FEDERAZIONE SISVET

AIPVET♦AIVI♦AMV♦ANIV♦ARNA♦ RNIV♦SICLIMVET♦SICV♦SIFTVET ♦SIRA♦SOFIVET♦SOIPA

Edizione virtuale

23-26 Giugno 2021

Con il supporto tecnico-scientifico
di

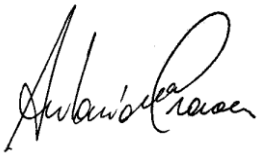
I contributi presenti negli Atti del
74° Convegno SISVet 2021
potranno essere citati utilizzando
il codice ISBN
9788890909290

Relazione del Presidente

Carissimi,
come la maggior parte di voi sanno il 21 Novembre 2018, a Brescia, con atto a rogito del Notaio Guido Cirilli, è stata formalizzata la Federazione SISVET con la sottoscrizione del nuovo Statuto da parte dei 12 Presidenti di Società Scientifiche Veterinarie che hanno aderito. La scelta di Brescia per la costituzione della nuova Federazione non è stata casuale, Brescia, infatti ha sempre rappresentato unmomento di comunione tra l'accademia e tutte le altre componenti della Veterinaria, grazie alla Fondazione Iniziative Zooprofilattiche e Zootecniche che ha sempre sostenuto la SISVET sia con iniziative editoriali di pregio sia garantendo sede e segreteria amministrativa alla Società sin dal lontano 1946, dopo che i nostri Maestri fondarono a Bologna la Società Italiana delle Scienze Veterinarie. A Brescia, dai Presidenti delle Società, che per il nuovo statuto, rappresenteranno i Soci della Federazione, è stato eletto anche il nuovo Comitato scientifico ed il nuovo Comitato esecutivo composto da me in qualità di Presidente, dalla Prof.ssa Adriana Ianieri, in qualità di Vice Presidente, dal Prof. Ezio Ferroglio, Amministratore Economo, dal prof. Gaetano Oliva, Segretario Generale, oltreche dai componenti Proff.ri Paolo Ciaramella, Giuseppe Iovane, Giovanni Lacalandra, Serenella Papparella e Giuseppe Radaelli, che insieme all'Assemblea dei Presidenti delle Società governerà per ilprossimo futuro la Federazione. Negli anni passati ci eravamo proposti di attuare per la SISVET un profondo rinnovamento volto principalmente a creare le condizioni che permettessero alla Società di costituire una sorta di collante tra le varie componenti del mondo delle Scienze Veterinarie, cercando di rafforzarne il ruolo "politico" anche fuori dello stretto ambito Accademico. Alcuni degli obiettivi sono stati raggiunti altri necessitano ancora di ulteriore definizione. Compito del nuovo comitato direttivo sarà quello di confermare quanto raggiunto e mettere le premesse per la realizzazione di nuovi traguardi in collaborazione con la Conferenza dei Direttori dei Dipartimenti di Medicina Veterinaria, con i Dipartimenti Universitari, con il Ministero dell'Istruzione, Università e Ricerca Scientifica, con il Comitato Universitario Nazionale, con il Ministero della Salute, con la Veterinaria Pubblica (Istituti

Zooprofilattici, ASL), con la Federazione Nazionale dei Medici Veterinari e con Sindacati, entità le cui finalità spesso coincidono e necessitano sempre più di integrazione e sinergia. Ulteriore particolare attenzione sarà dedicata ai giovani, ai rapporti internazionali e ad una maggiore integrazione tra la Federazione e le altre Società Scientifiche non accademiche e della libera professione per creare veramente le condizioni di una Veterinaria Unica. Primo banco di prova della nuova Federazione sarà il prossimo Congresso che si terrà ad Olbia dal 19 al 22 di Giugno 2019 il cui programma scientifico, concordato con il nuovo Comitato Scientifico, coordinato dalla Prof.ssa Maria Laura Bacci, dovrà essere arricchito da contributi frutto dell'impegno dei nostri migliori ricercatori con l'obiettivo di innalzare ulteriormente il trend, in costante crescita negli ultimi anni, sia in termini numerici che di qualità. In questo momento di transizione corre l'obbligo di ringraziare il Comitato direttivo e il Comitato scientifico che ci hanno preceduti e che tanto hanno fatto per il raggiungimento dei nuovi traguardi. In particolare, desidero ringraziare il prof. Bartolo Biolatti che fortunatamente continuerà a sostenerci con la sua esperienza, competenza e dedizione, nel suo nuovo ruolo istituzionale di *Past President*.
Un in bocca al lupo a tutti!

Antonio Crovace
Presidente SISVet



COMITATO SCIENTIFICO DELLA FEDERAZIONE SISVET

Coordinatrice

Maria Laura Bacci **(SOFIVET)**

Componenti

Antonio Crovace

Adriana Ianieri

AIPVET: Giacomo Rossi

AIVI: Anastasio Aniello

AMV: Francesco Abbate

ARNA: Andrea Formigoni

ANIV: Maurizio Mazzei

RNIV: Erminio Trevisi

SICLIM-VET: Micaela Sgorbini

SICV: Francesco Staffieri

SIFTVet: Petra Cagnardi

SIRA: Fausto Cremonesi

SOIPA: Giuseppe Cringoli

Conferenza dei direttori: Eraldo Sanna Passino

Direzione generale della sanità animale e dei farmaci veterinari:

Silvio Borrello

Direzione generale per l'igiene e la sicurezza degli alimenti e la nutrizione: Gaetana Ferri

FNOVI: Gaetano Penocchio

ABSTRACT

WORKSHOPS

and

Main Lectures

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

WORKSHOP 1

L'immunoterapia Nella Cura Dei Tumori Animali: Stato Dell'arte E Sviluppi Futuri

In collaborazione con
AIPVET e RNIV

Moderatori:
Dr.ssa Elisabetta Razzuoli, Prof.ssa Chiara Brachelente

9.45	Introduzione dei lavori
10.00	Ruolo del sistema immunitario nello sviluppo e nella progressione tumorale **Paola Allavena** Responsabile del laboratorio di immunologia cellulare – Humanitas Research Hospital –Milano
10.30	Sviluppo di vaccini a DNA anti-CSPG4 come nuova opzione terapeutica per la lotta contro il melanoma e l'osteosarcoma canino **Federica Riccardo** Università degli Studi di Torino
11.00	Sviluppo di approcci terapeutici alternativi nella lotta ai tumori: l'attività antitumorale di *S. thiphymurium* **Barbara Chirullo** Istituto Superiore di Sanità, Roma
11.30	Immunoterapia in oncologia veterinaria: la quarta strategia per combattere il cancro nel cane **Laura Marconato** Università di Bologna
12.00-12.30	Discussione

Role of the immune system in the development and progression of tumors

Paola Allavena

Dpt. Immunology and Inflammation, Lab. Cellular Immunology, IRCCS Humanitas Research Hospital, Rozzano, Milan, Italy

Corresponding author: Paola Allavena (paola.allavena@humanitasresearch.it)

The presence and functional activities of immune cells in tumors are raising increasing interest since they are relevant modulators of anti-cancer therapies and potential targets of specific treatments. In the last decades, our knowledge on their functional role and on their reciprocal interaction with tumor cells has remarkably increased. The amount and types of tumor-infiltrating leukocytes, commonly referred to as immune landscape, has been robustly shown to have prognostic value. For instance, the density of CD8+ T cells usually correlates with longer survival and better response to therapies. Strategies to stimulate effective immune responses against cancer have been explored since several decades. After years of modest outcomes, a significant breakthrough was achieved with the use of inhibitors to immune checkpoints (e.g., PD-1 and CTLA-4) that reactivate antitumor immunity, demonstrating the possibility of manipulating a patient's immune system to elicit significant responses. However, a significant proportion of patients do not respond to immunotherapies, or become resistant overtime. The identification of correlates of resistance and drivers of tumor progression is of paramount importance to optimize and consolidate the anti-tumor response elicited by immunotherapy.

Cooperation between innate and adaptive immunity is desirable for achieving a long-lasting, efficient anti-tumor response. However, cells of the innate immunity (e.g. macrophages) within the tumor micro-environment, are not only mostly inefficient against cancer cells, but actually promote tumor progression and hamper treatment efficiency. Tumor-associated macrophages (TAMs) are the most represented component of the innate immunity in the tumor stroma. TAMs support cancer cell survival and proliferation, distant spreading of metastases and angiogenesis; they also build an immunosuppressive milieu, which hampers the cytotoxic function of T lymphocytes. Therefore, there is a growing interest in the modulation TAM functions for therapeutic purposes (1).

During the last decades, several therapeutic approaches have been implemented in pre-clinical cancer models to neutralize the tumor-promoting roles of macrophages. Early attempts employed toxic compounds to reduce macrophage numbers in solid tumors; however, it is becoming increasingly clear that strategies aimed at reprogramming macrophages in tumors have a better chance of success. The re-education of TAMs has been investigated in the last years using different therapeutic approaches, such as agonist mAbs directed to surface receptors (e.g. CD40), synthetic compounds as agonists of Toll-like receptors, or blocking mAbs directed to inhibitory receptors (e.g. SIRP1a). So far, experimental results have indicated that the reprogramming of TAMs is feasible and promising. Furthermore, preclinical and initial clinical studies have shown that rather than monotherapies, combination therapies that tackle different mechanisms are more efficient for the appropriate stimulation of immune cells against tumors.

[1] Allavena P. et al. Therapeutic Manipulation of Tumor-associated Macrophages: Facts and Hopes. 2021 Clin Cancer Res 2021; 27:1–7.

Immunotherapy in veterinary oncology: the fourth strategy to fight cancer in dogs

Laura Marconato

Università degli Studi di Bologna, Dipartimento di Scienze Mediche Veterinarie

Corresponding author: Laura Marconato (laura.marconato@unibo.it)

The impetuous development of immunology and molecular oncology has led to a strong resumption of basic and clinical research in the field of immunotherapy for the treatment of cancer patients including, among others, lymphoma, osteosarcoma and melanoma, both in human as well as in veterinary oncology [1-3]. By simplifying very complex concepts, immunotherapy stimulates the host's immune system to recognize and eradicate the transformed cells, sparing the healthy ones, therefore acting selectively. In this sense, the patient's immune status is clearly fundamental in determining treatment response.

In general, there are four forms of immunotherapy: cytokine therapy, cellular therapy, antibody therapy (especially immune checkpoint blockade), and therapeutic vaccines.

Cytokines are molecular messengers that allow the cells of the immune system to communicate with one another; no data have been published regarding their efficacy in veterinary oncology patients. Monoclonal antibodies (MAbs) are directed against neoplastic cells, to which drugs with cytotoxic activity, cytokines or radioisotope substances that destroy cancer cells are linked [4]. In particular, MAbs bind to tumor antigens (TAAs - tumor-associated antigens) or to antigens that are overexpressed in neoplastic cells, causing their death through various mechanisms (apoptosis, antibody-dependent cytotoxicity, complement-dependent cytotoxicity, phagocytosis, inhibition of growth pathways or immune checkpoints). In particular, regarding immune checkpoints, growing knowledge of the factors responsible for protecting cancer cells from immune destruction has led to the development of novel, immune-based, anti-cancer treatment modalities.

The use of monoclonal antibodies to block either PD-1 or PD-L1 has produced outstanding clinical responses. Thus, the most widespread treatment approach using immune checkpoint inhibitors can cure patients with widely metastatic tumors. In veterinary oncology, immunotherapy is still at an embryonic stage. The only currently available weapon relies on cancer vaccines. Active immunotherapy triggers an antitumor response by stimulating the patient's immune system, typically through vaccination. Under normal conditions, TAAs are processed and presented by antigen-presenting cells to T lymphocytes, with secondary activation and proliferation of the same T lymphocytes, and elimination of neoplastic cells.

The main obstacle to this mechanism is the so-called immuno-editing, a remodeling process through which tumor cells, poorly immunogenic, evade immune surveillance. Cancer vaccines present TAAs in a more immunogenic form, thereby correcting deficiencies in tumor surveillance. Furthermore, immunotherapy is also able to establish long-term antitumor immunity ("memory"), reducing the risk of relapse.

However, since these are therapeutic and non-prophylactic vaccinations, it is necessary to take into account the compromise of the immune system of the patients themselves, caused by both the tumor and any previous therapy. Indeed, inducing an immune capacity in a host that has failed to develop an effective response for the first time requires special procedures that allow the presentation of TAAs to the host's effector cells. Intact tumor cells, well-characterized tumor antigens or generic immunostimulants can be used as a source of antigens. Lymphomas are particularly suitable for vaccine production. Considerable experience has accumulated in human oncology, and active immunotherapy has recently entered the therapeutic landscape of dogs with lymphoma as well [5-7].

Ongoing clinical trials at the University of Bologna are focused on vaccination against canine osteosarcoma, melanoma and hemangiosarcoma, with very promising results.

[1] Anderson KL, Modiano JF. Progress in Adaptive Immunotherapy for Cancer in Companion Animals: Success on the Path to a Cure. Vet Sci. 2:363-387, 2015.
[2] Dow S. A Role for Dogs in Advancing Cancer Immunotherapy Research. Front Immunol. 10:2935, 2020.
[3] Tarone L, et al. Naturally occurring cancers in pet dogs as pre-clinical models for cancer immunotherapy. Cancer Immunol Immunother. 68:1839-1853, 2019.
[4] Beirão BC, et al. Challenges and opportunities for monoclonal antibody therapy in veterinary oncology. Vet J. 218:40-50, 2016.
[5] Marconato L, et al. Randomized, placebo-controlled, double-blinded chemoimmunotherapy clinical trial in a pet dog model of diffuse large B-cell lymphoma. Clin Cancer Res. 20:668-77, 2014.
[6] Marconato L, et al. Enhanced therapeutic effect of APAVAC immunotherapy in combination with dose-intense chemotherapy in dogs with advanced indolent B-cell lymphoma. Vaccine. 33:5080-6, 2015.
[7] Marconato L, et al. Opportunities and challenges of active immunotherapy in dogs with B-cell lymphoma: a 5-year experience in two veterinary oncology centers. J Immunother Cancer. 7:146, 2019.

WORKSHOP 2

Consumo di carni e salute umana

In collaborazione con
ARNA – AIVI -SOIPA

Moderatori:
Prof. L. Bailoni, Prof. G. Bertoni

9.00	Saluto e presentazione di ARNA **L. Bailoni/G. Bertoni**
9.15	Evoluzione della carne e dei salumi italiani negli ultimi 50 anni **Giuseppe Pulina** Università di Sassari
9.45	Caratteristiche nutrizionali delle carni in rapporto alla salute umana **Franca Marangoni** Nutrition Foundation of Italy
10.15	Rischi parassitologici delle carni in Italia **Laura Kramer** Università di Parma
10.45	Pausa Caffé
11.00	Effetto dei nuovi sistemi di maturazione sulle caratteristiche sensoriali e nutrizionali della carne **Raffaele Marrone** Università degli studi di Napoli
11.30	Carni rosse, ossidazione e colesterolo **G. Lercker e M. Cocchi** Università degli studi di Bologna
12.00	Esperienze tecnologiche di additivazione dei salumi **Giuliano Dallolio** Consulente Industrie carni
12.15	Il consumo di carne negli ultimi vent'anni e tendenze future **C. Truzzi** - METRO **M. Tassinari** - Università degli studi di Bologna
12.30	Discussione **L. Bailoni** Università degli studi di Padova

Evolution of Italian meat and cured meats over the last 50 years

Giuseppe Pulina (1), Andrea Rossi (2), Davide Calderone (2), Gianni Battacone (1)

(1) Università degli Studi di Sassari Dipartimento di Agraria; (2) ASSICA, Rozzano (MI)

Corresponding author: Giuseppe Pulina (gpulina@uniss.it)

Italy is on of the main high quality cured meats producers in EU 27, accounting for 23% of the total PDO (no. 21) and PGI (no. 22) recognised by the Union. The 70% of the national pig-stock is destined for this type of production. Driven by consumer needs, pork produced in Italy has become increasingly lean: in the period 1990-2011 [1], yields in lean cuts increased by over 10%, while those in lard fell by 75-80%. However, the slaughter weight from 2007 to 2017 increased by 11%, with animals currently slaughtered at 164 kg live weight. The production of leaner meats was reflected in the fat content of cured meats which generally decreased. For example, the fat content of Cotechino Modena PGI was reduced by 34%, that of Mortadella Bologna PGI, by 11%, that of Prosciutto di San Daniele PDO. By 19%, that of Speck Alto Adige PGI, by 8%. The caloric content e has consequently decreased and is currently averaging (for the 22 main cured meat) 165±60 kcal/50g of products, with an average protein content of 12.4±3.0 g. Parallel to the reduction of the fat content, the Italian cured meats have improved the composition of fatty acids: SFA of Cotechino Modena PGI was reduced by 38%, that of Mortadella Bologna PGI by 11%, that of Prosciutto di San Daniele PDO by 14%. If fact, the SFA/UFA ratio in Italian cured meat is below 0.5. The salt (NaCl) content of the main Italian cured meats dropped on average 20%, as well as that of nitrates which collapsed by 83% in average. Summarising, in 20 years Italian cured meats have dramatically improved their nutritional value and lowered the health risk factors. The production of Italian cured meats has also improved in terms of environmental impacts. Higher body weight of litter size finished per sow reared resulted in lower GHG, eutrophisation and acidification impacts per kg of pig sold (2). These positive results for the consumer are the result of the combined efforts of the breeding and processing phase. In the breeding phase, the improvement of feeding techniques and genetic selection have profoundly affected the type of pig that is currently used by the industry. In the transformation phase, the advancement of conservation technologies and the rigorous control of hygienic processes have made it possible to reduce the salt content and practically cancel the nitrate content. Italian cured meats are part of Mediterranean diet and consumed responsably they represent a dietetic source of high value proteins and nutritional cofactors. Because of the processing methods, Italian cured meats maintain the qualities of the meat they derive unaltered, to which they are also comparable for risk factors. Actually, Italian cured meats consumed responsibly can be classified, as fresh meat, no having risk factors for health, according to Global Burden Disease Diet group [3].

[1] Istituto Nazionale di Ricerca per gli Alimenti e la Nutrizione (INRAN), Stazione Sperimentale per l'Industria delle Conserve Alimentari (SSICA), Istituto Valorizzazione Salumi Italiani (IVSI), Istituto Salumi Italiani Tutelati (ISIT) , 2011. Salumi italiani: nuovi valori, nuovo valore.
[2] Bava L., Zucali M., Sandrucci A., Tamburini A., Environmental impact of heavy pig productin in Italy 2017, Journal of Cleaner Production, 140: 685-691.
(3) Global Burden Disease Diet Contributors, Health dietary risck in 195 countries, 1990-2017: a systematic analysis of Global Burden Disease study 2017, 2019, The Lancet, 393: 1958-72.

Risk of food-borne parasites in meat: current situation in Italy

Laura Kramer, Marco Genchi, Alice Vismarra

Università degli Studi di Parma, Dipartimento di Scienze Medico-Veterinarie

Corresponding author: Laura Kramer (laurahelen.kramer@unipr.it)

Food-borne parasitic zoonoses represent an important challenge for the meat production chain. Control and prevention depend on both farm management and meat inspection at slaughter. The increasing consumer demand for high quality and residue-free meat has stimulated research of new control strategies. The following review will illustrate the current epidemiological situation in Italy of the most common food-borne parasitic zoonoses transmitted by meat.

Toxoplasma gondii is a food-borne zoonotic parasite causing toxoplasmosis. Prevalence of human infection in Italy as surveyed in pregnant women, ranges from 40-80% (1) and risk factors include living in rural areas and consuming raw/undercooked meat (2). Lamb, mutton, pork and chicken are most common meat products containing tissue cysts of *T. gondii*, while beef is less important in transmission (3). Infection with *T. gondii* in livestock is usually through ingestion of oocysts that contaminate pasture, feed and water. Prevalence in sheep in Italy range from 20%-80% (4, 5). Recent studies in Italy have reported that *T. gondii* prevalence in free-ranging pigs can be as high as 90%, while confinement-reared pigs are at a very low risk of infection (6, 7). Prevalence in beef is relatively low (8). Control of *T. gondii* in livestock is based on farm management measures including cat and rodent control and adequate protection of feed and water from contamination with cat faeces. Post-slaughter treatment of meat (freezing, cooking, and aging) can disactivate the parasite.

Trichinella spp is a group of nematodes that are transmitted through ingestion of raw/undercooked meat. Human trichinellosis is a potentially life-threatening disease. Pigs are the most common hosts of *Trichinella spiralis*, but prevalence has been greatly reduced by confinement rearing. However, backyard and free-ranging pigs may be at risk. Imported horse meat is also a well-known source of infection. Over 750 cases of human trichinellosis have been reported in the last 25 years in Italy. *T. spiralis* was responsible for over 80% of these. The major sources of infections were: horsemeat (82.2%); wild boar fresh sausages (11.9%), meat from pigs slaughtered without any veterinary control (5.9%). *Trichinella britovi* is the most widely distributed species within sylvatic life cycles of Europe (9) and the most common etiological agent of infection in Italy. *T. britovi* is maintained in nature by a sylvatic cycle in which the red fox (*Vulpes vulpes*) is the main reservoir. The most recent outbreaks in Italy have been linked to ingestion of wild boar sausages. Inspection remains the only control measure.

Taenia saginata is the most common human tapeworm in Europe, while *T. solium* is very rare (10). Infection is through ingestion of raw/undercooked beef containing cysticerci. The prevalence of cysticercosis in cattle in Italy ranges from 0-1.1%. Risk factors for cattle include importation from endemic areas (France), open water sources, the use of human fertilizer on pasture and the lack of personal hygiene amongst farm employees (10). Current monitoring should continue to be based on visual meat inspection, because more sensitive methods are not yet commercially available or fully validated for a routine diagnosis.

An example of best practice is the regional monitoring programme in Veneto, which is aimed at identifying affected farms and implementing farm management measures of prevention.

Consumption of undercooked pork from home slaughtered pigs could pose a risk for exposure to *T. solium* and the conditions necessary for the transmission between pigs and humans still persist in some European countries (11).

1) Martini et al., Toxoplasmosis and knowledge: what do the Italian women know about?, Epidemiology and Infection, 148, e256, 1–11, 2020.
2) Thaller et al., Risk factors for toxoplasmosis in pregnant women in central Italy, Le Infezioni in Medicina, 4:241-247, 2011.
3) EFSA Panel on Biological Hazards, Public health risks associated with food-borne parasites, EFSA Journal, doi: 10.2903/2018.
4) Gazzonis et al., *Toxoplasma gondii* infection in meat-producing small ruminants: Meat juice serology and genotyping, Parasitol Int,6:10-20, 2020.
5) Vismarra et al., *Toxoplasma gondii* in the Cornigliese sheep breed in Italy: Meat juice serology, in vitro isolation and genotyping. Vet Parasitol, 243:125-129,2017.
6) Papini et al., Occurrence of *Toxoplasma gondii* in Carcasses of Pigs Reared in Intensive Systems in Northern Italy. J Food Prot, 3:515-522, 2017.
7) Bacci et al., Detection of *Toxoplasma gondii* in free-range, organic pigs in Italy using serological and molecular methods, Int J Food Microbiol, 202:54-56, 2015.
8) Gazzonis et al., *Toxoplasma gondii* seroprevalence in beef cattle raised in Italy: a multicenter study, Parasitology Research, 119:3893–3898, 2020.
9) Troiano and Nante, Human Trichinellosis in Italy: an epidemiological review since 1989, Prev Med Hyg, 60:E71-E75, 2019.
10) Laranjo-Gonzalez et al., Epidemiology of taeniosis/cysticercosis in Europe, a systematic review: western Europe, Parasit Vectors, 10:349-356, 2017.
11) Meester et al., A quantitative risk assessment for human *Taenia solium* exposure from home slaughtered pigs in European countries Parasit Vectors, 12:82-96, 2019.

Effect of the new ageing systems on the sensory and nutritional characteristics of meat

Raffaele Marrone

Università degli Studi di Napoli Federico II, Dipartimento di Medicina Veterinaria e Produzioni Animali

Corresponding author: raffaele.marrone@unina.it

Meat is a good source of nutrients for human beings and provides high quality protein, fats, minerals and vitamins [1]. Red meat particularly, is a good source of zinc, iron, selenium, calcium, phosphorus and lipids, followed by vitamin A and B-complex vitamins [2]. Ageing processes in meat are associated with the development of enhanced characteristics, as increased tenderness, flavour and overall palatability [3]. Recently, it has been seen that consumers are more attracted in unprocessed food, such as raw ground meat (ex. tartare) or rare cooked meat steak, and tenderness, colour and freshness are considered to be among the most important value-determining factors affecting consumers' meat purchasing decision [4,5]. Controlled ageing process improve the sensory characteristics of meat, such as tenderness, aromas and flavors, making them more attractive to restaurant owners. This results in a product with high added value for gastronomic appreciation, thus enabling the provision of tools to food chain operators to improve the economic yield of food they market. Therefore, a major modern food technology goal is to guarantee safe and specific ageing processes with innovative methods by monitoring the physical and chemical state of meat during the transformation process (bacterial alterations, acidification, pH). Industry interest is growing and further information is needed to fully assess the potential of these methods in allowing for the also increase in shelf life in accordance with regulations in force on food safety, in a natural way and without additives. The impact of ageing and following tenderization on meat quality depends on many factors such as species, animal age, diet, breed, type of muscle, marbling characteristic and ageing conditions [6]. Innovative concepts regarding beef ageing has been described in many studies [7-8]. These systems improve post-mortem proteolysis and calpains myofibrillar processing that both have an important role in influencing meat tenderness and represent a marker of meat quality [9]. These benefits are less trimming loses, a high diminution of the shrinkage rates, as the own development of flavours and organoleptic characteristics. The assessment of modern ageing chambers leads us to clarify the actual conditions when meat is exposed to ageing process. The knowledge of these parameters allows us to determine corrective measurements, allows to monitor the physical and chemical state of the meat during the process and satisfies international food safety standards. Moreover, recent studies in progress explain that the use of this ageing innovative systems enhances the amount of active biomolecules (δ-Valerobetaine and γ-Butyrobetaine) in buffalo meat during maturation time without affect meat microbiological profile. In particular, the functional quality of raw ground buffalo meat has improved and the product could be directly sold in restaurant as a ready-to-eat food (e.g. tartare, carpaccio) that are very requested by the consumers. The emerging technologies employed for achieving meat tenderness could also be used in conjunction with the aging process to obtain high nutritional value meat products desired by the consumer. Future research in the field of postmortem aging should be focused on the applications of the new emerging physical technologies also in the stages prior to aging, in order to shorten the aging time and achieve the desired characteristics in the aged meat.

[1] Pereira et al. Vicente, A.F. Meat nutritional composition and nutritive role in the human diet. Meat Sci. 2013, 93, 586–592. [2] Tamburrano et al. Biochemical and nutritional characteristics of buffalo meat and potential implications on human health for a personalized nutrition. Ital. J. Food. Saf. 2019, 8, 8317.

Red meats, oxidation and cholesterol

Giovanni Lercker, Massimo Cocchi

Alma Mater Studiorum-Università di Bologna, Dipartimento di Scienze Veterinarie

Corresponding Author: G. Lercker (giovanni.lercker@unibo.it)

Red meats

Red meats have a negative health reputation based on correlations obtained in the past and to be reconsidered in the light of scientific developments over the past 30 years. Current knowledge identifies the most important aspects to consider in the methods of production, maturation, transformation and conservation.

Oxidation

Almost all molecules can oxidize, but more easily if reactive "sites" are present in their structure. Many biological oxidations are problematic, but many others occur due to the need for the proper functioning of living organisms. In the maturation of meats and their conservation pending sale and transformation into food, the role of temperature is decisive. It is known that each increase of about 10 ° C doubles the oxidation rate of the lipid components, but is halved with cooling. Little-known scientific discoveries, even to experts, have conditioned the positive innovations on the subject, as often happens in applicative topics. For example, for the photosensitized oxidation in the initiation phase, the oxidation rate is not known, which is of the order of magnitude of about 1,600 - 32,000 times with respect to the classical one, it does not occur by radicalic route and it is not buffered with the most commonly used antioxidants. of "chain breaking" type. The importance of the photosensitized oxidation reaction has increased exponentially due to the discovery of the isomeric transformation of the two hydroperoxides of the monoenes, obtained and purified (from photosensitized oxidation), into eight hydroperoxides, in quantitative proportions similar to those of the hydroperoxides obtained by thermooxidation (high temperature).

Cholesterol

Named after the “molecule of life”, cholesterol has long had a negative health reputation as it is linked to its quantitative "correlation" in the blood with cardiovascular disease and cancer. "Bad" cholesterol and "good" cholesterol are still mentioned today in blood test results and are responsible for the misconceptions of populations. Unfortunately, the popular-biological management of cholesterol in humans has had the greatest responsibility for created beliefs. Oxidized cholesterol: partly biologically produced to generate particular oxygenated molecules, but largely derived from oxidative stress mechanisms suffered or acquired from the diet [case of minimally-oxidized LDL (mmLDLox)]. The most recent scientific knowledge has shown that the real culprits are other molecules derived from the oxidation of polyunsaturated fatty acids and cholesterol, aiming at the reduction of oxidative effects as a real remedy.

Technological experiences of additives to cured meats

Giuliano Dallolio

Università Cattolica del Sacro Cuore di Piacenza, Dipartimento di Scienze e Tecnologie Alimentari per una Filiera Agro-alimentare Sostenibile (DiSTAS).

Corresponding author: G. Dallolio (giuliano.dallolio@gmail.com)

Over the past 50 years, developments in food science and technology have led to the discovery of many new substances, called additives, that can perform various functions in food. We know that food products are subject to many environmental stresses, such as temperature fluctuations, oxidation, exposure to microbial developments, which can change their original composition. Food additives are therefore in many cases essential to preserve the qualities and characteristics of the food that consumers require, to ensure food safety.

In the meat sector, in recent years, some food ingredients and additives have been put under the magnifying glass by the media as they are considered “generally” dangerous. A particular discussion should be made regarding the addition of nitrites and nitrates to meat that can lead to the production of nitrosamines which are potentially carcinogenic molecules. Potassium nitrite (E 249), sodium nitrite (E 250), sodium nitrate (E251) and potassium nitrate (E 252) are additives, having a secondary conservative effect.

The main technological function is to form red pigments with myoglobin. The inhibiting effect of nitrites is expressed not only on *Cl. botulinum* also on other clostridia such as *Cl. perfringens*. The use of nitrates and nitrites in sausages is undoubtedly essential today for the specific action against botulinum toxinfection. In the technology of meat products in recent years, however, there is more and more research into the reduction or elimination of the use of these additives due to the possible risk in the development of nitrosamines. For this reason, the legislator has also updated the legislation on the use of nitrites and nitrates in cured meats. The decree of 27 February 2008 in fact takes up the provisions of Directive 2006/52/EC on the use of nitrites and nitrates in meat products, regulating the maximum doses that can be added during the manufacturing phase. More precisely, the new decree allows the use of: Potassium or sodium nitrite (alone or in combination) at the maximum dose of 150 mg/kg in meat products, both raw and cooked, with the exception of products based on sterilized meat (Fo>3.00) for which the maximum dose is 100 mg/kg; it should be remembered that nitrites cannot be sold pure due to their high acute toxicity and are sold mixed with at least 50% sodium chloride. For potassium or sodium nitrate (alone or in combination) the maximum addition dose is 150 mg/kg for non-heat-treated meat products. The use of nitrates in heat-treated products is therefore no longer permitted. For organic products the maximum additional dose is 80 mg/kg. The products notwithstanding this legislation are Bresaola IGP of Valtellina and speck IGP of Alto Adige. The potential danger of using nitrites and nitrates has prompted some producers to offer cured meats and in particular salamis, "without nitrites and nitrates". In these products, however, the red color is ensured by the use of plant extracts, liquid or powder, obtained from green leafy vegetables, especially spinach, lettuce and celery, but also from roots such as beetroot and carrot. These vegetables contain nitrate in varying concentrations, and can reach up to four to five thousand milligrams per kilogram. It is clear that the addition of plant extracts and not of nitrite or "chemical" nitrate allows the producer a "cleaner" labeling. However, plant extracts have several critical points. One of these is the knowledge of the exact concentration of nitrates and nitrites present in vegetable extracts intended for sausages in order not to exceed the indirect addition of these products. Furthermore, plant extracts have a different behavior depending on whether they have been heat treated or not. In untreated plant extracts, the nitrate acts as a nitrite "reservoir". The reduction from nitrate to nitrite occurs by nitrator-reducing bacteria, such as those of the genus

Staphylococcus. Which possess nitrate reductase. For this reason, in meats treated with heat, for example mortadella, cooked ham and other precooked meats, such as zamponi and cotechini, frankfurters, etc. the addition of nitrate from vegetable sources does not ensure an adequate amount of nitrite, except by inserting nitrate reducing bacteria or modifying the production process. For this reason, there are plant extracts in which nitrite obtained from pre-fermentation is present. In conclusion, even for vegetable extracts rich in nitrates and nitrites and proposed or used in meat preparations, it is necessary that those who use them know the exact concentration, to ensure that the legal limits established for these products are not exceeded. For consumers, who clearly cannot be aware of all these details but who must gain increasing trust in both institutions and food producers, maximum transparency is essential. For this reason, an ethical commitment of producers, mixers of ingredients and additives and of the bodies responsible for control is necessary so that “clean” labels are truly an indication of safe products. In the case of nitrates and nitrites it is necessary that the consumer be allowed to be clear whether these are present directly or indirectly. To date, if nitrates or nitrites are added to the label, it must not appear "without added nitrites", but the words "nitrates or nitrites from a vegetable matrix". At the beginning of my speech, I tried to explain how the safety of a food additive is scrupulously established by EFSA and other scientific authorities. For this reason, my personal belief, especially for the addition of nitrates and nitrites, is to use them respecting the doses established by current legislation and declare them on the label. The search for a "clean" label, the so-called "clean label", is correct only if the non-use of an additive deemed safe by scientific studies is not replaced by products that indirectly contain it. This in my opinion can only create doubts and confusion in the consumer.

Meat consumption in the last twenty years and future trends

Marco Tassinari (1), Claudio Truzzi (2)

(1) Università degli Studi di Bologna, Dipartimento di Scienze Mediche Veterinarie; (2) Metro Italia C & C, San Donato Milanese (MI)

Corresponding author: Marco Tassinari (marco.tassinari@unibo.it)

The production and consumption of meat in the world have grown dramatically fron the post-war period to today. Thus the quantity produced today is about 5 times greater than that of the early sixties of the twentieth century: it went from about 70 million tons in 1960 (with about 3 billion people) to 337.3 million tons in 2020 (with over 7.5 billion people). In Italy in 1960 the per capita consumption of meat was 29.4 kg, of wich 13 kg represented beef. Since the 1980s, consumption of beef stabilized, while those of poultry and pork increased significantly, which fueled the growth in meat consumption: in 2000 it reached 98.4 kg per capita of which 25.6 kg of beef, 37 kg of pork and 17.7 kg of poultry meat. In the last 20 years, the consumption of beef has gradually decreased, we must not forget the effect on consumption of the spread of the epidemic of bovine spongiform encephalopathy (BSE), which confused consumers by pushing them towards alternative meat consumption, in favor of poultry and pork meat. The change in the lifestyle of the italians has also brought about changes in food consumption: the younger generations were the avant-gardes of this change in lifestylles, not only because in those age groups the rejection of meat, especially beef, was marked compared to the adult population, but also because in this demographic range there was a rapid spread of vegetarian, if not even vegan, dietary practices. Thus, consumption varied from 24.8 kg for beef in 2007 to 17.2 kg per capita in 2018, from 39.2 kg to 32.5 for pork and from 18.3 to 19.7 kg for poultry meat. But the “real” consumption of meat is very different, as calculated by Russo et al. [1]. According to the apparent consumption data currently available (FAO and Ismea) [2, 3], on average an Italian inhabitant eats 237 g per day of all types of meat annually (about 87 kg per year). The real per capita consumption, on the other hand, corresponds to less than half, or 104 g per day of meat (38 kg per year). This consumption includes all meat, regardless of how and where it is consumed. Considering only the consumption of red meat (beef and pork) and cured meats, the real consumption is 69 g per day, equal to just over 25 kg per year. As for beef only, real consumption drops to 24.8 g per day per capita (just over 9 kg per year). The consumption of beef in recent years has also changed: in particular, only some fine cuts (fillet and ribs) or products such as hamburger are preferred. Another very important aspect on meat consumption was the growth of ready to cooks and ready to eat, specially prepared with poultry and/or pork (fourth and fifth processing). These “alternative” products are a very interesting solution for the consumer who does not want to “waste time” in the kitchen and buys pre-cooked products, mainly chicken and pork. According to the report by the Boston Consulting Group (BCG) and Horizon Corporation (BHC) [4], the menu could soon change: the run-up of vegetable alternatives to meat, such as fake meat, burgers made by Beyond Meat and Impossible Foods, and “clean meat” (meat grown in the laboratory starting from stem cells taken from animals) has already started and will not stop. The peak of meat consumption is expected in Europe and in the United States in 2025 year, then it will start to decline. In 2035 year,

11% of all meat, seafood, eggs and dairy products consumed worldwide are highly likely to be alternative. By that time, Europe and North America will have reached “peak meat” and animal protein consumption will begin to decline. In 2050 year we will be more than 9 billion people and insects are one of the possible answers to animal proteins: according to the FAO, more than 2 billion people already use insects for food purposes and the edible species on the market are over 1,900, therefore also these “alternative” proteins could replace part of the animal proteins.

[1] Russo V., De Angelis A., Danieli P.P. Consumo reale di carne e di pesce in Italia. Dal consume apparente al consume reale con il metodo della Detrazione Preventiva delle Perdite. Franco Angeli, Milano, 2017.
[2] FAO 2020. Food Outlook - Biannual Report on Global Food Markets. November 2020. Rome. https://doi.org/10.4060/cb1993en.
[3] ISMEA, 2014. Piano di settore, Studio sui consumi di carne bovina in Italia. http://www.pianidisettore.it/flex/cm/pages/ServeBLOB.php/L/IT/IDPagina/1059
[4] Björn Witte, Przemek Obloj, Sedef Koktenturk, Benjamin Morach, Michael Brigl, Jürgen Rogg, Ulrik Schulze, Decker Walker, Elfrun Von Koeller, Nico Dehnert, and Friederike Grosse-Holz. Food for Thought. The Protein Transformation. March 2021, BCG, Blue Horizon.

Workshop A – SOIPA

Le parassitosi nel gatto

23 Giugno 2021

Programma

Moderatori:
Antonio Frangipane di Regalbono (Università degli Studi di Padova)

Marco Genchi (Università degli Studi di Parma)

Fabrizio Bruschi (Presidente SOIPA)

14.00	Saluto e introduzione dei moderatori
14.10	Le filariosi nel gatto **Marco Genchi** Università degli Studi di Parma
14.40	Nuove acquisizioni sul controllo delle parassitosi polmonari del gatto **Angela Di Cesare** Università degli Studi di Teramo
15.10	La Leishmaniosi felina ai giorni nostri **Emanuele Brianti e Roberta Iatta** Università degli Studi di Messina e Università degli Studi di Bari
15.40	Il gatto come ospite definitivo dei cestodi **Vincenzo Musella** Università di Catanzaro
16.10	Le zecche… un problema anche per il gatto **Domenico Otranto** Università degli Studi di Bari
16.40	Diagnosi parassitologica nella pratica clinica, errori ed orrori **Luigi Venco** Libero Professionista
17.10	Parassitosi del gatto: le linee guida ESCCAP **Ezio Ferroglio** Università degli Studi di Torino
17.40	Discussione

Workshop 3

Cosa ci resterà dell'esperienza di didattica a distanza?

24 Giugno 2021

Programma

Moderatori: Prof. M. Forni, Prof. G. Crescenzo

10.00	Riflessioni sulla valutazione della didattica a distanza e saluti autorità **G. Crescenzo** Università degli studi di Bari
10.10	L'indagine ANVUR sulla DAD - risultati preliminari **M. Sabella** ANVUR
10.25	Esperienze raccolte dai docenti sul territorio nazionale **Relatori multipli**
11.10	Pausa caffè
11.20	Esperienze raccolte dagli studenti sul territorio nazionale **Relatori multipli**
11.50	Pratica didattica e valutazione online **P. Raviolo** Università eCampus
12.05	Le implicazioni pedagogiche dei modelli di didattica sincrona e mista **E. Luppi** Università degli studi di Bologna
12.20	Riflessioni sul futuro del ruolo docente e nuove esigenze di formazione e presentazione della SIDVet **M. Forni** Università degli studi di Bologna
12.30	Discussione **M. Forni** Università degli studi di Bologna

Workshop B – SOIPA

Il paradigma del controllo delle elmintosi degli equini. Cosa è cambiato in 30 anni.

24 Giugno 2021

In collaborazione con
SOIPA

Programma

Moderatori:
Vincenzo Veneziano (Università degli studi di Napoli)
Fabrizia Veronesi (Università degli studi di Perugia)

14.00	Saluto e introduzione dei moderatori
14.10	Ascaridiosi negli equini: aspetti morfo-biologici, clinici ed epidemiologici **Antonio Scala** Università di Sassari
14.40	Aggiornamenti sulle strongilosi negli equini **Sergio Zanzani *e* Maria Teresa Manfredi** Università di Milano
15.10	L'abronematidosi degli equini: i progressi nella conoscenza, le sfide future **Annunziata Giangaspero** Università di Foggia
15.40	Diagnosi coprologica negli equini **Maria Paola Maurelli** Università degli studi di Napoli
16.10	Piano di Assistenza Parassitologica negli Equini: una esperienza in campo **Viviana Caracciolo** Medico Veterinario Ippiatra
16.40	Parassiti ed equini: le linee guida ESCCAP **Ezio Ferroglio** Università degli studi di Torino
17.10	Discussione

Workshop 4

Medicina traslazionale

25 Giugno 2021

In collaborazione con
SICV e SICLIMVET

Programma

Moderatori:
Prof. Mauro Di Giancamillo (Università degli studi di Milano)
Dr.ssa Michela Bullone (Università degli studi di Torino)

9.30	Introduzione dei lavori
10.00	Meniscus salvage from basic science to preclinical studies **Giuseppe Peretti** Responsabile dell'Équipe Universitaria di Ortopedia Rigenerativa e Ricostruttiva dell'IRCCS Istituto Ortopedico Galeazzi di Milano
10.45	Modelli traslazionali di danno renale acuto e terapie sostitutive **Giuseppe Castellano** Direttore della scuola di specializzazione in Nefrologia Università degli Studi di Foggia
11.30	Ricerca traslazionale ed applicazioni cliniche: impianti personalizzati per ricostruzione e rigenerazione ossea **Donato Monopoli Forleo** Head Biomedical Engineering at ITC and R&D Advisor en osteobionix Las Palmas de Gran Canaria
12.15	The equine asthma model **Jean-Pierre Lavoie** Faculty of Veterinary Medicine, Université de Montréal, St-Hyacinthe, QC, Canada
13.00	Discussione

The equine asthma model

Jean-Pierre Lavoie (1), Michela Bullone (2)

(1) Université de Montréal, Faculté de médecine vétérinaire. (2) Università degli Studi di Torino, Dipartimento di Scienze Veterinarie.

Corresponding author: Jean-Pierre Lavoie (jean-pierre.lavoie@umontreal.ca)

Equine asthma is a syndrome that encompasses a number of clinical entities all leading to a similar clinical presentation. Horses previously diagnosed as suffering from recurrent airway obstruction (RAO or Heaves) and inflammatory airway diseases (IAD) represent the vast majority of the cases now labelled, respectively, as severe and mild/moderate equine asthma (SEA and MEA). This new terminology was proposed to facilitate the communication between all shareholders and because of the many similarities found between equine lower airway chronic inflammatory conditions and human asthma. To date, most of the research has been conducted on horses with the severe form of asthma, and SEA is now proposed as a spontaneous animal model for human asthma.

