 0,1% ointment twice a day for 4 weeks in 4 dogs and with prednisone 1mg/kg for one week, then gradually tapered off, in 3 dogs. The treatment response was classified as complete, partial, stable or progressive taking the RECIST system as a reference. All dogs had a minimum follow-up of 60 days.

Within 4 weeks from day 0, complete response was observed in 2 dogs and partial response in 3 dogs: among these, one dog passed away due to causes unrelated to PAF on day 13 from the start of treatment (at that point, total diameter lesions had regressed by over 75%). No dogs underwent PAF recurrence or progression at the end of the follow-up period.

These case series suggest that adjuvant topical autologous PRP infiltration should be considered as a rescue strategy for the treatment of canine PAF refractory to standard therapy. This emphasizes the need for future research to assess the effectiveness of PRP in combination with standard protocols through randomized case-control studies.

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SIFTVET

Concentrations of heavy metals in bees, wax and honey collected from Molise Region: preliminary results.

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Heavy metals are particularly persistent and harmful environmental pollutants.
Some heavy metals, such as Fe, Cu, Cr, Zn, are essential micronutrient for human metabolism and are only toxic in high concentrations, others, such as Pb, Cd, Hg and As, are xenobiotic and toxic even in small quantities.
Due to their negative impact on the environment and living organisms, it is necessary to constantly monitor heavy metal pollution.
Bees (*Apis mellifera*) are considered excellent biological indicators thanks to their ethological and behavioral characteristics which allow constant contact with the surrounding environment. During foraging, bees come in contact with different environmental substrates [1]. In this way, bees can transport heavy metals from the environment into the hive by absorbing them from the atmosphere with their hairy bodies, collecting them through water and soil or collecting pollen and nectar on vegetation.
The aim of this study was to determine the concentration of xenobiotic heavy metals (Pb, Cd, As, Hg) and trace elements (Cu, Ni, Cr) in bees, wax and honey samples collected from 7 different sites in the Molise region (Italy). For each apiary, the samples were: about 100 foraging bees; 20 g of fresh wax and 50 g of fresh honey. Sampling was carried out in the summer of 2023; each sample was placed in a sterile container and stored at -20°C until further analysis.

The samples were homogenized by freezing with liquid nitrogen and ground with a porcelain laboratory pestle and mortar. The honey was thawed and filtered to remove bee and wax residues.
Heavy metals analysis was performed using inductively coupled plasma mass spectrometry (ICP-MS) after pressure digestion, according to test methods UNI EN 13805:2014 and UNI EN 15763:2010.

Preliminary results of the present study showed higher average concentrations of Pb than Cd, As and Hg: the highest values for Pb, Cd, As and Hg were 995, 185, 103 and 6.4 µg/kg, respectively.
The highest Pb content in honey was 139 µg/kg, which is above the maximum level of 100 µg/kg set in Regulation (EU) N°. 915/2023.
Among the trace elements, Cu was detected in all 3 matrices in higher concentrations than Cr and Ni, the highest Cu content was 30352 µg/kg in bees from the Isernia site.
Looking at the matrices, the highest average values overall were found in bees, followed by wax and finally honey. This result could be due to the different chemical composition and the different levels of contaminants in the 3 matrices [2].
The average values of all heavy metals showed overall higher concentrations at the Isernia site, which is probably due to the greater urbanization of this area compared to the other sampling sites.

The preliminary results of the present study show the presence of chemical contaminants in the selected bioindicators and in the honey food; this emphasizes the importance of conducting more in-depth studies to protect the environment and its inhabitants.

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Determination of Bisphenol A in ready-to-eat food with plastic packaging: preliminary results

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Bisphenol A (BPA) is an additive of plastic, used in the manufacturing of various materials, including food packaging. Due to incomplete polymerization and the noncovalent interaction between the additive and the plastic polymer, BPA is able to migrate easily from the packaging to the food; in fact, ingestion of contaminated foodstuffs is the most relevant form of exposure to this substance. BPA is an endocrine disruptor and alters the normal function of the endocrine system, posing serious risks to human health. Recently, the increased prevalence of disorders such as obesity, neurobehavioral deficit, diabetes, hypothyroidism, endometriosis, autism, breast, prostate, testicular, thyroid and endometrial cancers, have also been associated with exposure to endocrine disruptors [1]. In 2015, the European Food Safety Authority (EFSA) reduced the tolerable daily intake (TDI) of BPA from 50 to 4 µg/kg/day, but in 2023, EFSA re-evaluated the TDI of BPA and reduced it 20000-fold from 4 µg/kg/day to 0.2 ng/kg/day [2]. The purpose of this study was to evaluate the presence of BPA in ready-to-eat food products with plastic packaging such as dairy products (whole milk, skim milk, yogurt, cottage cheese, ricotta cheese, Galbanino cheese, provolone cheese, and parmesan cheese) and cured meats (mortadella, salami, ham and cooked ham). Sampling was carried out in November 2023, and each sample was placed in a sterile container and stored at -20°C until analysis. Subsequently, the sample was homogenized and weighed (2 g) and a solid-phase extraction (SPE) was performed. The determination of BPA was conducted using a high-performance liquid chromatography (HPLC) coupled to fluorescence detection (HPLC-FLD). The mobile phase as a mixture of H_2O (constituent A) and MeCN (constituent B) was pumped at a flow rate of 0.3 ml/min and the excitation and emission wavelengths of the fluorescence spectrometric detection were set at 230 and 315 nm, respectively. The preliminary results of the present study showed that no BPA was detected in dairy products, with the exception of provolone cheese samples, which showed median BPA values of 15.6 ng/g. A median BPA concentration of 43.31 ng/g was detected in cured meats. Based on preliminary data, the new TDI of BPA, is exceeded for all age groups in both the median and 95th percentiles. Further analyses will be necessary and will assist in subsequently evaluating the risk assessment related to BPA intake in humans.

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Concentration of non-essential (Cd and Pb) and essential trace elements (Zn, Fe, Cu) in tissues of 1 *Scyliorhinus canicula* and *Dalatias licha* collected in the Gulf of Naples

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Despite its status as the second-largest biodiversity hotspot globally, the Mediterranean region faces heightened vulnerability to climate change impacts, exacerbated by escalating human activities imposing substantial pressure on ecosystems. Positioned at the apex of the food chain, sharks play a pivotal role in preserving marine ecosystem equilibrium, contributing indispensably to biodiversity [1]. The absence of alternative species capable of fulfilling this ecological niche underscores the critical significance of sharks, and without these predators, the Mediterranean ecosystem could face potential transformation. The Mediterranean basin and northeastern Atlantic host an abundant population of the lesser-spotted dogfish, *Scyliorhinus canicula* [2]. This benthic species primarily inhabits the continental shelf. Its diet encompasses crustaceans, cephalopods, and polychaetes, contributing to its ecological significance within the region. Simultaneously, the kitefin shark, *Dalatias licha*, commonly found in the Atlantic Ocean, New Zealand, Australia, Japan, and the Mediterranean, serves as a deep-sea predator. Its versatile diet, including bony fishes, other elasmobranchs, cephalopods, crustaceans, and polychaete worms, underscores its ecological importance [3] This study aims to analyze and quantify trace elements, particularly non-essential (Cd and Pb) and essential trace elements (Zn, Fe, Cu), in tissues from *S. canicula* and *D. licha.*

Samples were collected between July and August 2020 in the fishing area between Ischia and Capri as the baycatch of scientific and commercial trawling operations (permit n. 0008453 issued May 15, 2020 approved by the ethics institutional review board of Italian Ministry of Agricultural, Food and Forestry Policies). Tissues (gills, liver, muscle, heart, intestine, stomach, spleen, gonads) from 15 specimens of *S. canicula* and the same tissues from 3 pregnant females of *D. licha* with their respective fetuses were digested in a microwave oven and concentrations of Cd, Pb, Zn, Fe, and Cu were determined using a High-Resolution Continuum Source (HR-CS) flame atomic absorption spectrophotometer (CONTRAA 300). Distribution and homoscedasticity was assessed through Levene test. Differences in the same tissue between the species in samples with normal distribution and homoscedasticity were tested by t-test, in samples with normal distribution and heteroscedasticity by robust t-test and in samples with not normal distribution by Mann-Whitney test. Statistical analyses were performed using R 4.3.2. A p-value < 0.05 was considered statistically significant.

The content of Cd was generally below the limit of detection (LOQ=0,40 mg/Kg) in both *S. canicula* and *D. licha*, except in the liver of both species, where Cd exhibited in*D. licha* higher content (p=0.016) (4,745 ± 3,282 µg/g) compared to *S. canicula* (0,199 ± 0,045 µg/g). This difference can be attributed to the trophic position of *D. licha*, an active predator occupying a higher position in the food chain, making it more susceptible to biomagnification compared to *S. canicula*. Lead content was below the limit of detection (LOQ=4.2 mg/Kg) in all tissues analyzed for both species. The liver of *D. licha* showed a significantly higher (p=0.014) content of Fe compared to *S. canicula*. Notably, Cu content was significantly higher in the stomach (p=0.036) and bowel (p=0.0008) of *S. canicula* than in *D. licha*, likely influenced by the crustaceans-based diet of *S. canicula*, given crustaceans' elevated Cu content. Furthermore, Zn content was significantly higher in the gills (p=0.048) and bowel (p=0.004) of *D. licha* compared to *S. canicula*. The content of trace elements in tissues of *D. licha* fetuses reflected those of the mothers and this suggests a possible maternal-fetal transmission through the yolk sac [4].

In conclusion, the different content of essential and non-essential trace elements in tissues reflects the peculiarities of specific tissue metabolisms, while the differences between *S. canicula* and *D. licha* are attributable to diet, position in the trophic chain and habitat occupied.

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Innovative therapy of influenza and respiratory infections caused by viruses through the use of siRNAs (short interfering RNAs)

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Influenza A viruses are a potential danger to human health due to viral recombination phenomena and the frequent appearance of pandemic biotypes. The animal host plays a crucial role in recombination events and in the hypothetical spillover of the virus [1]. These phenomena lead to the emergence of extremely virulent biotypes that are increasingly resistant to conventional therapies. The aim of this study is to propose new molecules that can be an alternative to vaccination plans and, in this perspective, RNA interference (RNAi) play a key role: this regulation mechanism of gene expression is addressed by small dsRNA molecules produced by the host itself but can be triggered also by the administration of exogenous siRNAs. In this work, a siRNA pool was generated in Escherichia coli starting from the pBAD-6xHis-BglII plasmid: the region between the SacI and NotI restriction sites was removed and replaced with the inverted repeats of the PCR product relating to the NP (nucleoprotein) gene. Subsequently, to improve the protein expression level and siRNA yield, the DNA fragment covering the p19 coding sequence and the inverted repeat was moved into a pBAD-6xHis-BglII vector, to generate the siRNA vector pBAD-6xHis-p19-NP. The operating principle of the vector is linked to the ability to produce the p19 protein, capable of sequestering siRNAs: after the expression of both transcriptional units (p19 and NP), endogenous ribonuclease III cuts the NP-related sequence into small dsRNA fragments, which are sequestered by the p19 protein and purified by NiNTA chromatography on gravity columns, according to NiNTA agarose protocol (Qiagen). The cell line used for the transfection was Madin Darby Canine Kidney (MDCK, code BS CL 64), stored in our biobank (www.ibvr.org). Transfection was performed by the reverse-transfection protocol, using Lipofectamine 2000 (Life Technologies) and BLOCK-iT™ Fluorescent Oligo (ThermoFisher Scientific) as a positive transfection control. The successful transfection was verified with the use of a fluorescence microscope. The influenza biotype used for the viral infection was: A/swine/Italy/1513-1/1998 H1N1 (VIR RE RSCIC 187). Then, 48h after infection, the resistance of the transfected cells to infection with increasing dilutions of the viral biotype was evaluated by observing the CPE and the results were compared with real-time PCR assays to detect the presence of non-degraded virus. The results showed a positive effect of siRNA treatment and the values obtained from molecular biology assays indicate a significant effect of siRNAs on viral replication. These data are encouraging for the production of innovative antiviral drugs based on this promising technology.

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77° CONVEGNO SISVET

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Detection of Ochratoxin A in Wild Boar Tissues

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Ochratoxin A (OTA) is a secondary metabolite produced by fungi of the genera *Aspergillus* and *Penicillium*, known for its toxic effects. OTA has been found to contaminate various food substrates such as cereals, spices, wine, beer, and dried fruit [1]. Its toxic effects include direct nephrotoxicity, as well as observed teratogenic, immunogenic, and carcinogenic effects. The International Agency for Research on Cancer (IARC) classifies OTA as a possible carcinogen for humans [2]. Recent increases in wild boar populations and their habitats in Italy, possibly attributed to climate change, may contribute to increased humidity and temperature, favoring fungal growth. Given the omnivorous nature of wild boars and their varied diet, they can serve as environmental bioindicators for contaminants like mycotoxins [3]. This study aimed to assess OTA concentrations in kidney, liver, and muscle tissue samples from 71 wild boars hunted in different areas of Avellino, Campania, from 2021 to 2022. Tissue samples underwent extraction, purification, and analysis using high-performance liquid chromatography with a fluorescence detector. Results revealed OTA presence in 40.8% of tested wild boars, with a median concentration of 0.56 μg kg^{-1} (range: 0.03-3.8 μg kg^{-1}). The highest OTA concentration was observed in the kidney (median: 0.7 μg kg^{-1}; range: 0.1-2.6 μg kg^{-1}) compared to muscle (median: 0.4 μg kg^{-1}; range: 0.03-3.8 μg kg^{-1}) and liver (median: 0.2 μg kg^{-1}; range: 0.03-2.1 μg kg^{-1}). OTA levels in the kidney positively correlated with those in muscle ($r=0.2$; $p=0.03$) and liver ($r=0.8$; $p=2.9e^{-020}$) (Spearman test). These findings underscore the substantial risk of OTA contamination in wild boar tissues, highlighting their potential as effective environmental indicators for mycotoxin prevalence. Moreover, considering the lack of control over wild animals' dietary intake and the incompletely understood chronic toxic effects of mycotoxin exposure, potential risks to human consumers necessitate urgent attention, emphasizing the need for acomprehensive legislative framework addressing meat contaminants.

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Implications of endocrine disruptor exposure: consequences for immunotoxicity and neurotoxicity

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Endocrine disruptors (EDs) are chemicals that can alter the normal functioning of the endocrine system by affecting the production, release, transport, metabolism, activity, or elimination of hormones. Here we investigated four different compounds in order to assess their toxicity. Atrazine is an herbicide widely used in agriculture to control weeds in corn and other crops. Scientific studies have shown that exposure to atrazine can interfere with the endocrine system, causing reproductive and hormonal problems in animals and potentially in humans. Imidacloprid is an insecticide in the neonicotinoid class, used to protect crops from insect infestations. It has been linked to harmful effects on the endocrine system of insects themselves, and there are growing concerns about its impact on non-target organisms, including humans. Glyphosate is the active ingredient in many herbicides, the best known of which is Roundup. Although glyphosate is not technically classified as an endocrine disruptor, some studies have raised concerns about its potential impact on the endocrine system, including possible hormonal and reproductive effects. PFOS is a chemical compound formerly used in a variety of products, including fire protection products, clothing products, paper and fabric coatings, and others. PFOS has been shown to interfere with the endocrine system, with detrimental effects on reproductive health, fetal development and the immune system. This study aimed to investigate the potential combined neurotoxic effects of these compounds on two types of neuronal cell lines, SHSY-5Y (murine neuroblastoma) and C6 (murine astrocytoma). The objective was to evaluate indicators of oxidative stress and inflammation, along with the release of pro-inflammatory cytokines such as IL-6 and INF-g, as well as anti-inflammatory cytokines like IL-10. The results indicate that the EDs exhibit inherent toxicity in neuronal cells, exacerbated by increased oxidative stress, and they synergistically amplify the overall effect. In a subsequent experiment, the murine macrophage cell line RAW 264.7 was utilized to further understand the immunotoxicity of these environmental pollutants, and their impact on immune response triggered by an inflammatory stimulus caused by lipopolysaccharide (LPS). The findings suggest that these chemicals can enhance the inflammatory response, even at non-toxic concentrations, by elevating intracellular ROS levels and altering pro-inflammatory cytokines such as IL-6, IL-10, and INF-g. The combined effects of these chemicals indicate a potential synergistic influence on regulating the inflammatory response. Further research into the potential impacts of these contaminants on animal brain and immune systems, including the mechanisms and timing of such impacts, may build upon this work..