The equine asthma model assumption is based on the following similarities with the human disease: clinical signs indicative of reversible airflow obstruction and airway hyperresponsiveness, pathological features revealing a remodelling of the lower airways, and evidence of chronic active inflammatory response. These findings are confirmed for SEA while reversible airflow obstruction, airway inflammation and hyperresponsiveness are present in the milder forms of asthma, the currently limited studies suggests that some remodelling of the airways may also be found in the milder forms of asthma.

Airflow obstruction has been extensively documented in SEA using different approaches, the most accurate being the use of lung function measuring devices based on pressure and flows generated at different levels of the respiratory system during tidal breathing. Indirectly, airflow obstruction has been attributed mostly to bronchospasm, due to its reversibility following administration of bronchodilators. Bronchospasm results from the activation of the airway smooth muscle, and the causes underlying its pathological activation in disease remains largely unknown, even in human asthma. The available evidence suggests that both remodelling and inflammation play a role, but data are scarce and direct confirmation is hardly achievable, which strengthens the need of appropriate asthma models.

In light of this information, it is not surprising that most of the translational research conducted until now using the equine asthma model is centred on the airway smooth muscle and its role in the disease. Early histomorphometric studies have revealed that the airway smooth muscle mass is increased in equine asthma, and this could lead to an increased ability to generate force and “squeeze” the airway upon activation. Importantly, studies in horses showed that this increased in airway smooth muscle mass is only partially reversible with current antiasthma medications. More recent studies have also highlighted how biochemical alterations in the myosin heavy chain exist in asthma, their reversibility, and their relationship with altered mechanical properties. One of the roles of the equine asthma as a model for human asthma is thus to allow a better understanding of the lung structure-function relationship in disease. This is important as it could pinpoint the treatment target associated with the best predicted response. From this point of view, the equine asthma model is unique, because it offers the possibility to harvest bronchial and lung samples more easily compared to human beings, and sequentially in time, which is impossible when murine or small mammal models are used. Moreover, the equine model is advantageous because it is chronic and spontaneous, reflecting the similar nature of the human disease.

The role of the equine asthma model as a proxy of the inflammatory milieu typical of human asthma is more debated. Indeed, although it is well recognized that human asthma patients have a predominant eosinophilic inflammatory profile, SEA is a neutrophilic disease. On the other hand, evidences exist in support of a neutrophilic inflammatory response, either in the presence or in the absence of eosinophilia, in roughly half of asthmatics, and neutrophilia seems to be linked with a more severe form of the disease. Certainly, horses and humans can be exposed to environments rich in potential toxicants for the lung, and this could elicit a similar neutrophilic response in both species. In this perspective, the equine asthma model can shed new light also in the fields of respiratory hygiene, immunity and disease pathophysiology.

Workshop C – SOIPA

Di terra, di acqua e di aria

La micologia veterinaria in una visione One Health

25 Giugno 2021

In collaborazione con
SOIPA

Programma

Moderatori:
Francesca Mancianti (Università di Pisa)
Roberta Galuppi (Università degli Studi di Bologna)

15.00	Saluto e introduzione dei moderatori
15.10	I lieviti del genere Malassezia: un problema di sanità pubblica **Claudia Cafarchia** Università di Bari
15.40	Dermatofiti e zoonosi: a che punto siamo **Andrea Peano** Università degli studi di Torino
16.10	Gli animali come sentinelle ambientali di Cryptococcosi **Patrizia Danesi** IZS Padova
16.40	Aspergillosi: una micosi condivisa **Roberta Galuppi** Università degli Studi di Bologna
17.10	Oomyceti acquatici: interazione uomo/animale/ambiente **Perla Tedesco** Università degli Studi di Bologna
17.40	Trattamenti alternativi: quali applicazioni **Francesca Mancianti** Università di Pisa
18.10	Discussione

POST CONGRESS

Entero-colopatie nel cane e nel gatto: internista e nutrizionista a confronto

26 giugno 2021

Programma

In collaborazione con

AIVPA GE-NUT e SICLIMVET

Moderatore: Deborah Cattaneo

9.00	Introduzione dei lavori **Dott.ssa Deborah Cattaneo** - Coordinatrice del Gruppo di Studio AIVPA di Gastroenterologia **Dott.ssa Eleonora Fusi** - Coordinatrice del Gruppo di Studio AIVPA di Nutrizione
9.30	Addison senza inversione elettrolitica, una problematica sovradiagnosticata? **Federico Fracassi** European Veterinary Specialist in Small Animal Internal Medicine, UNIBO
10.10	Con l'enteropatia… ci mancava l'eosinofilia! **Maria Veronica Giordano** Phd student UNITE - Endovet
	Pausa caffè
11.20	Ematochezia nel gatto… un sintomo mai banale! **Andrea Campanile** Libero Professionista - Endovet
12.00	Diarrea cronica e dimagramento in un cane: la diagnosi che non ti aspetti **Andrea Cocci** Libero Professionista
12.40	Pausa Pranzo

Moderatore: Giacomo Rossi

14.00	Qui colon ci cova **Chiara Costa Devoti** Libero Professionista
14.40	La piattaforma nutrizionale Purina per le condizioni gastroenteriche **Biagio Leopanto** Purina
15.00	Può la dieta essere di supporto nelle enterocolopatie croniche? Partiamo dai casi clinici appena affrontati **Monica Cutrignelli** Professoressa nutrizione alimentazione animale - UNINA
15.40	Integratori: sono efficaci in corso di problematiche a carico di piccolo e grosso intestino? **Eleonora Fusi** Professoressa nutrizione alimentazione animale - UNIMI
16.20-17.00	Discussione e Chiusura Lavori

Addison without electrolyte abnormalities, an overdiagnosed condition?

Federico Fracassi

Naturally occurring hypoadrenocorticism is an uncommon disease in dogs with prevalence ranges from 0.06 to 0.28%; this condition is characterized by low production of glucocorticoids, mineralocorticoids, or both. It is assumed that primary hypoadrenocorticism results from a slowly progressing immune-mediated destruction and consecutive atrophy of the adrenal cortex. Rare other causes include trauma and infiltrative damage by neoplasia, abscess, and granulomatous inflammation. A subset of dogs with hypoadrenocorticism can have normal sodium and potassium concentrations. Some authors reported that this condition can be observed in up to 30% of dogs with hypoadrenocorticism. These dogs are classified as having eunatraemic, eukalaemic hypoadrenocorticism (EEH) ("atypical" hypoadrenocorticism). Basal serum cortisol >2 µg/dL allows to exclude hypoadrenocorticism, and the ACTH stimulation test (ACTHst) confirms the disease. In the diagnosis of EEH two main diagnostic difficulties can arise: 1) the absence of typical biochemical abnormalities makes EEH difficult to suspect and can often be mistaken for other diseases processes such as gastrointestinal disorders; 2) the previous administration of corticosteroids (very commonly used in dogs with gastroenterological problems) can give false-positive results on the ACTHst. In most of the studies, the absent administration of corticosteroids was based only on the anamnesis, and this likely led to an overdiagnosis of EEH. In a recent study performed in our institution, where every dog was investigated also measuring the endogenous ACTH concentration, we observed that hypoadrenocorticism without electrolyte abnormalities has a prevalence of <1% in dogs with chronic gastroenterological signs.

Enteropathy...eosinophilia is the last thing you want!

Maria Veronica Giordano

DVM, Dipl. MU, PhD student, Faculty of Veterinary Medicine, University of Teramo, Italy

An 8-year-old male White Swiss Shepherd Dog was referred for severe weight loss, vomiting, mucoid diarrhea with hematochezia and a recent onset of hematemesis and melena. History suggests an inadequate response to anthelmintic, dietary and antibiotic trials; a BCS 3/9, pale mucous membranes and abdominal pain were detected during physical examination. Blood analysis evidenced marked eosinophilia, panhypoproteinemia, hypocholesterolemia and hypocalcemia. Endoscopy revealed a severe erosive-ulcerative gastroenteropathy with severe lymphoplasmacytic and eosinophilic infiltration observed at histological evaluation. Despite an initial improvement with a novel protein diet, antiacid and immunosuppressant therapy, the dog was presented afterwards for a pericardial effusion that revealed a predominant eosinophilic composition on cytological examination. The dog was then presented several times with recurrent episodes, characterized by relapsing gastrointestinal signs always accompanied by marked peripheral eosinophilia. In the light of the inadequate response to treatment of the chronic enteropathy (CE) the owner elected for euthanasia. Eosinophils are important components of the immune system and are often involved in hypersensitivity disorders and/or parasitic infestation. Recent studies demonstrate a number of canine eosinophilic gastrointestinal (GI) disorders unrelated to parasitic infestation. Eosinophilic infiltration of GI is unfrequently associated with circulating hypereosinophilia both in dogs and humans. The underlying pathophysiology behind eosinophilic infiltration of GI tract remains uncertain, and although the main clinical signs of eosinophilic gastroenteritis are similar to those described in other types of CE, the clinical response and prognosis are usually worst.

[1] Sattasathuchana and Steiner, 2014.
[2] Harris et al., 2013.
[3] Keeshen et al., 2016.

Hematochezia in cats, a never banal symptom

Andrea Campanile, DVM, M.Sc.

Libero Professionista, Freelance Endovet Campania

Feline Infectious Peritonitis (FIP) is an immune-mediated disease induced by an infection sustained by Feline Coronavirus (FCoV), a virus belonging to the *Coronaviridae* family, which affects domestic and wild felids, representing one of the main causes of mortality in this species. Affected cats can show granulomatous lesions affecting the nervous system, eyes and parenchymatous organs and, secondary to an immune mediate vasculitis, there can be accumulation of fluid in the body cavities including the pericardium. A rare manifestation of feline infectious peritonitis (FIP), poorly described in the bibliography, is characterized by the presence of isolated and palpable granulomas affecting the intestinal wall, and localized in the colon or at the level of ileocecal valve.

Ciro is a cat, castrated male of 10 months of age, 3 kg of weight, showing a progressive and worsening symptomatology characterized by chronic intermittent diarrhea and constipation. The following diagnostic work-up is carried out: haemogram; biochemistry; electrophoretic protein profile; FIV-FELV serology; coprological examination; PCR essay for *Tritrichomonas fetus*; ELISA test for *Giardia* spp; chest and abdomen radiographic study; abdominal ultrasound; endoscopical examination; histological, immunohistochemical and Real Time PCR examination on biopsies of the colonic granuloma. The positivity for feline coronavirus (FCoV/FIP) on IHC examination and PCR allow us to diagnose colonic granuloma secondary to feline infectious intestinal peritonitis (FIP).

The aim of the following work was to describe an uncommon manifestation of feline infectious peritonitis (FIP) which, although rare, must be considered in the differential diagnosis of a cat with chronic constipation, diarrhea and compatible blood-biochemical changes.

Megacolon.... but there is a catch!

Chiara Costa Devoti

DVM, Dipl. MU, MRCVS, Internal Medicine Clinician Ospedale Veterinario San Michele, Tavazzano con Villavesco (LO)

A nine years old male neutered domestic long-haired cat presented for a 3-month history of rectal tenesmus and obstipation after several medical treatment failures. At presentation his colon was dilated and painful at abdominal palpation. The anal sphincter was oedematous with severely hyperaemic mucosae. X-rays and CT scan revealed a megacolon with a severe thickening of the rectal mucosa. After rectal biopsies, a subtotal colectomy was performed. After surgery the cat was medically managed in hospital and then at home with rectal mesalazine, prucalopride, lactulose, steroids and fibres with a partial remission of the clinical signs. The histological diagnosis was of rectal adenocarcinoma. Owners refused further surgical therapy and chemotherapy and after one month elected for euthanasia. Even if idiopathic megacolon is the most common cause of constipation in cats, severe colonic dilation can also be an acquired condition caused by mechanical obstructions, intraluminal, extraluminal or intramural. Rectal and colonic neoplasia are un uncommon cause of constipation in cats. When diagnosed and cause of prolonged obstructions, both surgical and medical therapy are warranted. Colonic and rectal adenocarcinomas occur rarely in cats but according to literature, sub-total colectomy associated with carboplatin chemotherapy can be a good treatment option for feline colo-rectal adenocarcinoma.

Supplements: are they effective in the course of problems affecting the small and large intestine?

Eleonora Fusi

Dietary supplementation is a keystone in the treatment of gastrointestinal disease in dogs and cats. The use of the appropriate diet and feeding plan, associated with pre-probiotic, omega3 (EPA and DHA) and antioxidant supplementation is the most common clinical approach. The advanced frontier is the use of postbiotics. Well known in human medicine, postbiotics include substances released or produced by the metabolism of microorganisms, which could exert positive effects on the host.

In light of specific supplementation, fibre plays a pivotal role. Nutrient often neglected because of their chemical-physical characteristics (solubility and fermentability), fibre is an important component of various commercially available diets recommended for several diseases as well as of several dietary supplements used in case of gastrointestinal disorders.

In the choice of the dietary strategies, it should be remembered that each animal and its intestinal microbiota could react in different ways. Therefore, trials and inevitable errors are required to reach the correct amounts.

According to the scientific literature in dogs and cats, few are the studies on the efficacy of dietary supplementation. Moreover, they have been conducted on a small number of animals, using different methodologies. Further studies, based on larger randomized controlled caseload and rigid protocols are needed.

Nutritional management of chronic enteropathy

Monica Isabella Cutrignelli

University of Napoli Federico II, Department of Veterinary Medicine and Animal Production, Napoli, Italy

Nutrition plays the main role in gastrointestinal disease management because the intestinal physiological function is the digestion and absorption of nutrients. Consequently, the therapeutic approach for acute and chronic gastrointestinal diseases includes nutritional treatment. Sometimes the diet is the cause of gastrointestinal disease, and nutritional therapy is more important than pharmacological one. In other conditions, the use of a conventional diet can lead to diarrhea because the digestion and absorption are compromised and dietary treatment does not cure disease, but reduces the clinical manifestations, acting as an adjuvant to drug therapy.

Identifying the optimal combination of pharmacological and nutritional therapy could be difficult because the approach varies among the patients and it could be easy to make mistakes. The nutritional treatments might include one or several of these options: high digestibility, low fat, or the use of one or more novel protein sources. Many gastrointestinal diets for dogs and cats are more digestible and increasing nutrients absorption better compared to normal diets. When the ability of the intestinal tract is compromised, these diets could be particularly advantageous and could limit undesirable signs such as flatulence and diarrhea due to the presence of a high amount of undigested food in the large intestine. Moreover, the reduction of lipid content for gastrointestinal patients could limit discomfort due to the delay in food passage from the stomach to the intestine. Finally, when a diet adverse response is suspected, the use of diets formulated with lower antigenic protein sources is frequently the first-choice treatment.

ORAL COMMUNICATIONS

AIPVET

MAIN LECTURE

Comparative pathology and pathogenesis of zoonotic respiratory coronavirus infections

Judith M.A. van den Brand

Utrecht University, Faculty of Veterinary Medicine, Department of Biomolecular Health Sciences, Division of Pathology

Corresponding author: Judith van den Brand (j.m.a.vandenbrand@uu.nl)

For almost two decades, coronaviruses have caused several outbreaks of severe respiratory disease in humans. The current pandemic coronavirus, severe acute respiratory syndrome-coronavirus 2 (SARS-CoV-2), causes an acute respiratory syndrome, coronavirus disease 2019 (COVID-19). As of April 16th, 2021 over 137 million cases were confirmed worldwide with more then 2 million confirmed deaths as stated by the WHO [1]. The previously emerging coronaviruses, SARS-CoV in 2003 in East Asia and Middle East respiratory syndrome (MERS)-CoV in 2012, caused respiratory disease such as SARS-CoV-2, however, with varying severity. Most people show mild cold-like symptoms, while some people develop severe respiratory disease called acute respiratory distress syndrome (ARDS) [2]. In these severe cases the patients develop diffuse alveolar damage (DAD) with hyaline membrane formation and pneumocyte type 2 proliferation that may progress to a fibrotic stage DAD [3]. In human fatal cases, the samples taken mostly do not represent the full range of the different temporal stages of the disease and may be hampered by clinical treatments, concurrent pre-existing diseases, or secondary infections. To study the pathogenesis and to develop vaccine and therapeutic measures of human emerging coronaviruses, animal models are needed. Depending on the aim of the research question, the choice and design of the animal models for human disease are important and include the best animal species to compare the human situation, the appropriate age, inoculation routes and time points [4].

To determine the best animal species used in animal models, again dependent on the aim of the study, the choice is related to several (clinical) parameters that need to be compared to those human disease, as well as ethical, cost-related and housing factors. Comparative pathology of respiratory lesions of non-human primates with human cases infected with SARS-CoV, SARS-CoV-2 and MERS-CoV showed many similarities. The lesions in SARS-CoV and SARS-CoV-2 were more severe and were associated with the presence of virus in type I and II pneumocytes, while the lesions in MERS-CoV were less severe with associated with presence of virus mainly in the type II pneumocytes [5]. An important factor for the cell type and tissue tropism of the virus can partly be represented by the presence and the pattern of distribution of the specific receptors to which the viral spike proteins bind for infection. For SARS-CoV and SARS-CoV-2 this receptor is angiotensin converting enzyme (ACE)2 and for MERS the receptor is dipeptidyl peptidase 4 (DPP4) [6-8]. The difference in the distribution of the receptors is often closely related to the cell types that are infected in both humans and different animal species as is shown for the difference in disease of cats and ferrets infected with SARS-CoV. In cats ACE2 and SARS-CoV antigen is present in both type I and II pneumocytes while in ferrets both ACE2 and antigen are only seen in type II pneumocytes [9]. In importance of the presence of the receptor is also demonstrated in the resistance of ferrets to MERS-COV infection due to the inability of ferret DPP4 to bind to the spike protein [10].

Not only the presence of receptors is important but also the age and the difference in animal species are important factors in the development of disease. The increased severity of disease can be, as seen in humans with SARS-CoV-2 related to the increase in age. This was also seen in aged nonhuman primates that showed an exacerbated innate host

response to SARS-CoV and were more likely to develop disease [11]. Also, the different animal species can show differences in severity of disease as was shown in nonhuman primates where African green monkeys developed more severe disease than Cynomolgus macaques [12].

Many different animal species are now being used for vaccine and therapeutical development. Additionally, research in reservoir or intermediate animal species, such as bats, mink (for SARS-CoV-2) and Dromedary camels (for MERS-COV) is important in elucidation of the pathogenesis of emerging zoonotic coronaviruses [13-15]. The present SARS-CoV-2 pandemic has provided an excellent platform for extensive and rapid coronavirus research where the use of SARS-CoV and MERS-COV research is of great value. However, still many research questions are still unanswered and yet to be elucidated to conquer this pandemic and to prepare for future outbreaks.

[1] WHO Coronavirus (COVID-19) Dashboard, https://covid19.who.int/, viewed 16th of April, 2021.
[2] Zhang et al. An overview of current knowledge of deadly CoVs and their interface with innate immunity, Viruses, 26;13(4):560, 2021.
[3] Bosmüller et al. The pulmonary pathology of COVID-19, Virchows Archive, 478(1):137-150, 2021.
[4] J.M.A. van den Brand et al. The Pathology and Pathogenesis of Experimental Severe Acute Respiratory Syndrome and Influenza in Animal Models, Journal of Comparative Pathology, 151(1): 83-112, 2014.
[5] Rockx et al. Comparative pathogenesis of COVID-19, MERS, and SARS in a nonhuman primate model, Science, 368(6494):1012-1015, 2020.
[6] Yan et al. Structural basis for the recognition of SARS-CoV-2 by full-length human ACE2, Science 367, 1444, 2020.
[7] Li et al. Angiotensin-converting enzyme 2 is a functional receptor for the SARS coronavirus, Nature, 426, 450–454, 2003.
[8] Raj et al. Dipeptidyl peptidase 4 is a functional receptor for the emerging human coronavirus-EMC, Nature, 495:251–254, 2013.
[9] van den Brand et al. Pathology of experimental SARS coronavirus infection in cats and ferrets, Veterinary Pathology, 45(4):551-62, 2008.
[10] Raj et al. Adenosine deaminase acts as a natural antagonist for dipeptidyl peptidase 4-mediated entry of the Middle East respiratory syndrome coronavirus, Journal of Virology, 88(3):1834-8, 2014.
[11] Smits et al. Exacerbated innate host response to SARS-CoV in aged non-human primates, PLoS Pathogens, 6(2):e1000756, 2010.
[12] Smits et al. Distinct severe acute respiratory syndrome coronavirus-induced acute lung injury pathways in two different nonhuman primate species, Journal of Virology, 85(9):4234-45, 2011.
[13] Widagdo et al. Tissue Distribution of the MERS-Coronavirus Receptor in Bats, Scientific Reports, 2017 Apr 26;7(1):1193.
[14] Haagmans et al. An orthopoxvirus-based vaccine reduces virus excretion after MERS-CoV infection in dromedary camels, Science, 351(6268):77-81, 2016.
[15] Vergara-Alert et al. Livestock Susceptibility to Infection with Middle East Respiratory Syndrome Coronavirus, Emerging Infectious Diseases, 23(2):232-240, 2017.

Effects of different antimicrobials prophylaxis protocols on intestinal histology and microbiota composition in broiler chickens

Matteo Cuccato (1), Sara Divari (1), Frine Eleonora Scaglione (1), Cinzia Centelleghe (2), Alessandra Sereno (1), Paola Pregel (1), Francesca Tiziana Cannizzo (1)

(1) Università degli Studi di Torino, Dipartimento di Scienze Veterinarie (2) Università degli Studi di Padova, Dipartimento di Biomedicina Comparata e Alimentazione

Corresponding author: Matteo Cuccato (matteo.cuccato@unito.it)

Antimicrobials (AM) are routinely used in poultry farming to preserve avian health against bacterial infections. Furthermore, in the past, AM were used at growth promoter doses to improve growth performances and intestinal health status [1]. One of the undesirable consequences of AM overuse is the presence of their residues in edible tissues, with possible health risks for consumers and antimicrobial resistance spreading. AM treatments can also lead to negative consequences for chickens and their gut health [2]. However, the effect on chickens of AM administered at prophylactic doses is still not well-known. Thus, this study aimed to investigate the effect of prophylaxis with different AM, routinely used in poultry farming, on intestinal microbiota and gut tissues in broiler chickens.

A total of 240 male broiler chickens was raised in the poultry farm of the Department of Veterinary Sciences of the University of Turin, as previously described [3]. Concisely, chickens were divided according to different AM prophylaxis protocols: amoxicillin (AMX), amoxicillin + diclazuril (AMX + DCZ), thiamphenicol (THP), thiamphenicol + diclazuril (THP + DCZ), trimethoprim + sulfadiazine (TRIM) and diclazuril (DCZ). Animals receiving no treatment were used as controls. Withdrawal periods were respected and, at the end of the rearing cycle, chickens were regularly slaughtered. Ileal, cecal and colonic tissues and contents were collected and stored until further investigations. Histological evaluation of gut tissues was performed by applying a semi-quantitative multiple scoring system, that aimed to evaluate the presence, severity and diffusion of villi lesions and inflammatory infiltrate. Microbiota diversity analysis, already described [4], revealed that AM treatments modified the composition of microbial communities: alpha diversity was affected only in ileal samples and beta diversity was affected also in the caecum. Moreover, the taxonomic analysis showed that *Enterococcaceae* family was significantly over-represented in AMX and THP groups. Histological results showed that epithelial detachment and conglutination of intestinal villi were the main lesions in AM treated groups. Moreover, a diffuse lymphocytic infiltrate was found. Analysis of caecum and colon samples revealed that epithelial lesions and inflammatory infiltration were significantly augmented in AMX and THP groups, compared to control group.

In conclusion, these findings suggest that AM prophylaxes have a negative impact on gut health of broiler chickens. In particular, this study revealed an important association between microbiota modification, induced by AM treatments, and histopathological findings. Further studies are required to better clarify the role of altered gut microbiota in the pathogenesis of intestinal damage.

[1] Mehdi, Y et al. Use of antibiotics in broiler production: Global impacts and alternatives. Anim. Nutr. 4:170–178, 2018.
[2] Pascual, A et al. Effect of dietary supplementation with yeast cell wall extracts on performance and gut response in broiler chickens. J. Anim. Sci. Biotechnol. 11:40, 2020.
[3] Giannuzzi, D et al. Application of RNA-sequencing to identify biomarkers in broiler chicken prophylactic administered with antimicrobial agents. Animal, 15:100113, 2021.
[4] Cuccato, M. et al. 16S rRNA sequencing analysis of the gut microbiota in broiler chickens prophylactically administered with antimicrobial agents. Antibiotics 10:1–10, 2021.

Analysis of environmental samples for the evaluation of laboratory animal health status

Andrea Cacciamali, Chiara Romano, Riccardo Villa

IZSLER, Centro di Referenza Nazionale per i Metodi Alternativi, Benessere e Cura degli Animali da Laboratorio

Corresponding author: Andrea Cacciamali (andrea.cacciamali@izsler.it)

The protection of welfare and care of experimental animals are two cornerstones in the field of health surveillance in animal enclosures, which, if implemented through monitoring programs, guarantees optimal health conditions, reliability and reproducibility of the experimental results. The pathogens screening, implemented with a program in accordance with FELASA recommendations [1], fits in the 3Rs principle, in particular in the Refinement and Reduction concepts. In this way is highly probable to obtain an improvement in animal and environmental conditions [2]. It is known that the analysis of environmental samples allows to obtain results comparable to those carried out on biological samples. This approach would have an important impact not only on the generation of quality experimental data, but also on the reduction of the number of animals sacrificed, with the maintenance of their appropriate state of health [3]. Nowadays, a health-monitoring program for the identification of viral agents from biological samples has been carried out in the IZSLER animal experimental facility, performing PCR and RT-PCR.

The aim of the work was to refine the techniques for the extraction of nucleic acids starting from environmental matrices specifically from samples of mouse fur, sawdust and enrichment material.

A first DNA and RNA extraction test on raw materials using the commercial kits DNeasy PowerSoil, RNeasy PowerSoil DNA Elution and RNeasy PowerSoil Total RNA from QIAGEN (Milan, Italy), did not provide quantifiable nucleic acid concentrations. Then, fur samples and enrichment materials were infected with DNA viruses, (*Ectromelia Virus* and *Minute Virus of Mice)* at known concentrations (10^1-10^2 DC50/ml), while sawdust was infected with both viruses. DNA was extracted by DNeasy PowerSoil. At the same time, the samples were processed by the protocol applied to the solid biological samples and the DNA extracted by QIAamp DNA Mini (Qiagen, Milan, Italy). DNA concentrations by QIAamp DNA Mini were 0.025 ng/µl from fur, 0.27 ng/µl from sawdust and 0.17 ng/µl from enrichment materials, while by DNeasy PowerSoil was 0.083 ng/µl from sawdust and 0.10 ng/µl from enrichment materials. DNA from the fur was not quantifiable. Cq mean values obtained in Real-Time PCR from enrichment materials and fur reflect the DNA concentrations extracted with the different kits. Otherwise, the sawdust Cq values show an opposite trend probably due to the complexity of the matrix. It was not possible to obtain a sufficient quantity of nucleic acids from raw materials, useful for subsequent molecular investigations. Only after direct infection with high concentrations of viruses, a small amount of DNA was obtained. Different variables can be involved in the explanation of the weak yield obtained: a very low presence of viral agents in the environment, their different stability or the specificity of the commercial kits used. For this reason, these preliminary results will be further investigated in order to obtain a suitable tool for diagnostic practice.

[1] Russell WMS, Burch RL. The principles of humane experimental technique. London, UK: Methuen, 1959. [2] M Mahler et al. FELASA recommendations for the health monitoring of mouse, rat, hamster, guinea pig and rabbit colonies in breeding and experimental units. Laboratory Animals 48(3) 178-192, 2014. [3] Compton SR. PCR and RT-PCR in the Diagnosis of Laboratory Animal Infections and in Health Monitoring. J Am Assoc Lab Anim Sci. Sep 1;59(5):458-468, 2020.

Sirtuin 1 expression in canine mammary carcinomas: preliminary investigations

Mariafrancesca Sgadari, Nunzio Antonio Cacciola, Brunella Restucci

Università degli Studi Napoli "Federico II", Dipartimento di Medicina Veterinaria e Produzioni Animali

Corresponding author: Mariafrancesca Sgadari (m.sgadari@unina.it)

Sirtuin 1 (SIRT1) is a NAD- dependent hystone (Class III HDAC) involved in the epigenetic regulation of tissue homeostasis and many diseases by deacetylating both histone and non-histone targets. Therefore it has been reported to be a crucial regulator in many cellular pathways with strong relevance in genome stability maintenance, metabolism and cancer development and progression.

However, the role played by SIRT1 is still controversial having been proposed both as oncogene and as tumor suppressor. The aim of this study was to evaluate the expression of SIRT1 in a series of canine mammary carcinomas (CMC) by immunohistochemistry (IHC) and Western Blot (WB) analyses. These were conducted on spontaneous carcinomas surgically excised as routine diagnosis and treatment according to Directives 2010/63/EU.

21 samples of CMTs were classified and divided into grade 1 (8), grade 2 (7) and grade 3 (6). In addition, 6 normal mammary gland tissues (NMG) obtained from those cases in which the entire chain of mammary glands was excised, were used as healthy control tissues. Immunoreactivity was scored considering the number of positive cells in 10 HPF (grade 0: no positive cells, 1: <10%; 2: 10–30%; 3: 31–60%; 4: >60%) and the intensity of staining graded as weak (1), moderate (2), and strong (3). Then, a combined immunoreactivity score (IRS), ranging from 0 to 12, was calculated for each specimen by multiplying the values of these two categories. One-way ANOVA was used to compare differences between groups. Results with $p< 0.05$ were considered statistically significant. WB analysis confirmed the cross-reactivity of the anti-human SIRT1 antibody in canine mammary gland. Upon IHC, a decrease of SIRT1 protein expression was detected between NMGs and CMCs ($p=0.06$). Specifically, in normal mammary glands, strong nuclear SIRT 1 protein expression was found in epithelial ductal cells (mean IRS =10.5 range 9-12). In G1 carcinomas, the intensity of immunostaining was strong (mean IRS=9.75 range 6-12) and localized in the nucleus, even if, a moderate cytoplasmic immunoreactivity, was detected in some neoplastic cells. A progressive decrease of IRS values and a loss of nuclear localization was observed in G2 (mean IRS=7 range 4-9) ($p=0.03$) and G3 carcinomas (mean IRS= 4 range 0-4) ($p=0.007$), respectively. Based on these results, we proved, for the first time, the presence of SIRT1 in canine mammary tissues and its reduced expression in CMCs. Our results are in agreement with Wang et al. (2008), who demonstrated that, in several human cancer SIRT1 was expressed at lower levels compared to their healthy controls, by acting as an oncosuppressor through its role in DNA damage response, genome stability and tumor suppression. Our results are also in line with Song et al., (2012) who demonstrated that subcellular localization may account for a dual role of SIRT1 in normal versus cancer cells: SIRT1 may target its nuclear substrates to exert tumor suppressor function and target its cytoplasmic substrates to exert its tumor promoter function. However, further studies are necessary to better clarify the subcellular localization of SIRT1 in cancer cells and the role played also in veterinary oncology.

[1] Song et al. Janus-faced role of SIRT1 in tumorigenesis, Ann N Y Acad Sci, 1271:10-19, 2012.
[2] Wang et al. Impaired DNA damage response, genome instability, and tumorigenesis in SIRT1 mutant mice. Cancer Cells 14:312-323, 2008.

Programmed cell death-1, PD-ligand 1 and CD8α expression in canine diffuse large B-cell lymphoma by RNA-scope

Luca Aresu (1), Laura Marconato (2), Valeria Martini (3), Antonella Fanelli (1), Luca Licenziato (1), Arturo Nicoletti (1), Erica Melchiotti (4), Greta Foiani (4), Marta Vascellari (4)

(1) Università degli Studi di Torino, Dipartimento di Scienze Veterinarie (2) Università degli Studi di Bologna, Dipartimento di Scienze Mediche Veterinarie (3) Università degli Studi di Milano, Dipartimento di Medicina Veterinaria (4) Istituto Zooprofilattico Sperimentale delle Venezie

Corresponding author: Arturo Nicoletti (arturo.nicoletti@unito.it)

Immune checkpoints are a set of molecules known to be dysregulated in several human and canine cancers causing immune evasion and resistance. Aberrations of the PD1/PDL1 axis are often correlated with a worse prognosis and therapeutic strategies hitting this pathway are reported to block the intrinsic negative immunity regulation increasing the anti-tumor activity [1-2].

To gain insight into the role of immune checkpoints in canine diffuse large B-cell lymphoma (cDLBCL), we investigated the expression of PD1, PDL1 and CD8α by RNA-scope in de-novo cDLBCLs. RNA-scope signals were categorized into five grades according to the manufacturer's scoring guidelines. Results were correlated with several clinico-pathological features, including treatment, Ki67 index, immune signatures, tumor-infiltrating lymphocytes (TILs) and outcome.

A total of 33 dogs undergone complete staging work-up and treated with CHOP (n=12) or CHOP plus APAVAC (n=21) were included. Based on the RNA-seq profiles, 19 dogs presented a "hot" immune signature, and the remnants were characterized by a "cold" one [2].

Considering RNA-scope results, an association between PDL1 and PD1 was obtained ($p=0.002$), conversely CD8α did not correlate with either score. Ki67 index was correlated to PDL1 and PD1 scores ($p=<0.001$ and $p=0.006$, respectively). Furthermore, dogs with a "hot" signature were characterized by higher PDL1 and PD1 scores ($p=0.005$ and $p=0.033$, respectively).

A significantly higher risk of relapse and lymphoma-related death was found in dogs treated with chemotherapy alone, dogs with a "hot" signature and dogs with higher PDL1 and PD1 scores.

PDL1 expression was previously reported in canine cancer cells, including B-cell lymphoma, while PD1 expression was mainly associated to TILs. Here, the signal localization and the lack of correlation between PD1 and CD8α demonstrate that PD1 is also expressed in cDLBCLs by tumor cells rather than TILs. Even if the biological consequence of PD1+ tumor cells remains largely unknown, our findings suggest that PD1 intrinsic expression in cDLBCL might contribute to tumor growth escaping adaptive immunity. This hypothesis is further supported by the correlation obtained between Ki67 index and PD1 and PDL1 scores. Finally, the concordance of PD1 and PDL1 expression with prognostic immune signatures indicates that RNA-scope assay represents an alternative to more expensive technologies such as RNA-seq and to immunohistochemistry whose antibodies specificity represents a limitation in veterinary medicine.

[1] Havel J.J., Chowell D., Chan T.A. The evolving landscape of biomarkers for checkpoint inhibitor immunotherapy, Nature Reviews Cancer, 19**:**133–150, 2019.
[2] Aresu L. et al. New molecular and therapeutic insights into canine diffuse large B-cell lymphoma elucidates the role of the dog as a model for human disease, Haematologica, 104(6):e256-e259, 2019.

Prognostic role of PLK1-MYC signalling axis in canine appendicular osteosarcoma and *in vitro* targeting by BI 2536

Cecilia Gola, Luca Licenziato, Emanuela Morello, Selina Iussich, Paolo Buracco, Luca Aresu, Raffaella De Maria

Dipartimento di Scienze Veterinarie, Università degli Studi di Torino

Corresponding author: Cecilia Gola (cecilia.gola@unito.it)

Osteosarcoma (OSA) is the most common primary malignant bone tumour in dogs and is characterized by locally aggressive and highly metastatic behaviour. Despite the current standard of care most dogs succumb to the disease indicating the need for novel and specific treatment strategies.

The Polo-like kinase 1 (PLK1) and its main downstream transcription factor c-MYC are overexpressed in a variety of human cancer types, including OSA, and are associated with tumour cells growth *in vitro* [1]. The cross-talk between the two molecules coordinates a series of cellular processes, including growth, proliferation, differentiation, self-renewal and apoptosis. Given its pivotal role as regulator of the cell cycle PLK1 recently emerged as a potential target for cancer therapy. In particular, BI 2536 selective PLK1 inhibitor was shown to promote mitotic arrest and apoptosis in a variety of cancer cells [2]. Within this context PLK1 inhibition has been deeply investigated in human OSA, whereas little is known in canine OSA.

This research aims to evaluate PLK1 and c-MYC protein expression on 53 appendicular OSA, complete with follow-up data, and *in vitro* effects of BI 2536 on D17 canine OSA cell line, including Western Blot and qRT-PCR of the target genes.

A strong immunohistochemical labelling of PLK1 and c-MYC was detected in 38.3% and 50% of cOSA, respectively, but no correlation with clinicopathological data, such as histological grade and metastases, was found. However, c-MYC strong positivity was associated with significantly ($p = 0.003$) reduced overall survival (203 days) compared to dogs with low c-MYC positivity (553 days), resulting in a possible negative prognostic marker in canine OSA. Western Blot and qRT-PCR assays revealed that D17 and D22 expressed higher protein and transcript levels of both PLK1 and c-MYC compared to Penny and Wall cell lines. When treated with BI 2536 inhibitor at 2.5nM for 24h, D17 cell line showed a substantial decrease in cell growth. Interestingly, treated cells also showed decreased PLK1 transcript levels after 24-hour treatment (range 2.5-7.5 nM), suggesting a direct target inhibition in canine OSA as well. Consistent with human OSA, these preliminary data outline the prognostic value of c-MYC expression in canine OSA [3]. Furthermore, our *in vitro* assays highlight the anti-proliferative effect of BI 2536 and the role of PLK1 as a potential therapeutic target in canine OSA. However, further investigations elucidating the crosstalk mechanisms between PLK1 and MYC and the effects of PLK1 inhibition in canine OSA are warranted.

[1] Davis et al. Integration of genomic, transcriptomic and functional profiles of aggressive osteosarcomas across multiple species, Oncotarget, 8(44):76241-76256, 2017.
[2] Mo et al. PLK1 contributes to autophagy by regulating MYC stabilization in osteosarcoma cells. OncoTargets and Therapy, 12:7527-7536, 2019.
[3] Feng Wet al. Myc is a prognostic biomarker and potential therapeutic target in osteosarcoma. Therapeutic Advances in Medical Oncology, 12, 2020.

Digital and computer-aided pathology classification of canine mammary tumors

Giovanni P. Burrai (1), Andrea Gabrieli (2), Salwa Hawko (1), Salvatore Pirino (1), Marta Polinas (1), Elisabetta Antuofermo (2)

(1) Università degli Studi di Sassari, Dipartimento di Medicina Veterinaria (2) Università degli Studi di Sassari, Dipartimento di Chimica e Farmacia

Corresponding author: Giovanni P. Burrai (gburrai@uniss.it)

Cancer is the leading cause of death in companion animals, and mammary tumor (CMT), the most common neoplasm in female dog, represents a serious issue in veterinary practice [1]. Detection and diagnosis of CMT, can be accomplished by several imaging procedures, however the histopathological analysis remains the standard technique to differentiate benign from malignant tumors. Histopathological examination is a time-consuming process, requiring trained specialists, and can be influenced by several intrinsic and extrinsic factors. Moreover, diagnosis based upon examination of slides encounters inter-observer variability, with approximately a diagnostic concordance of 75% between specialists [2]. Digital (DP) and Computer-aided pathology (CAD), are emergent fields that will deeply change the temporal and spatial domains of pathologic diagnosis, improving the overall classification accuracy and reducing the inter-observer variability. In this study, a CMT database of 1056 jpeg Hematoxylin and Eosin (H&E) images, acquired from 44 cases of CMTs, was explored with three different CAD systems. Each system is based on the combination of a convolutional neural network (VGG16, Inception v3, EfficientNet), which acts as a feature extractor, and a classifier [Support Vector Machines (SVM) or Random Forest (RF)] placed on top of the neural net. Different strategies of data augmentation (i.e random cropping - rotation) and testing were explored to test the ability of the CAD systems to distinguish benign from malignant tumors. A 2 minutes recorded footage was obtained using an optical microscope, equipped with a digital camera and the most representative 24 images were selected from a pool of frames. Thus, 1024x768 high-resolution 24–bit color depth RGB images were captured from 20 benign and 24 malignant CMT. The performance of our models was validated on the standard BreakHis dataset [3] reaching an accuracy ranging from 0.86 to 0.91 considering all combinations (feature extractors, classifiers, and testing strategies). Test accuracies of our framework applied to the CMT dataset for distinguishing between benign and malignant tumors ranged from 0.63 to 0.85 across all architectures. In particular, the framework using EfficientNet as a feature extractor coupled with SVM and the simple augmentation strategy, resulted in the best performances, with testing accuracies fluctuating from 0.82 to 0.85 based on data augmentation strategies. Considering the importance of correct diagnosis in patient management, substantial efforts have been made for developing robust, precise, and automated DP and CAD systems for humans. The application of similar DP and CAD systems in CMT provides encouraging results taking into account the breakthroughs in artificial intelligence and machine learning technologies in the mainstreaming of cancer-related research.

[1] Sorenmo KU et al. Tumors of the Mammary Gland. In Withrow & MacEwen's Small Animal Clinical Oncology, 6th ed., Elsevier, 604–615, 2020.
[2] Elmore et al. Diagnostic concordance among pathologists interpreting breast biopsy, Jama-Journal of the American Medical Association, 313:1122-1122, 2015.
[3] Breakhis et al. A dataset for breast cancer histopathological image classification, IEEE Transactions on Biomedical Engineering, 63:1455-1462, 2016.

EGFR, feline squamous cell carcinoma and cetuximab: bridging the gap between benchside and bedside

Gennaro Altamura, Manuela Martano, Paola Maiolino, Karen Power, Bianca Cuccaro, Giuseppe Borzacchiello

Università degli Studi di Napoli Federico II, Dipartimento di Medicina veterinaria e Produzioni animali

Corresponding author: Giuseppe Borzacchiello (borzacch@unina.it)

Oral squamous cell carcinoma (OSCC) is among the most common malignancies in cats [1]. Despite different therapeutic approaches (surgery, radiation therapy (RT) and chemotherapy (CT)) are available, it is often characterized by high rate of recurrence and poor prognosis, therefore feline OSCC (FOSCC) represents a major challenge in veterinary oncology [2]. In humans, over the 90% of head and neck SCC (HNSCC) show overexpression of epidermal growth factor receptor (EGFR) in association with a more aggressive disease and lower survival rate [3]. Preclinical and clinical studies have validated the use of the anti-EGFR monoclonal antibody Cetuximab for the treatment of HNSCC, so that it is currently employed in the clinical practice in combination with CT and RT [3]. EGFR is aberrantly expressed also in FOSCC and has been shown to play a key role in FOSCC cells proliferation and survival [4,5,6]. The aim of this study was to assess the potential anti-cancer activities sustained by treatment with Cetuximab in preclinical models of FOSCC expressing EGFR [6]. FOSCC cells (SCCFs) were treated with Cetuximab at different concentrations and collected at different time points, along with Cetuximab sensitive human OSCC cell line CAL-27. Growth curves, relative growth inhibition and cell viability were calculated by cell counting, trypan blue exclusion assays and commercially available colorimetric kit. Cells pellets were collected and analysed by western blotting (WB) for EGFR and its downstream molecules in their total and phosphorylated forms. Results showed that incubation with Cetuximab caused the inhibition of cell growth coupled with a decrease in cell viability in SCCF cells as well as in control cell line CAL-27, as a confirmation of the reliability of the experiments. WB analysis confirmed that Cetuximab downregulated EGFR molecular pathway in feline and human cell lines. Our preliminary results suggest that Cetuximab counteracts EGFR signalling and exerts potential anti-cancer activities in preclinical models of FOSCC by impairing cell growth and cell viability. Further characterization of the molecular mechanisms and validation of the efficacy on additional cell models would confirm our data, paving the way for future translational studies aimed at evaluating the therapeutic potential of Cetuximab for treatment of FOSCC. The use of Cetuximab in combination with RT and CT would be conceivable, similarly to human counterpart [2].

[1 Bilgic et al. Feline Oral Squamous Cell Carcinoma: Clinical Manifestations and Literature Review, Journal of Veterinary Dentistry, 32(1):30-40, 2015.
[2] Supsavhad et al. Animal models of head and neck squamous cell carcinoma, Veterinary Journal, 210:7-16, 2016.
[3] Concu R., Cordeiro MNDS. Cetuximab and the Head and Neck Squamous Cell Cancer, Current Topics in Medicinal Chemistry, 18(3):192-198, 2018.
[4] Wypij J.M. A naturally occurring feline model of head and neck squamous cell carcinoma, Pathology Research International, 2013:502197, 2013.
[5] Bergkvist et al. Studies on the inhibition of feline EGFR in squamous cell carcinoma: enhancement of radiosensitivity and rescue of resistance to small molecule inhibitors, Cancer Biology & Therapy, 11(11):927-937, 2011.
[6] Altamura et al. The Small Molecule BIBR1532 Exerts Potential Anti-cancer Activities in Preclinical Models of Feline Oral Squamous Cell Carcinoma Through Inhibition of Telomerase Activity and Down-Regulation of TERT, Frontiers in Veterinary Science, 7:620776, 2021.

Preliminary assessment of Mesenchymal To Epithelial Transition in canine perivascular wall tumors (PWTs): E-cadherin, β-catenin and cytokeratins immunohistochemical expression

Francesco Godizzi (1), Federico Armando (2,3), Matteo Gambini (1,4), Silvia Dell'Aere (1), Lavinia Elena Chiti (1), Roberta Ferrari (1), Damiano Stefanello (1), Attilio Corradi (2), Giancarlo Avallone (5), Paola Roccabianca (1)

(1) Università degli Studi di Milano, Dipartimento di Medicina Veterinaria (DIMEVET). (2) Università degli Studi di Parma, Dipartimento di Scienze Medico-Veterinarie (3) University of Veterinary Medicine Hannover, Department of Pathology (4) I-VET Laboratorio di Analisi Cliniche – Flero, Italy (5) Università degli Studi di Bologna, Dipartimento di Scienze Mediche Veterinarie

Corresponding author: Francesco Godizzi (francesco.godizzi@unimi.it)

Epithelial to mesenchymal transition (EMT) has a major role in tumor progression and metastasis of carcinomas in humans and dogs. Recent findings suggest that the reverse process, mesenchymal to epithelial transition (MET), occurs in sarcomas and has been associated with decreased cell proliferation, invasion, and migration leading to a better prognosis.[5] Mesenchymal cells undergoing MET acquire an epithelial-like phenotype by gaining expression of typical epithelial markers (cytokeratins, β-catenin, and E-cadherin). Studies regarding MET in canine sarcomas are scarce[1] and the correlation between EMT or MET and prognosis has not been fully investigated. Canine perivascular wall tumors (PWTs) display a general more favourable behaviour compared to other soft tissue sarcomas.[2] Our hypothesis is that in PWTs the development of MET phenotype may be one of the factors involved in their distinctive behaviour. Thus, the aim of this work was to explore the occurrence of MET by assessment of specific marker expression by immunohistochemistry (IHC) in canine PWTs. A series of 36 canine PWTs were routinely processed and stained with anti-pan-cytokeratin (AE1/AE3), -β-catenin, and -E-cadherin. IHC results and histologic grades were recorded for all tumors. Cases resulted grade 1 (23/38), grade 2 (12/38) and grade 3 (3/38). All cases were pan-cytokeratins negative. Nuclear and cytoplasmic expression of E-cadherin (38/38) and β-catenin (31/36) were observed. E-cadherin is involved in cell-to-cell adhesion and normally absent in normal vascular mural cells.[3,4] E-cadherin and β-catenin expression may either reflect MET transition in PWTS or be indicative of a dysregulation of their corresponding pathways.

1. Armando et al. Mesenchymal to epithelial transition driven by canine distemper virus infection of canine histiocytic sarcoma cells contributes to a reduced cell motility in vitro, Journal of Cellular and Molecular Medicine, 24(16):9332-9348, 2020.
2. Avallone et al. The controversial histologic classification of canine subcutaneous whorling tumours: The path to perivascular wall tumours, Veterinary Comparative Oncology, 18(1):3-8, 2020.
3. Frismantiene et al. Cadherins in vascular smooth muscle cell (patho)biology: Quid nos scimus? Cellular signalling, 45: 23–42, 2018.
4. George et al. Cadherin:catenin complex: a novel regulator of vascular smooth muscle cell behaviour, Atherosclerosis, 188(1): 1–11, 2006.
5. Yang et al. Mesenchymal to epithelial transition in sarcomas, European Journal of Cancer, 50(3):593-601, 2014.

Pilot study on the possible immunohistochemical discrimination of the origin of canine ovarian carcinoma

Eleonora Brambilla (1), Alice Crippa (2), Barbara Banco (3), Debora Groppetti (1), Alessandro Pecile (1) Grieco Valeria (1)

(1) Università degli Studi di Milano, Dipartimento di Medicina Veterinaria (2) Libero professionista, (3) MyLav Laboratorio Analisi La Vallonea

Corresponding author: Eleonora Brambilla (eleonora.brambilla@unimi.it)

Ovarian epithelial tumors are rare in canine species, presenting variable gross and microscopic features which could reflect different diagnosis and are poorly described in literature[1]. Considering the various ovarian epithelial structures and the different possible prognosis of the corresponding tumors, discriminating immunohistochemical tests would be required.

Aiming to give a contribute to this field, for the present retrospective study, 18 cases of ovarian carcinoma and two normal canine ovaries were considered. Related paraffin blocks were retrieved from the archive of the Pathology Unit of the Veterinary Medicine Department of the University of Milan. Serial sections were obtained and tested immunohistochemically (ABC method) for the following markers CK7, CKAE1\AE3, and CK19.

In normal ovaries, CK AE1/AE3 was intensely and diffusely expressed by all epithelial structures. CK7 was intensely and diffusely expressed by the surface epithelium (SE) as already demonstrated in a previous report[2] and less expressed in subsurface epithelial structures (SES). CK19 was intensely expressed by both SE and SES. All tumors were positive for CKAE1\AE3, confirming the epithelial origin of the neoplasms. CK7 was intensely and diffusely expressed by the tumors growing on the ovarian surface while it was less and inconstantly expressed in tumors located in the ovarian parenchyma that, conversely, were intensely and diffusely stained by CK19, that were also expressed by SE.

The results of the present pilot study, seem to suggest that immunohistochemistry could be a possible tool for individuating the origin of ovarian epithelial tumors. Further studies on a larger number of samples, considering benign and malignant ovarian epithelial tumors, possibly including *rete ovarii* derived tumors and the use of a larger panel of immunohistochemical markers are required to confirm the results and to investigate possible prognostic differences.

[1] Akihara Y et al. Immunohistochemical evaluation of canine ovarian tumors, Journal of Veterinary Medicical Science, 69:703-708, 2007.
[2] Riccardi E et al. Immunohistochemical diagnosis of canine ovarian epithelial and granulosa cell tumors, Journal of Veterinary Diagnostic Investigation, 19:431–435, 2007.

Tumor-associated macrophages (TAMs) and tumor-associated neutrophils (TANs) in canine melanocytic tumors

Ilaria Porcellato (1), Monica Sforna (1), Alice Musi (1), Ilaria Bossi (1), Serenella Silvestri (2), Luca Mechelli (1), Chiara Brachelente (1)

(1) Università degli Studi di Perugia, Dipartimento di Medicina Veterinaria (2) Università degli Studi di Perugia, Dipartimento di Medicina e Chirurgia

Corresponding author: Ilaria Porcellato (ilariaporcellatodvm@gmail.com)

Despite recent advance in human melanoma immunotherapeutic approach, few studies have been conducted on the immune environment of canine melanocytic tumors. [1] Different studies highlight the role of innate immunity in the development, growth, and prognosis of human malignant melanoma through the release of pro- and/or anti-inflammatory cytokines, and tumor growth factors [2,3], but to the best of the authors' knowledge, this has never been investigated in canine counterpart.

This study aims at characterizing tumor-associated macrophages (TAMs) and tumor-associated neutrophils (TANs); moreover, the association among the expression of these markers and histological prognostic markers have been evaluated.

Sixty-five cases of melanocytic tumors (24 oral melanomas, 26 cutaneous melanomas, and 15 cutaneous melanocytomas) were retrospectively selected from our archives. After diagnosis confirmation and histopathological evaluation, immunohistochemistry for MAC387, IBA1, and MPO was performed on serial sections. Positive cells were counted by three operators in 10 high power fields, avoiding areas near ulceration and necrosis; the mean was calculated.

Results from our study showed that both neutrophils (MPO^+ and $MAC387^+$) and macrophages ($IBA1^+$ and $MAC387^+$) were more numerous in oral melanomas, when compared to cutaneous melanomas and melanocytomas ($P<0.001$ and $P=0.011$; $P=0.001$ and $P=0.002$ respectively). Moreover, an increased number of $IBA1^+$ cells was associated with macroscopic and histological negative prognostic factors, such as the major diameter ($P<0.05$), the tumor thickness ($P<0.05$), the mitotic count ($P=0.001$), and the nuclear atypia ($P<0.05$). The number of MPO^+ cells was associated with the mitotic count ($P<0.001$), the nuclear atypia ($P<0.05$), the major diameter ($P<0.05$), the degree of pigmentation ($P<0.05$), and the tumor thickness ($P<0.05$). These associations were not observed instead with the number of $MAC387^+$ cells. TANs and TAMs seem to be associated with malignant histological features in canine melanocytic tumors, but further studies to validate these results are needed.

Our results seem to confirm that the innate immune system may contribute substantially to biological behavior in canine melanomas and that TAMs and TANs could represent a feasible target for future immunotherapeutic strategies.

[1] Tarone L et al. Naturally occurring cancers in pet dogs as pre-clinical models for cancer immunotherapy. Cancer Immunol Immunother. 68:1839-1853, 2019. [2] Mignogna C et al. Innate immunity in cutaneous melanoma. Clin Exp Dermatol.; 42:243-250, 2017 [3] Tucci M et al. Immune System Evasion as Hallmark of Melanoma Progression: The Role of Dendritic Cells. Front Oncol.; 9:1148, 2019.

The role of dogs as sentinels of environmental contamination in Campania region

Davide De Biase (1), Valeria Baldassarre (1), Nicola Ambrosio (2), Giuseppe Piegari (1), Francesco Prisco (1), Ilaria d'Aquino (1), Serenella Papparella (1), Orlando Paciello (1)

(1) Università degli Studi di Napoli, Dipartimento di Medicina Veterinaria e produzioni animali (2) U.O.C. Sanità Animale, Azienda Sanitaria Locale Napoli 2 Nord

Corresponding author: Davide De Biase (davide.debiase@unina.it)

In the last decades, many concerns have raised about the possible adverse effects of environmental contaminants on human population. However, there are several difficulties when performing epidemiological studies to assess a correlation between chronic exposure to environmental chemicals and significant health risks; for instance, environmental diseases have long latency periods with multiple exposure routes and often non-specific health outcomes [1]. Thus, it has been recognized the importance to complement human epidemiology with animal studies. Dogs represent a warning sentinel for human health because they share the human environment and respond to many toxic insults in ways analogous to humans; they have physiologically compressed life spans, and they are free from some important lifestyle risk factors for disease [1-2]. The aim of this work was to standardize and validate methods to monitor environmental damage through the evaluation of biological markers such as genotoxic DNA damage by Comet assay [2] and the immunocytochemical expression of iNOS as a marker for oxidative stress [3]. We conducted a cross-sectional study employing exposed dogs living in a shelter in Caivano, a small city located in the infamous "triangle of death". The study was conducted with the approval of the Ethics Committee of the University of Naples (PG/2021/0030881). The study population included 15 clinically healthy mixed breed dogs, aged 4 to 10 years old, randomly sampled, with at a minimum two-year presence in the shelter. The control group consisted of 5 healthy dogs living in a domestic environment from a less polluted nearby area and using age/sex matching. Blood samples were collected for Comet assay to assess genotoxic damage and for immunocytochemistry to investigate iNOS expression. Semi-quantitative evaluation of iNOS expression was carried out counting the number of immunolabeled cells on a total of 100 cells for each slide at 40x magnification. The results were compared with environmental pollution data from the studied areas. The expression of iNOS and the genotoxic DNA damage were significantly higher in dogs from Caivano compared to controls. Moreover, a significative correlation was found between age and the used genomic and oxidative stress biomarkers. Conversely, no significant variations were found between the sexes. Our data show that dogs living in a highly polluted area and chronically exposed to environmental contaminants have a higher risk to develop genotoxic DNA damage. Moreover, we confirm that iNOS expression and comet assay may be reliable tool to assess environmental-related oxidative stress and genotoxic damage. We support the idea that dogs should be considered as an early sentinel for human environmental disease; the assessment of the expression of DNA damage and oxidative stress biomarkers in canine population could justify a more detailed examination of potential exposure in the corresponding human community.

[1] Backer L.C et al. Pet dogs as sentinels for environmental contamination. Science of the Total Environment, 274.1-3: 161-169, 2001.
[2] Kimura K.C. et al. Evaluation of DNA damage by the alkaline comet assay of the olfactory and respiratory epithelia of dogs from the city of Sao Paulo, Brazil. Experimental and toxicologic pathology, 62.3: 209-219, 2010.
[3] Letizia Tarantini et al, Effects of Particulate Matter on Genomic DNA Methylation Content and iNOS Promoter Methylation. Environmental health perspectives, 117.2: 217-222, 2009.

MAIN LECTURE

Respiratory pathology of swine and Porcine Respiratory Disease Complex: Are they synonims?

Giuseppe Sarli

Università degli Studi di Bologna, Dipartimento di Scienze Mediche Veterinarie

Corresponding author: Giuseppe Sarli (giuseppe.sarli@unibo.it)

Common examples of pig respiratory disease are rhinitis, pneumonia and pleuritis, which may occur associated or not. Pathogens responsible of respiratory diseases reach the targets by aerogenous or hematogenous routes that in the lung develop different patterns and distribution of the lesions. Aerogenous lung involvement induces a cranioventral pattern of which examples are swine enzootic pneumonia and bronchopneumonia. The etiological agents of these cranio-ventral swine pneumonias are respiratory viruses (e. g. SuHV-1, influenza viruses), and *Mycoplasma hyopneumoniae,* often associated with secondary bacterial agents. Macroscopical features typical of aerogenous pneumonias include: (a) variation in consistency (from hepatization to carnification); b) colour variation: from dark red in hepatized areas, to a whitish colour in chronic lesions. The chromatic variation may also be accentuated by inflammatory oedema (acute phases) of the perilobular connective tissue and by fibrous thickening (chronic phases); c) presence of exudate in the airways, from scarce and dense catarrhal exudate (enzootic pneumonia) to collection of pus in complicated enzootic pneumonia and in bronchopneumonia; d) whitish mural thickening of small airways present in *Mycoplasma hyopneumoniae* infections. There are some exceptions to what above stated: *Actinobacillus pleuropneumoniae* reaches the lung through the airways but the involvement of these latter is not a typical feature except in cases complicated by other bacteria. The lesions are not strictly cranioventral and both the classical acute fibrinous pneumonia with pulmonary and pleural involvement, and the chronic features (a large monolateral area of necrosis or its suppuration), are frequently recognized. Flu pneumonia is another exception: the pathogenesis is aerogenous but, if not complicated, the distribution of the lesions is often diffuse.

The spread of pathogens by the haematogenous route implies a different distribution of the lesions. If the etiological agents enter the lung through this portal of entry, the lesions are mainly located in the dorsal areas of the caudal lobes. Haematogenous lung involvement may appear as embolic pneumonia (arrest in the lung of septic thromboemboli originated from inflammation located elsewhere) or interstitial pneumonia (arrival of pneumotropic or endoteliotropic agents to the lung through the blood circulation as it happens in porcine reproductive and respiratory syndrome virus (PRRSV) and porcine circovirus type 2 (PCV2) infections and in massive nematode invasion or septicaemias from gram-negative bacteria). If in the first case the prominent macroscopic character is the presence of one or more abscesses, mainly in the best vascularized dorsal areas of the lung, in the second the macroscopic appearance is that of an interstitial pneumonia characterized by slight increase in consistency, color ranging from red in the acute stages to a pale whitish colour in the chronic forms and missing the airways involvement. Pulmonary hematogenous pathology also has examples in *Metastrongylus* and ascarid pulmonary invasion in which lung lesions are due to alveolar-septal damage by the larvae and, only for *Metastrongylus*, also from adults located in small bronchi in the dorsal areas of the lung, where they cause catarrhal-purulent bronchitis to which bacterial complications can also contribute.

However, these latter lesions are complex as they are also sustained by interstitial and granulomatous pneumonia directed against the parasites. They are associated to emphysema and atelectasis depending on whether the presence of exudate and parasites in the small airways causes obstruction or occlusion, respectively.

Pleuritis may be associated with pulmonary disorders (a classic example is in fibrinous pneumonia by *A. pleuropneumoniae*), but in the pig often the pleuritides (acute serofibrinous evolving in chronic fibrous pleuritis) may not be concomitant with pneumonia, but with other serosites (pericarditis, peritonitis) and arthritis in systemic pathology and supported etiologically *by Haemophilus parasuis, Streptococcus suis, Mycoplasma hyorinis, Actinobacillus* spp.

The term "Porcine respiratory disease complex" (PRDC) is used to indicate the current way of presentation of respiratory pathology in the modern pig farming. PRDC includes pneumonias with variable pictures, mixed of both aerogenous and haematogenous forms with variable etiology, often multimicrobial, and influenced by management factors. Well-founded today is also the concept that many etiological agents of respiratory pathology of the pig are ubiquitous in the airways and their isolation/identification is not always associable objectively to the current pathological features. In this complex context lung lesions registered at the slaughterhouse or at necropsies, and supplemented by histological investigations, must be considered as powerful tools to assign the proper role to aetiological agents. In recent years, the goal of co-localizing the causative agent with the lesions it produces has been frequently applied, and valid examples in routine diagnostics are those indicating pulmonary involvement during PRRSV and PCV2 infections.

DIDES: Italian diagnostic database for exotic animals

Arianna Meletiadis, Cristina Biolatti, Valentina Campia, Giuseppe Terribilio, Alessandro Dondo, Simona Zoppi, Pier Luigi Acutis

Istituto Zooprofilattico Sperimentale del Piemonte, Liguria e Valle d'Aosta, Italia

Corresponding author: Arianna Meletiadis (arianna.meletiadis@izsto.it)

Exotic species held in captivity are a well-established and widespread phenomenon: in Italy, there are approximately 16 million exotic animals in private houses, more than 13,000 in zoos, about 2000 in circuses and, finally, an unknown number in rescue centres. Despite this diffusion, their number is insufficient to allow the professionals, involved in the diagnosis of their diseases (from clinical veterinarians to diagnostic laboratories), to collect on their own a significant amount of diagnostic data for the different species involved.

With the idea of creating a bridge that can fill this communication gap, we started the project "DIDES" (Italian Diagnostic Database for Exotic Animals) which is a web portal aimed at facilitating the collection and organization of data emerged from the exams carried out on exotic animals by the various stakeholders. The DIDES home page is made available for free to all users but a registration on the portal is mandatory in order to get access to the entire database which consists in news, diagnostic protocols and 'DIDES' sections. The "News" section can be used for communicating issues that require rapid sharing among all subscribers. In order to offer a diagnostic reference tool, another section has been created where *ad hoc* diagnostic protocols, subdivided by animal species, are published: such protocols contain information on clinical signs, diagnostic techniques and anatomopathological signs from post-mortem examinations. The 'DIDES' section is dedicated to the storage of past diagnostic data for consultation purposes and offers the possibility to the users to register and share their own results of diagnostic investigations carried out on exotic animals, both alive or dead. The registration processes take place through the compilation of data regarding the identification of the animal, the necropsy, the laboratory tests and the diagnosis.

The Competent Authority, i.e. the Ministry of Health, can use the database to monitor the health situation of the various exotics animals and the eventual spread of pathogens. The actors that are supposed to be involved in the project are the Experimental Zooprophylactic Institutes (IZS), the Departments of Veterinary Sciences and veterinarians' freelancers.

Through a widespread use by professionals, DIDES can represent an effective and valid tool for having a real-time picture of the health situation of exotic animals and of the major health issues they can encounter.

Effects of insect live larvae on mucin dynamics in poultry: histochemical and biomolecular investigations

Elena Colombino (1), Sara Bellezza Oddon (2), Ilaria Biasato (2), Marta Caramori (1), Marta Gariglio (2), Stefania Zanet (1), Elena Battisti (1), Ezio Ferroglio (1), Laura Gasco (2), Achille Schiavone (1), Maria Teresa Capucchio (1)

(1) Università degli Studi di Torino, Dipartimento di Scienze Veterinarie (2) Università degli Studi di Torino, Dipartimento di Scienze Agrarie, Forestali e Alimentari

Corresponding author: Elena Colombino (elena.colombino@unito.it)

The gastrointestinal tract is covered by a mucosal layer which represents the first line of defence against pathogens. The top layer of the mucus gel surface is composed by glycoproteins called mucins (MUC), which are secreted by goblet cells [1]. Particularly, MUC-2 gene encodes for secretory MUC-2, which is the primary gel-forming mucin in the gut [1]. Mucin composition is considered one of the key elements contributing to gut health and it can be modulated through diet [2]. This study aims to investigate the effect of the dietary inclusion of *Hermetia illucens* (HI) and *Tenebrio molitor* (TM) live larvae on chicken mucin dynamics using histochemistry and MUC-2 gene expression. The experimental protocol (ID: 814715) was approved by the Ethical Committee of the University of Turin (Italy). A total of 180 four-day-old male broiler chickens (Ross 308) were randomly allotted to 3 dietary treatments (6 replicates/treatment; 10 animals/replicate): i) control (C); ii) C + HI iii) C + TM. Live larvae were distributed based on 5% of the expected daily feed intake. At slaughter (39 days of age), samples of duodenum, jejunum and ileum were collected from 12 animals/diet (2 animals/replicate) and submitted to histochemistry to evaluate the staining intensity of the three main mucin types (neutral, acidic sialilated and acidic sulphated mucins). MUC-2 gene expression was quantified on frozen samples of jejunum through quantitative real-time PCR. Data were analysed by R software ($P<0.05$). Mucin staining intensity was not influenced by diet ($P>0.05$) but it depends on intestinal segment ($P<0.01$) showing a proximo-distal increasing gradient from duodenum to ileum. Conversely, previous studies reported that low levels of HI and TM insect meal inclusion (<10%) in broiler's diet can positively modulate mucin dynamics. However, the same authors also observed that mucins are more abundant in the ileum compared to duodenum as recorded in the present study [3,4]. This is probably due to an increase in goblet cells density, suggesting that distal ileum may be a preferred region for bacterial colonization [3,4]. Considering MUC-2 gene expression, non-significant differences were observed among dietary treatments ($P>0.05$). No study is available on MUC-2 gene expression in insect-fed poultry but recently it has been demonstrated that HI meal inclusion in pigs' diet increased the MUC-2 expression in gut [5]. In conclusion, insect live larvae seemed to be less effective than insect meal in positively regulating gut health, showing no effect on mucin dynamics in broiler chickens.

Morphological and immunological evaluations performed in chickens subjected to “in ovo” microbiota enrichment; a preliminary study

Lucia Biagini, Livio Galosi, Sara Mangiaterra, Sara Berardi, Subeide Mari, Giacomo Rossi

Università degli Studi di Camerino, Scuola di Bioscienze e Medicina Veterinaria

Corresponding author: Lucia Biagini (lucia.biagini@unicam.it)

In the post-antibiotic era, poultry industry has focused on the use of probiotics, live bacteria capable of beneficial effects to the health of the host [1], and on their administration techniques [2, 5]. The aim of this study is to evaluate the effects of the *in ovo* administration, into the amnion, [4] of the probiotic mixture Slab51® (comprised of the following strains: *Streptococcus thermophilus* DSM 32245, *Bifidobacterium lactis* DSM 32246, *Bifidobacterium lactis* DSM 32247, *Lactobacillus acidophilus* DSM 32241, *Lactobacillus helveticus* DSM 32242, *Lactobacillus paracasei* DSM 32243, *Lactobacillus plantarum* DSM 32244, and *Lactobacillus brevis* DSM 27961), considering the hatchability of eggs, growth performances, intestinal morphology and development of main organs of the immune system. At 18 days of incubation, 135 Ross-308 eggs were divided into three groups. The eggs of groups P1 and P2 were inoculated with $1x10^6$ and $1x10^5$ CFU of probiotic bacteria, diluted in 0.5 ml of saline sterile solution respectively. Control group eggs (C) were inoculated with 0.5 ml of saline sterile solution. The hatchability is reduced in P1 due to the greater probiotic concentration. The weight of the chicks, evaluated weekly, was significantly higher in P1 and P2 than in C, throughout the duration of the experiment. The birds were conventionally slaughtered at 28 days of age for human consumption. Samples from duodenum, ileum and caecum were processed for the evaluation of the intestinal morphology. When compared to group C the parameters of villus height and crypt depth are both increased in duodenum and ileum of treated groups, indicators of an increased absorbent surface, while villus width is only increased in ileum of P2 group. In treated groups is also observed a constant functional increase of the lymphopoietic system, element resulting from the evaluation of thickness of the lamina propria, area of the splenic, bursal and intestinal lymphoid tissue, all parameters related to a greater cellular mitotic index. Preliminary results demonstrate the benefits of the treatment in all observed parameters, confirming the safety of the technique [5], and its enormous potential in preventing perinatal and early rearing period bacterial and parasitic infections, also in association with standard protocols of anti-viral vaccination [3] which are carried out in the poultry industry.

(1) Awad, W. A., Ghareeb, K., Abdel-Raheem, S., & Böhm, J. (2009). Effects of dietary inclusion of probiotic and synbiotic on growth performance, organ weights, and intestinal histomorphology of broiler chickens. Poultry science, 88(1), 49-56.
(2) Bai, S. P., Wu, A. M., Ding, X. M., Lei, Y., Bai, J., Zhang, K. Y., & Chio, J. S. (2013). Effects of probiotic-supplemented diets on growth performance and intestinal immune characteristics of broiler chickens. Poultry science, 92(3), 663-670.
(3) Bar-Shira, E., Sklan, D., & Friedman, A. (2003). Establishment of immune competence in the avian GALT during the immediate post-hatch period. Developmental & Comparative Immunology, 27(2), 147-157.
(4) Cheled-Shoval, S. L., Amit-Romach, E., Barbakov, M., & Uni, Z. (2011). The effect of in ovo administration of mannan oligosaccharide on small intestine development during the pre-and posthatch periods in chickens. Poultry Science, 90(10), 2301-2310.
(5) Cox, C. M., & Dalloul, R. A. (2015). Immunomodulatory role of probiotics in poultry and potential in ovo application. Beneficial microbes, 6(1), 45-52.

Administration of Protein Hydrolysates from Anchovy (*Engraulis encrasicolus*) waste for twelve weeks decreases Metabolic Dysfunction-Associated Fatty Liver Disease severity in ApoE$^{-/-}$ mice

Jessica Maria Abbate (1), Francesco Macrì (1), Fabiano Capparucci (2), Carmelo Iaria (2), Francesca Arfuso (1), Giovanni Briguglio (2), Orazio Cavallo (3), Giovanni Lanteri (2)

(1) Università degli Studi di Messina, Dipartimento di Scienze Veterinarie (2) Università degli Studi di Messina, Dipartimento di Scienze Chimiche, Biologiche, Farmaceutiche ed Ambientali (3) ASP Siracusa, Dipartimento Prevenzione Veterinario

Corresponding author: Jessica Maria Abbate (jabbate@unime.it)

Metabolic dysfunction-associated fatty liver disease (MAFLD), the most common cause of chronic liver disease in Western countries, ranges from simple steatosis to hepatic fibrosis and cirrhosis [1]. Nutritional interventions are key factors in preventing liver disorders, and, in this regard, fish-rich diets are considered helpful in the prevention of MAFLD, especially for cholesterol-lowering and antioxidant properties associated to the protein content [2]. Fish industry processing waste contains a relatively high amount of proteins, and enzymatic hydrolysis has been successfully explored to obtain added-value protein hydrolysates, proven to promote hypocholesterolemic, anti-inflammatory and antioxidative effects [2,3]. This study assessed the effect of the administration of protein hydrolysates from anchovy waste (APH) for 12 weeks in ApoE-knockout mice (ApoE$^{-/-}$) on attenuating high-fat diet-induced MAFLD (Approval no. 771/2018-PR). Thirty female ApoE$^{-/-}$ mice were divided into two groups (n=15/group) and fed a high-fat diet (HFD) with or without the addition of 10% (w/w) APH. After 12 weeks, mice were euthanized and serum lipid profile and hepatic enzyme activities were determined, whereas nuclear magnetic resonance was performed for hepatic lipid content. Liver sections were processed for staining with HE, Oil-Red O and Mallory's trichrome stains, and for immunohistochemistry with F4-80 and CD3. Two-way ANOVA for repeated measures was applied to evaluate the effect of diet and time on body weight values, whereas unpaired Student's t-test was performed to assess differences in serum and hepatic lipids levels, hepatic enzymes, and histological and immunohistochemical findings between groups. An increasing trend in body weight was observed in both groups (p<0.05), with a significantly lower percentage increase in APH-fed mice (*i.e.* 40.47%) compared to mice of the control group (*i.e.* 50.47%) (p<0.0001). Animals on a 10% (w/w) APH-diet for 12 weeks had a reduced serum total cholesterol and triglyceride levels, hepatic enzyme activity and hepatic triacylglycerol content, and a reduced hepatic lipid accumulation and macrophage recruitment compared with controls (p<0.0001). Hepatic fibrosis and lymphocytic infiltration were not observed in liver sections of mice enrolled in both groups. Results suggest that APH administration produces an anti-obesity effect, improves lipid metabolism and mitigates the effect of the high-fat diet on hepatic steatosis and hepatocytes injury. Anchovy by-product protein hydrolysates could be employed as a useful nutritional strategy in MAFLD prevention and treatment in the near future. The opportunity of utilizing protein rich processing by-product wastes in several diseases is worthy of future investigation and represents a key factor in reducing fish-industry derived pollution.

[1] Eslam et al. A new definition for metabolic dysfunction-associated fatty liver disease: An international expert consensus statement, Journal of Hepatology, 73:202-209, 2020.
[2] Chalamaiah et al. Fish protein hydrolysates: Proximate composition, amino acid composition, antioxidant activities and applications: A review, Food Chemistry, 135:3020-3038, 2012.
[3] Giannetto et al. Protein hydrolysates from Anchovy (*Engraulis encrasicolus*) Waste: in Vitro and in Vivo Biological Activities, Marine Drugs, 18:86, 2020.

Anti-Atherosclerotic effect of a 10% (*w/w*) Anchovy (*Engraulis encrasicolus*) Protein Hydrolysate diet in ApoE$^{-/-}$ mice

Jessica Maria Abbate (1), Carmelo Iaria (2), Fabiano Capparucci (2), Francesca Arfuso (1), Giovanni Briguglio (2), Giovanni Lanteri (2)

(1) Università degli Studi di Messina, Dipartimento di Scienze Veterinarie (2) Università degli Studi di Messina, Dipartimento di Scienze Chimiche, Biologiche, Farmaceutiche ed Ambientali

Corresponding author: Jessica Maria Abbate (jabbate@unime.it)

Atherosclerotic cardiovascular disease is a primary cause of mortality in humans worldwide, annually accounting for at least 30% of all causes of death [1]. The beneficial effect of fish consumption in decreasing the risk for cardiovascular disease has been well-established, with several beneficial metabolic effects associated to proteins from fish [2]. Noteworthy, normally discarded fish industry processing waste contains a relatively high amount of proteins, and utilization may offer the opportunity for generating protein-rich compounds which are useful for human health [3]. In particular, protein hydrolysates from fish waste beneficially influence the pathways involved in body composition, exerting anti-inflammatory and antioxidant activities, and making their potential usefulness in chronic human diseases of increasing interest [4]. This study assessed the anti-atherosclerotic properties of a 10% (w/w) anchovy waste protein hydrolysates (APH) diet in ApoE-/- mice for twelve weeks (Approval no. 771/2018-PR). Thirty female ApoE$^{-/-}$ mice were allocated into two groups (n=15/group), and fed a high-fat diet with or without the addition of 10% (*w/w*) APH. Monitoring of plaque growth in the abdominal aorta was assessed at 8 and 12 weeks, performing a high-frequency ultrasound and magnetic resonance imaging test. After 12 weeks, mice were euthanized and hearts were processed for staining with HE, Oil-Red O and Mallory's Trichrome stains, and for immunohistochemistry with F4-80, CD3, BDNF, TrkB and FNDC5. Plaque area and histochemical and immunohistochemical labelled tissue areas were assessed using Image J Software. Unpaired Student *t*-test was performed to assess differences in histological and immunohistochemical findings between groups. Overall, 12-weeks on an APH-diet attenuated plaque development in the aorta, with a regression of the arterial lesions at the end of the study compared to previous follow-up. A significant reduction in the plaque area was observed in the aortic sinus of APH-fed mice, displaying a significant reduction in lipid content compared to the control mice (*i.e.* 53.84 ± 1.97 *vs.* 81.79 ± 4.05%) (P<0.001); whereas no differences in extracellular matrix content (*i.e.* 9.21 ± 0.70 *vs.* 9.37 ± 0.20%), and macrophages recruitment were observed (*i.e.* 32.00 ± 0.80 *vs.* 32.33± 0.64%). Expression of BDNF, TrkB, and FNDC5 was moderate and diffuse in the atherosclerotic plaque of mice of both groups; whereas no CD3 expression was observed. The results obtained showed that 12 weeks on a 10% (w/w) APH-diet significantly attenuated atherosclerosis in ApoE-/-, exerting a lipid-lowering activity. Additionally, an APH-diet seems to induce plaque regression over time in atherosclerotic-prone mouse model. The opportunity to use protein hydrolysates from anchovy waste as a nutraceutical in atherosclerotic cardiovascular disease is worthy of future studies, also representing an inexpensive, sustainable nutritional strategy with minimal environmental impact.

[1] Barquera et al. Global Overview of the Epidemiology of Atherosclerotic cardiovascular disease, Archives of Medical Research, 46:328-338, 2015.
[2] Sirtori et al. Nutritional and nutraceutical approaches to dyslipidemia and atherosclerosis prevention: Focus on dietary proteins, Atherosclerosis, 203:8-17, 2009.
[3] Villamil et al. Fish viscera protein hydrolysates: Production, potential applications and functional and bioactive properties, Food Chemistry, 224:160-171, 2017.

Pathological findings in symptomatic and asymptomatic honeybees infected with Deformed Wing Virus (DWV)

Karen Power, Manuela Martano, Gennaro Altamura, Giuseppe Borzacchiell, Paola Maiolino

Università degli Studi di Napoli, Dipartimento di Medicina Veterinaria e Produzioni Animali

Corresponding author: Karen Power (karen.power@unina.it)

Over the past decades, many causes of honeybee decline have been identified, above all pesticides, incorrect beekeeping practices, reduced availability or quality of food resources, climate change and pathogens [1]. Bacteria, parasites and viruses can all infect honeybees and cause different degrees of impairment, often silently causing the collapse of the colony, due to asymptomatic infections. Viruses are significant threats to the health of honeybees and, although in most cases they cause asymptomatic infections, the association with the mite *Varroa destructor* often results in the loss of many individuals, probably following a reduction in the immune response [2]. The DWV is a single-strand RNA virus widely spread in beekeeping farms across the world. It is capable of infecting bees at every stage of development, from brood to adults, causing peculiar lesions as wings deformation, swollen and discolored abdomens [3]. Given the incidence of DWV in apiaries, 40 honeybee samples were randomly collected form a naturally DWV infected hive. All samples were subjected to anatomopathological examination to discriminate between symptomatic and asymptomatic individuals. Subsequently, 15 honeybee samples with symptomatic (n=10) and asymptomatic (n=5) infections, were frozen at-80° and analyzed by biomolecular techniques (PCR and RTqPCR) to investigate the presence/absence of the virus and determine the relative viral load. Moreover, 25 honeybee samples (19 symptomatic and 6 asymptomatic) were 10% formalin fixed and analyzed by an innovative histopathological processing technique [4]. The biomolecular results showed that a fragment of the expected size (69bp) of DWV was successfully amplified from 10/10 (100%) symptomatic samples and 5/5 (100%) asymptomatic samples and the viral load was higher in symptomatic honeybees compared to the asymptomatic group. The histopathological results showed degenerative lesions of the hypopharyngeal glands (19/19; 100%) and flight muscles (6/19;31%) in symptomatic samples while 4/6 (66%) asymptomatic samples highlighted the presence of plasmatocytes and granulocytes, as well as melanisation phenomena in the hemocele and in the midgut, suggesting an inflammatory response. Taken together, the results suggest a possible pathogenic action of DWV in both symptomatic and asymptomatic infections, and a possible role of the immune cellular response in keeping under control the virus in asymptomatic infections. However further studies are needed to better define the mechanisms underpinning the evidenced histological lesions.

[1] O'Neal S.T., Anderson T.D., Wu-Smart J.Y. Interactions between pesticides and pathogen susceptibility in honey bees, Curr Opin Insect Sci, 26:57-62, 2018.
[2] Nazzi F., Pennacchio F. Honey Bee Antiviral Immune Barriers as Affected by Multiple Stress Factors: A Novel Paradigm to Interpret Colony Health Decline and Collapse, Viruses,10(4):159, 2018.
[3] Bowen-Walker P.L., Martin S.J., Gunn A. The transmission of deformed wing virus between honeybees (*Apis mellifera* L.) by the ectoparasitic mite *Varroa jacobsoni* Oud, J Invertebr Pathol, 73(1):101-106, 1999.
[4] Power K. et al., Histopathological findings in testes from apparently healthy drones of *Apis mellifera ligustica*, Vest Sci, 2;7(3):124, 2020.

Pathology in under human care wild felids in North-East Italian zoological gardens

Cinzia Centelleghe (1), Alessandro Calore (1), Laura Cavicchioli (1), Giulia Maria De Benedictis (2), William Magnone (3), Camillo Sandri (3), Lucia Bono (4), Laura Voltan (5), Giuseppe Palmisano (1), Letizia Moro (1), Sandro Mazzariol (1)

(1) Università degli Studi di Padova, Dipartimento di Biomedicina Comparata e Alimentazione (2) Università degli Studi di Padova, Dipartimento di Medicina Animale, Produzioni e Salute (3) Parco Natura Viva Garda Zoological Park S.r.l. (4) Parco Faunistico Cappeller (5) Parco Faunistico Valcorba

Corresponding author: Cinzia Centelleghe (cinzia.centelleghe@unipd.it)

This retrospective study provides an overview on spontaneous diseases occurring in 33 under human care wild felids submitted for necropsy by zoological gardens located in North-East Italy between 2007 and 2020. Species included 10 tigers, 8 leopards, 7 lions, 4 caracals, 1 lynx, 1 bobcat, 1 serval, and 1 cheetah with an age ranging from 1 day to 20 years.

Inflammatory changes were frequently encountered in the gastro enteric system of 24 out of 33 felids (73%). The most common lesion was different type of enteritis found in 16 animals (chronic lympho-plasmacytic inflammation, hemorrhagic or catarrhal enteritis, associated with numerous intestinal parasites). Ulcerative and/or erosive gastritis was recorded in 7 animals, 3 of which were young ones and harboring foreign bodies in the gastric lumen. Renal lesions, predominantly interstitial (lympho-plasmacytic inflammation and fibrosis) followed by tubular (intra-tubular mineralization, tubular degeneration and/or necrosis, proteinaceous casts) and glomerular lesions (glomerulonephritis and glomerulosclerosis) were detected in 23 out of 33 animals (70%). The renal lesions prevalence increased with age. Pulmonary lesions were frequently encountered (19 out of 33 animals, 57%), mainly inflammations (interstitial or fibrino-necrotizing pneumonia, and bronchopneumonia), alveolar edema and anthracosis. Seventeen felids (51%) showed hepatic alterations, mainly vacuolar hepatopathy, various degree and type of hepatitis and hepatic cysts. Degenerative and inflammatory changes were detected in the articular surface and/or synovia of 6 adult or old animals (18%), while other 6 felids exhibited dilated cardiomyopathy. Three young animals displayed cardiopulmonary filariasis with microfilaremia. Tumors were found in 2 (both leopards) of 33 felids (6%) and the neoplasms originated from thyroid (adenoma) and mesothelium. One cheetah presented systemic amyloidosis and one caracal pup exhibited severe hydrocephalus with compression of the peripheral white matter, associated with severe diffuse purulent meningitis and encephalomyelitis. The statistical analysis, performed using Fisher-Yates and chi-squared tests, suggested a significative difference ($p<0.05$) between tigers and caracals for age related lesions, showing how the first are more predisposed to this category of injuries. It shows also that puppies are more affected by infective/parasitic lesions than adults and they present less chronic degenerative diseases than old animal. Moreover, males are more often interested by traumatic lesions than female felids.

Summarizing, lesions of the gastrointestinal tract followed by renal inflammatory and degenerative changes as well as pneumonia represent the most frequent findings in captive wild felids living in Northern-East Italy zoological parks. Compared to other American and European zoological gardens, neoplastic changes did not represent one of the most common lesions in these animals [1,2].

[1] Harrenstien et al. Mammary Cancer in Captive Wild Felids and Risk Factors for Its Development: A Retrospective Study of the Clinical Behavior of 31 Cases, Journal of Zoo and Wildlife Medicine, 27 (4): 468-476, 1996.

[2] Junginger et al. Pathology in Captive Wild Felids at German Zoological Gardens, PLoS ONE 10(6): e0130573, 2015.

In vitro evaluation of macrophages phagocytosis activity in cats with feline infectious peritonitis: preliminary results

Sara Mangiaterra, Alessandra Gavazza, Valentina Grifantini, Evelina Serri, Matteo Cerquetella, Giacomo Rossi

Università degli Studi di Camerino, Scuola di Bioscienze e Medicina Veterinaria

Corresponding author: Sara Mangiaterra (s.mangiaterra@unicam.it)

Feline coronavirus (FCoV) is the major pathogen of *Felidae* family with a worldwide distribution [1]. In cats it is highly prevalent in multi-cat environments; FCoV replicates in the intestines and can spread by oral-fecal transmission [1]. FCoV is separated into two pathotypes that are referred to feline enteric coronavirus (FECV) and feline infectious peritonitis virus (FIPV) [1]. Following the infection of the enteric epithelium, the virus can spread systemically developing the feline infectious peritonitis (FIP) [2-3]. Previous studies suggested that responses of macrophages to the virus followed by depletion of CD4+and CD8+T-lymphocytes are crucial for understanding the virus-host interactions [4-5]. The aim of this study was to evaluate the phagocytosis activity of monocyte-derived macrophages in FIPV-infected cats. The study population consisted of 15 cats with FIP and 13 cats positive for FcoV (control). Venous blood samples remaining from medical procedures were used to isolate monocytes. Two methods of isolation were used: a) peripheral blood mononuclear cells (PBMC) were separated from the buffy coat within 24 hours after obtaining the blood specimens [6]; b) magnetic-separation of CD14-positive cells from whole blood. Monocytes were allowed to attach to the slide and culture medium containing phorbol 12-myristate-13-acetate was added to induce macrophage differentiation [6]. After 24 hours, a solution with *Saccharomyces cerevisiae* was added to allow phagocytosis. Phagocytosis was measured by counting, microscopically, the number of ingested yeasts within the macrophages [7]. The mean of the number of phagocytic cells undergoing yeast phagocytosis was evaluated on 3 microscopic fields between the two groups. Medium ± SD percentage of macrophages phagocytosis in cats with FIP was 14.4 ± 5,8 and in the control group was 39,3 ± 17,7. Our preliminary results showed that in cats with FIP the percentage of macrophages phagocytosis is lower than the control group suggesting an "anergy" of phagocytic system cells that may allow the virus to use macrophage or monocyte as a *"trojan horse"* to evade the host defence.