Improving veterinary Pharmacovigilance of Dog's monoclonal antibodies: Reflections from Post-Market Surveillance of the Use of the Lokivetmab and Bedinvetmab

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Abstract

Lokivetmab and Bedinvetmab are two monoclonal antibodies (mAbs) recently widely utilized in veterinary medicine, particularly in the treatment of various conditions in dogs. Lokivetmab is primarily employed in managing atopic dermatitis, a common skin disorder characterized by itching and inflammation. This mAb, which binds to and neutralizes the pruritogenic cytokine Interleukin-31 (IL-31) specifically, is virtually completely caninized. Bedinvetmab is utilized to alleviate symptoms associated with osteoarthritis, a degenerative joint disease prevalent in aging canines. The action of this monoclonal antibody is by preventing the binding between NGF and tropomyosin kinase A, which is its receptor: this disrupts the signaling and results in the pain-relieving action.
Our study is aimed at analyzing reports of adverse effects associated with Lokivetmab and Bedinvetmab administration that have been documented after their release to the market. The data all refer to a time span from 2017 to 2024 for Lokivetmab and from 2021 to 2024 for Bedinvetmab. To conduct this analysis, we utilized the EudraVigilance database, which compiles and monitors adverse event reports submitted by healthcare professionals, veterinary practitioners, and pharmaceutical companies. Our findings from the analysis of adverse event reports revealed a spectrum of adverse effects attributed to Lokivetmab and Bedinvetmab. The number of suspected adverse reactions (SARs) for Lokivetmab is 2473 and for Bedinvetmab is 24635. Post-marketing surveillance observations revealed a variety of SARs, mainly including systemic disorders (13765), urinary (3060), digestive (6125), neurological (4774), musculoskeletal (2016) and cutaneous reactions (3131). Less serious adverse reactions were also noted.
These adverse effects encompassed various systems of the body, including gastrointestinal disorders, immune-related reactions, respiratory and urinary tract disorders, as well as behavioral alterations in some cases. Despite these reported adverse effects, it is important to note that the overall safety profile of Lokivetmab and Bedinvetmab remains relatively acceptable, especially when considering the therapeutic benefits they provide in managing challenging medical conditions in dogs. In conclusion, our study underscores the importance of ongoing pharmacovigilance efforts in monitoring the safety of veterinary drugs, particularly monoclonal antibodies, such as Lokivetmab and Bedinvetmab. While these medications demonstrate efficacy in treating specific canine ailments, their use should be accompanied by careful monitoring and adherence to recommended dosage regimens to minimize the risk of adverse events. Continued surveillance and reporting of adverse reactions are essential for ensuring the continued safe and effective use of these medications in veterinary practice.

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The effects of ocean warming on the biochemical and transcriptional responses of Mediterranean mussels exposed to the fluoroquinolone antibiotic enrofloxacin

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Ocean rising temperature and antibiotic contamination are recognized stressors that can lead to significant impact in aquatic environments [1]. Despite this recognition, there is a notable lack of comprehensive information regarding the impacts of antibiotic contamination in bivalves and, in particular, its combined effects with ocean warming. Additionally, there is limited understanding of the ability of bivalves to restore their biochemical equilibrium following exposure to these stressors.
To address this issue, the present study focused on understanding how the Mediterranean mussel *Mytilus galloprovincialis* responds to environmentally relevant concentrations (5 ng/L and 500 ng/L) of fluoroquinolone antibiotic enrofloxacin (ENR) under different temperature scenarios, mimicking normal and predicted global-warming temperatures (20°C and 25°C). Additionally, this study investigated the subsequent responses during a post-exposure recovery period. Understanding the effects of ENR exposure on marine organisms, particularly filter feeders like mussels, may contribute to the evaluation of the potential ecological risks associated with antibiotic contamination. Responses were assessed after a 14-day exposure period (EXP) followed by a 14-day recovery period (REC) through a combination of biomarker analyses and gene expression profiling. The study elucidated the physiological impacts of ENR exposure on mussels' gills, shedding light on the potential synergistic effects of antibiotic pollution and rising temperatures in marine environments.
Detectable ENR concentrations were found in mussels only after EXP at 500 ng/L, regardless of temperature. Unexposed mussels kept at 25°C exhibited biochemical impairments, including reduced metabolic capacity, increased energy reserve utilization, and enanched detoxification mechanisms. Similar patterns were observed in ENR-exposed mussels both at 20°C and 25°C, together with a decreased antioxidant activity. After REC, mussels restored their metabolic capacity, energy reserves, and antioxidant defences, gradually returning to control conditions, except for GST, which remained elevated. RT-PCR revealed variations in gene expression due to ENR exposure and temperature conditions. The selected genes of interest (GOIs) displayed upregulated (i.e. Catalase, SOD, GST, Isocitrate dehydrogenase) and downregulated (i.e. ABCB, Caspase, P53, CYP4y1, PKPYR) expression, although statistical significance was achieved only in some cases. Overall, these findings indicated that both stressors, acting alone or in combination, may represent a risk for marine bivalves like mussels.

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SIRA

ULTRASOUND MEASUREMENT OF THE URETHRO-PROSTATIC ANGLE FOR ESTIMATING PROSTATIC SIZE IN DOGS

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Ultrasound is currently considered the method of choice for assessing the size of the canine prostate[1]. Although some formulas for predicting prostate volume have already been published[1], it is not always possible to visualize and measure the gland in its entirety using ultrasound, even for an expert operator[2]. Objectively defining an enlarged prostate can be challenging, considering the huge variability in dog sizes. This study aimed to explore the reliability of a new, smart, practical, and less operator-dependent parameter, the urethro-prostatic angle (UPa), in estimating prostate volume. In longitudinal scanning, the UPa is formed by the line passing from the urethra and the line tangential to the cranial pole of the gland, at the point where the prostate crosses the urethra.

Fifty intact, pubertal male dogs of different ages (6.75 ± 3.79 years) and body weight (25.71 ± 13.63 kg) were enrolled. Ultrasound images of both transverse and longitudinal projections of the prostate were recorded (MyLab X90-Vet Esaote, multifrequency micro-convex probe 3-11 MHz). The degree of the UPa was measured and compared to the prostate volume calculated using the equation proposed by Ruel et al[3].

The UPa was easy to measure in all dogs. Its amplitude was positively correlated to the prostate volume ($p = 0.001$) (non-parametric correlation Rho Spearman) and allowed discriminating between normal (UPa $\leq 96.5°$) and enlarged (UPa $> 96.5°$) prostate based on the formula[3], with sensitivity of 88.9% and specificity of 70.7%, assessed with a ROC curve analysis.

The UPa appears to be a promising and useful parameter for estimating prostatomegaly in dogs. It is easy to acquire, even when the caudal pole of the prostate is located in the pelvis, it is independent of the size of the dog and does not require specific ultrasound skills and tools.

Although preliminary and worthy of further investigations, these results have shown interesting practical applications in canine andrology, also addressing less experienced technicians.

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GESTATION LENGTH IN MARES OF SALERNITANO AND PERSANO BREEDS

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In Italy, some horse breeds are submitted to the programmes for biodiversity conservation, such as the Salernitano and Persano breeds. These breeds are saddle meso-dolichomorphic horses reared in the south of Italy, especially in the Campania region, morphologically similar and with aptitudes in jumping show.
The conservation programmes include all aspects of increasing reproductive efficacy.
The knowledge of normal gestation length and duration of parturition stages is necessary for rationale management of birth, especially in monotocous species with long gestations.
In the literature, the gestation length of most widespread breeds has been studied with an average of 342 days and a variable range [1] demonstrated by the different factors influencing the duration of pregnancy [2]. Correct prediction of foaling and management of phases can be a discriminating factor for the survival of foal [2]. In order to diagnose any problems, it is necessary to know the average duration of the various stages of foaling [3].
This study was aimed to analyse the gestation length (GL) in Persano and Salernitano breed mares correlated to determining factors such as the foetal gender (FG), the age and reproductive conditions of the mares (RM), and the month of conception and birth (CB). Furthermore, the evaluation of the stages of foaling included the dilating stage (DS), the expulsive phase (EP), and the foetal membrane expulsion (SP).
Data were obtained through video surveillance from 39 eutocic foalings of Persano and Salernitano healthy mares with normal pregnancies and the birth of mature, healthy, and viable foal.
GL in these breeds is 331.2 days on average, ranging from 313 to 350 days. The mean is significantly lower than the literature reports in other breeds, despite the range being physiological [1,2]. Among the factors influencing gestation length the statistically significant parameter was FG, with an average of 329 in females and 334 in males ($p<0.05$). The various phases of foaling had an average duration of 30 minutes (SD), 31 minutes (EP) and 75 minutes (SP). The results showed that the data in the Salernitano and Persano mares agree with the data reported by other authors [3] showing a longer average duration of PE.
By correlating the GF to the duration of the various phases of foaling, the statistical analysis of the data showed a real significance between the GF and EP ($p<0,05$), showing an increase in this phase in females (36.7 minutes) compared to males (17.7 minutes).
In conclusion, the gestation length in the Persano and Salernitano breeds showed a shorter mean duration of gestation than in most of the previous studies [1,2]. The reasons for this phenomenon probably are linked to genetic, management, nutrition, climatic conditions, and latitudes.
In any case, by correlating the GL to the different factors considered, it can be concluded that the only factor influencing this parameter is the foetal gender, with longer GL in males and longer EP in females.

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Radio Electric Asymmetric Conveyer (REAC) treatment during prolonged ovarian cold storage improves *in vitro* embryo production in the domestic cat model

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Assisted Reproductive Technologies (ARTs) including in vitro embryo production (IVEP) and banking of genetic resources can contribute to the conservation of wild felids species, as the majority of them are considered to be at potential risk of extinction. The transport of ovaries at 4°C can be used to retrieve immature oocytes for IVEP from gonads of wild, valuable animals after they die or are castrated far from specialized laboratories. The optimal time of ovarian cold storage to sustain the viability of preantral follicles and the competency of domestic cat oocytes to develop into blastocysts following in vitro fertilization has been determined to be 24 h [1].
The Radio Electric Asymmetric Conveyer (REAC) is a novel a technology platform for bio-modulation which optimizes the ions fluxes and the mechanisms driving cellular asymmetry and polarization in biological structures and counteracts the biological mechanisms linked to aging and to degenerative process [2]. REAC treatment during liquid storage of stallion spermatozoa at 4°C for 72 h preserved spermatozoa acrosome membrane and DNA integrity, likely due to the enhancement of sperm antioxidant defences [3].
Considering the abovementioned REAC effects, this study aimed to investigate the impact of treating domestic cat ovaries with REAC for 48 hours at 4°C on the ability of oocytes to develop in vitro. Ovaries were harvested from domestic queens following routine ovariohysterectomies and randomly assigned to the REAC-treated (R: n= 13) and untreated (C: n=13) groups. In detail, ovaries were maintained in 4 ml of PBS at 4°C for 48h. The REAC device was set at 2.4GHz and its conveyer electrodes were immersed into the PBS. After 48h, ovaries were sliced to release cumulus-oocyte complexes which were selected according to their morphological characteristics [1] for in vitro maturation (IVM; R: n=130; C: n=133). Matured oocytes were fertilized (IVF) with frozen-thawed domestic cat epididymal spermatozoa and presumptive zygote were in vitro cultured (IVC) for 7 days. On day 2 and day 7 of IVC, respectively, the number of embryos cleaved and developing to the blastocyst stage was determined. IVM, IVF, IVC were performed according to the procedure of Piras et al [1, Piras]. Data were analyzed by Chi-square test with STATA\IC 11.0. Maturation rate of oocytes didn't differ between groups (R: n=59/130, 45.4%; C: n=66/133, 49.6%). Cleavage rate was higher ($P<0.05$) in R group (n=34/59, 57.6%) compared to C group (n=25/66, 37.9%). The percentages of blastocyst formation relative to the number of cleaved embryos (R: n=12/34, 35.3%; C: n=3/25 12.0%) and to the total number of MII oocytes (R: n=12/59, 20.4%; C: n=3/66, 4.5%) increased ($P<0.05$) after REAC treatment compared to untreated counterpart. According to our findings, REAC technology might offer a practical means of extending the period of ovaries storage for fertility preservation especially for endangered feline species.

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77° CONVEGNO SISVET

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Antimicrobial resistance profile of Escherichia coli strains isolated from healthy breeding bitches from kennels in Northern Italy

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Increasing antimicrobial resistance is a worrisome consequence of the large antimicrobial use, and misuse, both in humans and domestic animals. It is considered a threat to the global health and an emergency by the European Union. In breeding animals housed in kennels, there can be inappropriate antimicrobial administration with the hope to increase fertility or to reduce neonatal mortality. Escherichia coli is part of the canine intestinal bacteria flora and can show increased resistance in response to antimicrobial use, especially for those strains that are multi-drug resistant (MDR), i.e. resistant towards three or more antimicrobial classes, and Extended Spectrum Beta Lactamase producers (ESBL). The resistance profile of this bacterium has then been chosen as an indicator of the antimicrobial pressure in dog breeding facilities in Northern Italy. Rectal swabs were collected from five healthy breeding bitches in each of 16 medium-size dog breeding facilities in Northern Italy. The animals had not been treated with antimicrobials 30 days before swab collection. A bacteriological examination was performed at the Istituto Zooprofilattico Sperimentale delle Venezie within 48 hrs. E.coli isolation was done using blood agar and MacConkey agar. Selective media containing cefotaxime were also used to detect the presence of ESBL-producing E.coli. Species identification was performed by MALDI-TOF Mass Spectrometry (Bruker Daltonics). Antimicrobial susceptibility was evaluated by Minimum inhibitory concentration (MIC) using broth microdilution method. E.coli strains grown on selective media were analysed using the disk diffusion assay to phenotypically confirm ESBL production. 85 E.coli strains were isolated from the 80 examined bitches, due to the coexistence of more than one E.coli strain with different phenotypic characteristics: haemolytic, non-haemolytic and suspected ESBL producers, resistant to cefotaxime. Antimicrobial susceptibility testing was carried out on 58 strains prioritising haemolytic strains (N=20/58) and those grown on selective media. The percentage of resistance towards the tested antimicrobials was the following: ampicillin 100%, amoxicillin-clavulanic acid 100%, cephalexin 96.6%, cephazolin 41.4%, cefpodoxime 34.5%, cefovecin 29.3%, tetracycline 31.0%, doxycycline 22.4%, gentamycin 8.6%, erythromycin, kanamycin 13.8%, amikacin 1.7%, enrofloxacin 8.6%, pradofloxacin 8.6%, trimethoprim sulfamethoxazole 22.4%. Nineteen (19/85=22.3%) E.coli strains were phenotypically ESBL-producers and 10.3% (6/58) resulted multi-drug-resistant. Our was highlight complete loss of susceptibility towards ampicillin and amoxicillin-clavulanic acid, and almost complete towards cephalexin. These resistance rates are higher than those recently reported in healthy dogs from different geographical areas [1, 2]. Also the isolation of ESBL and MDR strains represents a warning sign of excessive antimicrobial use, although the distribution of these resistant strains varied among kennels, suggesting a different intensity of antimicrobial use.
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Clinical presentation of pyometra in the bitch at the veterinary teaching hospital of the University of Padua

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Pyometra is the most frequent pathology of the reproductive tract in bitches. The anamnesi s may include specific (vaginal discharge) and unspecific symptoms (e.g., depression, a norexia). Diagnosis is made through ultrasound examination in combination with clinical pre sentation. Treatment may either be conservative or surgical. Age has been considered a predisposing factor with the majority of cases presenting in older bitches [1].
A total of 74 bitches affected by pyometra were presented at the Veterinary Teaching Hospital (VTH) of the University of Padua between March 2017 and February 2024. The retrospectively collected data include signalment, clinical presentation and blood exams. Descriptive statistical analysis was performed using Microsoft® Excel (Version 16.78). The age of bitches at presentation ranged between 1 and 17.7 years (9.3±3.9 years). Of all bitches 43.7% were between 10 and 15 years, 19% were mixed breed whereas 81% were of a recognized breed. Diagnosis allowed to distinguish closed and open pyometra (based on the presence or absence of vaginal discharge). Of all patients 65.6% were affected by open pyometra. The most frequently described symptoms include vaginal discharge, depression and anorexia. Polyuria and polydipsia were present in 27.1% of cases. Creatinine concentration and BUN were increased in 19.3% and 51.8%, respectively. White blood cell count (WBC) was increased in 85.9% of cases and hypoalbuminemia was encountered in 80%. No difference in recovery time was noticed in cases of hypoalbuminemia. Creatinine levels were higher in closed pyometra (1.7±1.3 mg/dl) compared to open pyometra (1.2±1.4 mg/dl). Polyuria and polydipsia were not correlated to increased creatinine levels. Changes within hematologic and blood biochemistry exams improved after the resolution of the primary pathology. Although the interval between the last heat and presentation did not influence blood chemistry levels, a difference in WBC was observed with 27.9±14.5 (10^3/µl) and 21.4±9.7 (10^3/µl) in bitches with the last heat less than 1.5 months and more than 1.5 months prior to presentation, respectively. The majority of cases (76.7%), were treated with ovariohysterectomy combined with antibiotic treatment. Treatment with aglepristone in combination with antibiotics was used in 3 cases of closed and 9 cases of open pyometra. Creatinine levels were higher in animals treated with aglepristone (1.1±0.7) compared to animals treated with ovariohysterectomy (0.8±0.7). In 70% of cases ovariohysterectomy was performed after pharmacological treatment at owners request.
Changes in creatinine and BUN concentration were present, yet were not correlated to the presence of polyuria and polydipsia. Increased creatinine levels in closed pyometra may be due to delayed presentation at the VTH. The interval between the last heat and presentation at the VTH may have influenced WBC. Hypoalbuminemia was common most likely due to prolonged time of anorexia and losses due to the intrauterine accumulation of purulent material. Low albumin levels are considered a marker for recovery [2]; no such influence was observed in this population. Although pharmacological treatment was successful owners frequently opted for ovariohysterectomy after the resolution, most likely due to reduced risks of al elective surgery compared to an emergency intervention. None of the treated patients died due to the disorder, neither in the group of surgically treated patients nor in bitches treated conservatively.

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

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The Martina Franca donkey breed primarily inhabits the rural areas surrounding the eponymous municipality, as well as Mottola and Massafra in the province of Taranto. It is also bred in the neighboring municipalities such as Locorotondo in the province of Bari, and Cisternino, Ceglie Messapica and Ostuni in the province of Brindisi, all located in the Apulia region [1].
Typical characteristics in three-year-old Martina Franca donkey are: minimum height at the withers of at least 135 cm in males and 127 cm in females; a minimum chest circumference of 145 cm in males and 140 cm in females; minimum tibial circumference of 19 cm in males and 17 cm in females. The coat is dark bay (morello) with fur, while the abdomen, inner thighs and muzzle are grey. The snout and eye sockets have a flushed halo, while the tongue and nasal mucosa are pinkish. Anus, vulva, scrotum, and prepuce are dark, and the mane and tail are black [2, 3].
The primary objective of this study was to assess the current phenotype through various measurements, including height at withers, height of rump, height at tail attachment, trunk length, head length, width between auditory meatuses, width between temporal angles of eyes, width of cheeks, intermandibular distance, length of ears, width of chest, circumference of thorax, height of thorax, chest width, chest length, rump length, rump angle, front rump width, rear rump width, sternum-to-ground distance, shoulder length, shoulder angle, knee-to-ground distance, knee circumference, shin circumference, hock-to-ground distance, hock circumference, body weight, and the Body Condition Score (BCS).
This preliminary study also aims to identify the criteria for accurate reproductive selection through gamete conservation programs, with the secondary objective of preserving the typical characteristics of Martina Franca donkey stallions and mares. Despite recent population growth, the Martina Franca donkey still requires meticulous genetic management to mitigate inbreeding. The adoption of molecular characterization using genome-wide Single Nucleotide Polymorphisms (SNPs) offers numerous advantages. These include comparing genealogical-estimated inbreeding coefficients with genomic data, assessing the accuracy of genealogical relationships, determining genomic structure and diversity, and potentially developing a selective breeding program to prioritize desirable traits.
The study was conducted on 92 female and 15 male breeding animals from 8 different herds located in central and southern Italy. Measurements were taken using a tape measure, Hauptner's hippometer, and digital animal scales.
Upon comparing the measurement averages with those from Montanaro's 1930 study [2], statistical approach revealed significant variations. Notably, for females, there was a decrease in the width of auditory meatuses, sternum-to-ground distance, knee-to-ground distance, and body weight, while there was an increase in the width between temporal angles of the eyes, ear length, and thorax width. Conversely, males exhibited increases in trunk length, head length, thorax circumference, and various other measurements, alongside a decrease in sternum-to-ground distance and live weight.
In conclusion, our findings suggest that the phenotype of the Martina Franca donkey has been largely preserved over time. While no substantial differences were observed in female donkeys; male breeding stock displayed greater robustness, albeit with a decrease in weight across both sexes.

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Mammary fibroadenoma in a 3.5 month female cat

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Mammary tumours are common in female cats and occur mainly in cats aged between 10-12 years [1]. The rate of malignant neoplasms reaches even 95%, and they are usually represented by carcinomas. Although infrequent, benign mammary lesions have also been reported, mainly represented by fibroadenomatous change (FAC). FAC is a benign proliferation of the mammary ducts and mainly of the periductal connective tissue under progesterone dependence, occurring after puberty, during pregnancy or pseudopregnancy, or in animals, females, and males of any age, under probable improper use of progestins [2]. There is no clear evidence of the existence of FAC in dogs, in which similar lesions are classified as fibroadenomas. The feline fibroadenoma was present in the previous classification of World Health Organization, but, due to inconsistent cases, was removed. Recently, in a retrospective study [3], 9 archive cases of feline fibroadenoma were reclassified. Main features include single, well-circumscribed, uni- or multi-nodular mass that may affect one or rarely more mammary glands in the absence of hormonal sensitivity. This study reports a new clinical case of feline fibroadenoma observed in a 3-month-old female Maine Coon cat. She had not been treated with exogenous progestin and had been never observed in heat. Also, accidental expositions to drugs, steroids, o similar chemical compounds were excluded. The patient was admitted for a single, well-circumscribed, uni-nodular mass, 11x9 cm in size, at the left cranial thoracic mammary gland, which had increased in volume rapidly. Progesterone was 0.43 ng/ml. On ultrasonographic examination, the neoplasm appeared mainly as a well-circumscribed solid mass of granular texture, with anechoic areas in the parenchyma and outside the margins, consistent with peritumoural oedema. Cytologic examination revealed a population composed mainly of abundant spindloid mesenchymal cells. Despite basal value of circulating progesterone, a progesterone receptor antagonist was administered to block eventual progestin-like compunds. Aglepristone was administered at the dose of 15 mg/Kg with a 1, 2, 8, 15-day schedule, but no reduction was observed. Considering the reproductive value, ovariectomy was not allowed. Then, the lesion was approached with regional mastectomy. Samples were promptly fixed in 10% neutral buffered formalin and subjected to histopathological investigation. Microscopically, the lesion consisted of benign proliferation of ducts, tubules, and stromal cells. The neoplastic epithelial cells were arranged in elongated ducts that arborized into lobular units of small ducts and tubules. The stromal component was prevalent and divided into the interlobular and intralobular stroma, often oedematous or myxomatous. The pathological findings were consistent with FAC, however, considering the history, clinical manifestations, and lack of response to therapy, a diagnosis of fibroadenoma was made according to the recent reclassification paper [3]. Since the architectural and cytological features of the epithelial and stromal elements of the two lesions are comparable, the distinction can be based on morphological and clinical features, as well as the extent and delimitation of the lesion. Probably many cases reported in the literature as well-demarcated nodules affecting a single mammary gland and in young cats, whose diagnosis was FAC, should be reclassified as fibroadenomas. The distinction of the two entities impacts on the clinical approach. While aglepristone is the elective treatment for FAC, surgical excision is probably the only therapeutic option for fibroadenoma.

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Umbilical Coiling Index (UCI) in normal spontaneously delivered trotter foals

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The equine umbilical cord (UC) is characterized by a helical pattern of coils that make it a flexible and elastic structure able to resist external forces that could compromise blood flow. This particular structure makes it similar to the human UC (1).
The Umbilical Coiling Index (UCI) is obtained by dividing the total number of umbilical coils by the total length of the umbilical cord (cm) (2).
In a previous study, coils number and UCI in physiological equine pregnancies in Thoroughbred, Standardbred and Warmblood mares were described (3).
The aim of this study was to correlate the different characteristics of UC and the UCI with the foal's perinatal health in Italian Trotter horses.
Ten Italian trotter mares, aged between 8 and 21 years, were included in the study; for each mare, a form was prepared in which maternal factors such as age, number of previous births, gestation length, intrapartum events and neonatal factors were recorded.
At the time of foaling, the length of the UC and the number of coils were assessed and the UCI was calculated. Within five minutes after foaling, modified APGAR score for foals was evaluated.
UC total length was greater in males (81,67 ± 16,04 cm) than in females (68 ± 17,32 cm) and this result agrees with (3) Mariella et al. (2018), although with higher values in Italian Trotter horses than Thoroughbred, Standardbred and Warmblood mares. Gestation length was longer in mares that gave birth to female foals (345,85 ± 10,49 days) than in those that gave birth to male foals (332,67 ± 9,07 days).
No correlation with foal sex was observed as the number of coils and UCI. Furthermore, the total number of coils present on UC was lower (3,4 ± 0,84) than that found in the breeds studied by (3) Mariella et al. (2018) (5,33 ± 1). The APGAR score of the foals was between 8 and 14 and no correlation between UCI and APGAR score of foals was observed.
To validate these results, however, it will be necessary to implement the number of subjects included in the sample to understand whether the different parameters, assessed in the Italian trotter horse, confirm the data of (3) Mariella et al. (2018) or, as these preliminary results suggest, are actually different. This would allow a more complete analysis of the possible correlation between the morphological characteristics of the UC, the UCI and the APGAR score in foals. It would therefore be interesting to establish reference ranges for the Italian trotter breed to determine which values do not fall within the physiological range.

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FIELD OVARIECTOMY IN SOWS: APPLICATION OF AN INNOVATIVE SURGICAL TECHNIQUE

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The ovariectomy in the sow is a surgery performed to promote the improvement of weight gain (1), to avoid unwanted pregnancies and to eliminate a sensory defect in the meat known as "boar taint" (2). Right lateral approach laparotomy is the surgical technique commonly used to perform ovariectomy in the sow (1).
The aim of the present study was to perform the ovariectomy in sows comparing the standard technique with the affixing of traditional ligatures and the innovative technique, using a vessel dissection and coagulation device (Aesculap Caiman®).
The surgical times, the clinical parameters and the intra and post-operative complications were taken into consideration and the evaluation of the pain by the Piglet Grimace Scale (PGS) was performed.
The study (approved by the ethics committee with number 19/2022) involved 28 commercial hybrid nulliparous sows, aged between 4 and 10 months and weighing between 50 and 120 kg. The anesthesiological protocol provided for sedation with Azaperone 4mg/Kg administered intramuscularly deep (IM) in the retro-auricular region. After 15 minutes, general anesthesia was induced with ketamine 10mg/kg and detomidine hydrochloride 0,08 mg/kg, IM (3). The animals were randomly divided into 2 groups: Caiman Group (K) composed of 14 ovariectomized subjects using the Caiman® vasal dissection and coagulation device and Control Group (C) composed of 14 ovariectomized subjects, using the standard technique, with the affixing of traditional ligatures. The sows were positioned in left lateral decubitus and the surgical site was sterilized by surgical scrub. The flank approach was performed according to the technique described by Debbarma (2019) and always by the same surgeon. In Group K subjects, the Caiman forceps were placed at the base of the ovary, at the level of the ovarian pedicle, thus performing the ovariectomy. In Group C subjects, a single pass-through ligation was performed on the ovarian pedicle, using a synthetic-absorbable USP 2 thread. To exteriorize the left ovary, the uterine horn was followed up to the bifurcation and the contralateral horn was highlighted, resulting in the gonad being visible. The same procedure on the right ovary was repeated on the left ovary. Antibiotic and anti-inflammatory coverage for the post-operative period were administered. Regarding the duration of surgery, a significant difference was found between the two study groups. In Group C, in fact, the average surgery time was 25.80 ± 3.79 minutes, whereas in Group K, was 21.11 ± 5.56 minutes. The shorter duration of surgery led to a reduction in anaesthesia time and, therefore, a reduction in the occurrence of anaesthesia-related complications. In the evaluation of pre, intra and post-operative parameters, no statistically significant differences were observed between the two groups. The results of this study have shown that ovariectomy in the sow, performed with the Caiman® device, is a safe and fast technique, very important characteristics for field interventions.