(1) Jaimes JA, Whittaker GR. Feline coronavirus: Insights into viral pathogenesis based on the spike protein structure and function. Virol., 517:108-121, 2018.
(2) Pedersen NC. An overview of feline enteric coronavirus and infectious peritonitis virus infections. Feline Pract., 23:7–20, 1995.
(3) Tasker S. Diagnosis of feline infectious peritonitis: Update on evidence supporting available tests. J Feline Med Surg., 20(3):228-243, 2018.
(4) Kipar A, Meli ML, Baptiste KE, Bowker LJ, Lutz H. Sites of feline coronavirus persistence in healthy cats. J. Gen. Virol., 91, 1698–1707, 2010.
(5) Drechsler Y, Vasconcelos E, Griggs L M, Diniz P. CRFK and Primary Macrophages Transcriptomes in Response to Feline Coronavirus Infection Differ Significantly. Front. Genet., *11*, 584744, 2020.
(6) Goto-Koshino Y, Ohno K, Nakajima M, Mochizuki H, Kanemoto H, Tsujimoto H. A rapid and simple method to obtain canine peripheral blood-derived macrophages. J Vet Med Sci. Jun;73(6):773-8, 2011.
(7) Carneiro V M, Bezerra A C, Guimarães M, Muniz-Junqueira M I. Decreased phagocytic function in neutrophils and monocytes from peripheral blood in periodontal disease. J. Appl. Oral. Sci., 20(5), 503–509, 2012.

Diffusion of methicillin-resistant coagulase-positive Staphylococci in dogs and cats: prevalence changes over a 10-year follow up

Elena Spagnolo (1), Michele Berlanda (2), Ilenia Drigo (1), Karin Trevisiol (1), Monia Cocchi (1), Roberto Perin (1), Silvia Deotto (1), Ilda Idrizi (1), Daniela Pasotto (2), Michela Corrò (1)

(1) Istituto Zooprofilattico delle Venezie, Legnaro (PD) (2) Università degli studi di Padova, Dipartimento di Medicina Animale, Produzioni e Salute, Legnaro (PD)

Corresponding author: Michela Corrò (mcorro@izsvenezie.it)

Coagulase positive Staphylococci (CoPS) belong to the saprophytic bacterial flora of humans and animals, the most frequently isolated species being *S. aureus* (SA) and *S. pseudintermedius* (SP). Among dogs and less frequently in cats, CoPS are usually associated with skin and mucosal infections. Over the last years, the diffusion of methicillin-resistant coagulase-positive Staphylococci (MRCoPS) has reached worrying levels in terms of public health, especially since many of these clones are multi-resistant. Furthermore, the presence of MRCoPS in pets could be a source of contamination for humans and lead to therapeutic failure [1]. The aim of this study was to evaluate the prevalence of MRCoPS isolated from animals over a ten-year follow up. We compared results of two investigations on the presence and spread of MRCoPS isolated from clinic samples and analysed at the diagnostic laboratory of the IZSVe. The animals included in both studies suffered from skin infection (superficial and deep pyoderma, otitis externa, etc.) or respiratory/intestinal/urinary/genital tract infections, and a bacteriological examination was carried out on the collected samples (skin swab, faeces, urines, etc.). Basically, suspected *Staphylococcus spp.* colonies from culture plates were checked by Gram stain (cocci Gram positive) and catalase test (positive result), while confirmed *Staphylococcus spp.* colonies were tested for the presence of coagulase enzyme and their ability to grow in selective/differential media to detect methicillin-resistant (MR) clones. Suspected MR colonies were confirmed by molecular biology technique (PCR) in order to detect the presence of *mecA* and *mecC* genes, which confer resistance to methicillin. Species were identified by mass spectrometry (MALDI-TOF). The first study was carried out between 2011 and 2014, over which period 1624 samples were analysed from 1375 dogs (85%) and 249 cats (15%). CoPS were isolated from 24.3% of animals, specifically 361 dogs (26%) and 1 cat (0.4%). Among these, methicillin resistance was found in 5.1% of isolates. Species identification revealed a prevalence of SP (92.3%) respect to SA (7.7%) among MRCoPS. In the more recent study (2019 - 2021), 1101 samples were analysed: 938 came from dogs (85%) and 163 from cats (15%). CoPS were isolated in 35.7% of samples, with a higher frequency in dogs compared to cats: 38.2% (358/938) vs 21.5% (35/163). Preliminary data of species identification show a prevalence of SP (76%) whereas SA was rarely isolated (4%). From the genetic point of view, gene encoding for methicillin resistance (*mecA*) was found in 20% of the CoPS, while *mecC* gene was not found. It is noteworthy to underline that in both studies MRCoPS in cats was isolated in two cases only. In conclusion, statistical analysis confirms a significant difference in MRCoPS prevalence between the two study periods ($p<0.0001$ C.I.99%). Such relevant increase in methicillin-resistant clones in pets could be due to a wider use of antibiotics and resistance genes exchanges between bacteria [2].

[1] Van Duijkeren et al. Transmission of methicillin-resistant *Staphylococcus pseudintermedius* between infected dogs and cats and contact pets, humans and the environment in households and veterinary clinics, Vet Microbiol, 150:338-43, 2011.
[2] Epstein et al. Methicillin-resistant commensal staphylococci in healthy dogs as a potential zoonotic reservoir for community-acquired antibiotic resistance, Infect. Genet. Evol, 9:283-285, 2008.

Plasmodium matutinum causing Avian Malaria in lovebirds (*Agapornis* sp.)

Cristiano Cocumelli (1), Manuela Iurescia (1), Elena Lavinia Diaconu (1), Valentina Galietta (1), Caterina Raso (1), Francesco Grande (2), Letizia Fiorucci (3), Raffaella Parmigiani (1), Tiziana Palmerini (1), Claudio De Liberato (1), Andrea Caprioli (1)

(1) Istituto Zooprofilattico Sperimentale del Lazio e della Toscana, Roma (2) Loro Parque Fondación, Spain. (3) Jungle Park Y Aqualand, Spain

Corresponding author: Critiano. Cocumelli (cristiano.cocumelli@izslt.it)

Avian malaria is a worldwide distributed, vector-born infection of birds, caused by *Plasmodium* parasite. In spite of its distribution, *Plasmodium* is rarely a direct cause of mortality in wild birds, probably due to its co-evolution with the host, but in captive and exotic “naive” birds, or under stress conditions, avian malaria can become a life-threatening infection. Morphological and genetic characteristics of the *Plasmodium* detected in wild bird species are available [3], but very little is known about its presence and pathogenetic role in *Psittacidae* family. Sparse reports have described the occurrence of chronic infection in few genera, sustained by unspecified species of *Plasmodium* [1]. Here we report two cases of deadly avian malaria infection in lovebirds (*Agapornis* sp.), with the genetic characterization of the *Plasmodium* species involved. The birds were host in a zoo located in central Italy and were submitted for necropsy after sudden death two weeks apart. The first submission comprised three subjects died in October 2017; two of them were diagnosed with causes of death unrelated to malaria infection (data not shown). At gross examination, the third bird had splenomegaly and miliary necrotic foci on the liver. At histology, round to oval 20 to 80 µm in size schizonts, filled with numerous merozoites, were observed in the cytoplasm of endothelial or reticuloendothelial cells of liver, spleen, brain and lung. In the liver multifocal foci of necrosis were also observed; in the brain mild congestion and capillaries obstructed by schizonts were evident. In the second submission, a group of 9 frozen lovebirds died in August 2017 were retrospectively analyzed. Unfortunately, most of the carcasses were in poor degree of conservation that allowed to perform only PCR analysis on a pool of organs from three of the nine subjects. Target organs from both lovebirds submissions underwent PCR targeting the *cytochrome b* gene and resulted positive for *Plasmodium* sp. Positive PCR-products were sequenced and the resulting sequences were compared using BLAST and analyzed for similarity to sequences available at the MalAvi database. Phylogenetic analysis demonstrated a nucleotide identity with *P. matutinum* for all the obtained sequences (MALAVI lineages LINN1). To our knowledge, this is the first report describing avian malaria in lovebirds corroborated with the description of gross and histopathological lesions and identification of the *Plasmodium* species (*P. matutinum* LINN1) involved. Interestingly, this cluster of infection in lovebirds was detected in the same zoo, with simultaneous mortality in African penguins (*Spheniscus demersus*) due to avian malaria caused by *P. matutinum* LINN1, with *Culex pipiens* being the most probable vector [2]. Zoos maintaining captive birds, including Psittacine, in temperate areas where mosquitos are abundant should be aware of the risks of avian malaria and should put every effort to prevent outbreaks.

[1] Belo et al. Avian malaria in captive psittacine birds: Detection by microscopy and 18S rRNA gene amplification. Preventive Veterinary Medicine, 88(3), 220–224, 2009.
[2] Iurescia et al. *Plasmodium matutinum* Transmitted by *Culex pipiens* as a Cause of Avian Malaria in Captive African Penguins (*Spheniscus demersus*) in Italy. Front. Vet. Sci. 8:621974, 2021
[3] Nourani et al. Dynamics of prevalence and distribution pattern of avian *Plasmodium* species and its vectors in diverse zoogeographical areas - A review. Infect Genet Evol. 8:1042–44, 2020

Feline Immunodeficiency Virus-Associated Inflammatory Myopathy and Myocarditis in Naturally Infected Cats

Francesco Prisco (1), Davide De Biase (1), Valeria Baldassarre (1), Josep M. Monné Rodríguez (2), Lorena Cardillo (3), Giovanna Fusco (3), Pasquale Santoro (4), Serenella Papparella (1), Anja Kipar (2), Orlando Paciello (1)

(1) Department of Veterinary Medicine and Animal Production, University Federico II of Naples, Napoli, Italy (2) Institute of Veterinary Pathology, University of Zurich, Zurich, Switzerland (3) Istituto Zooprofilattico Sperimentale del Mezzogiorno, Portici, Italy (4) Veterinary Diagnostic Laboratory (Di.Lab.), Napoli, Italy

Corresponding author: Francesco Prisco (francesco.prisco@unina.it)

Inflammatory myopathy (IM) associated with Feline Immunodeficiency Virus (FIV) infection has been documented in experimentally infected adult cats [1]. Similarly, IM and myocarditis have been reported in humans infected with Human Immunodeficiency Virus (HIV) [2]. Moreover, HIV-positive patients, and particularly those with cardiomyopathy, show circulating cardiac-specific autoantibodies [3]. The present study investigated naturally FIV infected cats for the presence and morphological characteristics of IM and myocardiopathy, to gain knowledge on the underlying pathomechanisms.

Snap-frozen skeletal muscle (*Vastus lateralis* of *M. quadriceps femoris* [QF], lateral head of *M. triceps brachii* [TB]), and myocardium (interventricular septum) samples from 20 cats negative by PCR for Feline leukemia virus (FeLV) and Feline Coronavirus (FCoV) infection and FIV-positive by PCR, and from 20 triple-negative controls were examined histologically, using a standard muscle panel of histological stains. Immunohistochemistry for CD3, CD20, MHC I, MHC II, and FIV-p24-gag was also performed. Corresponding frozen QF and myocardial samples were assessed by qRT-PCR for the transcription of IFN-γ, TNF-α, IL-1β, IL-4, IL-6, IL-10, IL-13, IL-17, TGF-β expression. Furthermore, sera from 10 serologically FIV-positive, FeLV- and FCoV-negative cats and 5 triple-negative controls were tested for circulating anti-skeletal muscle autoantibodies, using an established indirect immunofluorescence (IIF) assay [4]. IM and myocarditis were observed in 8/20 (40%) and 6/20 (30%) FIV-infected cats; they were associated findings (p=0.012). In both skeletal muscles and myocardium, the inflammatory infiltrate was dominated by lymphocytes; these were mainly T cells (CD3+), with and fewer B cells (CD20+); at least 50% of the infiltrating inflammatory cells were FIV-p24-gag-positive. MHC class I and II were overexpressed in scattered skeletal muscle fibers and cardiomyocytes of FIV-positive cats. Moreover, total inflammatory cell numbers (r_s=0.614, p=0.006) and T cell numbers (r_s=0.534, p=0.018) in QF and myocardium were positively correlated. QF samples from FIV-positive animals showed significantly higher transcription levels for INFγ (p=0.010), TNFα (p=0.041), IL-17 (p=0.041), IL-6 (p=0.038), IL-13 (p=0.048), TGFβ (p=0.006) and IL-10 (p=0.041) compared to controls. There was no difference in the transcription of IL-1β and IL-4. Interestingly, apart from a significantly lower IL-13 transcription level (p=0.003), the myocardium of FIV-positive cats did not show any significant difference in the transcription of any studied cytokine compared to the control myocardia. Sera from FIV-positive cats showed a higher IIF-positivity up to a dilution of 1:300 compared with controls (p<0.05). The results show that a T cell dominated IM and myocarditis are common in naturally FIV-infected cats. There is evidence of an underlying autoimmune process, with circulating autoantibodies directed against muscle antigens. Further studies are now required to identify the autoantigen and the role of virus-infected lymphocytes in the process.

[1] Podell, M. et al. *Muscle Nerve* 1998, *21*, 1680–1685. [2] Hiniker, A. et al. *J. Neuropathol. Exp. Neurol.* 2016, *75*, 239–245. [3] Currie, P.F. et al. *Heart* 1998, *79*, 599–604.
[4] Pasolini, M.P. et al. *Vet. Pathol.* 2018, *55*, 133–143.

DIDES: Italian diagnostic database for exotic animals

Arianna Meletiadis, Cristina Biolatti, Valentina Campia, Giuseppe Terribilio, Alessandro Dondo, Simona Zoppi, Pier Luigi Acutis

Istituto Zooprofilattico Sperimentale del Piemonte, Liguria e Valle d'Aosta, Italia

Corresponding author: Arianna Meletiadis (arianna.meletiadis@izsto.it)

Exotic species held in captivity are a well-established and widespread phenomenon: in Italy, there are approximately 16 million exotic animals in private houses, more than 13,000 in zoos, about 2000 in circuses and, finally, an unknown number in rescue centres. Despite this diffusion, their number is insufficient to allow the professionals, involved in the diagnosis of their diseases (from clinical veterinarians to diagnostic laboratories), to collect on their own a significant amount of diagnostic data for the different species involved.

With the idea of creating a bridge that can fill this communication gap, we started the project "DIDES" (Italian Diagnostic Database for Exotic Animals) which is a web portal aimed at facilitating the collection and organization of data emerged from the exams carried out on exotic animals by the various stakeholders. The DIDES home page is made available for free to all users but a registration on the portal is mandatory in order to get access to the entire database which consists in news, diagnostic protocols and 'DIDES' sections. The "News" section can be used for communicating issues that require rapid sharing among all subscribers. In order to offer a diagnostic reference tool, another section has been created where *ad hoc* diagnostic protocols, subdivided by animal species, are published: such protocols contain information on clinical signs, diagnostic techniques and anatomopathological signs from post-mortem examinations. The 'DIDES' section is dedicated to the storage of past diagnostic data for consultation purposes and offers the possibility to the users to register and share their own results of diagnostic investigations carried out on exotic animals, both alive or dead. The registration processes take place through the compilation of data regarding the identification of the animal, the necropsy, the laboratory tests and the diagnosis.

The Competent Authority, i.e. the Ministry of Health, can use the database to monitor the health situation of the various exotics animals and the eventual spread of pathogens. The actors that are supposed to be involved in the project are the Experimental Zooprophylactic Institutes (IZS), the Departments of Veterinary Sciences and veterinarians' freelancers.

Through a widespread use by professionals, DIDES can represent an effective and valid tool for having a real-time picture of the health situation of exotic animals and of the major health issues they can encounter.

Effects of insect live larvae on mucin dynamics in poultry: histochemical and biomolecular investigations

Elena Colombino (1), Sara Bellezza Oddon (2), Ilaria Biasato (2), Marta Caramori (1), Marta Gariglio (2), Stefania Zanet (1), Elena Battisti (1), Ezio Ferroglio (1), Laura Gasco (2), Achille Schiavone (1), Maria Teresa Capucchio (1)

(1) Università degli Studi di Torino, Dipartimento di Scienze Veterinarie (2) Università degli Studi di Torino, Dipartimento di Scienze Agrarie, Forestali e Alimentari

Corresponding author: Elena Colombino (elena.colombino@unito.it)

The gastrointestinal tract is covered by a mucosal layer which represents the first line of defence against pathogens. The top layer of the mucus gel surface is composed by glycoproteins called mucins (MUC), which are secreted by goblet cells [1]. Particularly, MUC-2 gene encodes for secretory MUC-2, which is the primary gel-forming mucin in the gut [1]. Mucin composition is considered one of the key elements contributing to gut health and it can be modulated through diet [2]. This study aims to investigate the effect of the dietary inclusion of *Hermetia illucens* (HI) and *Tenebrio molitor* (TM) live larvae on chicken mucin dynamics using histochemistry and MUC-2 gene expression. The experimental protocol (ID: 814715) was approved by the Ethical Committee of the University of Turin (Italy). A total of 180 four-day-old male broiler chickens (Ross 308) were randomly allotted to 3 dietary treatments (6 replicates/treatment; 10 animals/replicate): i) control (C); ii) C + HI iii) C + TM. Live larvae were distributed based on 5% of the expected daily feed intake. At slaughter (39 days of age), samples of duodenum, jejunum and ileum were collected from 12 animals/diet (2 animals/replicate) and submitted to histochemistry to evaluate the staining intensity of the three main mucin types (neutral, acidic sialilated and acidic sulphated mucins). MUC-2 gene expression was quantified on frozen samples of jejunum through quantitative real-time PCR. Data were analysed by R software ($P<0.05$). Mucin staining intensity was not influenced by diet ($P>0.05$) but it depends on intestinal segment ($P<0.01$) showing a proximo-distal increasing gradient from duodenum to ileum. Conversely, previous studies reported that low levels of HI and TM insect meal inclusion (<10%) in broiler's diet can positively modulate mucin dynamics. However, the same authors also observed that mucins are more abundant in the ileum compared to duodenum as recorded in the present study [3,4]. This is probably due to an increase in goblet cells density, suggesting that distal ileum may be a preferred region for bacterial colonization [3,4]. Considering MUC-2 gene expression, non-significant differences were observed among dietary treatments ($P>0.05$). No study is available on MUC-2 gene expression in insect-fed poultry but recently it has been demonstrated that HI meal inclusion in pigs' diet increased the MUC-2 expression in gut [5]. In conclusion, insect live larvae seemed to be less effective than insect meal in positively regulating gut health, showing no effect on mucin dynamics in broiler chickens.

[1] Jiang Z, Applegate TJ., Lossie AC. Cloning, annotation and developmental expression of the chicken intestinal MUC2 gene, PLoS One, 8:1-14,2013. [2] Tsirtsikos et al. Dietary probiotic inclusion level modulates intestinal mucin composition and mucosal morphology in broilers, Poultry Science, 91:1860-1868, 2012. [3] Biasato et al. Black soldier fly and gut health in broiler chickens: insight into the relationship between cecal microbiota and intestinal mucin composition, Journal of Animal Science and Biotechnology, 11:1-12,2020. [4] Biasato et al. Gut microbiota and mucin composition in female broiler chickens fed diets including yellow mealworm (Tenebrio Molitor, L.), Animals, 9:1-12,2019. [5] Yu et al. Hermetia illucens larvae as a potential dietary protein source altered the microbiota and modulated mucosal immune status in the colon of finishing pigs, Journal of Animal Science and Biotechnology, 10:1-16,2019.

Morphological and immunological evaluations performed in chickens subjected to “in ovo” microbiota enrichment; a preliminary study

Lucia Biagini, Livio Galosi, Sara Mangiaterra, Sara Berardi, Subeide Mari, Giacomo Rossi

Università degli Studi di Camerino, Scuola di Bioscienze e Medicina Veterinaria

Corresponding author: Lucia Biagini (lucia.biagini@unicam.it)

In the post-antibiotic era, poultry industry has focused on the use of probiotics, live bacteria capable of beneficial effects to the health of the host [1], and on their administration techniques [2, 5]. The aim of this study is to evaluate the effects of the *in ovo* administration, into the amnion, [4] of the probiotic mixture Slab51® (comprised of the following strains: *Streptococcus thermophilus* DSM 32245, *Bifidobacterium lactis* DSM 32246, *Bifidobacterium lactis* DSM 32247, *Lactobacillus acidophilus* DSM 32241, *Lactobacillus helveticus* DSM 32242, *Lactobacillus paracasei* DSM 32243, *Lactobacillus plantarum* DSM 32244, and *Lactobacillus brevis* DSM 27961), considering the hatchability of eggs, growth performances, intestinal morphology and development of main organs of the immune system. At 18 days of incubation, 135 Ross-308 eggs were divided into three groups. The eggs of groups P1 and P2 were inoculated with $1x10^6$ and $1x10^5$ CFU of probiotic bacteria, diluted in 0.5 ml of saline sterile solution respectively. Control group eggs (C) were inoculated with 0.5 ml of saline sterile solution. The hatchability is reduced in P1 due to the greater probiotic concentration. The weight of the chicks, evaluated weekly, was significantly higher in P1 and P2 than in C, throughout the duration of the experiment. The birds were conventionally slaughtered at 28 days of age for human consumption. Samples from duodenum, ileum and caecum were processed for the evaluation of the intestinal morphology. When compared to group C the parameters of villus height and crypt depth are both increased in duodenum and ileum of treated groups, indicators of an increased absorbent surface, while villus width is only increased in ileum of P2 group. In treated groups is also observed a constant functional increase of the lymphopoietic system, element resulting from the evaluation of thickness of the lamina propria, area of the splenic, bursal and intestinal lymphoid tissue, all parameters related to a greater cellular mitotic index. Preliminary results demonstrate the benefits of the treatment in all observed parameters, confirming the safety of the technique [5], and its enormous potential in preventing perinatal and early rearing period bacterial and parasitic infections, also in association with standard protocols of anti-viral vaccination [3] which are carried out in the poultry industry.

(1) Awad, W. A., Ghareeb, K., Abdel-Raheem, S., & Böhm, J. (2009). Effects of dietary inclusion of probiotic and synbiotic on growth performance, organ weights, and intestinal histomorphology of broiler chickens. Poultry science, 88(1), 49-56.
(2) Bai, S. P., Wu, A. M., Ding, X. M., Lei, Y., Bai, J., Zhang, K. Y., & Chio, J. S. (2013). Effects of probiotic-supplemented diets on growth performance and intestinal immune characteristics of broiler chickens. Poultry science, 92(3), 663-670.
(3) Bar-Shira, E., Sklan, D., & Friedman, A. (2003). Establishment of immune competence in the avian GALT during the immediate post-hatch period. Developmental & Comparative Immunology, 27(2), 147-157.
(4) Cheled-Shoval, S. L., Amit-Romach, E., Barbakov, M., & Uni, Z. (2011). The effect of in ovo administration of mannan oligosaccharide on small intestine development during the pre-and posthatch periods in chickens. Poultry Science, 90(10), 2301-2310.
(5) Cox, C. M., & Dalloul, R. A. (2015). Immunomodulatory role of probiotics in poultry and potential in ovo application. Beneficial microbes, 6(1), 45-52.

Anti-Atherosclerotic effect of a 10% (*w/w*) Anchovy (*Engraulis encrasicolus*) Protein Hydrolysate diet in ApoE$^{-/-}$ mice

Jessica Maria Abbate (1), Carmelo Iaria (2), Fabiano Capparucci (2), Francesca Arfuso (1), Giovanni Briguglio (2), Giovanni Lanteri (2)

(1) Università degli Studi di Messina, Dipartimento di Scienze Veterinarie (2) Università degli Studi di Messina, Dipartimento di Scienze Chimiche, Biologiche, Farmaceutiche ed Ambientali

Corresponding author: Jessica Maria Abbate (jabbate@unime.it)

Atherosclerotic cardiovascular disease is a primary cause of mortality in humans worldwide, annually accounting for at least 30% of all causes of death [1]. The beneficial effect of fish consumption in decreasing the risk for cardiovascular disease has been well-established, with several beneficial metabolic effects associated to proteins from fish [2]. Noteworthy, normally discarded fish industry processing waste contains a relatively high amount of proteins, and utilization may offer the opportunity for generating protein-rich compounds which are useful for human health [3]. In particular, protein hydrolysates from fish waste beneficially influence the pathways involved in body composition, exerting anti-inflammatory and antioxidant activities, and making their potential usefulness in chronic human diseases of increasing interest [4]. This study assessed the anti-atherosclerotic properties of a 10% (w/w) anchovy waste protein hydrolysates (APH) diet in ApoE-/- mice for twelve weeks (Approval no. 771/2018-PR). Thirty female ApoE$^{-/-}$ mice were allocated into two groups (n=15/group), and fed a high-fat diet with or without the addition of 10% (*w/w*) APH. Monitoring of plaque growth in the abdominal aorta was assessed at 8 and 12 weeks, performing a high-frequency ultrasound and magnetic resonance imaging test. After 12 weeks, mice were euthanized and hearts were processed for staining with HE, Oil-Red O and Mallory's Trichrome stains, and for immunohistochemistry with F4-80, CD3, BDNF, TrkB and FNDC5. Plaque area and histochemical and immunohistochemical labelled tissue areas were assessed using Image J Software. Unpaired Student *t*-test was performed to assess differences in histological and immunohistochemical findings between groups. Overall, 12-weeks on an APH-diet attenuated plaque development in the aorta, with a regression of the arterial lesions at the end of the study compared to previous follow-up. A significant reduction in the plaque area was observed in the aortic sinus of APH-fed mice, displaying a significant reduction in lipid content compared to the control mice (*i.e.* 53.84 ± 1.97 *vs.* 81.79 ± 4.05%) (P<0.001); whereas no differences in extracellular matrix content (*i.e.* 9.21 ± 0.70 *vs.* 9.37 ± 0.20%), and macrophages recruitment were observed (*i.e.* 32.00 ± 0.80 *vs.* 32.33± 0.64%). Expression of BDNF, TrkB, and FNDC5 was moderate and diffuse in the atherosclerotic plaque of mice of both groups; whereas no CD3 expression was observed. The results obtained showed that 12 weeks on a 10% (w/w) APH-diet significantly attenuated atherosclerosis in ApoE-/-, exerting a lipid-lowering activity. Additionally, an APH-diet seems to induce plaque regression over time in atherosclerotic-prone mouse model. The opportunity to use protein hydrolysates from anchovy waste as a nutraceutical in atherosclerotic cardiovascular disease is worthy of future studies, also representing an inexpensive, sustainable nutritional strategy with minimal environmental impact.

[1] Barquera et al. Global Overview of the Epidemiology of Atherosclerotic cardiovascular disease, Archives of Medical Research, 46:328-338, 2015.
[2] Sirtori et al. Nutritional and nutraceutical approaches to dyslipidemia and atherosclerosis prevention: Focus on dietary proteins, Atherosclerosis, 203:8-17, 2009.
[3] Villamil et al. Fish viscera protein hydrolysates: Production, potential applications and functional and bioactive properties, Food Chemistry, 224:160-171, 2017.

Administration of Protein Hydrolysates from Anchovy (*Engraulis encrasicolus*) waste for twelve weeks decreases Metabolic Dysfunction-Associated Fatty Liver Disease severity in ApoE$^{-/-}$ mice

Jessica Maria Abbate (1), Francesco Macrì (1), Fabiano Capparucci (2), Carmelo Iaria (2), Francesca Arfuso (1), Giovanni Briguglio (2), Orazio Cavallo (3), Giovanni Lanteri (2)

(1) Università degli Studi di Messina, Dipartimento di Scienze Veterinarie (2) Università degli Studi di Messina, Dipartimento di Scienze Chimiche, Biologiche, Farmaceutiche ed Ambientali (3) ASP Siracusa, Dipartimento Prevenzione Veterinario

Corresponding author: Jessica Maria Abbate (jabbate@unime.it)

Metabolic dysfunction-associated fatty liver disease (MAFLD), the most common cause of chronic liver disease in Western countries, ranges from simple steatosis to hepatic fibrosis and cirrhosis [1]. Nutritional interventions are key factors in preventing liver disorders, and, in this regard, fish-rich diets are considered helpful in the prevention of MAFLD, especially for cholesterol-lowering and antioxidant properties associated to the protein content [2]. Fish industry processing waste contains a relatively high amount of proteins, and enzymatic hydrolysis has been successfully explored to obtain added-value protein hydrolysates, proven to promote hypocholesterolemic, anti-inflammatory and antioxidative effects [2,3]. This study assessed the effect of the administration of protein hydrolysates from anchovy waste (APH) for 12 weeks in ApoE-knockout mice (ApoE$^{-/-}$) on attenuating high-fat diet-induced MAFLD (Approval no. 771/2018-PR). Thirty female ApoE$^{-/-}$ mice were divided into two groups (n=15/group) and fed a high-fat diet (HFD) with or without the addition of 10% (w/w) APH. After 12 weeks, mice were euthanized and serum lipid profile and hepatic enzyme activities were determined, whereas nuclear magnetic resonance was performed for hepatic lipid content. Liver sections were processed for staining with HE, Oil-Red O and Mallory's trichrome stains, and for immunohistochemistry with F4-80 and CD3. Two-way ANOVA for repeated measures was applied to evaluate the effect of diet and time on body weight values, whereas unpaired Student's t-test was performed to assess differences in serum and hepatic lipids levels, hepatic enzymes, and histological and immunohistochemical findings between groups. An increasing trend in body weight was observed in both groups (p<0.05), with a significantly lower percentage increase in APH-fed mice (*i.e.* 40.47%) compared to mice of the control group (*i.e.* 50.47%) (p<0.0001). Animals on a 10% (w/w) APH-diet for 12 weeks had a reduced serum total cholesterol and triglyceride levels, hepatic enzyme activity and hepatic triacylglycerol content, and a reduced hepatic lipid accumulation and macrophage recruitment compared with controls (p<0.0001). Hepatic fibrosis and lymphocytic infiltration were not observed in liver sections of mice enrolled in both groups. Results suggest that APH administration produces an anti-obesity effect, improves lipid metabolism and mitigates the effect of the high-fat diet on hepatic steatosis and hepatocytes injury. Anchovy by-product protein hydrolysates could be employed as a useful nutritional strategy in MAFLD prevention and treatment in the near future. The opportunity of utilizing protein rich processing by-product wastes in several diseases is worthy of future investigation and represents a key factor in reducing fish-industry derived pollution.

[1] Eslam et al. A new definition for metabolic dysfunction-associated fatty liver disease: An international expert consensus statement, Journal of Hepatology, 73:202-209, 2020. [2] Chalamaiah et al. Fish protein hydrolysates: Proximate composition, amino acid composition, antioxidant activities and applications: A review, Food Chemistry, 135:3020-3038, 2012. [3] Giannetto et al. Protein hydrolysates from Anchovy (*Engraulis encrasicolus*) Waste: in Vitro and in Vivo Biological Activities, Marine Drugs, 18:86, 2020.

MAIN LECTURE

Basic approach to Dermatopathology

Chiara Brachelente (1), Francesca Abramo (2)

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

Corresponding author: Chiara Brachelente (chiara.brachelente@unipg.it)

Since the late 1970's, the histopathological pattern analysis has been introduced in veterinary medicine for the diagnosis of inflammatory skin diseases. Compared to the classical etiological and pathogenetic approach, the pattern approach uses a morphological approach to the lesion, defining the findings identifiable at low magnification and grouping them into specific categories. Then, moving to higher magnification, more details regarding the type of inflammatory cells, changes in the epidermis and so on can be identified in order to generate a list of differential diagnoses. The different histopathologic patterns do not correspond to specific disease entities, but diseases belonging to the same pattern often share common pathogenetic mechanisms and have to be interpreted based on the clinical appearance and history of the case. The main histopathologic patterns recognized are: 1. Perivascular dermatitis: is usually the least diagnostic and present in many diseases, representing the classical stereotypic "reaction pattern" of the dermis to a generic insult. For this pattern, supplementary information is obtained by the dermatopathologist considering additional findings, such as the associated epidermal changes and the type of inflammatory cells involved. 2. Interface dermatitis: has a strong diagnostic utility as it implies a process targeting the dermoepidermal junction. This pattern is often associated to immune-mediated or auto-immune diseases. 3. Pustular and bullous dermatitis (intraepidermal and subepidermal): is characterized by the intraepidermal or subepidermal formation of pustules or vesicles. These forms can be further classified according to the level of formation and the cellular content of the pustule/vesicle in the epidermis or below it and are often associated to acquired or congenital disease targeting the cell-cell or cell-membrane adhesion molecules. 4. Nodular/diffuse dermatitis: is characterized by a dermal infiltration of inflammatory cells that can be found forming nodules - sometimes coalescing – or diffusely infiltrating the dermis. The type of inflammatory cells (neutrophils, eosinophils, lymphocytes/plasma cells, histiocyte/macrophages) points towards diverse differential diagnoses. 5. Folliculitis, perifolliculitis, furunculosis and sebaceous adenitis: is characterized by an inflammatory process which is centered on the hair follicles and/or sebaceous glands. Differential diagnoses for this pattern are infectious (bacterial, mycotic or parasitic) and non-infectious (immune-mediated or autoimmune) diseases of the hair follicles. As a consequence of the follicular targeting, follicular structures can be destroyed (furunculosis); however, different from the nodular pattern, in this case the inflammatory infiltrate will be centered around hair fragments, free keratin lamellae or sebaceous material and this can be used to distinguish this form from the nodular/diffuse pattern. 6. Vasculitis: as the name implies, the inflammation is centered on the vessel wall and around it. In veterinary medicine, the most common form of cutaneous vasculitis usually involves small caliber vessels and is typically characterized by vessel wall changes without a significant component of inflammatory cells. Immune-mediated diseases, drug reactions or, less commonly, infectious diseases can present with this pattern. 7. Non-inflammatory diseases of the hair follicles: hair follicles are targeted, however an inflammatory process centered around them is usually not evident. In these pattern hair cycle abnormalities, follicular atrophies associated with ischemia and diseases characterized by an abnormal development of hair follicles are included. 8. Miscellaneous dermal changes: the dermis can be targeted by several miscellaneous dermal changes such as degenerative/dysplastic diseases (acquired

or congenital changes in the number and composition of dermal collagen and/or elastin), accumulation of dermal deposits (amyloid, calcium, hyaluronic acid) or dermal fibrosis. 9. Panniculitis: the inflammatory infiltrate is found primarily in the lobules of the subcutaneous adipose tissue (lobular panniculitis), in the interlobular connective tissue (septal panniculitis) or both (diffuse panniculitis). The panniculitis is often an extension of a dermal inflammatory process (nodular/diffuse dermatitis) and shares with this the same pathogenetic mechanisms.

Not all patterns have the same diagnostic utility and there can be significant overlap between different patterns, especially between the perivascular and the nodular/diffuse pattern. Furthermore, more than one pattern can be present at the same time and a detailed knowledge of the history and clinical findings can help the dermatopathologist in distinguishing the causal and temporal relationships between them. Through the teaching course, each single pattern will be presented with its typical histopathologic features and the list of differential diagnosis associated to it.

(1) Yager JA, Wilcock BP. Color Atlas and Text of Surgical Pathology of the Dog and Cat. Dermatopathology and Skin Tumors. Spain, Mosby-Year Book Europe Ltd., 1994.
(2) Gross TL, Ihrke PJ, Walder EJ, Affolter VK. Skin Diseases of the Dog and Cat: Clinical and Histopathologic Diagnosis, 2nd ed. Oxford, Blackwell Science, 2005.
(3) Mauldin E, Peters-Kennedy J. Integumentary System, in Pathology of Domestic Animals, 6th ed. Maxie MG eds., Oxford, Elsevier Ltd., 2016.
(4) Hargis AM, Myers S. The Integument, in Pathologic Basis of Veterinary Disease, 6th ed. Zachary JF, eds. St. Louis, Elsevier, 2017.

Post-mortem bruising in veterinary medicine: an experimental study

Frine Eleonora Scaglione (1), Arturo Nicoletti (1), Paola Pregel (1), Alessandra Sereno (1), Fiammetta Lenarduzzi (1), Emanuele Ricci (2), Lorenzo Ressel (2)

(1) Università degli Studi di Torino, Dipartimento di Scienze Veterinarie (2) University of Liverpool, School of Veterinary Science

Corresponding author: Arturo Nicoletti (arturo.nicoletti@unito.it)

Forensic veterinary pathology is an emerging discipline which plays an essential role in the investigation of animal abuse and unlawful killing. The task is particularly challenging, as the discipline involves a vast multitude of species, such as companion animals, exotics, livestock and wildlife. Indeed, extrapolating results from one species to another may lead to unreliable outcomes, less so from human literature [1]. One of the most debated topic during litigations is the occurrence of intra-vitam or post-mortem trauma, where the presence of haemorrhage plays a pivotal role. The aim of this work is to evaluate the possible formation of post-mortem bruising in dead animals and try to differentiate ante and post-mortem gross lesions. The animals were eligible for study enrolment if the formal consent from the owners was obtained. Briefly, 104 deceased animals were included in the study: 46 dogs (44.2%), 36 cats (34.6%), 10 pigs (9.6%), 6 guinea pigs (5.7%), 4 rabbits (3.8%), 1 sheep (0.9%) and 1 ferret (0.9%). Out of 104 total cases, 9 were positive controls (5 dogs and 4 cats), representing animals that had a confirmed head trauma short before death, and have not been subjected to trauma during the study; 55 (52.9%) were frozen and 49 (47.1%) were preserved at 4°C. The post-mortem interval in the not-frozen group ranged from 1 to 96 hours (median 48 hours). The animals were put in the prone position and a 150 cm plastic tube was placed perpendicularly upon the head of the animals. A steel sphere of 660 grams with a diameter of 73 mm was allowed to fall freely through the tube onto the head to procure a blunt trauma. In 19 (20.2%) animals the blunt trauma was obtained by means of a hammer. An animal was considered positive if a rounded area of subcutaneous reddening, measuring >0.5 cm in diameter, was recorded at the point of impact. Overall, 35 (38.8%) animals were classified as positive and, despite even a small haemorrhage was considered a sign of positivity, this percentage is still higher than expected for a finding generally considered uncommon [2]. The obtained results show that formation of a gross contusion by post-mortem trauma is more likely to appear in not-frozen cadavers and the presence of evident subcutaneous fat in the region of impact is significantly associated with the formation of a post-mortem gross contusion. Moreover, the formation of post-mortem contusions is not influenced by the type of impacting object. The presence of bruising on both subcutis and fascia is more likely to be found in ante mortem blunt trauma. Lastly, eye contusions and scleral haemorrhages have been found only in control cases. Even though further analyses are required to obtain more robust conclusions, the results of this work may contribute to the emerging field of forensic veterinary pathology, stressing the importance of a holistic approach to the recording of pathological findings, including histopathological examination, when assessing the “vitality” of bruises while performing a forensic autopsy.

[1] Munro R, Munro HM. Some challenges in forensic veterinary pathology: a review. J Comp Pathol. 2013 Jul;149(1):57-73.
[2] Saukko, P. In Knight's Forensic Pathology. 3ed. Taylor & Francis, 2004.

Systemic amyloidosis in cats from catteries: description of organ distribution and histologic lesions

Valentina Moccia (1), Eric Zini (2), Silvia Ferro (1)

(1) Università degli Studi di Padova, Dipartimento di Biomedicina Comparata e Alimentazione (2) Università degli Studi di Padova, Dipartimento di Medicina Animale, Produzione e Salute

Corresponding author: Valentina Moccia (valentina.moccia@phd.unipd.it)

Amyloidosis refers to a group of protein-misfolding disorders characterized by the accumulation of amyloid in tissues. Amyloidosis is classified into localized or systemic. Amyloid A (AA) amyloidosis is a systemic amyloidosis that involves serum amyloid A (SAA) as precursor protein and it is also called secondary or reactive amyloidosis, as it is caused by chronic inflammatory diseases.[1] Systemic AA amyloidosis is not common in cats and just few cases have been described.[2], [3] At the same time, this disease is a growing problem in captive felids which live together, as in recent studies horizontal transmission has been suggested as a possible transmission route.[4]

Under this light, the aim of this study is to identify AA deposits in tissues, describing its distribution and the histological lesions in cats coming from three catteries. We identified 10 cases of feline systemic amyloidosis in 9 European shorthair cats and in 1 Abyssinian cat and we verified the presence of amyloid deposits with Congo Red staining (CR) in the available organs. To confirm the presence of AA, pre-treatment of slides with potassium permanganate was used. Histological evaluation of lesions was performed with H&E. The organs positive at CR were spleen (9/9), adrenal gland (8/8), small intestine (7/7), kidney (9/10), liver (8/10), gallbladder (6/7), heart (6/8), parotid (3/10), tongue (3/3), stomach (7/10), large intestine (7/10), lungs (2/10), bladder (3/10), pancreas (5/9), skin (1/9), lymph nodes (4/10) and muscle (1/8). After potassium permanganate pre-treatment, the congo-red staining resulted negative in all samples, confirming the presence of SAA in these organs. The most frequently associated lesions were chronic enteritis (5 in small intestine and 6 in large intestine), and chronic interstitial nephritis (7) but inflammatory or hyperplastic lesions have also been described in lymph nodes (5/10), pancreas (5/9), spleen (5/9), liver (5/10), adrenal gland (4/8), lungs (8/10) and tongues (2/3). In conclusion, this study describes the distribution of AA deposits in 10 cats and the concurrent histological lesions, adding new organs to the already known possible sites of accumulation of AA. Our results suggest a possible higher prevalence of AA amyloidosis in catteries than expected, maybe related to environmental conditions (overcrowding, stress) which may influence the health status or ease an eventual horizontal transmission. For these reasons, AA amyloidosis should be taken into consideration in the management and in post-mortem analysis of cats coming from catteries.

[1] Murakami T., Ishiguro N., Higuchi K., Transmission of Systemic AA Amyloidosis in Animals, Vet. Pathol., 51:363–371, 2014.

[2] Van Der Linde-Sipman J. S., Niewold T. A., Tooten P. C. J., De Neijs-Backer M., Gruys E., Generalized AA-amyloidosis in Siamese and Oriental cats, Journal of Small Animal Practice, 39:442-447, 1997.

[3] Asproni P., Abramo F., Millanta F., Lorenzi D., Poli A., Amyloidosis in association with spontaneous feline immunodeficiency virus infection, J. Feline Med. Surg.,15:300–306, 2013.

[4] Zhang B., Une Y., Fu X., Yan J., Ge F., Yao J., Sawashita J., Mori M., Tomozawa H., Kametani F., Higuchi K., Fecal transmission of AA amyloidosis in the cheetah contributes to high incidence of disease, PNAS, 105:7263-7268, 2008.