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Comparative study of the reproductive tract echotexture in buck and alpaca

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The study took into consideration a number of 40 angora bucks and 40 alpaca males raised in Italy and Turkey. The aim of the study was to identify scales of tissue echogenicity in the different tracts of the male reproductive system (MRT) in buck (B) and in alpaca (A). The echogenic variability of the glandular structures in the B and in A is analyzed via Pixel Gray Intensity (PGI) analysis with the aim of identifying an echogenicity scale of each individual ultrasound Region of Interest (ROI). All the subjects were included in monitoring programs and underwent andrological examination including semen analyses. The study took into consideration the ultrasound images collected from each individual subject and which take into account the testicles (TEST), epididymis (EPI), deferential ampullae (DA), seminal vesicles (SV), prostate (PROST) and bulbourethral glands (BUG) as ROI. Statistical analysis took in consideration a correlation indexes calculation (Pearson's correlation indexes) among all the ROIs intraspecies and a one-way ANOVA interspecies considering the TEST, EPI, DA and BUG as depended variables and comparable ROIs. Within the data regarding alpaca males, a two-way ANOVA was performed considering season (high vs low temperature) and TEST cystic degeneration. The highest values were found in the comparison between the ROIs of the right testicle and the right epididymis. Positive correlations were also found in both seminal vesicles with the left mediastinum and the right testicular parenchyma, but no echogenic correlation of the bulbourethral gland was found with the other ROIs analyzed. The sperm density generated an excellent correlation value with the seminal vesicles in B. Positive correlations (>0.6 with $P<0.05$) and a positive result between BUG-PGI and TEST-PGI, TEST-PGI and semen concentration and volume, finally between SV-PGI and TEST-PGI. BUG did not give any correlation results with any ROI considered except for seminal density, probably caused by the secretions of the gland released into the urethra. The study of alpaca eco-texture took into consideration in comparative terms the PGI of comparable tissues such as testicles and bulbourethral glands. The absence of seminal vesicles and the presence of a species-specific prostate are the main differences. A PGI-based rating scale of eco-texture for pathological (cystic degeneration) or environmental (high and low ambient temperature) conditions. The hypo-echoeogenicity of hyperplastic glandular tissues up to the hyper-echogenicity of basically sclerotic areas are the most similar information found in the comparative study.

SOFIVET

77° CONVEGNO SISVET

Stato: INVIATO - ID: 13069

DOES INTERLEUKIN 1BETA CONTRIBUTE TO SUCCESSFUL MEMORY AGING IN SENIOR CATS?

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Aging is a multifaceted process characterized by the progressive decline of physiological functions. Much of the aging phenotype can be attributed to increased levels of circulating proinflammatory cytokines, such as interleukin-1β (IL-1β), which contribute to chronic, low-grade inflammation, known as inflammaging [1]. This state is associated with age-related cognitive decline and memory disorders in humans. While IL-1β is traditionally seen as detrimental to cognitive function, there are reports suggesting neutral or even positive effects of IL-1β on learning and memory in both humans and small rodent models, including older adults [2]. Yet, this relationship has not been systematically explored in cats. In this multicentric study, we assessed 47 privately-owned senior cats (median age = 9 years, range = 7–15 years; 24 females, all spayed and of various breeds), free from clinical signs of disease. They underwent a short-term spatial memory test, previously identified as potentially predictive of cognitive decline in senior cats [3]. Each cat was presented with 1 baited out of 5 identical plastic containers, for which they had to recall the location after a 30-second break.The test was conducted consecutively for each container in a pseudo-random sequence. Additionally, cognitive function and behavior were evaluated, and standard complete blood count (CBC), biochemistry, and serum cytokine measurements were performed. The study was approved by the University of Pennsylvania's Institutional Animal Care and Use Committee (IACUC protocol 807030). A positive correlation emerged between the cats' performance on the memory test and their IL-1β serum levels (Spearman's $\rho = 0.4$, $p = 0.007$). Conversely, a negative correlation was observed between the cats' age and both their memory test performance and IL-1β serum concentrations (Spearman's $\rho = -0.4$, $p = 0.013$ for both). Essentially, the youngest cats in our sample exhibited superior performance in spatial memory tests and higher cytokine levels, with these elevated levels being positively linked to better memory test scores. Our findings suggest that higher IL-1β concentrations might support memory performance in healthy senior cats. Further research, embracing a more extensive range of variables, is in progress to elucidate the role of IL-1β in neurocognitive aging in felines.

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77° CONVEGNO SISVET

SOCIETA´ SCIENTIFICA DI RIFERIMENTO **SO.FI.VET**

TITOLO **Evaluation of blood levels of tau 181 protein in the Arabian horse: preliminary results**

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Testo e Riferimenti bibliografici

Extending the lifespan of a pet poses challenges related to brain aging, similar to those described in elderly people with Alzheimer's disease. In horses, a gradual increase in lifespan occurs. Some subjects, bred for affection or for sporting activity, are managed with care and are not used for food purposes.
While indicators of brain aging such as amyloid beta and tau protein in the blood of dogs have long been demonstrated, the biochemical aspect in horses remains unexplored. Tau protein (pTau) is a highly soluble protein found in neurons. Under physiological conditions, it plays a crucial role in maintaining the proper functioning of neurons and the brain by promoting the formation and stabilization of cellular microtubules. However, during tauopathies or other neurodegenerative diseases such as Alzheimer's, abnormal hyperphosphorylation and subsequent aggregation of pTau occurs. Pathological pTau can be hyperphosphorylated at multiple epitopes, including amino acid 181. Its altered and insoluble form is aggregated and deposited in neurofibrillary tangles formed by paired helical filaments, which are one of the main anatomopathological signs of Alzheimer's disease.
The aim of this research was to investigate the presence of hyperphosphorylated pTau 181 in the serum of horses clinically healty and without any evidence of cognitive impairment. For this purpose, a cohort of 30 Arabian horses was selected based on breed and age (Ethical committee authorization n°: 24/2024). A blood sample (collected during routine clinic examination) was drawn from each subject to determine serum pTau 181 concentrations using a horse species-specific sandwich ELISA kit (Horse Phosphorylated Tau 181, MyBioSource, Inc. San Diego, USA). The tested subjects were distributed into three age groups: Young (1-5 years; N=8); Adult (6-15 years; N=16); and Elderly (16-28 years; N=6). The mean pTau 181 values (± SD) were as follows: Young group 34.01 ± 12.86 pg/ml, Adult group 34.39 ± 21.43 pg/ml, Elderly group 39.69 ± 12.14 pg /ml. The statistical analysis, carried out using the non-parametric Kruskall-Wallis test, did not show statistically significant differences, however the trend towards an increase in pTau 181 content with increasing age was evident. The results of this preliminary study are in line with existing literature in humans. Numerous studies have been conducted in human medicine to identify plasma biomarkers useful for the early diagnosis of neurodegenerative diseases. Among these, pTau phosphorylated in different residues has been studied and a strong correlation emerged between their plasma concentration and cognitive dysfunction (1). Another interesting working hypothesis involves testing in the equine species, using a species-specific kit, of two plasma markers of neurodegenerative diseases previously validated in dogs: β-amyloid40 and β-amyloid42. In dogs, the score obtained from the Behavioral Scorecard for Aging, known as “CADES”, was correlated with the concentrations of β-amyloid40 and β-amyloid42. For both forms of β-amyloid, a negative correlation compatible with the intracerebral accumulation of the substance during neurodegenerative diseases was observed (2). Further studies are necessary to obtain scientific evidence to support the hypothesis of using pTau and the two forms of β-amyloid as biomarkers of neurodegenerative pathology and accelerate the possible use of therapies.
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77° CONVEGNO SISVET

SOCIETA´ SCIENTIFICA DI RIFERIMENTO **SOFIVET**

TITOLO **SEASONAL HEAT STRESS EFFECTS ON THE OVULATION PROCESS IN DAIRY COWS**

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Testo e Riferimenti bibliografici

In temperate areas, heat stress (HS) is a major factor impairing reproduction in animals, particularly in dairy cattle [1].
The objectives of this study were to: (a) gain information regarding the influence of HS prior to and during the day of artificial insemination (AI) on the rate of ovulation in primiparous cows showing spontaneous estrus in a herd without cooling systems, (b) assess the incidence of delayed ovulation, and (c) evaluate the influence of HS on the double ovulation rate.
During the study period, the mean maximum temperature-humidity index (THI) at AI was 78.1 units. This index on the day of AI was registered in 90 (96.8%) of the 93 days of the study period from a meteorological station located less than 500 meters away of the herd.
Ovulation was measured using ultrasound 7-13 days after AI. The number and location of corpora lutea (CL) were recorded at this time. A high pixel intensity was associated with a young CL [2]. Delayed ovulation was defined as the presence of one young CL 7 to 13 days post-AI.

Of the 274 cows included in the study, 51 (18.6%) and 55 (20.1%) experienced ovulation failure and delayed ovulation, respectively. The maximum THI at AI was a factor among the various factors we investigated influencing the delayed ovulation rate with an odds ratio of 1.1 (P = 0.003).
The present findings suggest that cows' ovarian function could be considered normal in cows reaching ovulation at the end of estrus despite severe HS conditions. Probably these cows were the most fertile on the herd. The only variables that contributed to double ovulation were days in milk (DIM) and photoperiod, reinforcing previous results [3] that climate variables had no influence on the incidence of double ovulation (12.8%) and that ovarian function was considered as normal in cows reaching ovulation at the end of estrus.
Special clinical relevance acquires the strong relationship between an increased THI at AI and the occurrence of delayed ovulation. These results suggest that application of an inductor of ovulation at AI should reduce the incidence of both ovulation failure and delayed ovulation.

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77° CONVEGNO SISVET

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mTOR is an essential gate in adapting the functional response of ovine trophoblast cells under stress-inducing environments

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During the early stage of pregnancy in sheep (Day 16-23 of pregnancy) normal embryo development depends on placenta functionality. As immature placentation, vascularization is still insufficient to provide adequate nourishment [1]. It means the placenta physiologically copes with a suboptimal environment, thus trophoblast cells adopt adaptive strategies for supporting embryo growth. Autophagy is an intracellular degradation process promoting cell survival in response to stressful conditions, such as nutrient and oxygen deprivation. Autophagy is mainly regulated by the mechanistic target of rapamycin (mTOR), which is also known as a placental nutrient sensor [2]. Here, we tested the hypothesis that trophoblast cells may adapt to adverse conditions through an mTOR-dependent regulation of cell survival. Therefore, the main aim was to shed light on how mTOR drives placenta adaptive response to low-nutrient environments.
A previously characterized in vitro model of 21-day-old primary sheep trophoblast cells (oTCs) was employed [3]. oTCs were cultured in a normal environment (nutrient-enriched DMEM/F12, CTR), then subjected to 24-h mTOR inhibitor (100nM rapamycin, RAPA) and low-nutrient conditions (MEM, STARV). Gene and protein profiles were explored to assess autophagy modulation from the beginning to the end of the process, including mTOR activation, autophagic markers mRNA expression (ATGs), and autophagosome detection (LC3BI-II). Moreover, cell motility (wound healing assay) and the expression of solute carriers' genes (SLCs) on treated-oTCs were studied to evaluate whether mTOR activation/suppression affects trophoblast functionality.
Autophagy activation was confirmed both in rapamycin-treated and low-nutrient conditions by LC3BII higher expression compared to the normal environment. However, mTOR activation seems to be severely modified only following rapamycin treatment whereas prolonged starvation allowed mTOR reactivation. Nutrient deprivation promoted trophoblast bi-dimensional movement, on the contrary, mTOR-inhibition reversed cellular functionality. Furthermore, mRNA expression of amino acids transporters remains largely undisturbed except for SLC43A2 and SLC38A4 which are downregulated in starved and rapamycin-treated cells, respectively.Present findings suggest that mTOR inhibition affects placenta adaptation to suboptimal environments. Despite this, sheep trophoblast cells display uncommon responses to pro-autophagic stimuli. A low-nutrient availability stimulates placenta functionality, instead of preventing it. Indeed, we observed that prolonged starvation induces mTOR reactivation in an autophagy-dependent manner. In conclusion, sheep trophoblast cells may adapt to adverse conditions in the early stage of placentation by balancing, in an mTOR-dependent manner, nutrient recycling and transport with relevant effects for in vitro functional properties, which could potentially impact conceptus development and survival.

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77° CONVEGNO SISVET

Stato: INVIATO - ID: 13367

Expression of Behavioural Traits in Bernese Mountain Dog

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The Bernese Mountain Dog (BMD) is a breed selected in Switzerland in the early 1900s and was used as a farm guard dog, cart puller and cattle dog. Today, the relationship between popularity of the dog breed and the breed-typical behaviour could change for selection pressure (1).
The purpose of this study is to describe the behavioural characteristics of this breed in Italy.The Club Italiano Amatori Bovari Svizzeri (CIABS), the Italian breed club, created an online survey based on the adaptation of the Canine Behavioural Assessment and Research Questionnaire (c-BARQ) (2). The survey results were used to analyse breed differences in these representative behavioural trait scores: Stranger-directed aggression, Owner-directed aggression, non-social fear, dog-directed aggression, touch sensitivity, separation-related behaviour, attachment/attention-seeking, chewing inappropriate objects.
Over 12 months, from March 2023 to March 2024, the survey collected 792 responses from owners, of which 46.9% (n=371) owned males and 53.1% (421) owned females. The BMD appear to be sociable, calm, gentle, and affectionate with family members (48.9%), with guests (44.19%), with people outside the home (44.3%) and with dogs outside the home (43.9%). Forty-eight percent of BMDs alert and bark at strange objects/noises, inside and outside the home.
Unlike what is reported by Van der Velden (3), our results show that the BMD seems to be a reliable breed for family life and management, using barking as a means of communication and showing no signs of aggression. More studies are needed to deepen in our results.

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77° CONVEGNO SISVET

SOCIETA´ SCIENTIFICA DI RIFERIMENTO **SOFIVET**

TITOLO **Use of hair in the analysis of the steroid profile of wild species: the case study of the Apennine wolf**

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Testo e Riferimenti bibliografici

The Grey Wolf (Canis lupus), once common in the northern hemisphere, faced decline and extermination in various regions during 18th and 19th centuries. Variability in physical traits led to the discovery of numerous subspecies, including the italian wolf population (Canis lupus italicus) [1]. In the last 40 years Italian wolves have begun to recolonize parts of their historic range. This growth in population can lead to potential conflicts with human activities, the main cause of wolf mortality. In order to ensure long-term conservation, it is important to monitor population parameters such as distribution and abundance. Wolves have been observed in more populated areas, resulting in livestock depredation and collisions with vehicles. It is crucial to implement appropriate management measures to ensure the conservation of Italian wolf population [2]. Hormones are indicators of various physiological states: glucocorticoids allow to examine stress and animal welfare, whereas sex steroids are useful to evaluate the health status of reproductive tissues, necessary for the growth and maintenance of secondary sexual characteristics.
Measuring their concentration would allow to assess the activity of the hypothalamic-pituitary-adrenal (HPA) and hypothalamic-pituitary-gonadal (HPG) axes [3].
The study aimed to analyze the steroid profile of Apennine wolves using hair as non-invasive matrix. The concentrations of cortisol, testosterone, Dehydroepiandrosterone (DHEA), progesterone, and estradiol, from 20 specimens deceased in Emilia-Romagna and Tuscany regions and deposited at the Department of Medical and Veterinary Sciences of the University of Bologna, were measured using radioimmunoassay techniques, upon methanol extraction. Antibodies cross reactivity, as well as intra variation coefficient, were calculated. For the research purposes, hair was shaven for its entire length from the rump area (20x20cm).
This novel approach that had not been previously explored in understanding the reproductive and corticosurrenal endocrine axes of the Apennine wolf, has made possible to quantify all hormones subject of this study.
The statistical analysis, listed as mean ± SD, shows for cortisol, a mean concentration of 1.81 (±1.17) pg/mg. For testosterone, the mean concentration is 2.74 (±1.6) pg/mg. Instead DHEA 62.92 (±24.99) pg/mg, P4 53.22 (±32.8) pg/mg. Finally, E2 presents a mean concentration of 0.46 (±0.23) pg/mg. Comparing the levels of various hormones categorized by sex and age there aren't statistically significant differences. This could be also partially due to the small numbers of specimens provided and moreover a low frequency of adult individuals (35%) and a high frequency of sexually immature individuals (65%), since mortality due to collisions with vehicles mainly involves pups and juveniles. Although the study presents limitations, highlights the potential for non-invasive matrices in studying endocrinology of wild species.
Further studies with larger sample sizes is necessary to analyze hormone trends throughout the year and explore differences based on factors such as sex, age, and causes of death. These studies could be valuable for wildlife monitoring efforts, providing insights into the health and responses of wolves to environmental changes and human interactions.

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77° CONVEGNO SISVET

SOCIETA´ SCIENTIFICA DI RIFERIMENTO **SOFIVET**

TITOLO **COMPARISON OF MITOCHONDRIAL BIOENERGETICS OF MITOCHODRIA ISOLATED FROM TUMOUR TISSUE AND A 3D CELL CULTURE SYSTEM: PRELIMINARY RESULTS**

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Testo e Riferimenti bibliografici

Mitochondria are the metabolic fulcrum of the proliferation, survival and metastasis of tumour cells thanks to their bioenergetic and biosynthetic functions. It is well known that they can contribute to cellular malignant transformation through several mechanisms such as the production of mitochondrial reactive oxygen species (ROS) and functional deficiency in mitochondrial permeability transition (MPT) [1]. The focus of this research was on *i*) the MPT event regulated by the permeability transition pore (PTP) formation inducing cell death, and *ii*) mitochondrial oxidative metabolism evaluation. The studies were developed on isolated mitochondria from primary tumour and VITVO of canine thyroid cancer or hepatic hemangiosarcoma, characterized by hypoxic or highly blood permeated environment, respectively, evaluating the PTP event in the former and mitochondrial respiration in the latter. Our group has developed expertise in producing 3D primary cell cultures with the VITVO devices, a bioreactor for creating 3D models of cell cultures. Our data demonstrate 3D primary culture grown in the VITVO device to share phenotypic and genotypic similarities with the primary tumour but with low amount of stromal cells allowing to obtain a substrate rich in neoplastic cells [2]. In addition to this, the hypothesis that mitochondrial Ca^{2+}-activated F_1F_O-ATPase forms PTP can provide clues as a possible target of the MPT phenomenon in tumorigenesis [3]. Therefore, we aim to evaluate the mitochondrial F_1F_O-ATPase hydrolytic activity, differently activated by the natural cofactor Mg^{2+} or Ca^{2+}, which is an indirect index of the PTP formation. Mitochondria were isolated by fractional centrifugation after having chopped and homogenized the tissue or VITVO matrix of canine thyroid cancer. The results obtained show that Ca^{2+}-activated F_1F_O-ATPase activity is 53% lower than Mg^{2+}-activated F_1F_O-ATPase, and although this behaviour is also maintained in VITVO, in the latter Ca^{2+}-activated F_1F_O-ATPase activity decreases by 95.4% than Mg^{2+}-activated F_1F_O-ATPase. The result showed a low propensity to PTP formation and the desensitizing effect is more highlighted in VITVO than by the primary tumour. In canine hepatic hemangiosarcoma, a tumour highly permeated by blood, whose cells show a highly oxidative metabolic pathway, a method of evaluating mitochondrial respiration was developed by studying the activity of the electron transport chain respiratory complexes. Data suggest that complex I activity is reduced than complex II and complex IV, although in isolated mitochondria from VITVO the overall activity of the different complexes is greater than that detected in primary tumour. The results of this preliminary investigation highlight the results obtained in the 3D systems as suggestive of those obtained in primary tissue and allow us to identify any differences in mitochondrial activity and indirectly correlate them to PTP formation in tumours.

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77° CONVEGNO SISVET

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The Crucial Dynamics of Lipid Droplets in Early Ovine Embryonic Development

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Our research has unveiled a significant aspect of lipid metabolism of pre-implantation sheep embryonic development. The presence, maintenance, and utilization of lipid droplets (LDs) were described as crucial in peri-implantation events [1]. However, their specific function during pre-implantation has remained elusive despite their abundance in early embryos, particularly in farm animals [2]. In our study, we found that in vitro fertilized (IVF) sheep zygotes, when subjected to mechanical delipidation by micromanipulation (DEL), developed into blastocysts at a significantly lower rate than the control group (CTR) (5/39 (12.82%) for DEL vs 16/39 (41.03%) of the control (CTR), P=0.0097, Fisher's exact test). Despite the lower blastocyst rate, we observed that delipidated zygotes could rescue the removal of lipid droplets, which showed no difference compared to the control when stained at the blastocyst stage with BIODIPY (Fluorescent signal: 0.92 in DEL fold change over CTR; P>0.05, one-way ANOVA). Our interest focused on determining the metabolic source from which the reformation of LDs originated. Therefore, we treated the in vitro produced embryos with inhibitors of ATP citrate lyase (ACLY) and Acetyl-CoA synthetase (ACSS2), enzymes crucial for cytoplasmic acetyl-CoA production, the upstream source for De Novo Lipogenesis (DNL). However, the inhibition of these enzymes did not significantly impact blastocyst development (6/32 (18.72%) of inhibited_DEL vs 5/39 (12.8%) of DEL, P>0.05, Fisher's exact test) and the rescued DNL (BODIPY fluorescent signal: 0.4995 in inhibited_DEL fold change over DEL, P>0.05). Thus, it suggests the reformation of LDs post-delipidation is derived downstream in the DNL pathway directly from the free fatty acids stored in the oocyte cytoplasm. We will further investigate this hypothesis in future studies. In conclusion, we have identified a delicate balance between LD utilization and renewal that is crucial for proper sheep embryonic development during the pre-implantation stages. Our future focus will be exploring the impact of the balance between free fatty acids and stored LDs on development. Our belief is that understanding this metabolic mechanism during early embryo development is crucial information to enhance embryo development in vitro.

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77° CONVEGNO SISVET

SOCIETA´ SCIENTIFICA DI RIFERIMENTO **SOFIVET**

TITOLO **THE ROLE OF PBMCS IN THE PATHOPHYSIOLOGY OF MYXOMATOUS MITRAL VALVE DEGENERATION IN DOGS BY MITOCHONDRIAL BIOENERGETIC EVALUATION**

Autori Patrycja Anna Glogowski1, Silvia Granata2, Chiara Bernardini1, Giovanni Romito1, Cristina Algieri1, Roberta Salaroli1, Antonia Cugliari1, Fabiana Trombetti1, Micaela Fabbri1, Augusta Zannoni1, Salvatore Nesci1

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Testo e Riferimenti bibliografici

Myxomatous mitral valve degeneration (MMVD) represents the most common canine heart disease and a common cause of death in this specie, primarily due to left-sided congestive heart failure [1]. It is known that cardiomyocytes contain an abundant number of mitochondria. Consequently, cardiovascular diseases are often associated with mitochondrial dysfunction, which results in energy deficits and metabolic abnormalities. A better comprehension of its physiopathology is still needed even though recent studies have focused on the mitochondrial respiration of peripheral blood circulating cells (PBMCs) shedding light on the relevance of inflammation and the potential role of mitochondrial impairment in circulating cells. Moreover, some studies have highlighted the importance of the expression of genes in peripheral blood nuclear cells that could be used for studies related to different stages of heart disease in dogs with CMVD, whereas it could be used as a non-invasive diagnostic method [3]. Researches have demonstrated that human PBMCs' mitochondrial impairment has been associated with the development manifestation of cardiac failure [2]. This is why our investigation aims to understand the bioenergetic profile and role of PBMCs in MMVD, studying if there are significant differences in isolated PBMCs from healthy dogs, which have been considered 5 French Bulldog, 1 Boxer, 1 Bernese Mountain Dog and dogs with the disease, which were 1 Cavalier King charles spaniel and 2 half-breed dog at different stages of disease. Thus, it might be possible to identify new therapeutic strategies. The bioenergetics of PBMCs were assessed using the Agilent Seahorse XFp analyzer, specifically, we evaluated ATP generation, cell respiration and related mitochondrial parameters. We evaluated the viability of canine PBMC through flow cytometry. Interestingly, we detected a drastic decrease (about 70%) in mitochondrial ATP production in dogs with MMVD without noticing a significant difference in glycolytic metabolism. Normally, PBMCs metabolism in healthy dogs is aerobic while in dogs with MMVD the aerobic state changes and becomes quiescent. Furthermore, it has been noticed that the mitochondrial metabolic profile decreased in dogs with MMVD; the bioenergetic parameters of basal respiration, maximal respiration, and ATP production were decreased by about 70%, 80%, and 75%, respectively. On balance, MMVD is characterized by mitochondrial dysfunction causing a metabolic rewiring of energy metabolism due to a low level of ATP production and mitochondrial respiration.

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77° CONVEGNO SISVET

SOCIETA´ SCIENTIFICA DI RIFERIMENTO **SOFIVET**

TITOLO **Does “silly” diet modulate endocrine pathways?**

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The common forms of obesity often origin in childhood. Dietary mistakes during the prepubertal period can be particularly dangerous with effects that may extend over the course of a lifetime. A food overload over long periods leads to a breakdown in the regulatory mechanisms of the hypothalamic-pituitary-adrenal (HPA) and hypothalamic-pituitary-gonadal (HPG) axes with consequent endocrine imbalances and reproductive disorders [1]. Recent studies focused on food bioactive compounds to counteract obesity that may represent an attractive approach to be considered in this pathology [2]. Silymarin is a substance naturally present in foods and plants, and is characterized by antioxidant, anti-inflammatory and hepatoprotective properties that may be useful to be included in strategies to prevent/revert the effect of a high fat and high carbohydrate (HFHC) diet [3]. Hair could provide retrospective information about the interaction between diet and steroidogenesis over long periods of time. Hence, aim of this study was to investigate the hair concentrations of adrenal steroids in in vivo mouse model of juvenile obesity fed control diet (CTRL), obesogenic diet (HFHC) and nutraceutical supplementation with silymarin (270 mg/kg) (HFHC+SIL). The experimental protocol was approved by the National Authority (Ministero della Salute, Italy, Approval N° 11072013). Juvenile mice (42 females and 45 males) were used as model of diet-induced obesity. Hair samples were collected at 4, 8 and 20 weeks of treatment from the prepubertal to the pubertal period and the hormones were measured by radioimmunoassay. Specifically, the experimentation started after weaning and the animals were divided in two groups, one fed CTRL diet and the other HFHC diet for a total of 8 weeks. After the 8 weeks of treatment, half of the HFHC group continued with the HFHC diet whereas the others received the HFHC diet added with silymarin (HFHC+SIL). The CTRL group kept the CTRL diet. At every checkpoint the animals fed HFHC and HFHC+SIL diets showed higher live weights than animals of the CTRL group ($P<0.01$). As regards the endocrine status linked to the two diets, after 4 weeks of treatment the HFHC diet interfered with the HPA axis in mice resulting in a significantly higher cortisol/DHEA ratio ($P<0.01$) than the CTRL group. Also, the two groups tended to differ ($p=0.06$) at 8 weeks of treatment. After 20 weeks of treatment the cortisol concentrations were higher in the HFHC+SIL group than the CTRL group ($P\leq0.05$). DHEA-S concentrations were higher in the HFHC group than the CTRL ($P<0.01$), whereas the cortisol/DHEA-S ratio was lower in HFHC than both CTRL and HFHC+SIL groups ($P\leq0.05$). The data obtained on hair with an obese mouse model, along with those described on plasma [4], add information about long-term effects of an obesogenic diet on steroid hormones concentrations. Our results show as the supplementation with silymarin to a HFHC diet could be a tool to modulate endocrine pathways by reducing DHEA-S concentrations and improving the cortisol/DHEA-S ratio. Indeed, several studies show an association between adiposity and DHEA-S. To the authors’ knowledge, this is the first report about the effect of an obesogenic diet supplemented with nutraceuticals on hair steroid hormone concentrations in prepubertal-pubertal mice.

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77° CONVEGNO SISVET

SOCIETA´ SCIENTIFICA DI RIFERIMENTO **SOFIVET**

TITOLO **PBMCs mitochondrial metabolism characterization of dairy cattle at different lactations**

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Testo e Riferimenti bibliografici

Dairy cattle present different milk production throughout their life, consequently having various energetic and nutrient demands as it has been observed in specific tissues (mammary gland, liver, skeletal muscles, adipose tissue)1. So far, no research has been done on peripheral blood mononucleate cells (PBMCs). PBMCs are involved in inflammation and their mitochondrial respiratory function is easily available2.
The research aim is to investigate variations in energy requirements of PBMCs as dairy cattle progress at different lactation. Moreover, a comparison of these data was carried out between cows with poor and normal milk production to evaluate whether there is a difference in the metabolism of their PBMCs. 12 cows with parity between 2 and 6 and within 60 and 90 days in milk (DIM) were involved in the study and divided in 2 productivity groups (high-productive and low-productive). The median daily milk yield (53 kg/day) recorded in the herd among all multiparous cows with the same lactation length was used to classify cows in the two groups. PBMCs were isolated by density gradient centrifugation using Ficoll-Paque from blood samples collected by coccygeal vein. Subsequently, PBMCs were seeded in XFp PDL miniplates to run the Cell Mito Stress Test on the Agilent Seahorse XFp analyzer, which allows to record cells' response to mitochondrial stress simultaneously measuring the oxygen consumption rate (OCR), the cellular respiration, and extracellular acidification rate (ECAR), an index of glycolysis, in basal conditions using the modulators oligomycin, FCCP, and rotenone/antimycin. Results were normalized on cells' viability obtained by flow cytometry and analyzed considering parity and productivity group.
Our preliminary findings show that PBMCs isolated from animals with normal milk production generate higher levels of ATP than those with reduced milk production. Furthermore, we discovered that dairy cattle adjust better to energy demands with the increased lactation number, boosting all mitochondrial parameters.
In conclusion, our investigations indicate that dairy cattle's reduced milk production might be linked to an impairment in their capacity to respond to ATP requirements, and that mitochondria perform better in multiparous cows. Our findings are crucial in discovering new metabolic biomarkers that can provide insight into the overall health and well-being of cattle, specifically through non-invasive analysis. This information is valuable for improving livestock health and welfare practices.