Detection of Canine Parvovirus type 2 in larvae of *Calliphora vomitoria* (Diptera: *Calliphoridae*) fed on infected tissues: applications in veterinary forensic pathology

Giuseppe Piegari (1), Letizia Ceglie (2), Ilaria d'Aquino (1), Davide De Biase (1), Valeria Baldassarre (1), Raffaele Ilsami (1), Orlando Paciello (1)

(1) Dipartimento di Medicina Veterinaria e Produzione Animali, Università degli Studi di Napoli "Federico II" (2) Istituto Zooprofilattico Sperimentale delle Venezie

Corresponding author: Giuseppe Piegari (giuseppe.piegari@unina.it)

Canine parvovirus type 2 (CPV-2) is a highly infectious and environmentally resistant virus. CPV-2 is also reported to be one of the most common and important cause of mortality in young dogs [1]. In veterinary pathology, the diagnosis of canine parvovirus infection is commonly based on clinic history of the subject, gross and histopathological examinations. Furthermore, immunohistochemical (IHC) and Real-Time PCR examinations are commonly performed to detect virus proteins or genome in target organs [2]. However, in bodies in advanced decomposition, the post-mortem cadaveric changes determine liquefaction and disintegration of tissues with loss of cadaveric materials; therefore, more traditional organs, such as intestine or lymph node, are often not available for gross and ancillary examinations. These stages of cadaver decomposition are also characterized by an extensive insect activity that feed directly on cadaver, or on fluids released from cadaver during the decay process [3]. In this study, we investigate the CPV-2 immunohistochemical and molecular detection in necrophagous insects feed on intestine of infected animals and tested our hypothesis that necrophagous insects can be use as alternative matrix for CPV-2 detection in advanced decay cadavers. To this aim, 240 third stage *Calliphora vomitoria* larvae (*Diptera: Calliphoridae*) were selected for the study and divided in 3 groups of 80 larvae each. The group A was composed by larvae bred for 8 days at room temperature on intestine tissue obtained from dogs dead for CVP-2 infection; the group B was composed by larvae bred for 2 days on CPV-2 positive intestine tissue and for 6 days on CPV-2-free muscle tissue; the group C was composed by control larvae (larvae bred for 8 days on CPV-2-free muscles). Every day, developing larvae and pupae were collected, washed 10 times to remove viral DNA from the external tegument and, subsequently, tested for CPV-2 by PCR and immunohistochemical examination. The immunohistochemical procedures were carried out on 4 µm thick tissue sections using monoclonal antibody anti capsid protein VP2. Furthermore, real-time PCR was performed using primers and probes specific for fragments encoding the capsid protein VP2. Our results showed CPV-2 immunoreactivity in all animals in groups A and B. Strong immunoreactivity was observed in Malpighian tubule cells, hemolymph cells and food material. Similarly, in all experimentally contaminated groups, a strong positivity in genome load was detected at 24h and until the end of the study (8th day). Finally, no positive results were observed in control group for both PCR and IHC analyses. Our findings show a high resistance of VP2 virus protein and genome in larvae experimentally contaminated with CPV-2. Therefore, our results suggest a potential use of necrophagous larvae as alternative matrix to detect CPV-2 by molecular or immunohistochemical analysis in veterinary forensic pathology.

[1] Mylonakis M.E., Kalli I., Rallis T.S. Canine parvoviral enteritis: an update on the clinical diagnosis, treatment, and prevention, Veterinary Medicine (Auckland, N.Z.), 11:91–100, 2016.
[2] Fagbohun et al. Pathology and molecular diagnosis of canine parvoviral enteritis in Nigeria: case report, Comparative Clinical Pathology, 29:887–893, 2020.
[3] Brooks J.W. Postmortem Changes in Animal Carcasses and Estimation of the Postmortem Interval, Veterinary pathology, 53:929-40, 2016.

Histological and immunohistochemical examination of kidney as new tools to estimate age of puppies in veterinary forensic pathology

Ilaria d'Aquino (1), Gianluca Miletti (2), Rosario Fico (3), Leonardo Meomartino (1), Giuseppe Piegari (1), Emanuela Vaccaro (1), Orlando Paciello (1)

(1) Università degli Studi di Napoli Federico II, Dipartimento di Medicina veterinaria e produzioni animali (2) Istituto Zooprofilattico Sperimentale del Mezzogiorno (3) Centro di Referenza Nazionale per la Medicina Veterinaria Forense, IZS Lazio e Toscana

Corresponding author: Ilaria d'Aquino (ilaria.daquino@unina.it)

Estimation of age represents a central focus of veterinary forensic pathology field. It has recently assumed significant relevance for correctly determine the age of puppies illegally imported to Italy as well as to other European countries. As a matter of fact, in some circumstance, puppies are separated from their litters and transported illegally within the European Community when they are too young to be moved. Because of these reasons, veterinarians are increasingly demanded to precisely estimate the age of suspected illegally imported puppies. In veterinary literature, the main methods to evaluate the age of puppies are the visual examination of the dentition, specifically completeness of dental eruption and extent of tooth wear, and the skeletal age [1]. However, both teeth and bones growth are affected by environmental factors, such as nutritional, hormonal, and pathological changes; moreover, these methods are based on subjected observations by the operator. In contrast, the kidney is characterized by a specific postnatal development in which the kidney continues to mature both from a functional and anatomical point of view; to be specific, glomeruli continue to mature in the nephrogenic zone. In human glomerulogenesis, fetal mesangial and capillary endothelial cells change their immunohistochemical phenotypes with maturation [2]. Therefore, we hypothesized that the kidney histological and immunohistochemical examinations can be used as an indirect parameter for age determination in puppies at least in dead animals. To this aim, puppies were divided in 4 groups defined by age: Group A included 12 cadavers with an age between 0 and 15 days, group B included 6 cadavers with an age between 15 and 45 days, group C included 6 cadavers with an age between 45 and 75 days and group D included 6 cadavers with an age between 75 and 105 days. For each case, kidney samples were collected and processed for routine histopathology. A histological and morphometrical study was performed in order to establish if there is a correlation between the number of glomeruli and age of puppies. Standard immunohistochemical procedures were performed for the immunolocalization of a-SMA. Statistical analysis was carried out using Mann-Whitney U Test. As regards to morphometrical study, Mann-Whitney U Test allowed us to observe statistically significant differences among assessed groups ($p<0.001$). In all animals in group A, aggregated mesenchymal cells at the root of immature glomeruli showed a strong immunopositivity to a-SMA; in the other groups, we observed a progressive migration of positive cells towards the periphery of glomeruli with a gradually loss of immunohistochemical positivity. Our findings suggest a potential use of kidney morphometrical and immunohistochemical examination as indirect parameter to assess the age of puppies in illegally imported animals.

[1] Modina SC. Age assessment in puppies: Coming to terms with forensic requests, Forensic Sci Int., 297:8-15. 2019.
[2] Naruse K. An immunohistochemical study of developing glomeruli in human fetal kidneys. Kidney Int., 57(5):1836-46. 2000.

ORAL COMMUNICATIONS

AIVI

Occurrence of antimicrobial resistance associated genes and mutations in *E. coli* collected from carcasses of broilers reared in Italian antibiotic-free farms

Lucia Gambi (1), Cecilia Crippa (1), Alex Lucchi (1), Antonio Parisi (2), Alessandra De Cesare (3), Gerardo Manfreda (1), Frederique Pasquali (1)

(1) Alma Mater Studiorum – Università di Bologna, Dipartimento di Scienze e Tecnologie Agro-Alimentari (2) Istituto Zooprofilattico Sperimentale della Puglia e della Basilicata (3) Alma Mater Studiorum – Università di Bologna, Dipartimento di Scienze Veterinarie

Corresponding author: Frederique Pasquali (frederique.pasquali@unibo.it)

The use of antibiotics is associated to a selective pressure which foster the growth and survival of antimicrobial resistant (AMR) bacteria harboring mobilizable genes which can be horizontally transferred boosting the spread of the AMR character among bacteria including pathogenic one. Globally, antimicrobial resistance (AMR) is responsible for an estimated 33,000 deaths per year in the European Union remaining, since several years now, one of the biggest threats for human health [1]. As a one-health approach, in recent years, several initiatives have been put in place at European and National level for a more prudent use of antimicrobial agents (AMU) not only in humans but also in pets and food producing animals [2]. One of the animal food productions under the lens, has been broiler production due to the high market value, volumes of productions and the impossibility to treat individual sick animals which forces farmers and veterinarians to apply a metaphylactic approach [3]. Along with EU and National regulations, an increasing consumer demand of antibiotic-free broiler meat has been rising in recent years, boosting the production of broilers without the use of antimicrobial agents. Although several studies addressed the impact on AMU of different preventive measures (such as biosecurity, health management (i.e. vaccination) and good husbandry practices) applied in antibiotic-free production, less is known on the effective impact on antimicrobial resistance associated genes and bacteria [4]. From a food safety point of view, this evaluation is particularly relevant on broiler carcasses which can act as potential vehicles of transmission of AMR genes and bacteria to humans through the food chain.

In the present study, *Escherichia coli* from carcasses of broilers reared in three Italian antibiotic-free farms were collected and their genetic relatedness and resistome compared by whole genome sequencing approaches. In particular, the genomes of 39 *E. coli* isolates were sequenced by paired-end reads approach on an Illumina Miseq platform. Genomes were de novo assembled and their genetic relatedness was assessed by 7 genes Multi Locus Sequence Typing (MLST) and single-nucleotide polymorphisms (SNPs) calling. Additionally, assemblies were screened for plasmids as well as antimicrobial resistance associated genes and mutations using freely available bioinformatic tools.

Results: although mobilizable in most of the cases, thus potentially transmissible through horizontal transfer, the number and type of AMR genes and mutations are relatively low. A point of attention should be kept on aminoglycosides and beta-lactam resistance associated genes corresponding to more than 50% of all AMR genes detected.

[1] Cassini et al. Attributable deaths and disability-adjusted life-years caused by infections with antibiotic-resistant bacteria in the EU and the European Economic Area in 2015: a population-level modelling analysis, Lancet Infect Dis, 19: 56–66, 2019.
[2] Pokharel, S., Shrestha, P. & Adhikari, B. Antimicrobial use in food animals and human health: time to implement 'One Health' approach. Antimicrob Resist Infect Control, 9:181, 2020.
[3] Adam et al. Epidemiological assessment of the factors associated with antimicrobial use in French free-range broilers. BMC Vet Res, 15:219,2019.
[4] Mehdi et al. Use of antibiotics in broiler production: Global impacts and alternatives, Animal Nutrition, 4, 170-178, 2018.

Enumeration of natural flora and survey of foodborne pathogens in artisanal salami and its production environment

Cecilia Crippa (1), Gerardo Manfreda (1), Lucia Gambi (1), Alex Lucchi (1), Frederique Pasquali (1), Alessandra De Cesare (2)

(1) Alma Mater Studiorum – Università di Bologna, Dipartimento di Scienze e Tecnologie Agro-Alimentari (2) Alma Mater Studiorum – Università di Bologna, Dipartimento di Scienze Veterinarie

Corresponding author: Gerardo Manfreda (Gerardo.manfreda@unibo.it)

The Mediterranean region is known for being rich in traditional food products representing a vital part of the local heritage. Small traditional food productions are sometime considered less safe than industrial productions because the combination of artisan practices in traditional settings, potentially instable hygienic conditions and variable production processes may result in the contamination and survival of foodborne pathogens that may persist throughout the chain until the time of consumption. To support the Mediterranean artisanal food producers PRIMA funded the ArtiSafeFood project [1]. Within the project enumeration of total bacterial count, lactic acid bacteria and *Enterobacteriaceae* along with identification of *Salmonella*, *Listeria monocytogenes* and *Staphylococcus aureus* was performed on two batches (July and September 2020) of artisanal salami named “salame gentile” produced in Emilia Romagna region. During the surveys a total of 140 samples were collected as raw materials, dried and fermented products and the related environment during the artisanal salami ripening period of six months.

Four *L. monocytogenes* and three *S. aureus* isolates were collected. No samples were positive for *Salmonella*. Results on the enumeration of native microflora as well as antimicrobial resistance of bacterial isolates will be presented and discussed.

[1] ARTISANEFOOD Project. Innovative Bio-interventions and Risk Modelling Approaches for Ensuring Microbial Safety and Quality of Mediterranean Artisanal Fermented Foods. http://www.ipb.pt/artisanefood

Observations on the veterinary certification process of frozen pork meat for the export to the People's Republic of China

Gaetano Liuzzo (1), Alfonso Rosamilia (1), Jessica Ginestreti (1), Stefano Benedetti (1), *Andrea Serraino* (2)

(1) Local Health Authority of Modena (2) Department of Veterinary Medical Sciences, University of Bologna, Ozzano dell'Emilia, Italy

Corresponding author: Gaetano Liuzzo (g.liuzzo@ausl.mo.it)

The veterinary certification process is ruled by the Regulation (EU) 2017/625 on the official controls and other official activities (articles 86–91) [1], in which the term "official certification" is defined as the procedure by which assurance concerning compliance with one or more requirements laid down in the rules is provided by the competent authorities.

As part of the "other official control activities", the issuing of export certificates of frozen pork meat represents the predominant activity in a Northern industrial slaughterhouse for fattening heavy pigs authorized for the export to China that processes 1 million animals a year.

Additional requirements are specified in the agreement reached between the Ministry of Health and the General Administration of Customs of the People's Republic of China [2]. The "certifying officer" also complies with the operating procedures that the local competent authority (LCA) has defined in terms of export certification to third countries in the field of food of animal origin [3]. The certification process is divided into 5 phases: the food business operator's request for issuing certificates, the process control applied to the product, the emission of the certificate, the sending of the pre-notification and the scanning of the certificate to the Ministry of Health by the LCA and its sending through official channels to the Chinese authorities by the Ministry of Health. The official certificate model is marked with the code CI 01 and written on security paper (watermarked).

The aim of this study is to explore the reasons that led to the request of reissuing certificates from the operators (exporters and importers).

For the year 2020, the reasons of the reissue of the China export certificates by the Competent Authority were collected and analysed. The total certificates issued were 259, while the reissues were 76 (29.3%). The reasons for the reissuing were 81 and they were mostly related to: change of the vessel (21,0%), errors in the compilation of the product name (13.6%), misprints (13.6%), change of the certifying officer (6.2%) and of the consignee (12.4%), errors in the compilation of the batch (7.4%). Other reasons were to be found in errors in the date of production (6.2%) and in lost/missing documents (6.2%). These different results affecting the efficiency of the certification process were due to variables of commercial nature which were independent of the certifying officer. They specifically concerned the changes in the content and the changes of consignee.

As regards formal errors, special attention on the documental review phases by the certifying officer is needed.

[1] Regulation (EU) 2017/625 of the European Parliament and of the Council of 15 March 2017 on official controls and other official activities. OJ L 95, 7.4.2017, p. 1–142.
[2] Ministero della Salute, DGISAN n. 57134 del 18.09.2019 Modalità di controllo ufficiale delle attività di macellazione finalizzate all'esportazione verso la Cina di carne suina e prodotti a base di carne suina.
[3] Dipartimento di Sanità Pubblica, A.U.S.L Modena, Certificazione export paesi terzi. Alimenti di origine animale, PO DSP SPV 44, rev. del 15/0272018, 1-13.

The green side of beef: detection of different *Sarcocystis* spp. in carcasses affected by bovine eosinophilic myositis, including a putative new species

Selene Rubiola (1), Tiziana Civera (1), Davide Vercellino (2), Francesco Chiesa (1)

(1) Università degli Studi di Torino, Dipartimento di Scienze Veterinarie (2) Servizio Veterinario area C, A.S.L. AT

Corresponding author: Selene Rubiola (selene.rubiola@unito.it)

Bovine eosinophilic myositis (BEM) is a rare inflammatory myopathy detected at slaughter and observed in striated muscle of affected cattle. The following carcass condemnation results in considerable economic losses. This fact has brought to an increase interest in the etiology of this myopathy, despite its low prevalence [1]. There is evidence linking these lesions to the presence of protozoan parasites belonging to the genus *Sarcocystis*, which includes six species using cattle as intermediate hosts [2]. Yet, the high prevalence of *Sarcocystis* spp. in cattle carcasses does not correlate with the low prevalence of BEM. Researchers have thus focused on the possible association between BEM and particular *Sarcocystis* species [1]. The goal of the present study was to use molecular methods to identify *Sarcocystis* spp. inside and outside BEM lesions in condemned cattle carcasses, to evaluate the possible role of different *Sarcocystis* spp. in BEM etiology.

For this purpose, from January 2019 to January 2020, we collected heart and striated muscle samples from 25 BEM condemned carcasses. We categorized gross lesions into two groups: "green focal lesions" (GFL) and "green diffused patches" (GDP). One to five samples with lesions and two without lesions were collected, for a total of 94 samples. Genomic DNA was extracted and analyzed by multiplex-PCR targeting 18S rDNA and cox1 genes [3]. PCR products amplified using the genus specific primer set in absence of the specific fragment for *S. bovifelis*, *S. hominis*, *S. cruzi* or *S. hirsuta*, were sequenced to achieve species identification. Unidentified species were molecularly characterized through the amplification and sequencing of the complete 18S rDNA gene and the partial cox1 gene.

Out of 25 carcasses, 24 revealed the presence of at least one *Sarcocystis* spp (96%; 95% C.I.: 78.86 - 99.99 %). The majority of intralesional *Sarcocystis* spp. were found to be *S. hominis*, followed by *S. bovifelis*, *S. cruzi* and *S. hirsuta*. The presence of *S. bovifelis* and *S. hominis* was significantly higher in intralesional samples (43.2% and 52.3%, respectively) than in samples without lesions (2% and 14%, respectively), while there was no significant difference between the presence of *S. cruzi* or *S. hirsuta* in intralesional (27.3% and 2.3%, respectively) and extralesional (30% and 2%, respectively) samples. Moreover, a putative *Sarcocystis* n. sp. was detected in one carcass and molecularly characterized.

The present study contributes to our understanding of the importance of different *Sarcocystis* spp. in the BEM pathogenesis. The results emphasize the association of *S. hominis* and *S. bovifelis* with bovine eosinophilic myositis and highlight the presence of a new *Sarcocystis* sp. using cattle as intermediate hosts.

[1] Vangeel L et al. Different *Sarcocystis* spp. are present in bovine eosinophilic myositis. Veterinary Parasitology, 197:543–8, 2013.

[2] Gjerde B. Molecular characterisation of *Sarcocystis bovifelis*, *Sarcocystis bovini* n. sp., *Sarcocystis hirsuta* and *Sarcocystis cruzi* from cattle (*Bos taurus*) and *Sarcocystis sinensis* from water buffaloes (*Bubalus bubalis*). Parasitology Research, 115:1473–92, 2016.

[3] Rubiola S et al. Molecular differentiation of cattle *Sarcocystis* spp. by multiplex PCR targeting 18S and COI genes following identification of *Sarcocystis hominis* in human stool samples. Food and Waterborne Parasitology, 18:e00074, 2020.

Decrease of *Salmonella enterica* occurrence in Sardinian pig slaughterhouses during twelve years

F. Piras (1), *G. Siddi* (1), *V. Spanu* (1), *M. Demontis* (1), *M.P. Meloni* (1), *R. Sanna* (1), *V. Cibin* (2), *E. P. L. De Santis* (1), *C. Scarano* (1)

(1) Università degli Studi di Sassari, Dipartimento di Medicina Veterinaria (2) Centro di referenza nazionale e Laboratorio di referenza OIE per le salmonellosi., Istituto Zooprofilattico Sperimentale delle Venezie

Corresponding author: F. Piras (fpiras@uniss.it)

Aim of the study was to evaluate the trend of *Salmonella* occurrence during three investigations carried out in a period of twelve years (2008-2020) in eleven Sardinian pig slaughterhouses. A total of 1280 samples were collected, 1101 from slaughtered pigs (lymph nodes, colon content, carcass surface) and 179 from slaughterhouse environment (surfaces in contact and not in contact with meat). As regard the origin of the animals 73.7% of tested pigs came from local farms, 26.4% were imported from other European Member States. Samples were tested for *Salmonella* presence according to ISO 6579:2002 (for the first two investigations) and ISO 6579-1:2017 (for 2020 survey). One *Salmonella* isolate from each positive sample was serotyped (ISO/TR_6579-3). 59 *Salmonella* strains, selected among the more frequently isolated serotypes, were submitted to PFGE analysis (PulseNet US protocol). *Salmonella* was isolated from 11.8% pig samples and 10% environmental samples. Occurrence showed a sharp decrease through the years in samples collected from pigs: 18.8% in 2008, 10% in 2014, 3.4% in 2020. Reduction of *Salmonella* prevalence was observed in all the samples. *Salmonella* was detected in 30.5%, 9.9% and 2.6% lymph nodes during 2008, 2014 and 2020, respectively. As regard colon content, *Salmonella* was isolated in 16.4% samples in 2008, 11.8% in 2014 and 5.1% in 2020. Carcass surface showed an occurrence of positive samples of 14.1% in 2008, 8.7% in 2014, and 2.6% in 2020. The same trend was observed for environmental samples: 34.1% positivity in 2008, 3.7% in 2014, and 0% in 2020. Also, prevalence of carrier pigs (positive at lymph nodes and/or colon content level) showed a progressive reduction: 47% in 2008, 21.7% in 2014 and 7.7% in 2020. Prevalence was lower in animals coming from local farms (9%) rather than those coming from other Member States (57.3%), probably indicating the role of stressful factors as transport in increasing *Salmonella* susceptibility and shedding. Overall, 13 different *Salmonella* serotypes were identified during the surveys: Derby (31.5%), Anatum (14.6%), Typhimurium (14%), Rissen (11.2%), monophasic Typhimurium (7.8%), Panama (10.6%), Livingstone and Infantis (2.8%), Muenchen, Newport and Holcomb (all the three <1%). The most prevalent serotypes (*S.* Derby, *S.* Typhimurium, *S.* Anatum, *S.* Rissen, monophasic *S.* Typhimurium) were among those the most often isolated from slaughtered pigs and human salmonellosis cases in Europe, confirming the potential role of pigs and pork products in the epidemiology of human salmonellosis in EU. For *S.* Derby strains, eleven PFGE profiles were identified (similarity value >88.6%). Among *S.* Typhimurium strains, fourteen PFGE profiles were identified (similarity value >86.1%). Between *S.* Rissen strains, five profiles were identified (similarity value >85.8%). Although it was not possible to detect the same PFGE profile through the years, this technique allowed to show a high similarity between *Salmonella* strains belonging to the same serotypes thus indicating common ancestors. The results of the surveys showed a reduction of the rate of carrier pigs and *Salmonella* occurrence at slaughterhouse during twelve years in Sardinia that could be related to improvements in application of preventive and control measures at farm level and in the GMPs and GHPs at slaughterhouse. In this context, the Food Business Operator (FBO) plays a crucial role ensuring that meat complies with the microbiological criteria established by the EU regulations.

Eco-Design packaging for vacuum-packed meat preparations: a response to needs of the food market

Marika Di Paolo (1), Rosa Luisa Ambrosio (1), Giorgio Smaldone (2), Lucia Vollano (1), Maddalena Stompanato (3), Raffaele Marrone (1)

(1) Università degli Studi di Napoli Federico II, Dipartimento di Medicina Veterinaria e Produzioni Animali (2) Università degli Studi di Napoli Federico II, Dipartimento di Agraria (3) Ufficio Qualità LOMA S.r.l

Corresponding author: Marika Di Paolo (marikadp98@@gmail.com)

Meat preparations are highly perishable foods and require protection to maintain their quality [1]. Large-scale retail trade markets meat preparations in modified atmosphere packaging (MAP) and vacuum packaging (VP) in order to extend the shelf-life [2]. Food industry interest in sustainable and recyclable packaging has increased [3] directing the research at development of Eco-Design tools. The aim of this study was to evaluate the influence of pre-formed recyclable cardboard base with coextruded film with PE liner (Bernucci s.r.l-K400BG100) on shelf life of vacuum skin-packed sausages and hamburgers (EP group). The plastic packaging (Faerch Group-PP HI EOST-9541 Black) was used as a control (PP group). Sausages and hamburgers were vacuum skin packaged using the same top plastic film (Cryovac®-VST 0250 SKIN TOP WEB). Samples were stored at refrigeration temperature (2±1°C) during experiment. Analyses were performed in laboratories of the DMVPA of the UNINA at different times: 0, 8, 12 and 15 days from the production. pH, activity water (a_w), instrumental texture profile analysis (TPA), colour (CIELAB), Thiobarbituric Acid (TBA) test and Free Fatty Acids (FFAs) and drip loss were measured. Microbiological analyses performed were total bacterial count (CBT) at 7 and 30°C, *Enterobacteriaceae*, total coliforms, *Escherichia coli*, *Pseudomonas* spp., Lactic Acid Bacteria at 30°C, yeasts and moulds. pH ad a_w values showed a decrease during storage. However, a_w trends appeared to be influenced by the packaging materials in sausages where PP samples showed the highest value of a_w at the end of storage. Higher values of drip loss were found in PP sausages at the end of storage due to WHC of proteins decrease caused by pH decrease [4]. TPA results rivealed low hardness values in PP sausages. Regarding the colour, the values of redness (a*) appeared higher in PP hamburgers. In EP sausage the values of lightness (L*) and yellowness (b*) were lower at the end of storage. Fat alteration index showed same trends, however PP samples accounted the highest level of FFA. Microbiological results highlighted perfect counts overlapping. It's worth noting the high levels of CBT and *E. coli* in hamburgers, which always overcame the respective maximum limits of 6.7 and 2.6 Log CFU/g fixed by the European regulation (Reg. EC 2073/2005) for minced meat. *E. coli* count decreased at the end of the storage, however keeping over 1 Log CFU/g. Overall, Eco-desing packaging do not influence the shelf life of meat preparations, as for traditional packaging system the initial microbiological status must be considered. The use of green packaging seems to be a good alternative to plastic packaging, satisfying the current demands of consumers and market.

[1] Karimian Z, et al., Physico-mechanical and antimicrobial properties of isolated soy protein film incorporated with peppermint essential oil on raw hamburger, J Agr Sci Technol, 21:1145-59, 2019.
[2] ŁOPACKA, et al., Effect of MAP, vacuum skin-pack and combined packaging methods on physicochemical properties of beef steaks stored up to 12 days, Meat science, 119: 147-153, 2016.
[3] Martinho, et al., Factors affecting consumers 'choices concerning sustainable packaging during product purchase and recycling, Resources, Conservation and Recycling, 103: 58-68, 2015.
[4] Friedrich, L., et al., Influence of various preservatives on the quality of minced beef under modified atmosphere at chilled storage, Meat science, 79.2: 332-343, 2008.

Development of a predictive model for the shelf life of Atlantic mackerel (*Scomber scombrus*)

Filippo Giarratana (1), Felice Panebianco (2), Luca Nalbone (1), Graziella Ziino (1), Davide Valenti (3), Alessandro Giuffrida (1)

(1) Università degli Studi di Messina, Dipartimento di Scienze Veterinarie (2) Università degli Studi di Torino, Dipartimento di Scienze Veterinarie. (3) Università degli Studi di Palermo, Dipartimento di Fisica e Chimica

Corresponding author: Luca Nalbone (lnalbone@unime.it)

Despite its commercial relevance and widespread consumption, the shelf-life of the Atlantic mackerel (*Scomber scombrus*) during refrigerated storage was poorly investigated [1]. In fresh fish, loss of freshness is related to post-mortem biochemical, physicochemical and microbiological processes as well as handlings on board and land. Quality Index Method (QIM) was proposed as a suitable method for freshness and quality sensorial estimation of fishery products [2]. Demerit points are assigned to a set of typical characteristic and the relative scores are added up obtaining an overall sensory score, the Quality Index (QI). Several authors have reported a linear correlation between the QI score and the time of storage at a specific temperature [3,4]. This study aims to develop a probabilistic mathematical model based on the use of varying environmental parameters, such as the temperature, and on a successive statistical analysis of the results obtained. This model will be exploited to predict the shelf-life of the Atlantic mackerel based on specific storage temperatures. A total of 60 fresh fishes were subdivided into two groups and respectively stored in ice for 12 days at a constant temperature of 1±0.5°C (Group A) and a fluctuating temperature ranging between 1 and 7°C (Group B). Microbiological analysis and sensory evaluation through the QIM were performed on each fish at regular time intervals. A critical value of Log 6 CFU/g of spoilage bacteria associated with a significant decay of the sensorial characteristics was exceeded after 9 days of storage for Group A and 3 days for Group B. A reliable prediction of fish freshness was obtained by modelling the QIM as a function of the spoilage bacteria behaviour. A coefficient β of correlation was determined to convert the spoilage bacteria load into a Quality Index scores. The adoption of probabilistic models to assess microbial behaviour under different environmental conditions is an interesting tool for food industries to maximize production and reduce waste.

(1) Andrade A, Nunes ML, Batista I. Freshness quality grading of small pelagic species by sensory analysis. In: Ólafsdóttir G, Luten J, Dalgaard P, Careche M, Verrez-Bagnis V, Martinsdóttir E, Heia K, eds. Methods to determine the freshness of fish in research and industry. Proceedings of the Final Meeting of the Concerted Action "Evaluation of Fish Freshness", AIR3CT94 2283, Nantes Conference, November 12-14 1997, International Institute of Refrigeration, Paris, France: 333-338, 1997.
(2) Bremner HA. A convenient easy to use system for estimating the quality of chilled seafood, in Proceedings of the fish processing conference - Fish Processing Bulletin, ed. by Scott DN and Summers C, Nelson, New Zealand 7: 59-703, 1985.
(3) Huidobro A, Pastor A, Tejada M. Quality Index Method developed for Raw Gilthead Seabream (*Sparus aurata*). Journal of Food Science, 65: 1202-1205, 2000.
(4) Warm K, Bøknæs N, Nielsen J. Development of Quality Index Methods for evaluation of frozen cod (*Gadus morhua*) and cod Fillets. Journal of Aquatic Food Product Technology, 7: 45-59, 1998.

Application of a portable near-infrared spectroscopic device for on-field monitoring of the country of origin labelling of musky octopus (*Eledone spp.*)

Maria Olga Varrà, Sergio Ghidini, Maria Pia Fabrile, Adriana Ianieri, Emanuela Zanardi

Università di Parma, Dipartimento di Scienze degli Alimenti e del Farmaco

Corresponding author: Maria Olga Varrà (mariaolga.varra@unipr.it)

Today, cephalopods are of increasing economic and nutritional importance, as highlighted by the increasing global productions which reached over 365 million tons in 2018 [1]. Among cephalopods, large scale production and distribution of musky octopus (*Eledone* spp.) originating from the Mediterranean and North-East Atlantic fishing areas are currently increasing, thus potentially questioning the truthfulness of the geographic indications reported on the label and, by consequence, qualities and reputation that are due to the specific origin. As a matter of fact, fraudulent mislabelling concerning the country of origin is becoming a pressing problem affecting the fishery sector both from an economic and a sanitary point of view. Hence, a growing need exists for tightening up fish controls on the one hand and shifting towards a risk-based control of seafood provenance on the other, through the implementation of efficient and standardised food inspection analytical tools, for which the requirements of rapidity, portability, cost-effectiveness, and easiness of application are met. In the present study, the objective was to verify the suitability of using a handheld and portable near infrared (NIR) spectrometer to characterize the geographic origin of musky octopuses in a non-destructive and simple way. Three different batches of frozen musky octopuses (*Eledone* spp.) of medium size were collected from each of the two sampling sites chosen, corresponding to the FAO fishing areas 37.1.1 (i.e., Mediterranean Sea) and 27.9.a (i.e., North-East Atlantic Ocean), for a total of 118 specimens. After defrosting, cleaning, and skinning, 4 replicate NIR spectra were acquired from four different area of the samples' mantle using the portable NIR device MicroNIR OnSite-W by VIAVI (VIAVI Solutions Inc., San Jose, CA, USA). The instrumental parameters and analytical conditions were set as follows: diffuse reflectance acquisition mode, 908–1676 nm wavelength range, 6.2 nm resolution, 50 spectral scans, 10 ms integration time. The spectral data were exported and elaborated by chemometrics using the SIMCA 16.0.2 software package (Sartorius Stedim Data Analytics AB, Umea, Sweden) to generate a qualitative model based on orthogonal partial least square discriminant analysis (OPLS-DA) to differentiate samples according to their origin. The OPLS-DA calibration model from NIR spectral data, created using 70 out of 118 octopus's specimens, showed excellent prediction statistics outcomes, with an estimate of the fit and the prediction ability of the model reaching 86% and 78%, respectively. In addition, when the accuracy of the model in recognizing the remaining unlabeled 48 octopuses was tested, it was found that 90% of the Mediterranean samples and 89% of the Atlantic samples were correctly identified. By way of conclusion, the results achieved clearly highlight the effective support provided by NIR spectroscopy in the early detection of fraud related to the seafood origin. In the near future, the shift towards miniaturization and portability of the analytical instrumentation, may therefore result in fish authenticity testing moving directly to the field, being particularly advantageous in the context of daily routine and screening analysis both at industrial and retail level.

[1] FAO. Fishery Statistical Collection Global Capture Production Database, 2021. Available from http://www.fao.org/fishery/statistics/global-capture-production/en.

A tool to support sanitary survey in areas for live bivalve molluscs: preliminary results for the area Vasta N. 2

Francesca Barchiesi (1), Francesca Leoni (1), Donatella Ottaviani (1), Elena Rocchegiani (1), Erica Calandri (Bruno Barchiesi (2), Giovanni Guidoni (3), Federica Savini (3), Silvia Piva (3), Andrea Serraino (3), Federica Giacometti (3)

(1) Istituto Zooprofilattico Sperimentale dell'Umbria e delle Marche "Togo Rosati" (2) Azienda Sanitaria Unica Regionale A.S.U.R., Area Vasta N.2 (3) Università degli Studi di Bologna, Dipartimento di Scienze Mediche Veterinarie

Corresponding author: Francesca Giacometti (federica.giacometti3@unibo.it)

Before classifying a production or relaying area for live bivalve molluscs, the Competent Authority has to carry out a sanitary survey of the area, that includes an inventory of the sources of pollution of human or animal origin likely to be a source of contamination for the production area, an examination of the quantities of organic pollutants released during the year, according to rainfall readings, waste-water treatment, etc., the determination of the characteristics of the circulation of pollutants. Based on sanitary survey findings, a monitoring programme is established (1). *Escherichia coli* is a common indicator organism of faecal contamination in aquatic systems that in the European Union is used for the classification of the mollusc production areas as A, B or C. The presence of *E. coli* in water environment is associated with biotic and abiotic factors and its abundance is associated with heavy rainfall events that could cause an overload and possible leakage from sewage systems or facilitate the arrival of an increased amount of fecal material from land living animals to the sea.

This study presents data on: i) official controls performed in the area Vasta n.2 from 2015 until 2019 on 2.701 bivalve samples, that were examined for *E. coli* enumeration or *Salmonella* spp. detection; of these, 924 were samples of mussels of the species *Mytilus galloprovincialis* (n=473 examined for *E. coli*; n=451 examined for *Salmonella*) and 1777 were striped clams of *Venus gallina* (n=945 for *E. coli*; n=832 for *Salmonella*); ii) visualization of the different sources of contamination of the investigated area by QGIS; iii) assessment of the impact of rainfalls on *E. coli* concentrations in molluscs in the 48 and 72 hours before official sampling. In the 5 years of sampling, only 9 (1.9 %) and 44 (4.7%) non-compliant *E. coli* samples were observed in mussels and striped clams, respectively, and a significant difference in the *E. coli* level of contamination was found between mussels and clams (Kruskal-Wallis test, $p<0.00001$). An overall occurrence of 1.1% was reported for *Salmonella* spp., detected in 5 and 6 samples of mussels and striped clams, respectively. The most commonly isolated serotypes were *S.* Derby (18%), *S.* Napoli (18%) and monophasic *S.* Typhimurium (9%). For the assessment of the impact of rainfall, five catchment areas (Cesano, Misa, Esino, Musone) and 19 bivalve mollusc harvesting areas were investigated and 1.418 rainfall observations collected by pluviometers. The investigated sources of contamination (rivers, production areas of molluscs, water discharges, flood risk areas, sewage treatment plant, and livestock populations) were presented in different maps by an open source GIS. A correlation was observed between the *E. coli* concentration in molluscs and rainfall (precipitation quantity expressed in mm) at both 24 and 96 hours before sampling, reflecting the impact of the sources of contamination located in the investigated area and its amplification after heavy rainfalls.

E. coli concentration in bivalve molluscs was found to be correlated with rainfall in the area at both 24 and 96 hours before sampling. The proposed tool could be useful to raise awareness of potential contamination of the bivalve mollusc production areas and used for a real risk-based monitoring available to Veterinary Competent Authority and National Health System.

[1] Regulation (EU) 2019/627.

Application of information technology to auditing system

Martina Sartoni (1), Ambra Berni (2), Francesco Riccioli (1), Roberta Moruzzo (1), Alessandra Guidi (1)

(1) Università di Pisa, Dipartimento di Scienze Veterinarie (2) Epta Lab, Firenze

Corresponding author: Alessandra Guidi (alessandra.guidi@unipi.it)

In recent years, the technological revolution has hit the agro-food sector requiring the development of quality management systems capable of competing in a globalized market oriented towards continuous improvement. The current “Industry 4.0 development plan” is leading to a digital transition of food industry production processes and the food safety management tools with the aim to increase interaction, transparency and communication among interested parties [1,2]. Innovation is also promoted by regulatory updates: Regulation (EU) no. 2017/625 and the UNI EN ISO 22000: 2018 international standard encourage the use of "Food Tech" for archiving and exchanging data [3]. In this context, we applied information technologies solution in first and second party audits and evaluated the potential advantages of using a computerized and interactive checklist compared to the traditional one [4]. The study, conducted at the points of sale of a Tuscan large supermarket chain, was based on the use of an “Audit" software, with an android application, developed by a private consulting firm. This platform manages audit planning, auditor database and the editing and acquisition of digitized checklists and non-conformities. The study, comparing traditional vs innovative audit from 2016 to 2019, shows that the system facilitates the execution of the audit and reporting activities; the use of the software involves an average reduction of back office and front office times with an optimization in the use of resources, timing and travel costs. Moreover, the greater transparency and communication effectiveness of the system indicates a positive impact on non-conformities management and deficiencies reappearance. Presented data confirm that digitization as well as the smart technologies within the 4.0 industry framework are a closer and important tool for the food world and in particular for food safety management.

[1] Guidi A., Sicurezza alimentare 4.0. Tecnologie Alimentari, Interprogetti editore, 8: 8-10, 2019.
[2] Grunert K.G., Food quality and safety: consumer perception and demand. European Review of Agricultural Economics, 32:369–391, 2005.
[3] Ferri M., Il Regolamento UE 2017/625 sui controlli ufficiali. Cosa cambia? Società Italiana di Medicina Veterinaria Preventiva, 2019.
[4] Manita R. et al. The digital transformation of external audit and its impact on corporate governance. Technological forecasting and social change,150, 119751,2020.

Evaluation of effectiveness of a predictive model on circulation of pollutants in some areas of bivalve production of Campania region

Giorgio Smaldone (1), Rosa Luisa Ambrosio (2), Raffaele Marrone (2), Valeria Vuoso (2), Maurizio Della Rotonda (3), Aniello Anastasio (2)

(1) Department of Agricultural Sciences, University of Naples, Federico II (2) Department of Veterinary Medicine and Animal Production, University of Naples, Federico II (3) Centro di Riferimento Regionale per la Sicurezza Sanitaria del Pescato Campania Region- CRiSSaP

Corresponding author: Rosa Luisa Ambrosio (rosaluisa.ambrosio@unina.it)

The quality of coastal marine waters depends strictly on the anthropogenic environmental impact; drains overflow, subsea pipelines sewer, river mouths could be considered as sources of pollution along the coastline. Mussels, because of filter feeding habits, are critically sensitive to marine coastal water quality and thus require continuous monitoring to enforce food safety and human health. To achieve this goal a decision-making tool based on numerically coupled models that can forecast atmospheric and marine dynamics has been implemented in order to predict environmental impact and test different scenarios [1]. This model can foresee pollutant spills, transport and dispersion in both inshore and offshore environments. According to Reg. EC 2073/05, food safety criteria for bivalve placed in the market are represented by *E. coli* and *Salmonella* spp. parameters correlated to a faecal contamination. Aim of the work was to correlate data obtained by predictive model on transport and dispersion of pollutants and the microbiological results of mussel and water sampled in two sites of Gulf of Pozzuoli - Campania region. During 2019 no. 34 water and no. 37 mussel samples in no. 2 farms were collected with the support of CRiSSaP (Centro di Riferimento Regionale per la Sicurezza Sanitaria del Pescato – Campania Region). The samplings of mussels were carried out on the same "sentinel" row in points at different depths in order to have a representative specimen along the water column. Both mussels and water samples have been subjected to research of *E. coli* with ISO methods [2,3]. Three days before and three days after the sampling the area was monitored every 6 hours with the predictive model. N. 7 not compliances (NC) in the mussel samples were recorded, while no NC have been recorded for water samples. In all cases in which no NC were highlighted, the model showed no diffusion of pollutants; NC were classified in mild (*E. coli* found between 230 and 700 MPN) and serious (*E. coli* found over 700 MPN) level. When mild NC occurred, the predictive model did not foresee an involvement of the area, in our opinion this could be due to recognized variability of the *E. coli* as marker parameter and not to a poor reliability of the model. When serious NC occurred, the model showed a slight involvement of the proximal area of the farm where was located the sentinel row. Even if further studies to confirm this data are needed, the results would be of great importance as it would allow for the identification of a restricted area affected by the circulation of pollutants before official mussels sampling.

[1] Montella et al. WaComM: A parallel Water Quality Community Model for pollutant transport and dispersion operational predictions. 12th International Conference on Signal-Image Technology & Internet-Based Systems, 2016.
[2] UNI EN ISO 9308.
[3] UNI EN ISO 16649.

Occurrence of microplastics in bivalves: can a systematic literature review support risk assessment?

Alice Giusti (1), Lisa Guardone (1), Marco Diano (1), Francesca Susini (2), Andrea Armani (1)

(1) FishLab, Dipartimento di Scienze Veterinarie, Università di Pisa (2) Istituto Zooprofilattico Sperimentale del Lazio e della Toscana

Corresponding author: Alice Giusti (alice.giusti183@outlook.it)

Microplastics (MPs) are a global environmental issue, particularly affecting the aquatic ecosystem. Due to their small size (<5 mm), MPs can be absorbed or ingested by aquatic organisms, and transferred through food webs. Toxic effects due to the ingestion of MPs, alone or contaminated with additives or pollutants, have been hypothesized. Human exposure is inevitable, also following accumulation in the food chain. Seafood, especially bivalves, being filter feeders and consumed as whole, are an important potential pathway. The scientific interest in the topic is rising, and several narrative reviews on MPs in food, including seafood, have been published since the publication of a statement on the presence of MPs and nanoplastics in food by EFSA in 2016 (1), highlighting a scarcity of data on MPs occurrence.

The aim of this review was to systematically revise scientific papers (SPs) to assess the occurrence of MPs in different categories of bivalves (mussels, clams, oysters and scallop) worldwide. A double-step filtration was used, applying increasingly stricter quality criteria. Data on MPs abundance were first discussed focusing on all the investigated species and geographical areas. Then, a subset of SPs selected in the second filtering step was used to calculate the weighted MPs mean abundance and the human exposure per serving size.

In the first filtering process 87 SPs, published between 2014 and 2020 in 30 different scientific journals, were retained. Overall, 67 species, 6 genera and 1 family of bivalves were analysed. Mussels were the most analysed (61 SPs), followed by clams (55 SPs), oysters (31 SPs), and scallops (7 SPs). Mytilus edulis and *M. galloprovincialis* were the most investigated species, followed *by P. viridis, Mytilus sp., R. philippinarum* and *C. gigas*. All these are commercial species, globally farmed and distributed. Marine FAO areas 61 and 27 were most investigated. Overall, MPs mean abundance was variably reported, as well as the use of different methods and procedural controls. Therefore, in this study, the weighted MPs mean abundance was calculated only including data from a subset of SPs (n=32; 37%). The overall weighted MPs mean abundance including data from all FAO areas was 1.19 MPs/g ww. The highest value was observed in FAO area 61 (2.33 MPs/g ww), while values <1 MPs/g ww were observed in FAO areas 27 and 57. Among bivalve categories, the highest weighted MPs mean abundance (overall FAO areas) was observed for scallops (1.99 MPs/g ww), followed by mussels (1.71 MPs/g ww), clams (0.84 MPs/g ww) and oysters (0.65 MPs/g ww). Thus, the consumption of standard portions of each bivalve category determines the ingestion of a different number of MPs depending on the FAO area; the highest value (~645 MPs) would be ingested with a portion of mussels from FAO area 61.

Our findings confirmed the existence of quality issues and the lack of analytical standardization. A disparity among investigated species and geographical areas was observed, and only three studies addressed processed products. These aspects affect the outcome of systematic reviews to support risk assessment; future studies should explore the issue of MPs adopting an interdisciplinary perspective, integrating different technical and scientific competences to collect evidences for risk assessment and management.

[1] EFSA. Presence of microplastics and nanoplastics in food, with particular focus on seafood. EFSA Journal, 14(6):4501, 2016.