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77° CONVEGNO SISVET

SOCIETA´ SCIENTIFICA DI RIFERIMENTO **SOFIVET**

TITOLO **MONITORING OF PRO-INFLAMMATORY REACTION IN ATHLETE HORSES DURING ROAD TRANSPORT**

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Testo e Riferimenti bibliografici

The horse can be considered among the best athletes for this reason are often transported to the competition field. Transport could treat homeostasis and could activate the inflammatory cascade in transported animals [1],[2]. In view of such considerations, the inflammatory response of athlete horses transported by road was investigated by monitoring the dynamic change of serum concentration of some pro-inflammatory interleukins (i.e. Il-1α, Il-1β, IL-2, IL-6). In the present study, 10 horses were enrolled. The horses took part in an outdoor jumping competition spent at the “ADIM Center” located in Augusta SR, Sicily. Transport 1 consisted of moving the horses from the training center (Messina) to the competition venue; after 3 days of competition, horse took the same route back to the training center (transport 2). Blood samples were collected from each horses before (Pre) the beginning of transport events (transport 1 and 2), within 5 minutes (Post) and after 1 hour (Post 1h) from the end of both transport 1 and 2. On blood samples the assessment of interleukins concentration was performed. The concentration of interleukins 1α, 1β, 2 and 6 (IL-1α, IL-1β, IL-2, IL-6) was assessed through enzyme-linked immunosorbent assay kits specific for equine species (Equine IL-1alpha ELISA Kit, RayBio®, sensitivity 0.82 pg/ml; Equine IL-1beta ELISA kit, sensitivity 3.5 pg/ml; RayBio®, Equine IL-2 ELISA kit, RayBio®, sensitivity 0.2 pg/ml; Equine IL-6 ELISA Kit, RayBio®, sensitivity 1.4 pg/ml) by means of a micro-well plate reader (Sirio, SEAC, Florence, Italy). All calibrators and samples were run in duplicate, and samples exhibited parallel displacement to the standard curve for each ELISA analysis.
According to the results gathered in the current study, the serum concentration of IL-1α decreased at Post and Post 1h compared to the values obtained at rest condition ($P < 0.05$). The other pro-inflammatory interleukins analysed (i.e. IL-1β, IL-2 and IL-6) showed increased levels at Post than Rest and Post 1h in transport 1 ($P < 0.05$). The mentioned dynamic changes in interleukins may be deputed to the animal's initial response to the stressful stimulus. Specifically, IL-1α acts as an inductor of IL-1β which is secreted in its non-active form (as pro-IL-1β) and its activation in IL-1β occurs with the onset of inflammation leading to the activation of the other pro-inflammatory interleukins. According to the results obtained in the present study, the return trip after the completion of the races (transport 2) did not show a statistically significant influence on the investigated parameters ($P > 0.05$). Despite this, differences on the serum concentration of some interleukins herein investigated were found between transport 1 and 2. Specifically, higher levels of IL-1α at Pre and higher IL-1β, IL-2 and IL-6 values at Post were found in transport 1 than transport 2 ($P < 0.05$). It must be taken into account that the investigated horses performed three-consecutive days of jumping competition before they underwent the transport 2, therefore, it could be hypothesized that physical exercise could have attenuated the inflammatory state of horses through the production of anti-inflammatory interleukins likely to occur following exercise [1].

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77° CONVEGNO SISVET

Stato: INVIATO - ID: 13606

A porcine model of abdominal aortic aneurysm: validation of feasibility

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Abdominal aortic aneurysms (AAA) lead to death up to 150000 people worldwide every year [1]. For larger AAAs or those at risk of rupture, surgery is the only option, using prosthesis to replace the iliac arteries and the aorta. Such approach requires intense follow up and is not feasible in all patients, and no therapies are currently available to prevent aneurysmatic degeneration once an initial dilation has occurred. Porcine models of cardiovascular disease are highly acknowledged due to morpho-functional analogies with the human species, and related literature is steadily increasing. The goal of the project was to recreate and characterize a novel porcine in vivo model of infrarenal AAA obtained by combining pharmacological and surgical approaches. The model relies on a surgical balloon injury alongside daily administration of β-aminopropionitrile (BAPN), a molecule typically found in plants of the genus Lathyrus and known to induce AAA in the human species [2]. This compound is indeed capable of inhibiting the action of the enzyme lysyl-oxidase, responsible for collagen cross-linking in the extracellular matrix of the aortic wall [3]. Four intact three-years-old Göttingen minipig boars were enrolled in the study. The decision to only use intact males comes from literature, that shows more severe scenarios of AAA in men than women. Due to the behavioral features of adult boars, particular attention was given to the training and habituation to human contact of these animals, that started month before. Animals were let out of their box every day and were rewarded sweets and juice upon positive interaction with the operator. This training routine allowed the boars to maintain a very cooperative and docile attitude throughout the entire trial. Animals were housed in individual slatted boxes and fed once a day with yogurt and fruit juice in addition to the appropriate feed with ad libitum water. Environmental enrichments were provided such as hanging chains, plastic toys and balls, daily rotated to avoid boredom. BAPN administration started 7 days before surgery to finish after 28 days, at the daily dose of 0.15 g/kg. Different attempts were made trying to find the best way to feed the animals BAPN, always considering individual preferences; this contributed significantly to the refinement of the model. On the day of surgery, transabdominal ultrasound scans were performed to collect aortic diameters, and then pigs underwent physical AAA induction through balloon injury, with simultaneous local treatment of collagenase and elastase. After one month, animals were sedated to repeat ultrasound examinations and computed tomography, and then euthanized. Blood was collected for hematology both at surgery and euthanasia. Histological evaluations were performed to precisely assess the morphological changes to the aortic wall. Overall, only 2 out of 4 animals developed relevant aortic dilation, suggesting that the induction protocol, despite being promising, needs further development. [1] Mokdad et al., Lancet. 2016 Jun 11; 387 (10036): 2383-401 [2] Barrow et al., The Quarterly Review of Biology. 1974 49:2, 101-128 [3] Cullen et al., Journal of Vascular Surgery. 2019 70(1):252-260.e2

77° CONVEGNO SISVET

Stato: INVIATO - ID: 13621

Exogenous expression of Late Embryogenesis Abundant (LEA) proteins confers long term cold stress tolerance to sheep fibroblasts

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Late Embryogenesis Abundant proteins (LEAs), commonly found in plants, certain bacteria, and extremophiles invertebrates, play a crucial role in resisting abiotic-inducing stresses, such as low temperature and dry environments. Here we investigated the potential in vitro effects of different LEAs exogenous expression in mammalian cells exposed to cold temperature. Firstly, we produced a tagged version for three different LEAs: pTag-WCOR410-RFP, Triticum aestivum cold acclimation protein WCOR410 - binds to cellular membranes; pTag-RAB17-GFP-N, Zea mays dehydrin-1dhn - expressed in the nucleo-cytoplasm; and pTag-LEA-BFP, Artemia franciscana LEA protein group 3, - targets the mitochondria. Sheep embryonic fibroblasts were transiently transfected with single LEAs and kept in a mechanical fridge for seven days. At the end of the cold treatment, we found that LEAs-positive fibroblasts displayed a higher viability compared to control (WCOR410: 58.3%, RAB17: 63%, LEA3: 66.7% vs CTR: 30.7%; $p<0.01$ CTR vs WCOR410; $p<0.001$ CTR vs RAB17 and LEA3). A rescue experiment, where previously cold stressed cells were cultured at physiological temperature, confirmed the protection exerted from LEA, highlighted by an higher proliferation rate of LEA expressing cells, comparing to untreated controls (WCOR410: 29.6%, RAB17: 24.1%, LEA3: 25.9% vs CTR: 14.9%, $p<0.01$).
Interestingly, mitochondria in LEA-positive cells maintained a physiological elongated morphology at low temperatures, in contrast to LEA-negative cells where mitochondria became fragmented and rounded in a higher proportion of cells, a trait indicative of inactive mitochondria that anticipates apoptosis. By the fluorescent CellROX-green staining, we further explored the role of reactive oxygen species (ROS) in LEA expressing cells subjected to cold stress, and we observed their significant reduction (fold change of Mean Fluorescence Intensity over control: CTR: 1 vs WCOR410: 0.16, LEA3: 0.19, $p<0.001$). These findings suggest that LEAp buffer the excessive production of ROS in response to cold stress. Accordingly, immunofluorescence analyses of phosphorylated gamma histone H2A.X - incorporated a sites of DNA repair - revealed a higher DNA damage in control group compared to LEA-positive cells (fold change of Mean Fluorescence Intensity over control: CTR: 1 vs WCOR410: 0.14, RAB17: 0.43, LEA3: 0.28, $p<0.05$). We found that cold temperatures we used (9-12°C) induced a metabolic standby state in cells, resulting in significant cell death, whereas LEA-expressing cells exhibited extended dormancy and reduced cell death, similar to anhydrobiotic organisms. Surprisingly, LEAs may also play a role in reducing ROS production/degradation, suggesting a novel physiological function beyond their known chaperone role in protein protection. Future research is needed to understand the mechanisms by which LEAs modulate ROS levels. In conclusion, this study highlights how decreasing oxidative stress during cold metabolic standby is of fundamental importance for improving cell viability, probably through torpor induction in mammalian cells.

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77° CONVEGNO SISVET

Stato: INVIATO - ID: 13654

THE FUNCTION OF INTERLEUKIN-1 AS A FACTOR THAT INDUCES OVATION IN RABBITS (ORYCTOLAGUS CUNICULUS)

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According to certain research, the seminal plasma's active molecules play a role in triggering both sexes' reproductive systems [1]. One well-researched substance that causes ovulation in mammals is nerve growth factor (NGF). However, it is possible that other seminal plasma cytokines have an impact on the tissues of the female genital tract and cause ovulation additionally.Through the use of RT-PCR, this study examined the expression of the interleukin 1 (IL1)/IL1 receptor (IL1R) system in testis, prostate, seminal vesicles, deferens ampullae, and uterine tissues. Research was conducted using tissues from five male and five female rabbits. Western blotting (WB) was used to assess the presence of IL1 in seminal plasma. By exposing uterine tissue to either NGF and IL1 alone or in combination with their cognate receptor antagonist, the crosstalk between IL1 and NGF was researched in vitro. Prostaglandin F2alpha (PGF2alpha) and prostaglandin E2 (PGE2) levels were measured using ELISA test. Rabbit polyclonal antibodies to IL1 and IL1R were used in immunohistochemistry research. Data were analyzed by one-way ANOVA followed by the Student-Newman-Keuls t-test. The deferens ampullae showed a larger IL1 gene transcript ($p<0.05$) than the other tissues, while the prostate showed a higher IL1R gene transcript ($p<0.05$). A 30-35 kDa strong band by WB showed the presence of IL1 in seminal plasma. In addition to increasing PGF2alpha and PGE2 uterine secretion, the in vitro system demonstrated that IL1 increased basal uterine NGF synthesis while NGF had no effect on IL1.The immunohistochemical technique highlighted a positive reaction for IL1 and IL1R in the cytoplasm of the epithelial cells, in the ampullar portion of the deferens vas and in the prostate. No reaction was observed in the negative controls. The results of this study show that the reproductive tissues of both sexes of rabbits express the IL1/IL1R system. Furthermore, the endocrine activity of the uterus is modulated by the amount of IL1 in the seminal plasma. Thus, our data suggests that IL1 and NGF may have a combined ovulatory function. Finally, our findings lend support to the theory previously advanced by Duffy et al. [2] that ovulation is an inflammatory response. A complete understanding of the mechanisms behind the effects of ovulation-inducing stimulation would be necessary to improve reproductive outcomes for all mammalian species. [1] Maranesi et al. New insights on a NGF-mediated pathway to induce ovulation in rabbits (Oryctolagus cuniculus). Biol Reprod. 2018 98:634-643, 2018 [2] Duffy DM et al. Ovulation: parallels with inflammatory processes. Endocr Rev. Apr 1, 40(2):369-416, 2019

77° CONVEGNO SISVET

Stato: INVIATO - ID: 13665

"Il banco degli asini" - Activities of a specialized center for animal assisted human therapy in Apulia.

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"Il banco degli asini" is a specialized center for assisted therapy by residential animals (I.A.A.) located in Alberobello (BA) within the agri-zootechnical educational farm of the "Basile Caramia-Gigante" Agrarian-Hotel Secondary Education Institute. This project has been funded by "Putignano Trulli and Grotte" Rotary Club.It has been started in March 2022 and it is the first center in the province of Bari and in Italy located inside an educational institution. The center currently employs a multidisciplinary team licensed to deliver Animal Assisted Education (E.A.A.) using the mediation of asinine and equine species of animals.The team consists of two project managers/ intervention leaders, a veterinary doctor expert in E.A.A., an animal helper (donkey and horse), adequately trained in the propaedeutic, basic, and advanced courses issued by authorized centers. The center's organizational chart includes a center manager, a veterinary health director, and an animal welfare officer. The activities are aimed at the Institute's adolescence students with learning, socialization and communication disorders, special educational needs, and autism spectrum disorders; recently the activity has also been extended to external users. The center has been included in the experimental project for socialization assistance pathways for children with autism spectrum disorders promoted by the Welfare Department of the Apulia Region.The human-animal relationship, whose benefits are widely witnessed by multiple scientific studies [1,2], acts positively in socialization, psyche, cognitive-intellectual levels, communication, and psychomotricity. The animal becomes a "distractional co-therapist" to overcome the patient's problem/deficit/disturbance through an introspective and emotional path that pushes to hook latent or hidden sides of oneself and to trace a new alternative life course. A.A.I. therapies have rehabilitative, educational, and recreational value and aim to promote, activate, and support the resources and potential for individual growth and planning relationships, and social inclusion of disadvantaged people, helping to improve their quality of life and strengthen the self-esteem of the involved guys.The bio-psycho-social approach to adolescent problems, through the human-nature-animal relationship, is a fundamental mission of the Center. Children with frailty and need for help, guided in this experience, by the mediation of donkeys and horses, showed at the end of the course improvement in self-esteem with acquisition of awareness about their own abilities, development of empathic relationship with the animal and other people, positively channeling moods and energies.E.A.A. activities are preferably delivered on individual projects calibrated according to the patient's problem/deficit/disorder scheduling of a minimum of ten sessions. The individual patient's activities are monitored at the end of each session by filling out the appropriate forms. Feedback on animal welfare is provided not only by the coadjutor, during the work on the setting, but also by the animal husbandry staff, during rest.The animals involved in the Center's activities are three mares of Lipizzaner and Hungarian saddle breed, and six donkeys, of Sarda and Martina Franca breeds (a breed indigenous to the Murgia area). Some of these animals were adopted and rescued from certain death (abandonment, malnutrition, and slaughter). Donkeys represent a species that best establishes social relationships with humans, due to ethological characteristics of sociability, curiosity, and docility, and possess the behavioral requirements essential to pet therapy activities. During the year, the Center organizes workshops and practical/training internships for students.

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77° CONVEGNO SISVET

SOCIETA´ SCIENTIFICA DI RIFERIMENTO **Sofivet**

TITOLO **Assessing Handler-Dog team welfare in Animal-Assisted Education through endocrine parameters**

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Testo e Riferimenti bibliografici

In recent years, Animal-Assisted Interventions (AAI) have received considerable attention due to the potential benefits they provide to different categories of people. At the same time, there has been an increased focus on the welfare of animals participating in these procedures. However, data on the welfare of handler-dog teams during (AAI) sessions are lacking. This research aims to examine the welfare of dogs and their handlers engaging in Animal-Assisted Education activities at two nursing homes in Lucca. The research was funded by the IN-HABIT project H2020 (grant number 869227).

Ten dyads consisting of 7 handlers (owners) and 10 dogs were evaluated for salivary concentrations of oxytocin and cortisol. Samples were collected at the beginning of the project (baseline) and at follow up (after 7-8 sessions), before and after activities (T0 and T1).

Statistical analyses comprise descriptive (percentiles and normality test) and non-parametric (Wilcoxon rank test; $p < 0.05$) analysis.

Dogs and handlers showed different results for oxytocin and cortisol concentrations before and after sessions. Dogs' oxytocin concentrations (pg/ml) tended to slightly increase after sessions both at baseline (p= 0.46 median T0 = 54.5; T1= 57.1) and at follow up (p=0.46 T0 =65.5; T1 = 66.7), while handlers oxytocin concentrations (pg/ml) showed a slight decrease at baseline (p=0.88 T0= 187.1; T1= 173.4) and a slight increase at follow up (p=0.86 T0 = 216.4; T0= 233.4).
Dogs' cortisol concentrations (ng/ml) decreased after session at baseline (p= 0.39 T0 = 1.30; T1 =0.90) and significantly decreased at follow up (p = 0.028; T0 = 0.80; T1 = 0.51). Contrarily to oxytocin results, handlers' cortisol concentrations (ng/ml) showed an increase between T0 and T1 samples at baseline (p= 0.39 T0 = 1.3; T1 = 1.7) and a significantly decrease at follow up (p= 0.01 T0 = 1.8; T1 = 0.78).