DNA Barcoding as screening tool in the labelling analysis of Caviar Products Sold on Chinese e-commerce market

Lara Tinacci[1], *Xia Zhang*[2], *Siyun Xie*[2], *Huiru Kang*[2], *Jing Wen*[3], *Andrea Armani*[1]

(1) Università di Pisa, FishLab, Dipartimento di Scienze Veterinarie (2) Shaoguan University, Henry Fok College of Food Science, Shaoguan, China (3) Lingnan Normal University, Department of Biology, Zhanjiang, China

Corresponding author: Lara Tinacci (lara.tinacci@libero.it)

Caviar is a valuable seafood delicacy consisting of salt-cured roe of sturgeon and paddlefish species included among CITES endangered species and extensively farmed internationally. To cope with both overexploitation and counterfeits due to substitute and mimicking products, CITES implemented a labelling system bearing the mandatory information for caviar traceability and identification [1]. Counterfeits triggered the development of DNA based authentication techniques and DNA barcoding, among the approaches, represents an effective screening method in fraud monitoring [2,3]. Thus, the present study was set to verify the labelling compliance of the available products on the Chinese e-commerce platforms, potentially exposed to counterfeiting, to the requirements of the Chinese labelling standard (GB7718-2011) in force for pre-packaged food and CITES requirements [1]. In the study, a DNA barcoding approach targeting COI and cytb genes was applied as screening method [3] to highlight the occurrence of counterfeit phenomena and to verify labelling information validity. Forty caviar products were collected from one major e-commerce platform and the labelled information were checked against both national and international requirements. The final COI or cytb barcodes were queried against BOLD and NCBI reference databases and final identity values >98% or 99-100% were respectively applied to designate potential species identification [4,5]. DNA barcoding outcomes were compared to the labels analysis results to assess the occurrence of substitution and mislabelling incidents. All products were found fully compliant to GB7718-2011 standard and were verified belonging to Chinese producing plants. Conversely, CITES requirements were never satisfied and no references to either the production method (wild catch or aquaculture) or the origin of the roes processed for caviar production was reported. Nevertheless, the 32.5% of the products presented an explicit reference to the roe sturgeon species or hybrid. No counterfeits emerged by Barcoding analysis and all the products were confirmed belonging to sturgeon. Despite Barcoding ascertained limits in *Acipenser* sp. species discrimination and in commercial hybrids identification (2, 3), 42.5% of the products were finally found not matching the product expected identity according to the label information. The need to promote the application of CITES labeling system on the Chinese market clearly emerged by the study to protect both consumer interests and products sustainability. Noteworthy, the effective role of DNA barcoding as screening tool to monitor labelling validity.

(1) CITES Res. Conf. 12.7-Rev. CoP17, Conservation of and trade in sturgeons and paddlefish, https://cites.org/eng/res/12/12-07R17.php, 2017.
[2] Boscari et al. Species and hybrid identification of sturgeon caviar: a new molecular approach to detect illegal trade. Molecular ecology resources, 14(3):489-498, 2014.
[3] Pappalardo et al. From fish eggs to fish name: Caviar species discrimination by COIBar-RFLP, an efficient molecular approach to detect fraud in the caviar trade. Molecules, 24, (13): 2468, 2019, 2017.
[4] Hebert et al. Biological identifications through DNA barcodes. Proceedings of the Royal Society of London. Series B: Biological Sciences, 270(1512): 313-321, 2003.
[5] Parson et al. Species identification by means of the cytochrome b gene. International journal of legal medicine, 114(1): 23-28, 2000.

Occurrence of antibiotics and non-targeted metabolite residues in raw bovine milk

Maria Nobile, Sara Panseri, Francesco Arioli, Luca Maria Chiesa

(1) Università degli Studi di Milano, Dipartimento di Scienze Veterinarie per la Salute, la Produzione Animale e la Sicurezza Alimentare.

Corresponding author: Maria Nobile (maria.nobile1@unimi.it)

Antibiotics have been widely used in animal husbandry for over 60 years for therapy of common pathologies, as well as mastitis, respiratory, neonatal diarrhea, podal diseases, etc. and for prophylactic purposes [1]. Moreover, overuse of antibiotics to increase growth performance and feed efficiency, or to synchronize and control the reproductive cycle and breeding performance [2], may result in the presence of residues of antibiotic in milk, a public health concern. As regard the above-mentioned main antibiotic uses, the presence of residues in milk may be due as direct administration of drugs to animals or indirectly, for example from the farming and production environments [3]. On the basis of the mentioned premises, despite the fact there are MRLs (maximum residue limits) for antibiotics intended for zootechnical use (those that do not have a limit are prohibited) [4], it is increasingly desirable to reach zero residues in milk, not only from a food safety point of view, but also to limit technological problems during cheese-making that can cause significant economic losses. This study aims to verify the absence of administered antibiotics after different therapeutic treatments due to medical conditions, taking into consideration the withdrawal period, and to evaluate the reliability of screening tests under field conditions after confirmatory HPLC-HRMS (High Performance Liquid Chromatography-High-Resolution Mass Spectrometry) Orbitrap analysis. A total of 141 raw bovine milk was analyzed. Moreover, the presence of expected or non-targeted metabolites was investigated. The presence of antimicrobial drugs was shown in 29% of the samples, and also sometimes their metabolites (for enrofloxacin and lincomycin), despite the fact that samples were collected at the seventh milking. Moreover, in 9% of the samples, undeclared treatments were found due to the presence of both parent drugs and metabolites. Finally, the putative identification of 2 new enrofloxacin metabolites, Enrofloxacin-N-methylacetamide and Enrofloxacin-ornithine, was proposed. In the light of these evidences, monitoring of metabolites is recommended, for potential implications on food safety and on technological dairy processes.

(1) Sachi et al. Antibiotic residues in milk: Past, present, and future, Journal of Advanced Veterinary and Animal Research, 6: 315–332, 2019.
(2) Nisha A.R. Antibiotic Residues—A Global Health Hazard, Veterinary World, 2: 375–377, 2008.
(3) Gaugain et al. A non-targeted LC-HRMS approach for detecting exposure to illegal veterinary treatments: The case of cephalosporins in commercial laying Hens, Journal of Chromatography A, 1599: 161–171, 2019.
(4) European Commission. Commission Regulation (EU) 37/2010/CE on pharmacologically active substances and their classification regarding maximum residue limits in foodstuffs of animal origin, Official Journal, 15: 1–72, 2010.

Characterization by Multi Locus Sequencing Typing (MLST) of *Bacillus* strains isolated from processed cheese

Angela Maria Catania, Maria Ausilia Grassi, Pierluigi Di Ciccio , Alessandra Dalmasso

Università degli Studi di Torino, Dipartimento di Scienze Veterinarie

Corresponding author: Angela Maria Catania (angelamaria.catania@unito.it)

Processed cheese is a widely used commercial product characterized by high microbiological stability and extended shelf life obtained through the application of severe heat treatment. However, spore forming bacteria can survive thermal process, causing quality defects in the final product and safety issue if contamination by pathogenic bacteria occurs. Among spore forming bacteria, microorganisms belonging to *Bacillus* genus have been reported (1, 2). In this study we examined the first hours' production of processed cheese, not commercialized, in an industrial dairy plant for a period of eight weeks, between June and October 2020. Matrix-assisted laser desorption ionization time-of-flight mass spectrometry (MALDI-TOF MS) was used to identify bacteria colonies, allowing the isolation of fourteen *Bacillus cereus* and twenty-one *Bacillus subtilis* strains, the results were further confirmed by amplification and sequencing of 16 rRNA bacterial region. A multi locus sequence type (MLST) analysis was performed to assess the genetic similarity among the isolated strains. Seven housekeeping genes were analyzed for both species, and the results were compared with data on the MLST website (www.pubmlst.org). Results showed that two sequence type were identified for *B. cereus* as previously described (3). ST-32 was observed in only one strain and the ST-371 in the remain thirteen isolates. On the contrary, all *B. subtlis* strains showed a new allelic profile for the *pycA* gene. The resulting new sequence type was submitted and registered in MLST database as ST-249. Based on the results, MLST approach has confirmed to be a rigorous method for genetic strain typing. Moreover, the identification of a genetically homogeneous bacterial population could probably be ascribable to the presence of a resident in-plant *Bacillus* population. Further studies will be necessary to assess the source of contamination and the ability of the bacteria isolates to adhere to surfaces in dairy plant and form biofilms. In fact, biofilms could become a reservoir of bacteria causing contamination of final dairy products.

[1] Oliveira et al. Processed cheese contamination by spore-forming bacteria: a review of sources, routes, fate during processing and control, Trends in Food Science & Technology, 57:11-19, 2016.
[2] Gopal et al. The prevalence and control of *Bacillus* and related spore-forming bacteria in the dairy industry, Frontiers in Microbiology, 6:1-18, 2015.
[3] Cardazzo et al. Multiple-locus sequence typing and analysis of toxin genes in *Bacillus cereus* foodborne isolates, Applied and Environmental Microbiology, 74:850-860, 2008.

Evaluation of isolation protocols for *Salmonella* detection in bovine milk samples: preliminary results

Daniela Cristiano (1), Maria Francesca Peruzy (2), Yolande T.R. Proroga (1), Antonio Rippa (2), Nicoletta Murru (2)

(1) Istituto Zooprofilattico Sperimentale del Mezzogiorno, Portici (NA) (2) Università degli Studi di Napoli, Dipartimento di Medicina Veterinaria e Produzioni Animali

Corresponding author: Maria Francesca Peruzy (mariafrancesca.peruzy@unina.com)

In 2019 Salmonellosis, mainly due to *Salmonella* (*S.*) *enterica*, was the second most commonly reported bacterial food-borne zoonoses in the European Union [1]. The reference method, EN ISO 6579:2017, for *Salmonella* isolation involves an enrichment in Buffer Peptone Water (BPW) and the use of selective media [2]. Although inexpensive and simple, the conventional method is long and time-consuming, taking up to 5 days [3]. The development of rapid and effective methods for the detection of *Salmonella* in foods is important to conduct a proper control of the contaminated products, to identify quickly outbreak sources, and to prevent the spread of illness. The aim of this work was to evaluate *Salmonella* detection in experimentally contaminated raw milk samples incubated in two enrichment media (BPW and modified BPW) for twenty hours at different temperatures. Bovine milk samples were collected in one farm in the Campania region, in southern Italy. Samples were experimentally inoculated with *S.* Thyphimurium previously activated twice in Tryptone Soy Broth and incubated at 37°C for 48 hours. In particular, each sample was divided into nine aliquots (1-9), one was used as negative control, fours were inoculated with a bacterial load within 1-10 CFU/mL (aliquots 1 to 4) and the other four with 10-10^2 CFU/mL (aliquots 5 to 8). Twenty-five ml of milk samples were added of 225 mL (1:10 wt/ wt) of sterilized BPW. In four aliquots (aliquots 1, 3, 5, 7) the RAPID'*Salmonella* Capsule was added to the BPW (BPWC). Seeded samples were then, incubated in parallel at 37 (aliquots 1, 2, 5, 6) and 42°C (aliquots 3, 4, 7, 8) for twenty hours. At time zero, and after 2, 4, 5, 6, 7, 8, and 20 hours of incubation each sample was streaked onto RAPID'*Salmonella* Agar Plate, incubated for 24 hours at 37°C. When the modified BPW was used (BPWC), regardless of the temperature of incubation *Salmonella* was detected after 20 and 8 hours in samples seeded with the lower and higher contamination level, respectively. In samples incubated in BPW, *Salmonella* was detected in all samples after eight hours, except in aliquot 4 (aliquot contaminated with 1-10 CFU/mL and incubated at 42°C) in which the pathogen was isolated after 20 hours. Based on the results, by reducing the incubation time to eight hours is possible to detect the pathogen in milk samples. No differences were observed in samples incubated at 37 or 42°C and the use of the RAPID'*Salmonella* Capsule did not improve the detection of the pathogen. These preliminary data could be also used in the future for the development of a molecular method for a quicker detection of *Salmonella* in food samples.

[1] EFSA and ECDC (European Food Safety Authority and European Centre for Disease Prevention and Control), 2021. The European Union One Health 2019 Zoonoses Report. EFSA Journal 2021;19(2):6406, 286 pp. https://doi.org/10.2903/j.efsa.2021.6406.
[2] EN ISO 6579-1:2017, Microbiology of the food chain — Horizontal method for the detection, enumeration and serotyping of *Salmonella* — Part 1: Detection of *Salmonella* spp.
[3] McGuinness S, McCabe E, O'Regan E, Dolan A, Duffy G, Burgess C, Fanning S, Barry T, O'Grady. Development and validation of a rapid real-time PCR based method for the specific detection of *Salmonella* on fresh meat. Meat Science 83(3):555–562, 2009.

Can Protective Cultures improve sheep's milk cheeses safety?

M.P. Meloni , F. Piras , V. Spanu , R. Sanna , G. Siddi , M. Demontis , C. Spanu , E. P. L. De Santis , C. Scarano

Università degli Studi di Sassari, Dipartimento di Medicina Veterinaria.

Corresponding author: M.P. Meloni (mariapinameloni@yahoo.it)

Protective cultures can be regarded as an interesting opportunity to minimize the risk of persistence or development of *L. monocytogenes* in sheep's milk cheeses. To introduce protective cultures on the production process of protected designation of origin (PDO) cheese from sheep's milk it is essential to use only autochthonous microorganisms in order to respect the specifications provided. At the same time, most of the commercial protective cultures are also mesophilic, including *Lactobacillus plantarum*, while some cheesemaking process are designed to promote primary fermentations from thermophilic strains. Previous experiment using a mesophilic commercial protective culture, like *L. plantarum*, showed that for semi-cooked cheese curd cheese the high temperatures provided in some steps of the production process (45-47°C) were not appropriate for mesophilic bacteria growth.

The aim of this study was to select lactic acid bacteria (LAB) from local sheep milk chain production that could be used as protective cultures in Pecorino Sardo PDO cheese production process. From raw sheep's milk samples 74 out of 220 isolates were obtained and after biochemical and phenotypical preliminary tests they were identified as presumptive LAB. The sequencing of 16s rRNA was used to identify the isolates at species level. The in vitro activity of LAB strains and 2 commercial protective cultures against 50 *L. monocytogenes* strains (serotypes 1/2a, 1/2b, 1/2c e 4b) was investigated. LAB were isolated using Man Rogosa Sharpe agar at pH 5.5, M17 agar with 0,5% of lactose and Elliker agar with 0,5% of Beef extract incubate aerobically and anaerobically at 45°C for 72 h. *L. monocytogenes* strains were previously isolated from different cheeses and dairy plants according to ISO 11290-1:2017 method. LAB isolates and commercial bacterial strains were examined for their ability to produce bacteriocin. The inhibitory activity against *L. monocytogenes* was investigated by Well Diffusion Assay method. *L. monocytogenes* was inoculated into Brain Hearth Infusion agar to obtain a final concentration of ca. 10^5 and it was poured onto the plates. To obtain the inoculum to be tested for inhibitory activity both commercial protective cultures and LAB were inoculated on "scotta", the liquid waste residual of Ricotta cheese production, and incubated at 37°C (optimum temperature for *L. plantarum*) for 72 h and at 45°C (optimum temperature for thermophilic bacteria) for 72 h respectively. Afterwards, wells of 8 mm diameter were cut into the medium using a sterile tip and 70 µl of protective cultures at a concentration of 10^8 were poured into the wells. Among LAB, 8 out of 74 isolates showed an in vitro inhibitory activity against *L. monocytogenes*, that was comparable to the 2 commercial protective cultures. The inhibition zones diameter was comprised between 1.6-2.2 cm and 1.1-1.7 cm, respectively for commercial protective cultures and LAB. The results of this study should be considered as preliminary and demonstrates that autochthonous thermophilic LAB isolates may be considered as candidates for further characterization and research for developing specific protective cultures for Pecorino Sardo PDO cheese production.

Effect of gaseous ozone against biofilm of *Listeria monocytogenes* isolates from dairy industries

Felice Panebianco, Selene Rubiola, Francesco Chiesa, Tiziana Civera, Pierluigi Aldo Di Ciccio

Università degli Studi di Torino, Dipartimento di Scienze Veterinarie

Corresponding author: Felice Panebianco (felice.panebianco@unito.it)

In the food industry, the control of bacterial biofilms is based on the use of chemical substances for routine sanitation. Among the newest anti-biofilm strategies, ozone may be a promising tool to control bacterial biofilm in food processing industries. The antimicrobial action of ozone has been documented on a wide variety of organisms (1). The interest in ozone as an alternative to chemical disinfectants is based on its advantage of being an environmentally friendly technology with low environmental impact (1). Ozone in gaseous form may be used to control biofilm in hard-to-reach areas within food processing environments. Among food-borne pathogens, *Listeria monocytogenes* (*L. monocytogenes*) can form biofilm and persist for months or years in food environment constituting a source of recurrent food contaminations (2). To date, data concerning the effect of ozone on biofilm of this pathogen are still scarce (1). In the present study, the effect of gaseous ozone against biofilm formed by 11 *L. monocytogenes* isolates (10 strains from dairy industries and 1 ATCC) was evaluated.

First of all, the biofilm capacity of all strains was assessed by using the micro-method assay. Then**,** the biofilm production index (BPI) of each strain was calculated by using the macro-method assay. Additionally, viable bacteria in biofilm state (macro-method assay) were counted in Brain Heart Infusion (BHI) agar after mechanical scraping of adherent cells in each well. The experiments (at room temperature with a high relative humidity) by using ozone gas at 50 ppm for 6 hours were carried out in an ozone-inert plexiglas chamber connected to an ozone generator to assess its impact in prevention (inhibition) and removal (eradication) of *L. monocytogenes* biofilm. BPIs and loads of viable adherent cells in the biofilm obtained after ozone treatments were compared to the control values.

Significant reductions in BPIs were observed for five dairy isolates after inhibition and eradication tests, whereas counts of live adherent cells in the biofilm state showed a slight loads decreases (~ 1 Log CFU/cm^2) for almost all strains after treatments.

These preliminary results have shown a partial and strain dependent effect of ozone gas on the biofilm biomass. Anyway, only a slight reduction of bacteria (live cells) in the biofilm was observed. Based on our outcomes, the ozone gas was not able to mitigate the *L. monocytogenes* biofilm. Further research is required to understand the potential application of gaseous ozone as an additional tool to improve the existing cleaning and disinfection procedures and to control the *L. monocytogenes* biofilm within the food processing environment.

[1] Marino M., Maifreni M., Baggio A., Innocente N. Inactivation of foodborne bacteria biofilms by aqueous and gaseous ozone, Frontiers in Microbiology, 9:2024, 2018.
[2] Fagerlund A., Langsrud S., Møretrø T. Microbial diversity and ecology of biofilms in food industry environments associated with *Listeria monocytogenes* persistence, Current Opinion in Food Science, 37:171-178, 2021.

ORAL COMMUNICATIONS

AMV

MAIN LECTURE

Carlo Ruini: the father of veterinary anatomy who made art of the horse body

Adalberto Merighi

Università degli Studi di Torino, Dipartimento di Scienze Veterinarie

Corresponding author: Adalberto Merighi (adalberto.merighi@unito.it)

1598 marks the end of the dark years of the veterinary art with the publication of the treatise *Dell'Anotomia et dell'Infirmità del Cavallo* (The Anatomy and Diseases of the Horse) by the Bolognese Carlo Ruini, a work which holds, among the monographs, a place of particular importance for the quality of the images and which can rightly be considered the first scientific treatise on veterinary anatomy. The most important and undeniable merit of Ruini's treatise lies in the fact that it offers, for the first time, a systematic description of the anatomy of an animal conducted in a scientific manner.

I here describe the main features of this stunning book and consider the many artistic aspect of the opera that not only was scientifically very accurate for its time but was also endowed of many excellent drawings of the horse anatomy. The artistic quality of Ruini's tables was so high that his drawings were attributed to some very famous painters of the time or to their workshops, including Leonardo, Titian, or the renowned incisor Agostino Carraci.

That the Ruinian iconography has been assimilated to the work of such artists testifies beyond any doubt the recognition of an intrinsic arty value of the woodcuts accompanying the treatise, beyond the purely anatomical worth. The choice of an engraver of indisputable artistic skills for a gigantic and very expensive work of illustration of his book, unusual at that time, offers the measure of how much Ruini believed the iconographic support was fundamental to the outcome of the experimental work.

Effect of essential oils on the oxyntopeptic, somatostatin and ghrelin immunoreactive cells in the European sea bass (*Dicentrarchus labrax*) gastrointestinal tract

Maurizio Mazzoni , Alessio Bonaldo , Giulia Lattanzio , Luca Parma , Serena Busti , Pier Paolo Gatta , Claudio Tagliavia , Paolo Clavenzani

Università degli Studi di Bologna, Dipartimento di Scienze Mediche Veterinarie

Corresponding author: Maurizio Mazzoni (m.mazzoni@unibo.it)

In order to increase fish production, fish farms can sometimes have stressful conditions, which favour the spread of bacterial, fungal, viral and parasitic disease with consequent reductions in production performance [1]. To reduce these consequences, the use of essential oils (EOs) derived from plants has been the focus of aquaculture studies due to their properties (e.g. antioxidant and antimicrobial), which have been shown to reduce biochemical and endocrine alterations and, consequently, to improve the welfare status and the fish growth performance parameters. In non-mammalian vertebrates, one cell type, the oxyntopeptic cells (OPs), secretes both hydrochloric acid and pepsinogen into the lumen to initiate protein digestion. In Teleosts, ghrelin increases food intake, regulates intestinal motility and energy balance. On the contrary, somatostatin inhibits gastric acid secretion, food intake and promotes catabolic processes [2]. Based on the above considerations, the aim of this study was to evaluate whether EOs are able to modify OPs expression, the number of enteroendocrine cells (EEC) and the absorbent surface in the gastrointestinal tract of European sea bass. For this study, 36 fish were used, divided into three experimental groups: control group (CTR) fed with basic diet, group E fed with basic diet supplemented with natural EOs and group E fed with basic diet supplemented with EOs composed of active ingredients obtained by synthesis (not natural). Experimental procedures were evaluated and approved by the Ethical-Scientific Committee for Animal Experimentation of the University of Bologna, Italy. At the end of the trial, for each fish, the stomach, cranial and middle intestine were sampled and then processed for histological and IHC methods. The Na^+K^+ATPase antibody was used to label OPs, while, for the EECs, anti-somatostatin (SOM) and anti-ghrelin (GHR) antibody were used. The cranial and middle part of intestine were stained with E&E. Threshold binarization was used to evaluate of the OPs immunoreactive (-IR) area in the gastric mucosa and the adsorbent surface of the intestine, while the EECs in 2.5 mm^2 of gastric mucosa were counted. We observed OPs in all parts of the stomach, distributed along the adenomere of the simple tubular gastric glands. The highest density of Ops-IR area was in the CTR group (0.66 mm^2±0.1). Ops-IR area was significantly reduced in the F diet (0.22 mm^2±1, CTR *vs* F, $p<0.003$), while in the E diet (0.39 mm^2±1) a trend was observed (CTR *vs* E, $p=0.08$). EECs were mainly distributed along the glandular adenomeres. Some SOM or GHR-IR cells had the morphological appearance of “open-type” EECs with an elongated homogenous cytoplasm and two cytoplasmic prolongations, while others SOM or GHR-IR cells had the “closed-type” EECs appearance with a round shape without cytoplasmic prolongations. In the gastric mucosa, the F group (15.6±4.2) exhibited a significant change in the mean number of SOM-IR cells respect to the CTR group (CTR 11.8±3.7) (F *vs* CTR $P < 0.03$). These observations will provide a basis for better understanding the digestive physiology and help pathologists and nutritionists in future studies on diet and diseases affecting this species.

[1] Walker P., et al. Emerging viral diseases of fish and shrimp. Veterinary Research, 41:1–24, 2010.
[2] Volkoff H., et al. Neuropeptides and the control of food intake in fish. General and Comparative Endocrinology, 142:3–19, 2005.

Vimentin expression in the zebrafish oral cavity: a potential role in taste buds regeneration

Maria Cristina Guerrera, Giuseppe Montalbano, Germana Patrizia Germanà, Maria Levanti, Rosaria Laurà, Caterina Porcino, Antonino Germanà, Francesco Abbate

Università degli Studi di Messina, Dipartimento di Scienze Veterinarie

Corresponding author: Maria Cristina Guerrera (mguerrera@unime.it)

Morphological features of the tongue provide insight into the feeding attitudes of fishes and are closely related to their evolution, particularly in zebrafish as a model organism for the study of vertebrate biology and physiology, including humans. In this study, morphological features of the dorsal surface of the tongue were investigated by scanning electron, confocal laser, and light microscopy. Scattered papillae of the lingual mucosa containing gustatory calyxes indicating taste perception were observed. Immunoreactivity to vimentin was demonstrated in a subpopulation of taste bud cells supporting the hypothesis that cells from the underlying connective tissue might migrate into the epithelium, undergo a mesenchymal-epithelial transition and contribute to the formation and renewal of zebrafish taste buds. The results suggest that the ability to perceive bitter and umami is present in zebrafish taste buds, providing the basis for understanding the physiological mechanisms of taste perception with potential clinical applications in the treatment of taste disorders using zebrafish as a model.

[1] Abbate et al. The oral cavity of the adult zebrafish (Danio rerio). Anatomia Histologia Embryologia, 35:299-304, 2006.
[2] Venkatesan et al. Distribution of α-Gustducin and Vimentin in premature and mature taste buds in chickens. Biochemical and Biophysical Research Communications. 479:305-311, 2016.
[3] Wang et al. Abundant proliferating cells within early chicken taste buds indicate a potentially "built-in" progenitor system for taste bud growth during maturation in hatchlings. Histology and Histopathology. 34:503-511, 2019.

TRPV4 expression on lingual taste buds of larval, juvenile, and adult stages of gilthead sea bream (*Sparus aurata*)

Kamel Mhalhel, Maria Levanti, Giuseppe Montalbano, Francesco Abbate, Rosaria Laurà, Maria Cristina Guerrera, Mauro Cavallaro, Antonino Germanà

Università degli Studi di Messina, Dipartimento di Scienze Veterinarie

Corresponding author: Kamel Mhalhel (kmhalhel@unime.it)

The fish sensory system includes specialized organs that can perceive external mechanical and chemical sensory stimuli necessary for life and survival. The gustatory system is a chemosensory system that plays an essential role in identifying nutrients and avoiding harmful substances by detecting changes in chemical composition of the environment [1]. Taste buds, the morphofunctional units for taste perception, are observed in both aquatic and terrestrial vertebrates. They perceive and transduce gustatory stimuli using G protein-coupled receptors, signaling cascades, and a complex arrangement of ion channels, among which the TRP superfamily in some fish species and in mammals [2]. The gilthead seabream (*Sparus aurata*, Linnaeus, 1758) is considered one of the most important aquaculture species in the Mediterranean Sea [3]. At first feeding, the larval buccal cavity is functional but is structurally and functionally less complex than that of adults [4]. However, during the juvenile stage, larvae show dramatic changes in anatomy, physiology, and behavior [5,6]. The tongue of gilthead seabream is formed by an enveloping mucous membrane hosting taste buds, a musculature, and an osteo-fibrous skeleton [7]. Therefore, in the present study, we evaluated, by immunohistochemistry, the expression of TRPV4, a member of the TRP superfamily, in taste buds during different developmental stages. TRPV4 expression in the tongue was observed in all considered stages. Immunoreactivity for the TRPV4 antibody was detected mainly in taste bud sensory cells, epithelial cells, and nerve fibers of the connective tissue underlying the mucosa. The TRPV4 expression pattern was different depending on the morphological changes of the taste buds observed at the different examined stages.

[1] Roper, S.D.; Chaudhari, N. Taste Buds: Cells, Signals and Synapses, *Nature Reviews Neuroscience*, *18*, 485–497, 2017.
[2] Samanta, A.; Hughes, T.E.T.; Moiseenkova-Bell, V.Y. Transient Receptor Potential (TRP) Channels, *Subcell Biochem*, *87*, 141–165, 2018.
[3] Arabaci, M.; Yilmaz, Y.; Ceyhun, S.B.; Erdogan, O.; Galip Dorlay, H.; Diler, I.; Akhan, S.; Kocabas, M.; Ozdemir, K.; Koyun, H.; et al. A Review on Population Characteristics of Gilthead Seabream (Sparus Aurata), *Journal of Animal and Veterinary Advances*, *9*, 971–981, 2010.
[4] Elgendy, S.A.A.; Alsafy, M.A.M.; Tanekhy, Mahmoud. Morphological Characterization of the Oral Cavity of the Gilthead Seabream (Sparus Aurata) with Emphasis on the Teeth-Age Adaptation, *Microscopy Research and Technique*, *79*, 227–236, 2016.
[5] Mhalhel, K.; Germanà, A.; Abbate, F.; Guerrera, M.C.; Levanti, M.; Laurà, R.; Montalbano, G. The Effect of Orally Supplemented Melatonin on Larval Performance and Skeletal Deformities in Farmed Gilthead Seabream (Sparus Aurata) 2020, *International Journal of Molecular Sciences*, *21*.
[6] Yúfera, M.; Halm, S.; Beltran, S.; Fusté, B.; Planas, J.V.; Martínez-Rodríguez, G. Transcriptomic Characterization of the Larval Stage in Gilthead Seabream (Sparus Aurata) by 454 Pyrosequencing, *Marine Biotechnology*, *14*, 423–435, 2012.
[7] Tina, P.; Nives, K.; Ivana, R.; Ivana, B. Histological and Biochemical Features of the Digestive System in the Cage-Reared Gilthead Sea Bream (Sparus Aurata), *International Journal of Biotechnology and Recent Advances*, *1*, 51–56, 2018.

Effects of light exposure on the calretinin and calbindin expression in zebrafish retina

Marialuisa Aragona, Giuseppe Montalbano, Germana Patrizia Germanà, Maria Levanti, Rosaria Laurà, Francesco Abbate, Antonino Germanà, Maria Cristina Guerrera

Università degli Studi di Messina, Dipartimento di Scienze Veterinarie

Corresponding author: Marialuisa Aragona (marialuisa.aragona@unime.it)

The incidence rates of light-induced retinopathies increased significantly in the last decades because of continuous exposure to the harmful effects of light from different electronic devices. Recent studies show that exposure to short-wavelength visible light in blue has been related to the pathogenesis of light-induced retinopathies. However, the biological effects of long-term exposure are not fully known yet. Calbindin and calretinin, two homologous cytosolic calcium-binding proteins implicated in the regulation of important processes in central neurons and peripheral nervous systems [1,2,3] were investigated to elucidate the potential role of these proteins in maintaining retinal homeostasis. The effects of exposure to light at different wavelengths with emission peaks in the blue light range (400-500 nm) on the expression of calretinin and calbindin, using double immunofluorescence with confocal laser microscopy, were studied. 20 adult zebrafish (*Danio rerio*), 3 months old were obtained from previous studies [4], divided into 5 groups, exposed to different lighting conditions (white light, blue-white light, blue light, darkness and control group) for ten days. 4 fish from each group were sacrificed and decapitated [4] and the heads processed for histological and immunohistochemical analysis. The localization of calretinin and calbindin in the retina of zebrafish was analyzed using immunofluorescence method. The results show a different expression of calbindin and calretinin in the zebrafish retina after various lighting conditions. In control group, calretinin immunoreactivity was observed only in a subpopulation of amacrine cells in the inner nuclear and ganglion cells layers while an exposure to white light, white-blue and blue light causes a decrease of immunostaining. No immunostaining was observed after exposition to darkness. Therefore, the results suggest that these calcium binding proteins may be involved in the maintenance of retinal homeostasis.

[1] Kovács-Öller et al. Expression of Ca 2+- Binding Buffer Proteins in the Human and Mouse Retinal Neurons, International Journal of Molecular Sciences, 20:2229, 2019.
[2] Kovács-Öller et al. Spatial Expression Pattern of the Major Ca2+- Buffer Proteins in Mouse Retinal Ganglion Cells, Cells, 9:792, 2020.
[3] Fosser et al. Calbindin-D28k and calretinin in chicken inner retina during postnatal development and
neuroplasticity by dim red light, Developmental Neurobiology, 73:530-42, 2013.
[4] Sánchez-Ramos et al. Light regulates the expression of the BDNF/TrkB system in the adult zebrafish retina, Microscopy Research and Technique, 76:42-49, 2013.

Structure and ultrastructure of the viper fish photophores, *Chauliodus sloani* Bloch & Schneider, 1801

Marilena Briglia, Mauro Cavallaro, Francesco Abbate, Maria Cristina Guerrera, Maria Levanti, Marzio Cometa, Giuseppe Montalbano, Antonino Germanà, Rosaria Laurà

Università degli Studi di Messina, Dipartimento di Scienze Veterinarie

Corresponding author: Rosaria Laurà (rosaria.laura@unime.it)

The viper fish (*Chauliodus sloani*) belongs to the family *Stomiidae*, which is represented by deep-sea ray-finned fish, including the barbeled dragonfishes. They are considerable components of the micronekton in the oceans, found in mesopelagic waters and include approximately 277 species in 27 genera. These fish reach high levels of biomass in the ocean, and their diel vertical migration plays an important role in the marine ecosystem in transferring energy from shallow to deep-sea waters. Moreover, they are considered key species in the pelagic environment and are main components in the diet of several top marine predators. The viper fish, are well adapted to living in the twilight zone and possess several photophores and other light organs, that produce intrinsic bioluminescence. Photophores are glandular cutaneous organs, present in several species of marine organisms, devoted to the production of chemical light and common in mesopelagic and deep-sea fish belonging to several families [1]. These light organs are ventrally or laterally arranged and located in different parts of the fish body such as the tail peduncle and head. In general, the main function of photophores located in ventral and lateral surfaces of the fish body is to counterilluminate the silhouette of the fish, so providing camouflage [2]. A contribution to the knowledge of the photophore structure of the mesopelagic fish C. sloani is given by means of a structural and ultrastructural study, to better identify the anatomical structures constituing these light organs. The present study showed that photophores of C. sloani consist of a deep photogenic chamber containing photocytes and support cells and a set of annexes as filter lens, gelatinous body, reflector and pigmented layer with a dioptric function. Particularly, the photocytes are characterized by numerous vesicles, abundant sarcoplasmic reticulum and granules, different both in size and electron density. The photocytes are placed in a radiated laminar system which converge towards the centre of the photogenic chamber communicating, by an extracellular channel, with lens filter. The latter is composed of contiguous cords of irregularly shaped cells characterized by a homogeneous cytoplasm and severals vesicles. Evident desmosomes are present along the cells borders. A gelatinous body, distally located in the photophore, near the basal membrane of the lining epithelium, covers the lens filter and shows granules which confers a typical mucous consistence. Laterally to the lens and around the photogenic tissue a reflector is present formed by cells containing crystals-like guanine arranged in layers.

The external surface of the reflector wall is surrounded by a thin pigmented layer composed by melanine granules. These data will be useful to provide a baseline to better understand the physiology, use and function of the photogenic system of this species in the mesopelagic environment.

[1] Cavallaro M., Guerrera M.C., Abbate F., Levanti M.B., Laurà R., Ammendolia G., Malara D., Stipa M.G., Battaglia P. Morphological, ultrastructural and immunohistochemical study on the skin ventral photophores of Diaphus holti Tåning, 1918 (Family: Myctophidae), *Acta Zool.*, 1–7, 2020.
[2] Mallefet J., Duchatelet L., Hermans C., Baguet F. Luminescence control of Stomiidae photophores, Acta Histochem, 121(1):7-15, 2019.

Environmental enrichment for the early larval stages of *Acipenser baerii* in captive environments

Lucia Aidos, Valentina Serra, Chiara Bazzocchi, Silvia Modina, Alessia di Giancamillo

Università degli Studi di Milano, Dipartimento di Medicina Veterinaria

Corresponding Author: Alessia Di Giancamillo (alessia.digiancamillo@unimi.it)

The Siberian sturgeon, *Acipenser baerii* Brandt 1869, is included in the IUCN Red Data List. Taking into account the continuous decrease of its natural populations, it is necessary to gather as much information as possible in order to better manage the populations and to enhance farming practises.

Larval stages are particularly sensitive to environmental conditions, as these can affect survival and potential growth (Johnston, 2006). In fish destined to production, a suitable environment is of great importance for growth efficiency at harvest. Environmental enrichment is considered to improve biological functioning of captive animals by improving their psychological and behavioural needs. Little information is available on habitat preferences of the free embryos of *Acipenser baerii* upon hatch. The aim of this study was to assess the environmental enrichment without compromising larval morpho-functional aspects.

After hatching and throughout the endogenous feeding period, larvae were reared in two types of substrate (Bioballs type 1 – BB1 and Bioball type 2 - BB2) vs. no substrate (CTR). Behavioural, larval growth parameters (weight and length) and histometrical analyses were studied in order to evaluate muscle development. The analysis of myogenesis and stress were carried out through the use of Real Time PCR, evaluating the relative expression of a pool of genes: Myod, Myog and Mrf4 (involved in myogenesis), Igf2 (involved in growth), Hsp70, Hsp90α, Hsp90β and Glut2 (markers of cells exposed to high stress conditions). This study was approved by the Ethic Committee of the University of Milan (OPBA_15_2018).

Larvae reared with BB1, when compared to those reared with BB2 or CTR, were heavier and longer at the end of the trial, and histometrical analyses showed larger areas of total muscle area, slow muscle area and fast muscle area ($P<0.05$). This is in accordance with studies performed with other species of sturgeon (Baker et al., 2014; Boucher et al., 2017). Moreover, CTR larvae showed an acceleration of muscle differentiation, which can have negative consequences on its growth potential. Despite this, gene expression analysis suggests, instead, that the larvae reared in BB2 are those with greater growth potential, which may eventually occur after the start of exogenous nutrition. The highest expression of stress-related genes in BB2 larvae may indicate some sort of metabolism activation of these animals and not necessarily be a negative result.

According to our results, it would seem more favourable to provide a substrate rather than a bare bottom, for Siberian sturgeon in these phases of development. Understanding the effect of the rearing environment on larval sturgeon development is vital for effective hatchery practices, particularly in conservation aquaculture, but also for habitat restoration to enhance natural propagation.

(1) Baker et al. Swimming performance and larval quality are altered by rearing substrate at early life phases in white sturgeon, *Acipenser transmontanus* (Richardson, 1836), Journal of Applied Ichthyology, 30, 1461-1472, 2014.
(2) Boucher et al. The effect of substrate rearing on growth, aerobic scope and physiology of larval white sturgeon *Acipenser transmontanus*, Journal of Fish Biology, 92 (6), 2017.
(3) Johnston, I.A. Environment and plasticity of myogenesis in teleost fish. The Journal of Experimental Biology, 209, 2249-2264, 2006.

NPY involvement in coupling food intake and ageing in teleost fishes

Daniela Giaquinto[1],[2], Valentina Schiano[2], Elena De Felice[1], Chiara Attanasio[2], Carla Lucini[2], Paolo de Girolamo[2], Livia D'Angelo[2]

(1) Università degli Studi di Camerino, Scuola di Bioscienze e Medicina Veterinaria (2) Università degli Studi di Napoli Federico II, Dipartimento di Medicina Veterinaria e Produzioni Animali

Corresponding author: Daniela Giaquinto (daniela.giaquinto@gmail.com)

Neuropeptide Y (NPY), first isolated from pig brain [1], is a 36-amino acid peptide highly conserved among vertebrates. It acts through the binding to five different receptors (Y1, Y2, Y4, Y5, Y6) [2]. NPY is abundantly expressed in central and peripheral nervous system, as well as in other tissues, particularly in gastrointestinal tract. The extensive distribution in all vertebrate taxa suggests that this peptide plays important roles in many physiological functions. NPY is considered a strong orexigenic factor, binding to the Y1 receptor and activating signaling pathways in the hypothalamus of the mammalian brain [3]. In fish, NPY promotes food intake and affects the psychophysiological functions, i.e. decreasing swimming activity [4]. Furthermore, some studies suggest that the NPY system is also linked to the ageing process [5] as mediator of nutrient-sensing pathways.

Here, we propose to expand our knowledge on the role of NPY during vertebrate ageing. To this aim, we compare, for the first time, the age-related central and peripheral expression of NPY in two teleost fishes, *Nothobranchius furzeri* (turquoise killifish) and *Danio rerio* (zebrafish), two consolidated model organisms with a different lifespan and feeding habits, although being both stomachless [6]. Experiments (Animal Welfare Body, University of Naples Federico II, PG/2018/0049615 - 22/05/2018) were performed on brain and gut of turquoise killifish, sampled at 5 and 27 weeks post-hatching, and zebrafish, sampled at 6 and 24 months post fertilization.

In turquoise killifish, we confirmed an increase of NPY expression in the brain upon ageing [7] and the highest expression in the brain compared to gut, also in old specimens. We observed comparable data also in zebrafish, corroborating the idea that in these two species NPY has a main central regulation. Remarkably, the pattern confirmed a wider distribution of NPY along the whole brain, not only in the hypothalamic region but also in the epithelium lining the bulb and the anterior gut. By using two different fish species, serving both as models in aquaculture and in biomedical research, we provide new insights on the role of NPY in regulating food intake during vertebrate ageing.

[1] Tatemoto K. et al. Neuropeptide Y: a novel brain peptide with structural similarities to peptide YY and pancreatic polypeptide, Nature 296, 659–660, 1982.
[2] Silva A.P. et al. The putative neuroprotective role of neuropeptide Y in the central nervous system, Curr. Drug Targets CNS Neurol. Disord, 4, 331–347, 2005.
[3] Baraban S.C. Neuropeptide Y and limbic seizures, Rev. Neurosci, 9 117–128, 1998.
[4] Matsuda K. et al. Neuroendocrine control of feeding behavior and psychomotor activity by neuropeptide Y in fish, Neuropeptides, 46 275–283, 2012.
[5] López-Otín C. et al. The hallmarks of aging, Cell, 153 1194–1217, 2013.
[6] D'Angelo L. et al. Anatomical features for the adequate choice of experimental animal models in biomedicine: I. Fishes, Ann Anat, 205:75-84, 2016.
[7] Montesano A. et al. Age-related central regulation of orexin and NPY in the short-lived African killifish *Nothobranchius furzeri*, J Comp Neurol, 527:1508–1526, 2019.

Reliability of melanomacrophage centres as indicators of stress response in teleost fishes

Letizia Passantino (1), Rosa Zupa (1), Chrysovalentinos Pousis (1), Gualtiero Basilone (2), Aldo Corriero (1)

(1) Università degli Studi di Bari Aldo Moro, Dipartimento dell'Emergenza e dei Trapianti di Organi, Sezione Cliniche Veterinarie e Produzioni Animali (2) Consiglio Nazionale delle Ricerche, Istituto per l'Ambiente Marino Costiero, Capo Granitola (TP)

Corresponding author: Letizia Passantino (letizia.passantino@uniba.it)

Melanomacrophage centres (MMCs) are aggregates of pigmented phagocytes, characterized by heterogeneous inclusions and located in hemolymphopoietic organs of various non-mammalian vertebrates [1].

In the present work, data from published and ongoing studies on MMCs were analysed in order to get insights on MMC reliability as response biomarkers to stress and environmental pollution.