The decrease observed in cortisol levels after sessions in dogs suggests that they were not stressed during the activities, and therefore their handlers were able to safeguard their welfare. However, the observed increase in oxytocin post-session levels in dogs goes further: indeed, oxytocin is usually released in case of interactions with humans perceived as positive by dogs (Handlin et al., 2012). Such results, taken together thus suggest that dogs were experiencing a positive state of welfare during the AAI, taking advantage of the interactions with patients.

Regarding the handlers, at baseline a decrease in oxytocin levels and an increase in cortisol levels suggest that the first session might have been a bit stressful for them: a possible explanation is that handlers initially might have experienced a bit of stress or pressure, due to being in charge of the dog welfare and safety but not knowing the users and the environment. However, at follow up, as they became more confident with the activities and experienced positive sessions over time, their oxytocin levels increased, and their cortisol levels decreased during AAI.

In conclusion, these findings shed light on the importance of considering not only stress but also positive state of welfare in the involvement of AAIs. Being beneficials for users, as well as for other parties involved, is key to work in a respectful manner and to optimize intervention outcomes.

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77° CONVEGNO SISVET

SOCIETA´ SCIENTIFICA DI RIFERIMENTO **SOFIVET**

TITOLO **Toxoplasma gondii Seropositivity: A Potential Link to Anxiety in Companion Dogs (*Canis lupus familiaris*)**

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Testo e Riferimenti bibliografici

Fear is an adaptive response to potential or real threats, crucial for the animal's survival, whereas anxiety is the anticipation of future danger or threats. Dogs may experience fear and anxiety in certain situations without necessarily having an anxiety disorder. However, these emotions can shift from being adaptive to maladaptive and pathological if they persist chronically and are inappropriate and disproportionate given the context. In anxiety disorders, an individual's capacity to adapt is compromised by an exaggerated perception of threat that diverges from reality, resulting in diminished quality of life and welfare [1]. This preliminary study investigates the potential link between the development of anxiety disorders and seropositivity to *Toxoplasma gondii* in companion dogs. Indeed, *T. gondii* is a widespread apicomplexan protozoan parasite that can infect a variety of warm-blooded species, and recent research has evidenced how the potential neural localization of bradyzoite cysts in intermediate hosts can lead to behavioural modifications [2].

124 adult dogs (above 2 years of age) referred to a veterinary clinic were randomly selected to participate in the study, without knowing their seropositivity status for toxoplasmosis. Blood samples were collected during routine veterinarian check-ups and were analyzed through an indirect fluorescent antibody test (IFAT) for IgG against *T. gondii*. Meanwhile, these animals underwent classification as either affected or unaffected by anxiety disorders through an interview conducted by a veterinary surgeon expert in animal behaviour, who took into consideration what suggested by PANAS (positive and negative activation scale) and the Lincoln Canine Anxiety Scale. The laboratory analyses to determine if there were or not seropositivity to *T. gondii* were conducted without knowledge of the anxiety diagnosis and vice versa, as double-blinded research. Statistical analysis was conducted using JMP 17, a software package developed by SAS. A Receiver Operating Characteristic (ROC) analysis was employed to divide dogs into small-breed (weighing <15 kg) and medium/large-breed (weighing >15 kg). Furthermore, multiple nominal logistic regression models were utilized to investigate the association (as odds ratio) between anxiety and both toxoplasmosis positivity and breed size.

The results showed that large/medium-breed dogs had a 2.34 times lower risk (p=0.01) of developing disorders than small dogs, irrespective of *T. gondii* previous exposure. In addition, larger dogs tended a higher likelihood (3.41 times; p=0.07) of anxiety disorder when testing positive for *T. gondii*. These findings suggest a potential association between *T. gondii* exposure and the emergence of anxiety disorders in dogs. Considering seropositivity to *T. gondii* as a risk factor for anxiety development could be useful during the diagnostic process of anxiety disorders. In cases of seronegativity, advising dog owners to avoid risky behaviors, such as the use of raw meat, could help prevent *T. gondii* infection in animals that are affected or predisposed to the development of anxiety. However, it is crucial to consider the multifactorial nature of anxiety, and further research, including anatomopathological studies, is recommended.

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77° CONVEGNO SISVET

SOCIETA´ SCIENTIFICA DI RIFERIMENTO **SOFIVET**

TITOLO **TUBULIN POST-TRANSLATIONAL MODIFICATIONS IN CANINE NEUROGENIC MUSCULAR ATROPHY**

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Testo e Riferimenti bibliografici

The impact of neural activity on skeletal muscle becomes evident when there is a rapid and substantial muscular deterioration resulting from disruption or lack of neuronal input. For instance, in cases of injury, severance, or compression of a nerve, atrophy sets in at a notably accelerated pace compared to other factors such as immobilization, cachexia, malnutrition, aging, and dystrophies. Dogs suffering from neurogenic atrophy endure a diminished quality of life because of sensory and motor impairments, including coordination abilities, twitching, and decreased ability to sense pain, touch, or temperature changes [1]. Tubulin posttranslational modifications (PTMs) have emerged as important regulators of the neuronal microtubule cytoskeleton. In particular, the PTM D2, the only irreversible PTM and a marker of hyperstable microtubules, is implicated in neurodegeneration, as well as axon regeneration failure [2-3]. Here we explored whether PTMs are involved in canine neurogenic muscular atrophy.
9 adult dogs with symptoms of neurogenic muscular atrophy had biopsies performed on the quadriceps femoris and triceps brachii. All surgical procedures were performed under anesthesia by a veterinarian and with owner consent.
The dogs with a diagnosis of neurogenic muscle atrophy were divided according to the severity of atrophy status: mild, moderate, and severe. Histopathological examinations of muscle fibers were performed to assess the morphology and to measure the activity and the distribution of mitochondria. Muscle biopsy sections with nerve end terminal were selected to measure the level of D2, acetylated, detyrosinated, and polyglutamylated tubulins. Our preliminary results show a correlation between the severity of the disease and the accumulation of $\Delta 2$, that appears more pronounced in cases of moderate and severe muscle atrophy compared with mild atrophy suggesting that hyperstable microtubules may contribute to axonal disfunction of nerve fibers approach the muscle fibres and terminate in them.

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77° CONVEGNO SISVET

SOCIETA´ SCIENTIFICA DI RIFERIMENTO **SOFIVET**

TITOLO **EFFECTS OF DIETARY SUPPLEMENTATION WITH OMEGA-3 FATTY ACIDS FROM EXTRUDED LINSEED AND ALGAE PADINA PAVONICA ON THE GROWTH PERFORMANCE OPF FATTENING RABBITS**

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Testo e Riferimenti bibliografici

Incorporating n-3 polyunsaturated fatty acids (PUFA) into rabbit nutrition is emerging as a promising strategy to enhance not only the animals' productive and reproductive performance but also the nutritional value of rabbit meat, potentially providing a functional food for humans [1]. While both plant and animal products rich in n-3 PUFA have been explored in rabbit nutrition, linseed-derived products have gained significant attention for yielding promising results [2]. This study investigates the effects of dietary supplementation with n-3 PUFA derived from extruded linseed alone and in combination with algae *Padina pavonica* extract on the growth performance and metabolic status of fattening rabbits. The experimental protocol was approved by the Ethical Committee of the Department of Veterinary Medicine of the University of Milano (OPBA_18_2021). Sixty New Zealand White growing rabbits, weaned at 35 days of age and slaughtered at 85 days, were divided into three groups (n=20 each): commercial diet (CNT group), commercial diet integrated with 5% extruded linseed (L5% group), and commercial diet integrated with 3.5% extruded linseed in combination with 0.2% algae *Padina pavonica* extract (LPP group). Growth performance parameters including live weight (LW), average daily gain (ADG), and feed conversion ratio (FCR) were assessed weekly, while feed intake (FI) was recorded daily. Metabolic status was evaluated by measuring plasma concentrations of insulin, glucose, and non-esterified fatty acids (NEFA) at 35, 60, and 85 days of age. The L5% group exhibited the highest marginal mean for LW and ADG with lower marginal mean for FCR (all $P<0.05$), indicating improved feed efficiency compared to the LPP group. Moreover, supplementation with extruded linseed and algae *Padina pavonica* extract did not significantly impact plasma concentrations of insulin, glucose, or NEFA, suggesting no adverse effects on the animals' energy metabolism.
Overall, the results suggest that both extruded linseed and algae *Padina pavonica* extract can be safely incorporated into rabbit diets at 5% and 0.2% respectively without compromising the productive performance and the energy metabolism of the animals. Future studies can provide a more comprehensive understanding of the benefits of incorporating extruded linseed into fattening rabbit diets, specifically focusing on its effects on growth rate, energy metabolism and feed efficiency.

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77° CONVEGNO SISVET

Stato: INVIATO - ID: 13851

Sex differences in Guinea Pigs' Performance in a Spatial Navigation Task

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The ability to orient oneself in space is a trait with ancient origins and is particularly well-developed in migratory birds and rodents, which have often served as models for the development of cognitivist theories applicable to the human domain. All vertebrates are endowed with a complex of cognitive modules associated with various brain areas that enable them to navigate space, memorize safe routes to shelters, hunt, and raise offspring. For this purpose, the subjects must possess the ability to perceive, preserve, and recall information concerning the spatial characteristics of the environment. Spatial navigation tests in animal studies have proven to be very useful for exploring learning and memory abilities (Lipp et al. 2001) and has been extensively investigated for detecting sex differences in mammals. The domesticated guinea pig (Cavia aperea porcellus) serves as a promising species for such studies, due to distinct parental investment between the sexes (Saucier et al. 2008): males have been proven to outperform females in orientation in voles, cervine mice, mice, rats and humans, although mixed results have been obtained in dogs (Scandurra et al. 2018). In our exploration of sex-related effects on spatial navigation, we focused on the learning performance and spatial strategy (that are not mutually exclusive: animals possessing the capacity to utilize multiple signals can integrate them based on the relative importance of these available signals) employed by guinea pigs in a plus maze paradigm: egocentric and allocentric navigation are two fundamental approaches to spatial orientation and understanding one's position in the environment. Results show that 50% of the animals completed the spatial navigation test. Regarding sex differences, 24.2% of females completed the test, while the completion rate for males was significantly higher at 64%. Males achieved the learning criterion with a significantly lower number of trials compared to females, indicating superior spatial abilities in the plus maze paradigm. Like their wild counterparts, male domestic guinea pigs exhibited better spatial skills than females. However, we observed no sex difference in the preference of strategy or the latency of task resolution.
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77° CONVEGNO SISVET

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Canine cutaneous hypersensitivity reactions: inflammatory and immune response-related gene signature

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Cutaneous hypersensitivity reactions (CHRs) are inflammatory skin disorders affecting both humans and dogs, characterized by diverse clinical presentations and responses to therapy. The underlying causes and mechanisms of CHRs are often difficult to assess, complicating diagnosis and treatment [1].
CHRs involve abnormal immune responses upon re-exposure to various triggers, such as allergens, irritants, or infections, leading to inflammation, skin damage, and irritation. The innate immune system plays a primary defense role through pattern recognition receptors (PRRs) on skin cells, crucial for detecting specific molecular patterns associated with pathogens or tissue damage [2]. The engagement of PRRs triggers the production of pro-inflammatory cytokines (e.g. IL-6) and chemokines, stimulating the synthesis of acute phase proteins (APPs), including haptoglobin (Hp) and lipopolysaccharide-binding protein (LBP), which contribute to systemic inflammation in CHR [3].
This study investigates the role of immune and inflammatory responses mediated by specific receptors and circulating proteins in CHR pathophysiology.
Using formalin-fixed paraffin-embedded (FFPE) samples from canine CHR cases (n=20) and healthy controls (n=3), RT-qPCR analysis was performed to detect the expression levels of seven genes, including PRR family members (CD209 and CLEC4G), chemokines (regakine-1-like), and APPs (LBP-like and Hp-like). Additionally, the involvement of IL-6 and signal transducer and activator of transcription 3 (STAT3) in the inflammatory JAK-STAT signaling cascade was examined to determine their involvement as key regulators of the acute phase response.
The study revealed a statistically significant increase in the expression levels of CD209, Hp-like ($p < 0.01$), LPB-like, regakine-1-like, and CLEC4G ($p < 0.05$) genes in CHR compared to healthy controls. Conversely, IL6 and STAT3 display no statistically significant difference between the two groups ($p > 0.05$).
To date, no single cause of canine CHR has been identified, as several factors, such as the properties of the skin epithelial barrier and the skin microenvironment, genetic and environmental factors contribute to the individual predisposition and phenotypical heterogeneity of CHR symptoms.
We, therefore, believe that a study based only on the central dogma of biology offers a limited understanding of the mechanisms driving the pathophysiology of canine CHR. The gene expression profile observed in this study is only the beginning. The research group is planning additional studies to explore the expression of proteins and microRNAs that could potentially aid in the diagnosis and treatment of this multifactorial disease in dogs.

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SOIPA

Prevalence and associated risk factors of *Ascaris suum* milk spot lesions in pigs slaughtered in Italy: the abattoir as a monitoring tool

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The monitoring of liver lesions caused by larval migration of *Ascaris suum* provides a useful tool for assessing the circulation of this parasite and for conducting large-scale epidemiological studies [1]. Considering the lack of updated data on the prevalence of milk spots in pigs slaughtered in northern Italy, a retrospective observational study was planned in an area characterised by a high density of intensive pig farms, particularly devoted to the production of cured meats, evaluating the plausible influence of several variables. The study focused on data on milk spot lesions recorded between October 2020 and September 2021 in one of the main national abattoirs located in the province of Mantua (northern Italy). In the survey year the livers of 754833 carcasses from 399 farms were analyzed during the post-mortem inspection. Farms were located in different regions of northern Italy, particularly 250 were in Lombardy, 51 in Emilia-Romagna, 47 in Piedmont, 38 in Veneto and 13 in Friuli-Venezia Giulia. For each recruited farm data concerning the region and province of rearing, the slaughtering season, the farm size and the type of farm production were collected. These variables were entered into generalized linear mixed models (GLMMs) and the likelihood of finding an animal with milk spots in relation to these factors was estimated. At the farm level, 368 out of 399 farms were positive (92.2%) and out of 754833 carcasses, 198964 showed liver lesions, with an overall prevalence of 26.4%. Lombardy was the region with the highest prevalence (27.9%), followed by Veneto (26.1%), Emilia-Romagna (24%), Piedmont (18%) and Friuli-Venezia Giulia (12.5%). The risk of finding animals with milk spots raised in spring and summer, when temperatures are higher and *A. suum* third-stage larvae hatch from the eggs in a shorter time interval, leading to an increase in the number of infected animals and consequent liver lesions [2]. Moreover, medium-sized farms showed a higher probability of liver injury than large and small ones, as in large farms the application of all-in/all-out systems and regular cleaning and disinfection protocols could reduce parasite exposure; at the same time, small farms could provide efficient management focused on each animal [2]. Finally, the number of lesions was significantly higher in farrow-to-finish farms and this aspect could be related to the presence of farrowing units in this type of farm production, where inadequate anthelmintic protocols could favour the persistence of *A. suum* in the following stages of the production cycle [3]. Given the high prevalence recorded and the massive economic damage in the pig industry caused by organ discarding, it is necessary to promote a better cooperation between abattoirs, veterinarians and farmers developing specific control plans. Further, the abattoir monitoring system could record current data on *A. suum* circulation from a One Health perspective, considering that, although human cases are rare, it is a zoonosis.