Liver samples from 31 wild and captive reared Atlantic bluefin tuna (*Thunnus thynnus*) [2, 3] and from 90 wild European anchovies (*Engraulis encrasicolus*) caught in areas differently exposed to industrial and agricultural pollutants [4], and liver and spleen samples from 47 wild and captive reared greater amberjack (*Seriola dumerili*) [5, unpublished data] were fixed in 10% buffered formalin and embedded in paraffin wax. Deparaffinized sections were stained with haematoxylin-eosin; Mallory's basic fucshin (Merck) and Perls VanGieson (Bio-Optica) stainings were used to identify lipofuscin–ceroids and ferric iron respectively; peroxidase detection was performed by a Leukocyte Peroxidase kit (Sigma). The terminal deoxynucleotidyl transferase-mediated d'UTP nick-end labelling (TUNEL) method was used to identify apoptotic cells and the immunohistochemical detection of cytochrome P450 monooxygenase 1A (CYP1A) was performed by means of polyclonal antibodies anti-fish CYP1A (Biosense Laboratories). Lipofuscin–ceroids and ferric iron were detected in MMCs of all the three examined species, whereas peroxidase was mainly detected in free macrophages. In Atlantic bluefin tuna, a high density of MMCs and liver apoptotic cells, and a strong CYP1A immunostaining were observed in young individuals reared in the central Adriatic Sea compared with adults caught from the wild or reared in the western Mediterranean. In European anchovy, a high density of MMCs and a strong CYP1A immunostaining were observed in fish sampled in the Gulf of Gela, a marine area dramatically affected by environmental pollution. In greater amberjack, MMC density was higher in spleen than in liver sections. Confinement in captivity did not affect MMC density, whereas differences were found between males and females and among fish in different reproductive conditions. The present study confirms that MMCs represent a useful biomarker of fish exposure to environmental pollution; however, sex and reproductive state may affect MMC density, possibly leading to data misinterpretations.

[1] Agius C., Roberts R.J. Melano-macrophage centres and their role in fish pathology, Journal of Fish Diseases, 26:499–509, 2003.

[2] Passantino et al. Liver melanomacrophage centres as indicators of Atlantic bluefin tuna, *Thunnus thynnus* L. well-being, Journal of Fish Diseases, 37: 241–250, 2014.

[3] Corriero et al. Increased liver apoptosis and tumor necrosis factor expression in Atlantic bluefin tuna (*Thunnus thynnus*) reared in the northern Adriatic Sea, Marine Pollution Bulletin, 71:23-28, 2013.

[4] Basilone et al. Liver melanomacrophage centres and CYP1A expression as response biomarkers to environmental pollution in European anchovy (*Engraulis encrasicolus*) from the western Mediterranean Sea, Marine Pollution Bulletin, 131:197–204, 2018.

[5] Passantino et al. Increased melanomacrophage centres in the liver of reproductively dysfunctional female greater amberjack *Seriola dumerili* (Risso, 1810), Journal of Fish Diseases, 43:503-514, 2020.

Ontogenetic regulation of D-Aspartate in the brain of the short-lived vertebrate *Nothobranchius furzeri*

Livia D'Angelo[1], Antonio Palladino[1], Alessandro Usiello[2,3], Paolo de Girolamo[1]

[1]Università degli Studi di Napoli Federico II, Dipartimento di Medicina Veterinaria e Produzioni Animali
[2]Università della Campania "Luigi Vanvitelli", Dipartimento di Scienze e Tecnologie Ambientali Biologiche e Farmaceutiche [3]CEINGE Biotecnologie Avanzate, Napoli

Corresponding author: Livia D'Angelo (livia.dangelo@unina.it)

D-aspartate (D-Asp) is the most abundant endogenous D-amino acid within mammalian brain during embryonic and early neonatal life. During adulthood, D-Asp levels drastically decrease due to the concomitant onset of D-aspartate oxidase (DDO) activity, a flavoenzyme that selectively degrades bicarboxylic D-amino acids [1]. Exaggerated higher D-Asp levels in Ddo knockout murine brains trigger a profound neuronal death, not seen in controls, suggesting that DDO may control the vulnerability to accelerated brain ageing process [2; 3]. We aim to explore for the first time the ontogenetic regulation of D-Asp and DDO in the brain of a non-mammalian species, the African turquoise killifish, *Nothobranchius furzeri*. This teleost fish is gaining new traction for ageing studies, thanks to its compressed lifespan during which it shows typical hallmarks of ageing process [4]. Up to date, DDO activity has been documented in the kidney and liver of some edible teleost fish, where D-aminoacids are metabolized upon food intake, and in the brain where it displays unchanged levels [5]. All experimental procedures performed in accordance with the Italian Law (D. Lgs. 26/2014) were approved by the appropriate Committee at the University of Naples Federico II (PG/2018/0049615). We report that D-Asp content drastically decreases in the brain of *N. furzeri* after hatching, differently from D-serine, whose levels are not significantly changed. Unexpectedly, DDO mRNA levels do not increase over time, similarly to other two conserved genes involved in D-aminoacids metabolism, namely, D-amino acid oxidase and Serine racemase. DDO mRNA transcripts are mainly expressed in neurons of diencephalic region. Moreover, in murine brain, postnatal DDO gene expression is paralleled by progressive demethylation within its putative promoter region in murine brain [6]. Consistent with no main changes in DDO mRNA expression, our preliminary data documents comparable methylation levels within the putative DDO promoter region in *N. furzeri*. These results shed light on a potential different regulation of D-aminoacids in the brain of mammalian and fish species and open the way to unravel the biological meaning of the prominent postnatal DDO activity in the vertebrate brain, which is still not clarified yet.

[1] Errico F. et al. Free D-Asp regulates neuronal dendritic morphology, synaptic plasticity, gray matter volume and brain activity in mammals. Transl Psychiatry 4:e417, 2014.
[2] Cristino L. et al. d-Aspartate oxidase influences glutamatergic system homeostasis in mammalian brain. Neurobiol Aging. 36(5):1890-902, 2015.
[3] Nuzzo T. et al. Free d-aspartate triggers NMDA receptor-dependent cell death in primary cortical neurons and perturbs JNK activation, Tau phosphorylation, and protein SUMOylation in the cerebral cortex of mice lacking d-aspartate oxidase activity. Exp Neurol. 317:51-65, 2019.
[4] D'Angelo L. et al. Anatomical features for the adequate choice of experimental animal models in biomedicine: I. Fishes. Ann Anat. 205:75-84, 2016.
[5] Sarower M.G. et al. Distribution and characteristics of D-amino acid and D-aspartate oxidases in fish tissues. J Exp Zool A Comp Exp Biol. 295(2):151-9, 2003.
[6] Punzo D. et al. Age-Related Changes in D-Aspartate Oxidase Promoter Methylation Control Extracellular D-Aspartate Levels and Prevent Precocious Cell Death during Brain Aging. J Neurosci. 36(10):3064-78, 2016.

Growth and stress evaluation in juvenile diploid and triploid Atlantic salmon (*Salmo salar*) during smoltification

Martina Bortoletti (1), Lisa Maccatrozzo (1), Stefano Peruzzi (2), Marta Vascellari (3), Erica Melchiotti (3), Giuseppe Radaelli (1), Daniela Bertotto (1)

(1) Università degli Studi di Padova, Dipartimento di Biomedicina Comparata e Alimentazione (2) The Arctic University of Norway, Department of Arctic and Marine Biology (3) Istituto Zooprofilattico Sperimentale delle Venezie, Dipartimento di Istopatologia

Corresponding author: Giuseppe Radaelli (giuseppe.radaelli@unipd.it)

In recent years, increasing commercial interest has been directed towards the use of artificially induced triploid Atlantic salmon (*Salmo salar*). Triploidization, a technique that produces sterile individuals, is a desired practice to prevent genetic interactions between farmed and wild fish and to avoid pre-harvest sexual maturation, which impairs growth and flesh quality. In salmon, triploidy may affect smoltification, a complex process of physiological changes regulated by the GH/IGF axis, which facilitate fish transition from freshwater to saltwater. Recent studies in triploid fish showed that growth impairments such as skeletal abnormalities can be reduced by changing diet formulations. In fact, triploids may show different dietary requirements than diploids, especially in terms of amino acids requirements and metabolism. The use of hydrolyzed proteins in aquafeeds for triploid fish may improve amino acid digestibility and utilization, supporting growth and health. Hence, the present study aimed to evaluate the effect of the dietary replacement of fish meal with hydrolized fish protein on growth and seawater adaptation of diploid and triploid salmon during smoltification. To this purpose, a total of 60 fish (30 fish per ploidy) were fed either a standard fish meal (STD) diet or a diet in which 45% of the fish meal was replaced with hydrolysed fish proteins (HFM) (15 fish per diet) (Skretting AS, Stavanger, Norway). To induce parr-smolt transformation, fish were reared under 12h of light (12L:12D) and at low temperature (10.0±0.5°C). Fish muscle and liver were sampled every month from October to December. Real Time PCR was used to assess growth hormone (GH) and its receptor (GHrec), insulin-like growth factor I (IGF-I), Myostatin (MSTN) and heat shock protein (HSP70) muscle gene expression in a total of 30 fish per ploidy. Livers were stained with RNAscope® in-situ hybridization, a novel and highly sensitive multiplex nucleic acid technology, which allowed to detect and localize IGF-I mRNA in formalin-fixed paraffin-embedded organs. GH, GHrec and IGF-I expression did not show significant differences according to diet or ploidy, whereas changes were found according to sampling time, where gene expression increased from October to November. This trend was well-reflected by the in-situ hybridization of liver sections, as the parenchyma showed a more evident positivity to IGF-I probe in the November samples than in the October ones. Indeed, during salmon smoltification, IGF-I along with GH and GHrec can improve salinity tolerance, as they are peculiar genes involved in fish osmoregulation and saltwater adaptation, highlighting the synergy between these hormones. As for MSTN expression, no significant differences were found, indicating that myogenesis was not affected by any of the three factors studied. The same result was obtained for HSP70, justified by the low temperatures and optimal conditions in which the animals were kept. Our results confirm that smoltification period was successfully induced, given the significant variation in GH, Ghrec and IGF-I expression over time. However, diet and ploidy seemed to not affect saltwater adaptation, growth and stress response. In conclusion, this study highlights the feasibility of using triploid salmons in aquaculture for reproductive control and genetic containment.

Stress response in meagre (*Argyrosomus regius*) juveniles: comparison between manual and mechanical grading

Martina Bortoletti (1), Cristina Ballarin (1), Stefano Caberlotto (2), Marta Vascellari (3), Erica Melchiotti (3), Daniela Bertotto (1), Giuseppe Radaelli (1)

(1) Università degli Studi di Padova, Dipartimento di Biomedicina Comparata ed Alimentazione (2) Valle Cà Zuliani Società Agricola Srl (3) Istituto Zooprofilattico Sperimentale delle Venezie, Dipartimento di Istopatologia

Corresponding author: Daniela Bertotto (daniela.bertotto@unipd.it)

In aquaculture, fish grading is an important procedure, often performed repeatedly during the rearing cycle. This ensures homogenous sized batches of fish, as even fish of the same age typically have a high variability in body weight (15–40% coefficient of variation vs. 7–10% in poultry or pigs) [1]. More homogeneous groups simplify feed management (e.g., similar pellet size) and reduce risks for cannibalism. Fish handling practices such as grading may cause stress, making the animals more vulnerable to diseases. The overall effect of stress is the activation of the hypothalamic-pituitary-interrenal axis (HPI) followed by the release of glucocorticoids, with cortisol as the main one. In stressful conditions, heat shock proteins (HSPs) display a protective function and play a role in cellular homeostasis in all living organisms [2]. High expression of *hsp70* mRNA has been observed in fish subjected to stressful conditions such as overcrowding [3], transport [4], and heat stress [5]. The present work aimed at investigating the effect of two different grading methods, manual and mechanical, on stress response of meagre (*Argyrosomus regius)* juveniles. For this purpose, whole-body cortisol level was evaluated at several time points, before and up to 48h after grading, by radioimmunoassay analysis. Moreover, HSP70 protein level and localization were detected by western blot and immunohistochemical analyses, respectively. Cortisol results confirmed that fish subjected to grading showed a stress response, with some differences between the two grading methods. Manual grading appeared to be less severe, as fish showed a shorter recovery time to basal levels compared to those mechanically selected (24h vs. 48h after grading, respectively; $P<0.001$). Noteworthy, recovery times varied according to the duration of the stress applied. In mechanical grading, fish were transferred to a series of tubes before reaching the grader and the tanks, probably making this procedure longer and slightly more stressful than the manual one. However, western blot analysis did not reveal significant differences in HSP70 level between the two methods and both groups showed immunoreactivity in several tissues and organs. Given the transient nature of fish physiological response after grading, neither of the two tested methods seemed to represent a severe stress, as reflected by a rather quick fish recovery.

[1] Gjedrem T., Olesen I. Basic Statistical Parameters. In: Gjedrem T. (eds) Selection and Breeding Programs in Aquaculture. Springer, Dordrecht, 2005.
[2] Tkáčová J., Angelovičová M. Heat Shock Proteins (HSPs): a Review, Animal Science and Biotechnology, 45:349-353,2012.
[3] Gornati R. et al. Molecular markers for animal biotechnology: sea bass (*Dicentrarchus labrax,* L.) HMG-CoA reductase mRNA, Gene, 344:299-305, 2005.
[4] Poltronieri C. et al. Quantitative RT-PCR analysis and immunohistochemical localization of HSP70 in sea bass *Dicentrarchus labrax* exposed to transport stress, European Journal of Histochemistry, 51:125-136, 2007.
[5] Bertotto D. et al. Whole body cortisol and expression of HSP70, IGF-I and MSTN in early development of sea bass subjected to heat shock, General and Comparative Endocrinology, 174:44-50, 2011.

Influence of Best Available Technique (BAT) farming systems on the enteric nervous system (ENS) of fattening pigs' ileum

Valentina R. Herrera M. (1,2), Katarzyna Palus (3), Annamaria Costa (4), Silvia Clotilde Modina (1), Alessia Di Giancamillo (1)

(1) Università degli Studi di Milano, Dipartimento di Medicina Veterinaria (2) IRCCS Istituto Ortopedico Galeazzi, Tissue Engineering and Biomaterials Lab (3) University of Warmia and Mazury in Olsztyn, Department of Clinical Physiology (4) Università degli Studi di Milano, Dipartimento di Scienze Veterinarie per la Salute, la Produzione Animale e la Sicurezza Alimentare "Carlo Cantoni" (VESPA)

Corresponding author: Valentina Herrera (valentina.herrera@unimi.it)

The enteric nervous system (ENS) is part of the autonomic nervous system and consists of two types of intramural ganglia[1]: 1) the muscular ganglia (MG) which create the myenteric plexus (MP) between the circular and longitudinal muscular fibers in the muscular layer of the intestine; 2) the submucous ganglia (SG) located in the submucosal layer forming the submucous plexus (SP)[2]. These neuronal cells are involved in the regulation of a large variety of gastrointestinal tract functions, both from a physiological point of view and in terms of adaptive responses to systemic and gastrointestinal disorders and to the impact of harmful substances [3–7]. Given these premises, the aim of this study was to evaluate whether two different farming systems (Best Available Techniques (BAT)-system vs not-BAT system) act differently on the enteric nervous system. To do this, six pig enteric traits per group (experimental group (EG) vs control group (CG) reared in BAT and non-BAT systems respectively) were collected at the slaughterhouse (University Ethical approval: OPBA 58_2016). Through histological and immunofluorescence analysis, we evaluated the number of neurons, the number of plexuses and the ratio between the area of the plexuses and the area of the section, as well as the thickness of the mucous layer that covers the intestinal epithelium. In EG, both the number of neurons and the number of plexuses increased in the muscular and in the submucosal layers. The area occupied by neuronal cells, evaluated thought Protein Gene Product 9.5 (PGP9.5), increased in SP but did not have any significant variation in MP. The area occupied by glial-like cells, evaluated through Glial Fibrillary Acidic Protein (GFAP), increased both in the SP and in MP. There was no significant change in mucus secretion in the two experimental groups. These data suggest that pigs reared in no BAT solutions during perinatal life exhibit increased vulnerability to ENS. Moreover, these findings mimic some of the pathophysiologic findings in human gut disorders, thus suggesting that the pig could be a valuable sentinel for human environmental exposure.

[1] Makowska K, Gonkowski S. Animals, 10(12):2445, 2020.
[2] Makowska K, Obremski K, Gonkowski S. Toxins (Basel), 10(4), 2018.
[3] Szymanska K, Makowska K, Gonkowski S. International Journal of Molecular Sciences, 19(3), 2018.
[4] Furness JB. Nature Reviews Gastroenterology & Hepatology, 9(5):286-294, 2012.
[5] Furness JB et al. Advances in Experimental Medicine and Biology, 817:39-71, 2014.
[6] Palus K, Całka J. International Journal of Molecular Sciences, 21(1), 2020.
[7] Vasina V et al. Autonomic Neuroscience: Basic and Clinical, 126-127:264-272, 2006.

Feed physical form affects anatomical traits of mandibular gland in growing pigs

Cecilia Dall'Aglio (1), Francesca Mercati (1), Elena De Felice (2), Federico Maria Tardella (2), Josef Kamphues (3), Maria Grazia Cappai (4), Paola Scocco (2)

(1) Università degli Studi di Perugia, Dipartimento di Medicina Veterinaria (2) Università degli Studi di Camerino, Scuola di Bioscienze e Medicina Veterinaria (3) Università di Medicina Veterinaria di Hannover, Istituto di Nutrizione Animal (4) Università degli Studi di Sassari, Dipartimento di Medicina Veterinaria

Corresponding author: Cecilia Dall'Aglio (cecilia.dallaglio@unipg.it)

The study was carried out on growing pigs fed with different dietary treatments based on different grinding intensities and compactions of the same diet, to test the effects of chewing acts on salivary production and fluidity. Therefore, the glycohistochemical profile and the presence of aquaporin 5, a canne protein modulating the saliva fluidity, were investigated. In addition, presence and localization of both apelin and its receptor were studied. Study was performed on the mandibular gland obtained from pigs enrolled in a wide research project [1, 2] aiming to test the effects of different physical forms of the diet on animal's health, production and welfare. The project was approved by the Ethics Committee on Animal Welfare of the Hannover District Government in accordante with the German legislation on animal welfare. 48 castrated male growing pigs (German Landrace x Large White on Duroc sires) were fed, for 4 weeks, with different forms of the same diet, namely coarsely ground meal (CM) finely ground pelleted (FP) and coarsely ground pelleted (CP) diets. Samples were analyzed by conventional histochemistry to identify the glycohistochemical profile, and by immunohistochemistry to localize aquaporin 5, apelin and apelin receptor. Statistical elaborations were performed using the *stats* R-package, version 3.5.3. Adenomere of pig mandibular gland increased both the quantity and acidity of produced glycoconjugates from CM to FP and CP diets; this probably call forth higher watery saliva, thus promoting a better feed softening facilitating the amalgamation of the bolus. Mandibular gland increased aquaporin 5 positivity in CP diet, supporting the hypothesis of an augmented demand for water. Findings suggest that the differenziate mechanical stimuli linked to different feed physical forms, likely allow to diverse physiological behavior of pig mandibular gland. The intense chewing activity linked to the highest feed compaction and hardness promotes an increase in pig mandibular gland secretion; in addition, saliva becomes more fluid and richer in acid glycoconjugates in order to better lubricate the bolus and protect the mouth mucosae. Based on apelin/receptor localization, it was hypothesized that in pig mandibular gland the apelinergic system likely performs an endocrine control on the demilunes activity and a paracrine control on ducts, facilitating the production of serous saliva by pig mandibular gland.

[1] Cappai et al., Different physical forms of one diet fed to growing pigs induce morphological changes in mandibular glands and local leptin (Ob) production and receptor (ObR) expression. Journal of Animal Physiology and Animal Nutrition 100: 106,7-1072,2016.
[2] Pirino et al., The presence and distribution of cannabinoid type 1 and 2 receptors in the mandibular gland: The influence of different physical forms of diets on their expression in piglets. Journal of Animal Physiology and Animal Nutrition, 102: 870-876,2018.

Effects of red orange and lemon extract (RLE) supplementation on NPY pattern in gastro-entero-pancreatic system of goat kids

Elena De Felice (1), Daniela Giaquinto (1), Sara Damiano (2), Angela Salzano (2), Roberto Ciarcia (2), Paola Scocco (1)

(1) Università di Camerino, Scuola di Bioscienze e Medicina Veterinaria. (2) Università degli Studi di Napoli Federico II, Dipartimento di Medicina Veterinaria e Produzioni Animali.

Corresponding author: Elena De Felice (elena.defelice@unicam.it)

Over the last decades extensive studies have been done to search for natural alternatives to in-feed antibiotics in farm animals, and plant compounds have been identified to have great potentials [1]. Among them, a growing interest in the consumption of anthocyanin-rich food has been observed. Anthocyanins (ANTs), which belong to the flavonoid family, are water soluble polyphenolic pigments widespread in the plant kingdom. Several researches highlighted the numerous health benefits of ANTs, like antioxidant, anti-inflammatory and immunostimulant properties [2]. Although many of the biological effects of polyphenols are well known, only a limited number of studies have addressed the influence of their supplementation in ruminant diet, focusing primarily on anti-oxidant capacity. This study aimed to examine the effect of a diet supplemented with a standardized powder extract, Red (blood) orange and Lemon Extract (RLE), rich in flavanones, anthocyanins and other polyphenols, on the neuropeptide Y (NPY) distribution in gastro-entero-pancreatic system of goat kids. We decided to focus our attention on NPY because in mammals this peptide is widely distributed in both the central and the peripheral nervous systems and it has been functionally implicated in the regulation of feeding behavior and gastrointestinal tract motility [3]. The research was approved with Protocol PG/2019/0028161 by the University of Naples Federico II. Sixty kids of Saanen bred, both males and females, after colostrum administration, were randomly divided into two homogenous groups of 30 kids each. The two experimental groups were fed for 40 days with: (1) standard diet made of hay (100 g) and kids starter (150 g) (CTRL group); (2) standard diet supplemented with RLE (90mg/kg) (TRT group). We carried out immunohistochemical analyses on samples of abomasum, duodenum (removed 4 cm from the pyloric sphincter) and pancreas collected from the two experimental groups. For the first time, we document that NPY is widely distributed in the gastro-entero-pancreatic tract of goat kids. Immunoreactive cells were detected in scattered cells of the epithelium and in typical varicosity in the muscular layer of the abomasum; in neuroendocrine cells and varicose fibers of the muscular layer in the duodenum and at the margin of pancreatic islet cells. The wider distribution was observed in the duodenum, and to a less extent in the pancreas and abomasum. In order to evaluate if RLE supplementation feed can affect NPY's distribution, we quantified the number of immunoreactive cells in all the three different selected segments. Remarkably, we described a strong significant increase of NPY immunolabeled cells in the abomasum and pancreas ($p>0.0001$) and a non-significant increase in the duodenum ($p < 0.05$) of TRT group compared to CTRL group. Our findings can be useful for further researches on the interaction between neuropeptides and polyphenols aiming to reduce antibiotic use and prevent antimicrobial resistance.

[1] Landers T. F. et al. A review of antibiotic use in food animals: perspective, policy, and potential, Public Health Reports, 127:4-22, 2012.
[2] Cisowska A. et al. Anthocyanins as antimicrobial agents of natural plant origin, Natural Product Communication, 6:149-156, 2011.
[3] Kageyama H. et al. Neuronal circuits involving neuropeptide Y in hypothalamic arcuate nucleus-mediated feeding regulation, Neuropeptides, 46:285-289, 2012.

Immunohistochemical detection of apelinergic system in the abomasum of sheep

Elisa Palmioli (1), Paola Scocco (2), Cecilia Dall'Aglio (1), Francesca Mercati (1)

(1) Università degli Studi di Perugia, Dipartimento di Medicina Veterinaria (2) Università degli Studi di Camerino, Scuola di Bioscienze e Medicina Veterinaria

Corresponding author: Francesca Mercati (francesca.mercati@unipg.it)

The growing summer drought stress given by global warming is pre-empting the moment of the maximum productivity of pasture and shortening the availability period of fresh forage for the animals with an adequate nutritional value to ensure animal productivity [2].

The adipokines are molecules mainly produced by adipose tissue related to the individual's nutritional status [3]. One of them is the apelin (AP), which with its receptor (APJ), has been detected in the gastrointestinal tract [1, 4] and it takes action in a series of physiological processes including energy metabolism and nutritional status. This work is a part of a wider project about the expression of AP and APJ in the digestive system of the sheep under different nutritional levels (No. of approval 95/2018-PR); here, the apelinergic system was investigated through immunohistochemistry in the abomasum of 15 Comisana x Appenninica adult female sheep. The flock was free to graze on the pasture from June to the pasture maximum flowering (MxF group) feeding on fresh forage. Throughout the period between the maximum pasture flowering (MxF) and the maximum pasture dryness (MxD), the MxD group has been grazing on pasture feeding only on fresh forage, while the experimental group (Exp) received also a feed supplementation of 600 g/day/head of barley and corn. Samples of the abomasum were collected for each group, fixed in formalin and embedded in paraffin wax. Dewaxed sections were microwaved for antigen retrieval. The primary antibodies, a rabbit polyclonal anti-AP (Novus Biochemical) and a rabbit polyclonal anti-APJ (Abnova), were diluted in PBS 1:200 and 1:400 respectively and incubation was performed overnight at room temperature. The immunological reaction was detected with the ABC kit (VectorLaboratories) and visualized with diaminobenzidine.

Preliminary data show the presence of AP and APJ in all analyzed samples suggesting an autocrine and paracrine action of the molecule. The positive cells have been found in the lining epithelium of the mucous layer and in the gastric glands mainly localized in their basal third. The comparisons performed among the three groups evidenced a lower intensity of immunopositivity in the MxD group for both AP and APJ. The detection of both molecules in the mucous layer of the abomasum and their different staining in the three analyzed groups suggests a role of the apelinergic system in the abomasum function regulation in the sheep. This role could be influenced by diet.

[1] Huang et al. Function and regulation of apelin/APJ system in digestive physiology and pathology, Journal of Cellular Physiology, 234(6), 7796–7810, 2019.
[2] Scocco et al. Effects of summer rainfall variations on sheep body state and farming sustainability in sub-mediterranean pastoral system, Spain Journal of Agricultural Research, 14:1-4, 2016.
[3] Trayhurn et al. Adipose Tissue and Adipokines—Energy Regulation from the Human Perspective, The Journal of Nutrition, 136(8), 2006.
[4] Wang et al. Apelin, a new enteric peptide: localization in the gastrointestinal tract, ontogeny, and stimulation of gastric cell proliferation and of cholecystokinin secretion, Endocrinology, 145: 1342-48, 2004.

Adiponectin system in the skin: a comparison between obese and normal-weight dogs

Mercati Francesca (1), Cecilia Dall'Aglio (1), Antonio Di Loria (2), Diego Piantedosi (2), Paolo Ciaramella (2), Elvio Lepri (1), Margherita Maranesi (1)

(1) Università degli Studi di Perugia, Dipartimento di Medicina Veterinaria (2) Università degli Studi di Napoli, Federico II, Dipartimento di Medicina veterinaria e Produzioni animali

Corresponding author: Francesca Mercati (francesca.mercati@unipg.it)

Adipokines are molecules involved in the energetic metabolism and linked to obesity, both in its genesis and in the appearance of related disorders in the different organs (1). There is an increased incidence of obesity in dogs and this represents an important health issue since obesity influences on a plethora of associated diseases including skin dermatological disorders (2). Considering the scarcity of information in pets, the purpose of this work was to evaluate the presence and distribution of adiponectin (ADIPOQ), a component of adipokines family, and the two related receptors (ADIPOR1 and ADIPOR2), in the skin of obese dog. This topic raises interesting questions on the modification and role of adipokines in the skin in obesity conditions, as already suggested in our previous investigation performed on the leptin system in the skin of obese dogs (3). The study was carried out on adult medium sized mixed breed dogs. Animals were divided in two groups consisting of ten obese (body condition score, BCS≥7/9) and ten normal weight (BCS of 4–5/9) dogs. BCS was evaluated according to the nine-point BCS system (4). Dogs were healthy and fed a homemade chicken protein based diet. Skin biopsies were collected during surgical neutering from the ventral region and used to perform immunohistochemistry and Real-time PCR. The study procedures were approved by the Ethical Animal Care and Use Committee (n. PG/2017/0099607) of the University of Naples Federico II. Immunohistochemistry was performed with rabbit polyclonal antibodies respectively anti-ADIPOQ (MyBiosource, CA, USA), anti-ADIPOR1 (LifeSpan BioSciences, WA, USA) and anti-ADIPOR2 (Aviva Systems Biology Corporation, CA, USA). The primer sequences used for the Real-Time PCR were as follows: ADIPOQ: TTCATCTGGAAGTGGGCGAC (F), AAGGAAGCCCGTAAAGGTGG (R); ADIPOR1: GCAGACAAGAGCAGGAGTGT (F), AGCCATGAGGAAGAACCAGC (R); ADIPOR2: GGTCTCCCGGCTCTTCTCTA (F), AATGCCCAGCACACAGATGA (R). Immunostaining of ADIPOQ was observed in the adipose tissue extending among follicular clusters, in the sweat and sebaceous glands, in the endothelium and some connective cells. Both receptors were observed in the epidermis, in the hair follicles, in the sweat and sebaceous glands. In addition, ADIPOR2 was observed in the adipose tissue, in the endothelium and some connective cells. ADIPOQ and ADIPOR2 transcripts were expressed 5.4-fold ($p<0.01$) and 2.3-fold less ($p<0.01$) respectively, in Obese than in Normal-weight dogs while ADIPOR1 expression did not change. Accordingly, ADIPOQ and ADIPOR2 expression in the skin appear negatively correlated with obesity. These findings evidence that the ADIPOQ system changes in the skin of obese dogs and suggest that the ADIPOQ effect on the skin is at least in part regulated by the reduced expression of ADIPOR2.

[1] Raffan E. The big problem: battling companion animal obesity, 2013, Veterinary Record; 28:287.
[2] Ohashi et al. Role of anti-inflammatory adipokines in obesity-related diseases, 2014, Trends in Endocrinology and Metabolism, 25(7):348-55.
[3] Maranesi et al. Leptin System in Obese Dog Skin: A Pilot Study, 2020, Animals (Basel), 10(12):2338.
[4] Laflamme D.P. Development and validation of a body condition score system for dogs, 1997, Canine Practice, 22, 10–15.

Morphological characterisation of preterm rabbit-derived Precision Cut Lung Slices as an alternative to bronchopulmonary dysplasia in vivo models

Luisa Ragionieri (1), Roberta Ciccimarra (1), Zoboli Matteo (1), Ferdinando Gazza (1), Antonio Cacchioli (1), Enrica Scalera (2), Matteo Storti (2), Costanza Casiraghi (2), Fabrizio Salomone (2), Francesca Ravanetti (1)

(1) Università degli Studi di Parma, Dipartimento di Scienze Medico-Veterinarie (2) Chiesi Farmaceutici, R&D Department, Parma, Italy

Corresponding author: Luisa Ragionieri (luisa.ragionieri@unipr.it)

Bronchopulmonary dysplasia (BPD) is the most common complication of preterm delivery, with significant morbidity and mortality in a neonatal intensive care setting. Research in this field is aimed at identifying the mechanisms of late lung development with possible therapeutic targets and the improvement of medical management.

The rabbit is one of the preclinical models with greater translatability to humans, as also term-born rabbits are in the alveolar phase of lung development and, if exposed to hyperoxic conditions show morphological, functional and vascular alterations similar to those characterizing human BPD. In preclinical research great attention is paid to optimize the experimental procedures, reduce the number of animals used in experiments and, where possible, replace animal models with alternative assays, following the principle of the 3 Rs (Replace, Reduce and Refine). The use of in vitro assays based on the ex vivo culture of Precision Cut Lung Slices (PCLS) goes in this direction, representing a good compromise between controlled and flexible in vitro models and the more physiologically relevant in vivo ones [1].

This work aims to morphologically characterise the PCLS derived from preterm rabbits cultured in different oxygen conditions up to 7 days, as model for preclinical studies of BPD lung development / alveolarization (Ethical approval no. 899/2018-PR – Italian Ministry of Health).

The characterization of rabbit PCLS for the study of normal lung development and of its alterations requires the analysis of morphological changes over a time course. It was necessary a preliminary optimization of pre / processing methods, inclusion, alignment, and cutting of the original sections of 300 microns. The morphological analysis was conducted evaluating a series of histomorphometric parameters related with the alveolarization process [2]. The Radial Alveolar Count, could not be applied to PCLS, due to the tissue changes following agar infusion and culture conditions. Parameters like Mean Linear Intercept and Septal Density did not allow to highlight significant differences between different oxygen conditions and time points. Shape Factor and Roughness, evaluated to highlight the increasing complexity of the airspaces during the alveolarization process, following the formation of septal crests, gave only some indications, without highlighting substantial differences. These results could be influenced by the stretching of the air spaces walls due to lung filling with agarose, which probably partially reduced the protrusions of the septal crests inside the air spaces. The only histomorphometrical parameters suitable for histologically evaluate the alveolar development in vitro resulted Tissue Density and Septal Thickness. For future pharmacological studies, we suggest to analyze them after 4 days of in vitro culture, when the greatest differences between the exposure to normoxia used as control, the alterations due to hyperoxic conditions and potential drug effects may be detectable.

[1] Liu et al. Use of precision cut lung slices as a translational model for the study of lung biology. Respiratory Research. 20:162, 2019.

Novel Findings in Genetically-Driven Enteric Neuropathy: The Rad21 Knock-In Mouse Model

Giulia Lattanzio (1), Francesca Bianco (2), Elena Bonora (2), Maurizio Mazzoni (1), Laura Calzà (3),
Luciana Giardino (3), Luca Lorenzini (3), Angelo Peli (1), Catia Sternini (4), Roberto De Giorgio (5), Paolo Clavenzani (1)

(1) Department of Veterinary Medical Sciences (DIMEVET), University of Bologna, Bologna, Italy (2) Department of Medical and Surgical Sciences (DIMEC), University of Bologna, Bologna, Italy (3) IRET Foundation, Ozzano Emilia, Italy (4) UCLA/DDRC, Division of Digestive Diseases, Departments Medicine and Neurobiology, David Geffen School of Medicine, UCLA, Los Angeles, CA, USA (5) Department of Morphology, Surgery and Experimental Medicine, University of Ferrara, Ferrara, Italy

Corresponding author: Giulia. Lattanzio (giulia.lattanzio2@unibo.it)

RAD21 is a double-strand-break repair protein, which exerts several key roles as a component of the cohesin complex and regulator of transcription processes [1]. Investigating a consanguineous family of Turkish origin with a clinical phenotype prominently characterized by severe enteric dysmotility, namely neurogenic chronic intestinal pseudo-obstruction (CIPO), our group identified a novel causative *RAD21* (Ala622Thr) missense mutation in two members [2]. Immunohistochemical studies showed Rad21 immunoreactivity (-IR) in a subset of neurons of the mouse enteric nervous system [3]. In order to understand how the identified RAD21 mutation impairs gut motility, we developed a genetically re-constructed Rad21 conditional knock-in (Rad21KI) mouse carrying the Ala626Thr mutation. This model is expected to recapitulate the main clinical and pathological features observed in the affected family members. In this study, we performed qualitative and quantitative characterization of myenteric neurons in the ileum and colon of Rad21KI *vs.* wild type (WT) mice. Immunohistochemical analysis was performed in whole mount myenteric plexus preparations using the pan-neuronal marker HuC/D, choline acetyltransferase (ChAT, a marker for cholinergic excitatory motor neurons) and neuronal nitric oxide synthase (nNOS, a marker for inhibitory motor neurons) (Experimental procedures were evaluated and approved by the Ethical-Scientific Committee for Animal Experimentation of the University of Bologna, Italy). Based on preliminary result on a small number of animals, we observed that there was an overall reduction of HuC/D myenteric neurons/field in of Rad21KI mice compared to WT (≈ 20% in the ileum and ≈ 30% in the colon). We also showed that HuC/D/ChAT-IR myenteric neurons/field were 20.4±2.6 in the ileum and 17.5±1.6 in the colon in WT *vs.* 5.8±1 ($P \leq 0.005$) and 15.9±2.8, respectively in Rad21KI mice; HuC/D/nNOS-IR myenteric neurons/field were 17.6±4.6 in the ileum and 17.5±3.4 in the colon in WT *vs.* 12.3±2.2 and 5.1±1.1 ($P \leq 0.005$), respectively, in Rad21KI. In the Rad21KI mouse model, there is an overall reduction of the HuC/D myenteric neurons, with the excitatory neurons being significantly reduced in the ileum but not the colon, whereas the inhibitory neurons were reduced in both ileum and colon. The reduction of neuronal populations might represent a mechanism underlying the impairment of motility in CIPO patients with the *RAD21* mutation. The elucidation of the mechanisms through which individual subsets of nitrergic and cholinergic myenteric neurons are affected in distinct gut segments by the Rad21 mutation requires further analysis.

[1] Guacci et al. Sister chromatid cohesion: the cohesin cleavage model does not ring true, Genes to Cells, 12:693–708, 2007.
[2] Bonora et al. Mutations in RAD21 disrupt regulation of APOB in patients with chronic intestinal pseudo-obstruction, Gastroenterology, 148:771–782, 2015.
[3] Bianco et al. Expression of RAD21 immunoreactivity in myenteric neurons of the human and mouse small intestine, Neurogastroenterol and Motility, 30: e13429, 2018.

A novel collagen skin-like scaffold improves skin regeneration in sheep

Luca Melotti (1), Tiziana Martinello (2), Andrea Venerando (1), Roberta Sacchetto (1), Anna Perazzi (3), Cinzia Ferrario (4,5), Ilaria Iacopetti (3), Michela Sugni (4,5,6), Marco Patruno (1)

(1) Università degli Studi di Padova, Dipartimento di Biomedicina Comparata e Alimentazione (2) Università degli Studi di Bari, Dipartimento di Medicina Veterinaria (3) Università degli Studi di Padova, Dipartimento di Medicina Animale, Produzioni e Salute (4) Università degli Studi di Milano, Dipartimento di Scienze e Politiche Ambientali (5) Università degli Studi di Milano, Dipartimento di Fisica, Centro della Complessità e dei Biosistemi (6) Università degli Studi di Milano, Dipartimento di Scienze e Politiche Ambientali, Centro GAIA 2050

Corresponding author: Marco Patruno (marco.pat@unipd.it)

Skin wound healing is a multi-phase dynamic process fundamental for the restoration of skin integrity. Skin wounds are a common occurrence in Veterinary Medicine and an inadequate wound care management might lead to a poor prognosis, with a consequent impact on the health of the animal and the economic sector. Conventional treatments (e.g. autografts) have many limitations and do not provide a proper wound healing. Tissue engineering techniques could provide a solution by the production of scaffolds able to support skin regeneration, in function and structure, and promote wound closure. In this work, we describe the application of a novel designed collagen-based skin-like scaffold (CBSS) [1], produced with collagen extracted from sea urchin food wastes, for the treatment of experimental second intention healing wounds in the sheep (approved by the Italian Ministry of Health no. 51/2015-PR, in accordance with the Body for the Protection of Animals (OPBA)). After 7, 14, 21 and 42 days from wound creation, clinical observations were performed along with skin biopsies collected for histopathology (H&E and IHC for Ki67 and α-smooth muscle actin (αSMA)) and molecular analysis (RT-PCR for collagen type I and III, VEGF and hair-Keratin (hKER)) for untreated (control) and treated (CBSS) wounds. CBSS-treated wounds showed a higher inflammatory cell infiltration than control wounds at 7 days; nonetheless, at 42 days treated wounds showed no inflammation while it was still present in control wounds. The CBSS application led to a higher deposition of granulation tissue (GT) at day 7 while in control wounds it was still low. In treated wounds, the amount of GT started to diminish by day 14 while increasing in control wounds. These results were reflected by the gene expression analysis of the immature (type III) and mature (type I) form of collagen: while in the control wounds type III expression was increasing, reaching its peak at 21 days, in CBSS-treated wounds the up-regulated one was the mature type I. This scenario resulted into an appropriate maturation (also supported by the induced gene expression of VEGF) of the GT in treated wounds at 42 days while the higher amount of collagen type III led to dermal fibrosis in control wounds, also characterized by αSMA immunopositivity. Moreover, the application of the CBSS led to a higher re-epithelialization rate at 14 and 21 days in comparison to the control; concomitantly, a higher immunopositivity for Ki67 (marker of proliferation) was observed in the basal layer of the newly formed epidermis. Histologically, in treated wounds along with the earlier presence of a neoepidermis at day 14, it was also observed the appearance of skin adnexa with the concomitant gene expression of hKER, absent in control wounds until day 42. In conclusion, the application of a collagenous biomaterial anticipated the inflammatory phase, promoted the maturation and remodelling of the GT into a mature and well-organized dermis along with skin adnexa, and accelerated the re-epithelialization process. Overall, these preliminary findings suggest that this marine collagen scaffold possesses regenerative properties, worthy of further investigations, might be a useful tool for the treatment of second intention healing skin wounds in Veterinary patients.

[1] Ferrario et al. From Food Waste to Innovative Biomaterial: Sea Urchin-Derived Collagen for Applications in Skin Regenerative Medicine, Marine Drugs, Aug 6;18(8):414, 2020.

A novel anatomical template for skin regeneration

Isabella Mavaro (1,2), Claudia Mazio (2), Antonio Palladino (1), Francesco Urciuolo (3,4), Costantino Casale (3), Paolo Antonio Netti (2,3,4), Giorgia Imparato (2), Paolo de Girolamo (1), Chiara Attanasio (1,2)

(1) Department of Veterinary Medicine and Animal Productions, University of Naples Federico II, Naples, Italy (2) Center for Advanced Biomaterials for Healthcare, Istituto Italiano di Tecnologia, Naples, Italy (3) Department of Chemical, Materials and Industrial Production Engineering (DICMAPI) University of Naples Federico II, Naples, Italy (4) Interdisciplinary Research Centre on Biomaterials (CRIB), University of Naples Federico II, Naples, Italy

Corresponding author: Isabella Mavaro (isabellamavaro@live.it)

Numerous advances have been made in the field of skin tissue engineering in recent years to overcome skin injuries, burns and pathologies. Most of skin substitutes are exogenous matrices requiring the contribution of both host fibroblasts and endothelial cells and, therefore, a long time to become functional after implantation. In view of this, here we investigate the potential of a specific pre-vascularized dermis (PVD) obtained by seeding freshly isolated fibroblasts onto gelatin microbeads and adding, at a certain culture time, endothelial cells (HUVECs) [1]. More in detail, we implanted our engineered skin on the back of nu/nu mice in a full thickness skin defect model which, respect to the subcutaneous pocket we previously experienced, is more functional for both general and wound healing applications.

At different timepoints (3, 7, 14, 21 and 42 days) we retrieved our skin biohybrids and analysed them by histology and immunofluorescence. Animal studies were performed following the guidelines of EU (2010/63/EU).

Our main objective is to study the behaviour and the degree of integration of our skin substitute in the host organism. First of all, an appreciable integration of a pre-vascularized substitute with host tissue results in a fast anastomosis between the two vascular networks. However, since integration is a complex process there are other aspects which have to be considered. For example, a major limitation of skin substitutes in clinical application is to mimic the physiological sensitivity of the host skin.

Regarding vascularization, we looked for the expression of the lectins *Griffonia Semplicifolia* and *Ulex Europaeus Agglutinin I* (UEA I) to mark murine and human vessels respectively. We noticed, starting from day 14 onwards the onset of numerous anastomosis between the two vascular networks (human and murine). Afterwards, from the innervation standpoint, we looked at the expression of Neurofilament-M, PGP9.5, NGF and BDNF along with the corresponding receptors TrkA and TrkB. Interestingly, we noticed a partial reinnervation of our skin substitutes already 42 days after implantation through the appreciable expression of PGP9.5, a promising result in comparison with other skin substitutes where the reinnervation is observed not earlier than after 8 weeks of implantation [2]. Therefore, since neurotrophins guide fibroblasts differentiation into myofibroblasts, also increasing skin tensile strength, we analysed the variation of the Young Modulus of our samples via indentation test. Further to better frame expression of our markers we are performing a quantitative analysis through PCR.

In conclusion, our skin substitute, in a full thickness skin defect model, shows an earlier vascularization and innervation time compared to other substitutes described in the literature leading us to investigate the connection between vascularization and innervation during tissue development and maturation.

[1] Mazio et al. Pre-vascularized dermis model for fast and functional anastomosis with host, Biomaterials, 192:159-170, 2019.

[2] Biedermann et al. Rebuild, restore, reinnervate: do human tissue engineered dermo-epidermal skin analogs attract host nerve fibers for innervation? Pediatric Surgery International, 29:71–78, 2013.