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In vitro efficacy of Red Lapacho (*Tabebuia avellanedae*) against *Giardia duodenalis*

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Giardia duodenalis is a widespread protozoan affecting mammals, including humans and dogs. Affected dogs exhibit a range of symptoms from subclinical to severe abdominal pain and diarrhea. Giardiosis may become chronic, thus requiring repeated treatment with synthetic drugs like fenbendazole (FBZ) and metronidazole (MTZ) [1]. In the last years, drug resistance is rising, especially in human medicine; although no cases of drug resistance are reported in the veterinary field, a recent study showed a lack of efficacy of FBZ in treatment of canine giardiasis [2]. Consequently, therapeutic alternatives are required. Medicinal plants as been traditionally used as anti-parasitic compounds, but systematic evaluation under controlled experimental condition is often lacking. Here we have examined the efficacy of *Tabebuia avellanedae* dry extract (TD) and hydroalcoholic extract (TH), as well as one of its active compounds, β-lapachone (β-lap), as potential treatment against *G. duodenalis* infection in dogs. In vitro anti-giardial activity of compounds (IC_{50} values after 48 h) was evaluated by ATP-content assay using reference isolates of *G. duodenalis* Assemblage A and B. MTZ was used as reference drug. In vitro cytotoxicity effects were evaluated using human Caco-2 and canine MDCK cell line (CC_{50} values at 6, 12, 24 and 48 h) and selectivity index (SI = IC_{50}/CC_{50}) evaluated at 48h. Therefore, we evaluated the in vitro cytotoxic effects of three concentrations of all the compounds on intestinal Organoid Derived Monolayers (ODMs) after 48h evaluating both alteration of transepithelial electrical resistance (TEER) and organoids viability. We observed good anti-*G. duodenalis* activity of all the compounds. Both the Caco-2 and MDCK cell viability assay produced similar results for TD, with only the highest concentration (2 mg/ml) showing toxicity at 12, 24 and 48 hours, while no cytotoxicity was recorded for TH. As expected due to his anticancer activity [3], β-lap was toxic against both Caco-2 and MDCK cells. A significant SI value was recorded for TH; however, the SI values for TD and β-lap warranted further assessment using alternative biosystems to verify the safety of the compounds. A remarkable low toxicity was observed for TD and β-lap on ODMs, while no toxicity was detected for TH. Our in vitro results pointed out on a potential therapeutic applicability of *T. avellanedae*. This is the first time that organoids were used for testing antigiardial compounds. Future studies will focus on evaluating *T. avellanedae* using an in vitro model of *Giardia*-host co-culture, as well as an animal model of giardiasis.

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Urban wildlife, a spiky issue: first detection of *Giardia duodenalis* in Italian European hedgehogs (*Erinaceus europaeus*)

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Giardia duodenalis is a widespread protozoan responsible for giardiasis, a disease that can cause gastrointestinal symptoms. Basing on the genetic analysis, *G. duodenalis* is classified in eight Assemblages (A to H) that display different host affinity [1]; Assemblage A and B specifically infect humans with select sub-genotypes showing the capacity for zoonotic transmission. Among the many species of wild animals that inhabit urban settlements, the European hedgehog (*Erinaceus europaeus)* is one of the few species that has adapted to live in close contact with humans; therefore, survey of potential zoonotic parasites shared from hedgehogs and humans could be of great public health concern, especially in urban areas with high hedgehog density [2].
Fecal flotations for coprological examination were routinely carried out on 48 symptomatic and asymptomatic hedgehogs admitted to a Wildlife Rescue Center in Central Italy. To confirm the suspect of infection with *G. duodenalis*, feces were also destined to a direct immunofluorescence assay (MERIFLUOR®) and DNA extraction followed by Nested PCR amplification of a 511 bp fragment of the beta-giardin gene to be used in a PCR-RFLP protocol that allow for Assemblage and sub-genotype characterization [3]. Furthermore, one of the two hedgehogs that tested positive to *G. duodenalis* died due to a severe diffuse interstitial and granulomatous pneumonia; samples of the small intestine were formalin fixed, processed with routine histological techniques, and stained with Hematoxylin and Eosin.
Cysts of *G. duodenalis* were detected in the fecal flotations of two hedgehogs (4.17%), both the immunofluorescence assays and the PCR amplification confirmed the presence of the cysts and DNA respectively. The RFLP protocol attributed the sample to the Assemblage A1. Trophozoites were also found on the histologic slides, confirming once more the presence of the parasite. *G. duodenalis* has been previously found in hedgehogs from other European Countries and New Zealand but this is the first description of Assemblage A1 from European hedgehogs in Italy. Assemblage A1 has the potential to infect humans as well as a range of other mammals. Is therefore important not to leave available food and water sources that can promote the spreading of zoonotic parasites from wildlife to humans and pets. The detection of a highly pathogenic zoonotic assemblage in hedgehogs can bridge the transmission of the parasite from the wild to the domestic environment and underlines the importance of maintaining appropriate hygiene measures when interacting with both symptomatic and asymptomatic animals.

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77° CONVEGNO SISVET

Stato: INVIATO - ID: 13662

Molecular analyses indicate Low Zoonotic Risk Associated with Giardia duodenalis infection in Dogs and Cats from a Veterinary Teaching Hospital

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Giardia duodenalis (syn. G. lamblia and G. intestinalis) causes giardiasis in humans and most mammals, representing one of the most common intestinal parasites in humans. Giardiasis has a significant impact on public health due to its high prevalence and tendency to cause severe epidemics mainly food and waterborne [1]. Although infections in dogs and cats are often asymptomatic, giardiasis can lead to clinical gastrointestinal manifestations in pets [2-3]. Considering the potential presence of zoonotic assemblages of G. duodenalis in companion animals and to assess the possible risk for pet owners, the aim of this study was the molecular identification of Giardia spp. in dogs and cats using the triosephosphate isomerase (TPI) gene, selected for its high genetic variability allowing differentiation of assemblages through sequencing. From June 26 to October 12, 2023, a total of 139 samples were microscopically analysed during parasitological diagnostic routine upon clinical suspicion in the Laboratory of Parasitology and Parasitic Disease of the Department of Veterinary Medical Sciences of the University of Bologna. These comprised 96 fecal samples from dogs and 43 from cats, that were investigated for Giardia presence using Lugol-stained sediment analysis. Positive samples underwent PCR targeting the giardia TPI gene for molecular characterization. Of the total 139 fecal samples tested microscopically for Giardia sp. presence during the study period, 5 (5.2%) samples from dogs and 4 (9.3%) samples from cats tested positive. A prevalence of 44.4% was observed in dogs ≤ 6 months old and 3.4% in dogs > 6 months old, with statistical significance ($P<0.01$). In addition to these samples, 28 previously positive samples by microscopy were also subjected to the same PCR, for a total of 37 samples (28 from dogs and 9 from cats) targeting the TPI gene resulted in amplification of 22 samples, from which 14 readable sequences were obtained. These confirmed the species as Giardia duodenalis and revealed the presence of Assemblage C in 9 dog samples, D in 1 dog sample, and F in 4 cat samples, all species-specific assemblages. Based on our laboratory diagnostic experience, no zoonotic assemblages were detected, although sporadic reports of zoonotic assemblages in pets have been noted in Italy [4-7]. Based on these preliminary results, we can conclude that in our specific case, giardiasis in pets is likely transmitted through species-specific routes that do not contribute significantly to the epidemiology of human giardiasis.

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77° CONVEGNO SISVET

Stato: INVIATO - ID: 13718

Unraveling implications of Neospora caninum infection in dairy cattle farms in Italy

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Neospora caninum, a protozoan parasite, is a major cause of bovine abortion worldwide. An epidemiological study on tank bulk milk reported a prevalence of 30.7% in farms located in the Po Valley [1]; moreover, a preliminary study on two dairy herds in Lombardy suggested an adverse effect of the parasite in early pregnancy and on milk yield [2].

This epidemiological study aimed to evaluate the seroprevalence (P) for N. caninum at herd level and the relationship of bovine serostatus on herd reproductive and productive parameters in dairy cattle farms in Italy.

In 13 selected herds with a positive result during the screening on tank bulk milk, 2576 blood samples from all cows above 24 months of age were analyzed by an immunofluorescence antibody test (MegaFLUO Neospora caninum, Megacor). These were medium-large farms with herd consistency between 120 and 860 animals raised in intensive production system based on Holstein Friesian cows. The herds were located in the provinces of Bergamo (n=1), Brescia (n=1), Cremona (n=8), and Milano (n=3). The abortion rate in these farms varied between 0.9 and 15.1%. Information on herd reproductive and productive performances and individual data of cattle were collected. Generalized linear models (GLMs) were developed.

834 individual samples showed N. caninum antibodies (P=32.4%); the intra-herd seroprevalence varied between 8.9 and 61.6%. Medium age and number of lactations of seropositive animals were slightly lower than negative ones (47.1 vs 47.3 and 2.17 and 2.23) but with no statistical significance. Overall, the number of inseminations for conception (2.4 vs 2.2), the days in milking (209.6 vs 198.6) and the daily milk production (34.4 kg vs 35.7 kg) and the 305mature equivalent milk yield (305ME) (11730.8 vs 12184.4) were respectively higher and lower in seropositive than seronegative cows.
According to the number of inseminations classes, the cows with 3 or more inseminations showed a higher seroprevalence (P= 37.5 %) if compared to those with less than 3 inseminations (P=29.2) (OR=1.4, p=0.000). Further, a higher seroprevalence of N. caninum was detected in cows with a daily milk production lower than or equal to 20 kg (P=40.6%) and between 21 and 25 kg (P =34.8%) (OR=1.8 and 1.4, p=0.027 and 0.049, respectively), whereas animals producing between 26 kg and 30 kg (P=29.2%) and more than 30 kg (P =27.3%) of milk were less often positive. Besides, a higher seroprevalence was detected in cows with 305ME lower than 10000 if compared to those with this value equal to or higher than 10000 (P=39.3 and 29.7%, respectively) (OR=1.5, p=0.011).

Antibodies to N. caninum resulted widely spread in Italian dairy herds. A relationship between the serostatus and reproductive and productive parameters of the dairy cows was demonstrated. However, even if in the majority of surveyed herds the number of abortions was limited, data seem to support an effect of N. caninum in early pregnancy.

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77° CONVEGNO SISVET

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Application of new sustainable devices and innovative technologies to control cystic echinococcosis.

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Cystic echinococcosis (CE), caused by the larval stage of Echinococcus granulosus, has a worldwide distribution and is considered one of of the most severe parasitic zoonosis of grazing sheep in the Mediterranean region. The lifecycle of E. granulosus involves canids as definitive hosts and usually sheep and other herbivore species as intermediate hosts. Free-roaming dogs (owned and unowned) are the major source of echinococcosis and the most challenging category in dog population management for the control of CE. New sustainable devices and technologies are needed to implement the efficiency of CE control programmes, especially for definitive hosts. In this study, conducted in a highly endemic area of southern Italy, it was explored the combined use of Geographical Information Systems (GIS) and innovative devices (e.g., GPS collars, drone, camera trap) to identify the spatiotemporal patterns of the free-roaming owned dogs and to design new anthelmintic treatment strategies for wild canids gravitating near the CE positive sheep farms.The use of wearable devices consist of Global Positioning System (GPS) makes it possible to track fine scale animal (sheep and dogs) movements and identify the most frequented locations within grazing areas. The anthelminthic bait delivery using unmanned aerial vehicles (UAVs) allows the development of deworming strategies specifically designed for capillary and automatic distribution of anthelmintics in study areas, saving time and resources. Furthermore, camera traps allow to remotely monitor in real-time the acceptance of the medicated baits (laced with praziquantel) by the targeting animals (e.g. stray canids).These innovative tools and technologies have been successfully used in southern Italy to implement control of CE within the actions of the Echino-Safe-Med project. This research was funded by: i) the project “New sustainable tools and innovative actions to control cystic ECHINOcoccosis in sheep farms in the MEDiterranean area: improvement of diagnosis and SAFEty in response to climatic changes-ECHINO-SAFE-MED”, supported by PRIMA (Partnership for research and innovation in the Mediterranean area); ii) the Regional Reference Centre for Animal Health (C.Re.San.), Campania Region, Italy

77° CONVEGNO SISVET

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Foodborne parasites in horse meat: investigating Toxoplasma gondii and Sarcocystis spp. presence in Large Scale Retail Products

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The increasing number of reported foodborne outbreaks and cases of infection in Europe [1] and the rise in consumption of raw or undercooked horse meat highlight the importance to evaluate the safety standards of horse meat [2]. While regulations and standardized inspections in slaughtered animals exist for some pathogens, such as Trichinella spp., protocols for detecting Toxoplasma gondii are lacking, and for Sarcocystis are limited to those species forming macroscopic cysts [3]. Hence, limited epidemiological data on T. gondii and Sarcocystis spp. infections in horse are available, further complicating risk assessment. Therefore, the aim of this study was to investigate the prevalence of these parasites in horse meat sold in large-scale retail trade and to estimate the associated risk for consumers.
A total of 110 pre-packaged 9 different cuts of meat samples from 5 commercial brands were collected in 12 supermarkets in Lombardy (northern Italy). The presence of anti-T. gondii antibodies was evaluated in meat juice samples using a commercial ELISA, while the presence of parasitic DNA in muscle samples was investigated using real-time PCR protocols targeting the B1 and 529 bp-RE genetic markers In the case of Sarcocystis spp., two end-point PCRs targeting 18S rDNA and COX1 genetic markers were performed. A subset of positive samples was subsequently Sanger sequenced. Statistical analysis was conducted utilizing the Chi-square test to assess potential risk factors (e.g., supermarket, commercial brand, and cut of meat) associated with the presence of these pathogens.
For T. gondii, a seroprevalence of 11.8% (12/102) was obtained, while any positivity for the presence of parasitic DNA was not found by both real time PCR protocols. The presence of Sarcocystis spp. was highlighted in 63.6% (70/110) of the tested samples using the 18S rDNA marker; BLAST analyses conducted on the COX1 sequences obtained from 20 different samples confirmed 99-100% identity with sequences of S. fayeri. Statistical analysis showed a significant difference among the different cuts of meat, with those to be consumed raw/undercooked more at risk of being positive for anti-T. gondii antibodies (p-value=0.019).
The study reveals the presence of T. gondii and Sarcocystis sp. in horsemeat destined to large scale retailing in Italy. A limited concordance between serological and molecular data for T. gondii was recorded, as previously reported in horses; statistical analysis suggested a potential suitability of certain typology of muscles for detecting anti-T. gondii antibodies. The presence of S. fayeri in above 60% of horse meat samples may suggest a potential risk of poisoning for consumers, especially considering recent food trends favoring the consumption of raw or undercooked horse meat. The obtained results underscore the necessity of drawing the attention of sanitary authorities to the presence of food-borne parasites that currently lack regulatory oversight during inspections. It is crucial to establish surveillance plans focused on detecting and identifying these pathogens, especially in ready-to-eat large-scale retail products intended for raw consumption.
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