In situ characterization of glycans in the horse bladder urothelium

Salvatore Desantis (1), Nicoletta Santamaria (1), Maria Mastrodonato (2), Giovanni Scillitani (2)

(1) Università degli Studi di Bari-Aldo Moro, Dipartimento dell'emergenza e dei trapianti di organi (DETO) (2) Università degli Studi di Bari-Aldo Moro, Dipartimento di Biologia

Corresponding author: Salvatore Desantis (salvatore.desantis@uniba.it)

The urinary bladder cavity is lined by a highly specialized epithelium, the urothelium, which prevents permeation of solutes and noxious agents back into the bloodstream and underlying tissues, serving also as a sensor and transducer of physiological and nociceptive stimuli [1]. In addition, in physiological conditions the urothelium can also function as a secretory tissue [2]. A mucous layer protects the urothelium of the urinary bladder from potentially harmful environmental substances, attachment of bacteria and proteolytic enzymes present in the urine [3]. Moreover, the glycan composition of urothelial mucous layer could have a role in the intravesical pharmacological treatments [4]. Despite their considerable importance in urinary bladder physiology, few studies are available about the glycoconjugates expressed in non-human species. In this study the glycoconjugate pattern of urothelium lining the horse urinary bladder was investigated. Tissue fragments from three horse stallions in good health status, aged 2.5-4 years, were fixed in 4% (w/v) PBS-buffered paraformaldehyde, embedded in paraffin wax and stained with a panel of twelve lectins, in combination with saponification and sialidase digestion (Ks). The urinary bladder urothelium has three distinct layers from the basal zone to the lumen consisting of basal, intermediate and superficial cells (umbrella cells). Cytoplasm of basal cells showed glycans ending with Neu5AcGal1-3GalNAc, GlcNAc and with terminal/internal Man (Ks-PNA, GSA II, and Con A II reactivity). A sub-population of intermediate cells also displayed terminal Neu5Ac2-6Gal/GalNAc, NeuNacα2-3Galβ1-4GlcNAc, Gal1-3GalNAc, Gal, terminal and sialic acid-linked GalNAc, internal GlcNAc and Fucα1-2Galβ1-4GlcNAc (MAL II, SNA, PNA, GSA I-B_4, SBA, ks-SBA, Ks-WGA, and UEA I reactivity). The cytoplasm of umbrella cell population contained all the above cited sugar residues. Moreover, LTA-reactive fucosylated glycans and Ks-DBA-positive sialoderivatives were found in some scattered umbrella cells. These sialoglycans were secreted in the bladder lumen. The bladder luminal surface stained with MAL II, SNA, PNA, Ks-PNA, and GSA I-B_4 displaying a coating of sialo- and galactose-terminating glycoconjugates. These findings show that different glycosylation patterns exist along the horse bladder urothelium, and different sub-populations of umbrella cells are present secreting the sialoglycans which constitute the protective gel layer lining the bladder. Compared to results of a similar study carried out onthe donkey bladder urothelium [2], the present research reveals a species-specific glycan pattern and could contribute to a better understanding of the differences between domesticated odd-toed ungulate mammals via comparative glycopattern investigation.

[1] Lasič et al. Properties of the urothelium that establish the blood-urine barrier and their implications for drug delivery, Reviews of Physiology, Biochemistry and Pharmacology Vol 168, 2015.
[2] Desantis et al. In situ characterization of glycans in the urothelium of donkey bladder: Evidence of secretion of sialomucins, Acta Histochemica, 115:712–718, 2013.
[3] Kreft et al. Formation and maintenance of blood–urine barrier in urothelium, Protoplasma, 246:3–14, 2010.
[4] Lopedota et al. Spray dried chitosan microparticles for intravesical delivery of celecoxib: preparation and characterization, Pharmaceutical Research, 33:2195–2208, 2016.

Defective SERCA1protein causing bovine Pseudomyotonia and human Brody disease: from pathogenic mechanism to a novel therapeutic approach

Roberta Sacchetto (1), Eylem Emek Akyurek (1), Francesca Busato (1,2)

(1) Università degli Studi di Padova, Dipartimento di Biomedicina Comparata e Alimentazione
(2) Clinica Veterinaria San Marco, Padova

Corresponding author: Roberta Sacchetto (roberta.sacchetto@unipd.it)

Cattle congenital pseudomyotonia (PMT) is an inherited recessive autosomal muscular disorder due to missense mutations in *ATP2A1* gene, encoding SERCA1 protein (Sarco-endo-plasmic reticulum Ca^{2+}-ATPase, isoform1) [1]. SERCA1 is responsible for transporting Ca^{2+} from cytosol back into the lumen of sarcoplasmic reticulum (SR) playing a crucial role in muscle relaxation. Bovine PMT has been described in the Chianina and Romagnola italian breeds. Even though clinical symptoms were homogeneous, PMT has been found genetically heterogeneous. All PMT Chianina animals were homozygous for a single point mutation leading to an Arg164His (R164H) substitution, while most of PMT Romagnola cases were compound heterozygous, carrying a mutation identical to that of Chianina breed, in addition to two point mutations leading to Gly211Val and Gly286Val (G211V/G286V) substitutions [2]. In spite of this heterogeneity of *SERCA1* gene mutations, a striking selective reduction of SERCA1 protein has been described in SR membranes isolated from bovine muscles of different PMT-affected cattle breeds. Recently we have clarified the pathogenic mechanism underlying Chianina PMT: the R164H SERCA1 mutation generates a protein functionally active but corrupted in proper folding that was ubiquitinated and prematurely degraded by the ubiquitin-proteasome system [3].

The relevance of cattle PMT is based on phenotypic and genotypic similarities with human Brody myopathy, a “rare” genetic muscular disorder. Clinical symptoms genetic and biochemical findings, clearly demonstrated that cattle PMT is the true animal model of Brody disease. This is not surprising since, in the last years, the counterparts of human pathologies have been found in many domestic mammalian species.

Using the heterologous cellular model HEK293 overexpressing SERCA1 mutants, we have deeply investigated SERCA1 mutations found in Romagnola breed. G211V and G286V mutations were introduced separately or together into bovine SERCA1 cDNA. Using both immunofluorescence and western blot analyses we have found that only the G211V mutation is responsible for the PTM phenotype of Romagnola and the treatment with proteasome inhibitor rescues the expression level of G211V mutated SERCA1 at ER membranes in HEK293 cell model. At present, neither specific therapy nor mouse model for Brody myopathy exists. Our findings opened new perspectives for a therapy of this rare disease. To this aim, in the same cell model we have tested small molecules known as “CFTR correctors” specifically developed for rescuing type II Cystic Fibrosis Transmembrane Regulator (CFTR) mutants, causing Cystic Fibrosis. We have observed, by western blot analysis, that treatments with these compounds induce an increase of SERCA1 mutant content. So, a possible pharmacological therapy could be hypothesized for the specific population of Brody patients in which, as in bovine PMT, *ATP2A1* mutations impair SERCA1 protein folding causing its rapid degradation.

[1] Sacchetto et al. A defective SERCA1 protein is responsible for congenital pseudomyotonia in Chianina cattle, Am J Pathol ,174:565-573, 2009.
[2] Murgiano, Sacchetto et al. Pseudomyotonia in Romagnola cattle caused by novel *ATP2A1* mutations, BMC Vet. Res, 8:186. 2012.
[3] Bianchini et al. Inhibition of ubiquitin proteasome system rescues the defective sarco(endo)plasmic reticulum Ca2+-ATPase (SERCA1) protein causing Chianina cattle pseudomyotonia, J Biol Chem. 289:33073-33082, 2014.

"Hacking Extracellular Vesicles": use of reporter protein to study the traffic of bio-molecules within extracellular vesicles

Gabriele Scattini (1), Laura Musa (1), Samanta Mecocci (1), Maria Rachele Ceccarini (2), Patrizia Casagrande Proietti (1), Stefano Capomaccio (1), Antonello Bufalari (1), Rodolfo Gialletti (1), Olimpia Barbato (1), Luca Avellini (1), Tommaso Beccari (2), Luisa Pascucci (1)

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

Corresponding author: Gabriele Scattini (gabriele.scattini@studenti.unipg.it)

Over the last two decades the study of extracellular vesicles (EVs) has broadened our knowledge about intercellular communication, suggesting new ways through which cells exchange information in physiological and pathological conditions. EVs are membrane-bounded micro and nanoparticles containing a large variety of biological molecules, from nucleic acids to small bioactive molecules. They are generated by parental cells that "package the cargo" and release the vesicles in the extracellular space. EVs diffuse in biological fluids and exert their functions in two principal ways: releasing the cargo into target cells by membrane fusion or interacting with surface receptors on recipient cells. The mechanisms that regulate the entry and transport of molecules into EVs, as well as the role of specific EV sub-populations are still poorly defined.

The overall purpose of this study is to increase our knowledge on the mechanisms and sub-structures involved in protein loading and transport by EVs. To this aim, in this first phase of the study, we studied the incorporation in EVs of a reporter protein (GFP) associated with different EV specific proteins (tags) with the aim to *hack* the molecular trafficking mechanisms of EVs. The study was carried out on Mesenchymal Stromal Cells (MSC) that are known to be involved in tissue regeneration, immunomodulation, inflammation and angiogenesis and to produce a huge amount of EVs through which they carry out their biological action.

cDNAs encoding EV specific proteins (CD63, TSG101 and Syntenin-1) were isolated and cloned into a green fluorescence protein (GFP) expression vector in order to obtain plasmids encoding for chimeric proteins. Canine MSCs were transfected with plasmids to allow the expression of labeled EV-related proteins. Images of fluorescent living cells were acquired 48 hours post-transfection and cell medium was collected to isolate EVs. Immunogold labeling was performed with anti-GFP antibody to detect chimeric proteins and their fine intracellular localization by Transmission Electron Microscopy (TEM). Western blotting was performed on lysed cells and isolated EVs to confirm and quantify chimeric proteins expression and concentration.

The use of genetically engineered molecular tags allowed to describe the localization of the reporter protein in living cells, without the perturbation of antibody labelling, and allowed its detection in immunogold analysis. The study, although in its early phase, confirmed the incorporation of GFP inside EVs. Different pathways and sub-structures of the endosomal system appeared to be involved depending on the different tags used. Further analyses are necessary to achieve a more detailed characterization.

[1] Fitzsimmons R. Mesenchymal Stromal/Stem Cells in Regenerative Medicine and Tissue Engineering, Stem Cells International, eCollection 2018.
[2] Crivelli B. Mesenchymal stem/stromal cells extracellular vesicles: From active principle to next generation drug delivery system, Journal of Control Release, 262:104-117, 2017.
[3] Corso G. Systematic characterization of extracellular vesicle sorting domains and quantification at the single molecule – single vesicle level by fluorescence correlation spectroscopy and single particle imaging, Journal of Extracellular Vesicles, eCollection 2019.

Morphological and morphometric study of the vascular system in the equine allantochorion

Claudio Tagliavia (1), Marco Canova (1), Maddalena Piai (1), Roberta Gini (1), Aliai Lanci (1), Carolina Castagnetti (1,2), Annamaria Grandis (1)

(1) Alma Mater Studiorum – University of Bologna, Department of Veterinary Medical Sciences
(2) University of Bologna, Health Science and Technologies Interdepartmental Center for Industrial Research

Corresponding author: Claudio Tagliavia (claudio.tagliavia2@unibo.it)

Normal fetal development is dependent on adequate placental blood perfusion. The placenta is a deciduous organ that originates from the apposition of the endometrium with the outermost part of the *conceptus*. In the mare, the placenta can be classified as diffuse, microcotyledonary, epitheliochorial [1] and it is peripherally constituted by the allantochorion, on which a dense vascular network is developed, fundamental for feto-maternal exchanges.

This study aims at performing a morphological and morphometric investigation of the equine allantochorial vascular network, to provide anatomical data useful for embryology and to a better understanding of perinatal diseases, as already done in the human sphere [2]. Fourteen healthy mares with normal pregnancies and healthy foals were included. Their placentas, free from gross abnormalities, were used to produce casts of the arterial and venous systems [3]. Seven arterial cast models were obtained by injecting a polyurethane foam mixed with a red or yellow nitro dye, respectively in the arteries supplying the pregnant and non-pregnant horn. The same procedure was performed to obtain seven venous casts using blue or yellow colored foam.

During the preparation of the casts, it was possible to identify a single umbilical vein, formed by the union of two large branches that ran parallel to the two arteries. The insertion of the umbilical cord turned out on the area coinciding with the dorsal wall of the uterus, opposite to the great curvature of the pregnant horn. In 12 out of 14 cases, the insertion was between the horns, in 2 samples at the entrance of the non-pregnant horn. Regarding the distribution of the umbilical vessels, it was observed that, more frequently, one artery and its satellite vein led to the pregnant horn and to the base of the great curvature of the allantochorion, while the other pair of vessels sprayed to the non-pregnant horn and the body of the allantochorion [4]. Basically, the areas supplied by the main vessels increased as their caliber increased. The orders of division reached by the veins and the arteries were on average higher in number at the body level. The average caliber of the vessels gradually decreased in size as the order of division increased. Regarding the morphology, almost all of the vessels showed a serpiginous trend, however, at the level of the convexity of the pregnant horn, the presence of vessels with a rectilinear pattern was seen. The most commonly observed type of branching pattern of the allantochorionic vessels was the dichotomous division. Finally, many anastomoses were noted at the level of the dense vascular texture, which ensured continuous exchanges between the different branches of the venous network of the allantochorion.

Despite the several affinities, from the comparison between arteries and veins, it emerged that the latter had a larger average diameter, more orders of division, and a denser texture of anastomosis.

In conclusion, the obtained data could represent a valid help for a better understanding of pathological findings.

[1] Morresey P.R. The placenta. In Equine Reproduction. II ed. Wiley – Blackwell, 2011.
[2] Gordon et al. Anthropometry of fetal vasculature in the chorionic plate, Journal of Anatomy, 211: 698-706, 2007.
[3] De Sordi et al. A new method of producing casts for anatomical studies, Anatomical Science International, 89: 255-265, 2014.

Morphological variability of the atrioventricular valve cusps in the dog heart

Margherita De Silva (1), Giulia Lacchini (1), Franca Rambaldi (1), Marco Canova (1), Cristiano Bombardi (1)

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

Corresponding author: Margherita De Silva (margherita.desilva2@unibo.it)

In the literature regarding humans and equines, cases of aberrant morphology of the atrioventricular valve cusps have been extensively reported, including the common presence of accessory leaflets [1-5]. The morphological and morphometrical studies of the atrioventricular valves of the dog, on the other hand, are limited [6]. The aim of the present anatomical study was to investigate the morphological variability and morphometrical features of the valvular leaflets of the mitral and tricuspid valves of the dog. We hypothesised that accessory leaflets commonly occur and exist as independent structures in the atrioventricular valves of the dog, and that, therefore, the nomenclature “bicuspid” and “tricuspid” would not be anatomically accurate.

Twenty normal hearts belonging to small-, medium-, and large-sized dogs of both sexes were used. The left and right atrioventricular valves were exposed by excision of the atria; the morphology of the leaflets was examined, and the number of leaflets for each atrioventricular ostium was then counted. The atrioventricular valves were isolated, and the width, height and thickness of each leaflet were measured.

In addition to the principal leaflets, 1 to 5 accessory leaflets were identified in the mitral valve and 1 to 2 in the tricuspid valve. In only 15% of hearts, the mitral and tricuspid valves consisted of 2 and 3 leaflets, respectively, as it is commonly known. In 85% of mitral valves, 1 to 5 accessory cusps were present. In 40% of tricuspid valves, the parietal and angular cusps were fused together, whereas in 45% of tricuspid valves, 3 to 5 total cusps were counted, of which 1 to 2 accessory cusps. All the accessory leaflets were separated from the adjacent leaflets at their insertion. They had lesser mean thickness, height and width values than the principal leaflets. Indentations along the free edges of the principal leaflets were identified in both atrioventricular valves, dividing each cusp into multiple scallops. Specifically, 2 to 3 scallops were counted in the parietal cusp of the mitral valve, and 2 to 6 in the parietal cusp of the tricuspid. The septal and angular cusps free edges tended to be smoother, with infrequent cleavages.

In conclusion, accessory leaflets occurred commonly in the atrioventricular valves of the dog and appeared as independent structures, whereas the principal leaflets frequently presented cleavages of their free edges, which gave them a multi-scalloped appearance. The clinical relevance of increased numbers of commissures resulting from accessory leaflets, as well as that of the indentations of the leaflets’ free edges, and their relationship with valvular regurgitation, need to be further investigated.

[1] Wafae et al. Anatomical study of the human tricuspid valve, Surgical and Radiologic Anatomy, 12:37–41, 1990.
[2] Ring et al. The prevalence and impact of deep clefts in the mitral leaflets in mitral valve prolapse, European Heart Journal Cardiovascular Imaging, 14:595–602, 2013.
[3] Lama et al. Morphometry and aberrant morphology of the adult human tricuspid valve leaflets, Anatomical Science International, 91:143-150, 2016.
[4] Athavale et al. Is tricuspid valve really tricuspid?, Anatomy and Cell Biology, 50:1-6, 2017.
[5] De Silva et al. Morphological variability of the atrioventricular valve cusps in the equine heart, Equine Veterinary Journal, 2021. doi: 10.1111/evj.13434.
[6] Alves et al. Morphometric study of the tricuspid valve in dogs, Anatomia Histologia Embryologia, 37:427-429, 2008.

A combined morphometric approach to feature mouse kidney vasculature

A. Palladino[1], C. Pizzoleo[1,2], I. Mavaro[1,2], C. Lucini[1], C. Attanasio[1,2]

(1) Department of Veterinary Medicine and Animal Productions, University of Naples Federico II, Naples, Italy (2) Center for Advanced Biomaterials for Healthcare, Istituto Italiano di Tecnologia, Naples, Italy

Corresponding author: A. Palladino (a.palladino1986@gmail.com)

Morphometric analysis of organ vasculature is widely recognized to provide essential insights into both physiological and pathological conditions [1]. In any case, vascular architecture is unique for each organ being strictly linked to its function. Because of this, a large part of cardiac output is directed to the kidney due to its main blood filtration role. Alterations, such as capillary rarefaction, predispose to chronic kidney disease (CKD) [2]. In this context, deepening the methodologies to study the renal vascular network can be of basic importance. To meet this need numerous animal models and, in parallel, several methods have been developed. In this work we propose a protocol to accurately feature kidney vasculature in mice, however, the same protocol is suitable to be applied also to other animal models. Animal studies were performed following the guidelines of EU (2010/63/EU). The approach is multiparametric and mainly based on the micro-computed tomography (µCT) technique. Micro-CT allows studying in detail the vascular network of any organ by exploiting the possibility to perfuse the sample with a contrast agent [3]. The proposed protocol provides a fast and reliable method to extract quantitative information from the µCT scan by using only the basic functions of the software supplied by the scanner without any additional analysis. Through iterative cropping of the scanned ROI and calculation of a sample-specific threshold, we calculated that the average volume of a female BALB/c kidney of 8 weeks is 147.8 mm^3 ±5.4%. We also pointed out that the average volume of the vascular network is 4.9% ±0.3%. In parallel, we investigate kidneys through histological and immunofluorescence techniques to integrate the information gained via µCT and to frame them in the tissue context. Vessel count on histological sections showed a different density in the different regions of the organ parenchyma. In detail, vessel density in the cortex was 19.03±2.51 vessels/ROI while in the medulla it was 10.6±1.7 vessels/ROI and 5.4±1.3 vessels/ROI in the outer and inner medulla respectively. We then studied vessel distribution in the renal parenchyma which showed that 55% of vascular component is included in the cortex, 30% in the outer medulla, and 15% in the inner medulla.

Collectively, we propose an integrated approach that can be particularly useful in the preclinical setting to characterize the vasculature of any organ accurately and rapidly.

[1] Nordsletten, D.A. et al., 2006. Structural morphology of renal vasculature. American Journal of Physiology-Heart and Circulatory Physiology. 291, 296–309.
[2] Fliser, D. et al., 2011. The dysfunctional endothelium in CKD and cardiovascular disease: Mapping the origin(s) of cardiovascular problems in CKD and kidney disease in cardiovascular conditions for a research agenda. Kidney international supplements. 1, 6–9.
[3] Rossi, L. et al., 2016. Vasculogenic potential evaluation of bottom-up, PCL scaffolds guiding early angiogenesis in tissue regeneration. Journal of Materials Science: Materials in Medicine. 27, 1–11. https://doi.org/10.1007/s10856-016-5720-7

ORAL COMMUNICATIONS

ANIV

Prevalence and antimicrobial resistances of *Salmonella* spp. isolated from wild boar in Liguria region

Valeria Listorti (1), Elisabetta Razzuoli (1), Isabella Martini (1), Laura Migone (1), Lucia Decastelli (2), Walter Mignone (3), Enrica Berio (3), Roberta Battistini (4), Carlo Ercolini (4), Laura Serracca (4), Tiziana Andreoli (5), Monica Dellepiane (5), Daniela Adriano (2), Monica Pitti (2), Daniela Meloni (2), Paola Modesto (1,2)

(1) Istituto Zooprofilattico Sperimentale del Piemonte, Liguria e Valle d'Aosta, Genova (2) Istituto Zooprofilattico Sperimentale del Piemonte, Liguria e Valle d'Aosta, Torino. (3) Istituto Zooprofilattico Sperimentale del Piemonte, Liguria e Valle d'Aosta, Imperia. (4) Istituto Zooprofilattico Sperimentale del Piemonte, Liguria e Valle d'Aosta, La Spezia. (5) Istituto Zooprofilattico Sperimentale del Piemonte, Liguria e Valle d'Aosta, Savona

Corresponding author: Valeria Listorti (valeria.listorti@izsto.it)

Salmonella spp. is one of the most important zoonotic agents, and antimicrobial resistant bacteria are one of the most challenging health problems worldwide [1]. Wild boar are an important reservoir of antimicrobial resistant bacteria including *Salmonella* spp. and might represent a risk for the transmission to humans [2]. The aim of the study was to investigate the presence of *Salmonella* spp. in liver samples of wild boar hunted in Liguria region and to analyse the profiles of antibiotic resistance of the isolated strains. The survey was conducted from 2013 to 2017. 4335 livers were collected and analysed for the presence of *Salmonella* spp. PCR-based method was used for screening analysis using the iQ-Check Salmonella II PCR Detection kit (Bio-Rad, Milan, IT). The isolation of *Salmonella* strains from PCR positive samples was made according to ISO 6579:2002/COR. 1, 2004. While the serotype identification of the isolated strains was carried out according to ISO/TR 6579-3, 2014. Antimicrobial susceptibility against 16 molecules was investigated using the Kirby-Bauer disk diffusion test performed according to Clinical and Laboratory Standard Institute (CLSI) guidelines. A total of 260 *Salmonella* strains belonging to all the six *Salmonella* subspecies have been isolated, with a prevalence of 6%. The 60.4% of the isolated strains were typed as *S. enterica* subs. *enterica;* in particular, 31 serotypes were identified. Among them, seven out twenty of the most frequently *Salmonella* spp. serotypes isolated in human illness [1] have been identified: *S.* Enteritidis (7.7%), *S.* Typhimurium (3.8%), *S.* Typhimurium monophasic variant 1,4,[5],12:i:- (1.5%), *S.* Infantis (1.2%), *S.* Newport (3.1%), *S.* Napoli (8%), and *S.* Coeln (3.5%). The overall other subspecies of *Salmonella* spp. represented the 40% of the isolated strains. The 94.6% of the analyzed strains resulted resistant to at least one of the tested molecules. In particular, the 96% of the tested strains resulted resistant against sulfadiazine + sulfamerazine + sulfamethazine, the 21.9% of the strains to trimethoprim-sulfamethoxazole and the 20% to tetracycline. The 10.8% of the strains tested against streptomycin resulted resistant. A percentage of tested strains ranging from 5 to 10% resulted resistant to ampicillin, amoxicillin + clavulanic acid, cefalotin and kanamycin. A percentage of tested strains, ranging from 1 to 5%, resulted resistant to cefotaxime and gentamicin. Less than the 1% of tested strains resulted resistant to chloramphenicol, colistin, ceftazidime, enrofloxacin, and nalidixic acid. No one strain resulted resistant to ciprofloxacin. Despite the presence of multi-drug resistances, the isolated strains present a high sensitivity rate against antimicrobials considered "Highest Priority Critically Important" [3].

[1] EFSA and ECDC (European Food Safety Authority and European Centre for Disease Prevention and Control), The European Union One Health 2019 Zoonoses Report. EFSA Journal, 19: 6406, 2021. [2] Zottola T., Montagnaro S., Magnapera C., Sasso S., De Martino L., Bragagnolo A., D'Amici L., Condoleo R., Pisanelli G., Iovane G., Pagnini U. Prevalence and antimicrobial susceptibility of *Salmonella* in European wild boar (*Sus scrofa*); Latium Region – Italy. Comp Immunol Microbiol Infect Dis., 36: 161-168, 2013.

Phenotypic and genotypic resistance to colistin in *E. coli* isolated from wild boar (*Sus scrofa*) hunted in Italy

Fabrizio Bertelloni, Giovanni Cilia, Filippo Fratini, Valentina Virginia Ebani, Luca Turini, Domenico Cerri, Barbara Turchi

University of Pisa, Department of Veterinary Science

Corresponding author: Fabrizio Bertelloni (fabrizio.bertelloni@unipi.it)

Colistin, an antibiotic belonging to the class of polymyxins, recently regained attention as one of the last resort antibiotics against some multidrug-resistant bacteria. In 2015 a plasmid-located colistin resistance determinant (*mcr-1*) was identified in *E. coli* and subsequently different *mcr* gene variants were documented [1]. Nowadays, information on colistin resistance in bacteria from wildlife is scarce. The aim of the present study was to evaluate the potential role of wild boar as a reservoir and carrier of colistin resistant bacteria and mobile colistin resistance genes. A total of 168 *E. coli* was isolated from rectal swabs collected from hunted wild boar, during hunting season 2018-2019, in 4 provinces of Tuscany, Italy. Minimum Inhibitory Concentrations (MIC) was employed to evaluate phenotypic resistance against colistin; isolates with MIC values >2 µg/mL were considered resistant. Susceptible and resistant *E. coli* were tested for the presence of *mcr-1* and *mcr-2* genes. Overall, 27.9% of *E. coli* scored resistant to colistin, with MIC values ranging between 4 µg/mL and >256 µg/mL. Obtained results showed a high percentage of colistin resistant *E. coli* in wild boar. This could be linked to a possible "accumulation" of resistant bacteria and/or resistance genes in this omnivorous, semi-synanthropic wild ungulate. Genes *mcr-1* and *mcr-2* were equally distributed in the studied population: 13.6% of *E. coli* harbored both genes, 15.4% scored positive only for *mcr-1* and 15.4% for *mcr-2*. This is in contrast with other studies carried out in Italy on livestock isolates, where *mcr-1* resulted the predominant gene [2]. The presence of both genes was mainly associated with higher MIC values: 21 *mcr* positive isolates had a MIC value >256 µg/mL. Whereas the presence of *mcr-1* or *mcr-2* alone was detected also in susceptible *E. coli* (19.6%). Most of the available studies evaluated the presence of *mcr* genes only in resistant isolates, and few works reported the occurrence of susceptible phenotype in *mcr*-positive strains [2]; however, the lack of expression of resistance genes is well documented, recently also for colistin [1]. A percentage of 53.3% isolates did not show resistance genes and most of them exhibited a low MIC value. Some isolates (3.0%) scored negative for both *mcr-1* and *mcr-2*, but resulted phenotypically resistant; this could be due to the presence of other mobile colistin resistance elements or to chromosomal resistance genes [1]. In conclusion, wild boar could be considered a potential reservoir of colistin resistant bacteria. In the light of the possibility of contacts with domestic animals and humans this wild species could have an important role in the diffusion of this emerging problem and the monitoring programs on wildlife should also include this aspect.

[1] Nang S.C., Li J., Velkov T. The rise and spread of *mcr* plasmid-mediated polymyxin resistance. Crit Rev Microbiol, 45:131–161, 2019.
[2] Magistrali C.F. et al. Mobile colistin resistance genes in *Escherichia coli* from pigs affected by colibacillosis. Int. J. Antimicrob. Agents, 52:744–746, 2018.

Identification of different circoviruses in wild carnivores, Italy

Linda A. Ndiana, Gianvito Lanave, Costantina Desario, Violetta Vasinioti, Giovanni Sgroi, Nicola Decaro

Department of Veterinary Medicine, University of Bari, Valenzano, Bari, Italy

Corresponding author: Nicola Decaro (nicola.decaro@uniba.it)

Canine Circovirus (CaCV) is a relatively newly discovered member of the Family *Circoviridae*. The virus has had conflicting reports regarding its role in enteritis and its clinical manifestations in dogs are still debated (1, 2). Viruses displaying a genome identity up to 80% to CaCV have been identified in different wildlife carnivores from Europe, mainly in wolves (*Canis lupus*) and red foxes (*Vulpes vulpes*) (3, 4). This suggests that an interspecies transmission of circoviruses between wildlife and dogs can occur (3). However, there are limited sequence data of CaCVs from wild animals to clearly depict the portrait of the genetic relatedness to strains detected in domestic dogs.

In view of the multi-host nature of CaCV and in order to further clarify the role of wildlife in the epizootology of this virus, we investigated the presence of circoviruses in wild carnivores in Italy.

A total of 262 samples consisting of spleen (255) and intestines (7) were collected from wild animals found dead, including foxes (*Vulpes vulpes*) (n=232), wolves (*Canis lupus*) (n=8), and Eurasian badgers (*Meles meles*) (n=22). Sampling was carried out in different regions of central and southern Italy during years 2014- 2020 (5). DNA extracts were screened by a real-time PCR (qPCR) assay with specific primers and probe targeting the Rep encoding gene of CaCV. For all positive samples, partial Rep gene amplification was performed by conventional PCR and subsequently sequenced.

CaCV DNA was detected in a total of 8 animals, including 4/22 (18%) Eurasian badgers and 4/8 (50%) wolves, while no red foxes (0/232, 0%) tested positive. Successful amplification and sequencing of partial Rep gene was obtained from 7/8 positive samples. Blast analysis revealed that 4 sequences (2 from Eurasian badgers and 2 from wolves) displayed a 92.8-98.5% nt identity to CaCV detected in domestic dogs. Surprisingly, one sequence (strain 145.20-5432) displayed 68.6% nt identity to feline cyclovirus (KM017740) whilst the other (145.20-1329) was 79.4% nt identical to a cyclovirus of bat (HQ738637); both cyclovirus sequences were identified from wolves.

This study demonstrates the circulation of different circoviruses in wild carnivores, the implication of which should be investigated. Full genome sequencing of the strains detected in this study would give more insight into the genetic composition of circoviruses circulating in wild carnivores. Continuous surveillance of circoviruses in wildlife will help understanding the ecology of these viruses and the interspecies transmission between wild and domestic carnivores.

1) Decaro N, et al. Genomic characterization of a circovirus associated with fatal hemorrhagic enteritis in dog, Italy. PLoS ONE, 9:e105909, 2014.
2) Van Kruiningen HJ, et al. Canine circoviral hemorrhagic enteritis in a dog in Connecticut, J. Vet. Diagn. Investig, 31:732–736, 2019.
3) Zaccaria G, et al. Circovirus in domestic and wild carnivores: An important opportunistic agent?, Virology, 490:69–74, 2016.
4) De Arcangeli et al. Genomic Characterization of Canine Circovirus Detected in Red Foxes (*Vulpes vulpes*) from Italy using a New Real-Time PCR Assay, J Wildl Dis, Jan;56(1):239-242, 2020.
5) Ndiana, Linda A., et al. Circulation of diverse protoparvoviruses in wild carnivores, Italy, Transboundary and Emerging Diseases, 2020.

Hepatitis E virus in a wild-domestic interface

Maria Irene Pacini (1), Mario Forzan (1), Marcello Periccioli (2), Maurizio Mazzei (1)

(1) Università degli Studi di Pisa, Dipartimento di Scienze Veterinarie (2) Azienda USL Toscana Sud Est, Dipartimento di Prevenzione, Unità Funzionale di Sanità Pubblica Veterinaria e Sicurezza Alimentare

Corresponding author: Maurizio Mazzei (maurizio.mazzei@unipi.it)

Hepatitis E virus (HEV) is a non-enveloped, single-strand, positive-sense RNA virus belonging to the *Hepeviridae* family. Molecular analysis has revealed several genotypes (Gt), of which Gt 3 and Gt 4 have a wide host range and are zoonotic agents, both responsible for sporadic and epidemic outbreaks in industrialized countries [1]. Humans can become infected with HEV through the consumption of raw or undercooked meat derived from infected animals or through direct contact with reservoir animals and usually develop acute, self-limiting hepatitis [1]. Although suids are recognized as the main reservoirs of HEV, several other species may act as HEV hosts, including domestic and wild animals [1-4]. Therefore, in ecosystems shared by wild and domestic animals and humans, especially hunters, HEV epidemiology could result in a complex network [2,5]. Moreover, in Italy, and particularly in Tuscany, hunting is a largely diffused activity. The aim of this study was to investigate the circulation of HEV in the most hunted wild species in Tuscany (wild boar and deer), and in hunting dogs.

Sera, organs, and faecal swabs were sampled from 2019 to 2021 in Tuscany from wild boar (n=64), red reed (n= 9), fallow deer (n=66), roe deer (n=79) and hunting dogs (n=76). In detail, liver samples from wild boar, red deer, fallow deer, and roe deer were collected during slaughtering procedures following hunting activity regulated by the Regional Hunting Law (Legge regionale 1994 no. 3 DPGR 48/R/2017). Serum samples and faecal swabs were obtained from dogs undergoing clinical examination in veterinary clinics (Pratica clinica non sperimentale art 2 comma 1 lett b, D.lgs.vo 26/2014). Serological analyses were conducted by HEV Ab Ultra (DIA.PRO), and virological analysis by ELISA antigen kit (HEV-Ag ELISA Kit; XpressBio) and/or by One-step RT-qPCR [6]. Positive virological results were confirmed by sequence analysis. Results confirmed the presence of HEV in the studied area since a total of 5% of sera samples and 3% virological samples resulted positive. Our data indicate the circulation in a specific ecosystem of HEV in different species. Besides, the detection of HEV in hunting dogs raises questions regarding the possible diffusion of the pathogen in domestic animals and its zoonotic potential.

[1] Wang B., Meng XJ. Hepatitis E virus: host tropism and zoonotic infection, Current Opinion in Microbiology, 59:8–15, 2021. [2] Serracca et al.. Molecular Investigation on the Presence of Hepatitis E Virus (HEV) in Wild Game in North-Western Italy, Food and Environmental Virology, 7:206–212, 2015. [3] Di Bartolo et al., Presence of Hepatitis E Virus in a RED Deer (Cervus elaphus) Population in Central Italy, Transboundary and Emerging Diseases, 64:137–143, 2017. [4] Sarchese V., di Profio F., Melegari I., Palombieri A., Sanchez SB., Arbuatti A., Ciuffetelli M., Marsilio F., Martella V., di Martino B. 2019. Hepatitis E virus in sheep in Italy. Transboundary and Emerging Diseases, 66:1120–1125, 2019. [5] Meng XJ. Hepatitis E virus: Animal reservoirs and zoonotic risk, Veterinary Microbiology, 140:256–265, 2010. [6] Jothikumar N., et al., A broadly reactive one-step real-time RT-PCR assay for rapid and sensitive detection of hepatitis E virus, Journal of Virological Methods, 131:65–71, 2006.

Diversity of CRESS DNA viruses in synanthropic squamates

Paolo Capozza (1), Gianvito Lanave (1), Georgia Diakoudi (1), Francesco Pellegrini (1), Roberta Cardone (1), Violetta Iris Vasinioti (1), Nicola Decaro (1), Alberto Alberti (2), Jairo Alfonso Mendoza-Roldan (1), Krisztián Bányai (3), Domenico Otranto (1), Canio Buonavoglia (1), Vito Martella (1)

(1) Università degli Studi di Bari, Dipartimento di Medicina Veterinaria (2) Università di Sassari, Dipartimento di Medicina Veterinaria (3) Institute for Veterinary Medical Research, Centre for Agricultural Research, Budapest, Hungary

Corresponding author: Vito Martella (vito.martella@uniba.it)

The family Circoviridae (CVs) contains viruses with covalently closed, circular, single-stranded DNA (ssDNA) genomes, and includes the smallest known autonomously replicating, capsid-encoding animal viral pathogens. Members of this viral family are known to cause fatal diseases in birds and pigs [1]. Also, CVs have been identified in the stools, blood and cerebrospinal fluid (CSF) of humans, although a possible pathogenic role, if any, has not been demonstrated [2, 3].Squamata reptiles (i.e., snakes and lizards) have become more synanthropic due to the anthropic pressure (i.e., urbanization, habitat lost and deforestation), which has shifted forested animal behavior to an urban and peri-urban adaptation. Synanthropic reptiles in the Mediterranean basin are represented by geckoes (i.e., *Tarentola* spp., *Hemidactylus* spp.) and lacertid lizards (i.e., *Podarcis* spp.) [4]. Using a degenerate PCR protocol and direct sequencing, we identified Circular rep-encoding single-stranded (CRESS) DNA viruses in about 31.7% (33/104) of lizards and geckoes sampled in different Italian areas (Sicily, Basilicata and Apulia). Different CRESS DNA viruses likely reflected dietary or environmental contamination, and included avian-like (N=3), dog (n=4), bat-like (n=1), rodent-like (n=4), goat-like (n=1) and insect-like (n=2) viruses. Rep sequences of at least two types of human-like cycloviruses (CyCV) were identified consistently, regardless of geographic location, namely TN9-like (n=10) and TN12-like (n=6). A third human-like CyCV, TN25-like, was detected in a unique sample. A potential recombinant CyCV (TN9xTN25) strain was also recognized. CRESS DNA viruses were not detected in sera of volunteers (n=100) from an island where sampling in squamates revealed human-like CyCV sequences. After enrichment of circular DNA with rolling cycle amplification (RCA), we generated by inverse PCR the complete genome of TN9-, TN12- and TN25-like CyCVs, of a rodent-like CV, of an insect-like CyCV and of a bat-like CyCV. Whether the CRESS DNA viruses identified in squamates are only contaminant viruses of the intestinal content or they can replicate to some extent in these animals remains unclear. Interestingly, since these animals are synanthropic, and CyCVs have been identified repeatedly in humans with and without clinical signs, squamates could play a role in sustaining CyCV circulation and increasing CyCV pressure in the ecosystem.

[1] International Commettee on Taxonomy of Viruses. Available online: https://talk.ictvonline.org/ictv-reports/ictv_online_report/ssdna-viruses/w/circoviridae/659/genus-circovirus. [2] Li L., et al. Multiple diverse circoviruses infect farm animals and are commonly found in human and chimpanzee feces. J Virol. 2010 2;84(4):1674-82. [3] Smits S., et al. Novel cyclovirus in human cerebrospinal fluid, Malawi, 2010-2011. Emerg Infect Dis. 2013;19(9):1511–3. [4] Todd B., et al. The global status of reptiles and causes of their decline. Ecotoxicology of Amphibians and Reptiles, second ed. Taylor and Francis, Hoboken, pp. 47–67, 2010.

Identification of new astroviruses in synanthropic squamates

Georgia Diakoudi (1), Paolo Capozza (1), Francesco Pellegrini (1), Violetta Iris Vasinioti (1), Gianvito Lanave (1), Cristiana Catella (1), Michele Camero (1), Antonio Parisi (2), Loredana Capozzi (2), Jairo Alfonso Mendoza-Roldan (1), Domenico Otranto (1), Canio Buonavoglia (1), Vito Martella (1)

(1) Università degli studi di Bari Aldo Moro, Dipartimento di Medicina Veterinaria (2) Istituto Zooprofilattico Sperimentale di Puglia e Basilicata

Corresponding author: Vito Martella (vito.martella@uniba.it)

Astroviruses are small, non-enveloped, positive-sense, single-stranded RNA viruses and they are associated with acute gastroenteritis in children worldwide. Since their first description in 1975 in children with diarrhea, astroviruses have been identified in several animals including avian, mammal, reptile, amphibian and fish species (1, 2). Astroviruses have also been associated with hepatitis and nephritis in birds and with encephalitis in human and animal hosts (2, 3). Squamates have become synanthropic reptiles due to the urbanization and are very common and abundant in the urban and peri-urban areas of the Mediterranean basin, represented mainly by geckoes and lacertid lizards.

We screened fecal samples from lizards and geckoes collected in different Italian regions (Apulia, Basilicata, Calabria and Sicily) using a degenerate PCR protocol and direct sequencing and we identified novel astrovirus species in 11% (11/100) samples. The presence of the virus was also detected in the brain of a lizard. Using a 3'RACE RT-PCR protocol, we generated information for the complete capsid protein (ORF2) and partial RdRp genomic (ORF1b) regions for 6 of the 11 positive samples. Upon sequence analysis, the astroviruses displayed up to 48.2% identity at the nucleotide level to other astroviruses identified in squamates (1). The Italian strains shared 41.8-97.7% nucleotide and 29.3-99.1% amino acid identity to each other in the ORF2 gene. Upon phylogenetic analysis, the viruses formed three distinct clusters highlighting the variability of the circulating astroviruses. Interestingly, similar astrovirus strains were identified in different geographic locations, a pattern suggestive of species-specificity. The pathogenic role, if any, of the astroviruses identified in squamates remains to be elucidated. Even though astroviruses are considered to infect in a species-specific manner, lately these viruses have been related to cross-species transmission and recombination events (2, 4). Since some reptiles are synanthropic and often part of the diet of feral and domestic cats, they might play a role as carriers, maintaining the circulation of some pathogens in the ecosystem.

[1] Shi et al. The evolutionary history of vertebrate RNA viruses. Nature, 556:197–202, 2018.
[2] Wohlgemuth N., Honce R., Schultz-Cherry S. Astrovirus evolution and emergence. Infection, Genetics and Evolution, 69:30-37, 2019.
[3] Johnson et al. Astrovirus Pathogenesis. Viruses, 9(1):22, 2017.
[4] De Benedictis et al. Astrovirus infections in humans and animals - molecular biology, genetic diversity, and interspecies transmissions. Infection, Genetics and Evolution, 11(7):1529-1544 (2011).

Antibiotic resistance in *Escherichia coli* from wild birds recovered in a wildlife rescue centre

Ilaria Prandi, Patrizia Robino, Patrizia Nebbia, Ugo Ala, Alessandro Bellato, Maria Cristina Stella, Mitzy Mauthe von Degerfeld, Giuseppe Quaranta

Università degli Studi di Torino, Dipartimento di Scienze Veterinarie.

Corresponding author: Patrizia Robino (patrizia.robino@unito.it)

Commensal bacterial strains isolated from wild birds can be used to monitor changes in antimicrobial resistance since those animals usually do not receive antibiotic treatments, but they suffer the effect of anthropogenic activities. The aim of this work was to evaluate the consequence of a hospitalization period on antimicrobial resistance in fecal *Escherichia coli* isolated from wild bird species (raptors and synanthropic birds) admitted in the wildlife rescue centre of the Department of Veterinary Sciences (Turin University, Italy). In addition, the level of anthropization of the collection areas of the different rescued species was assessed. From 2017 to 2019, cloacal samples were collected from 145 birds, including 101 raptors and 44 synanthropes, at time of arrival as well as 5 and 10 days afterwards, searching for *E. coli* strains and testing their susceptibility to a panel of seven antibiotics.

At admittance, *E. coli* were found in 93 animals, which were included in this study. Out of 93 isolated strains, 83 (89.2%) were resistant towards at least one antimicrobial agent, 36 (38.7%) were multi-drug resistant (MDR) and 8 (8.6%) were Extended spectrum beta-lactamase (ESBL)-producing *E. coli*. Antimicrobial resistance increased after 10 days of hospitalization (OR=1.35, $p<0.01$), especially in animals treated with antimicrobial therapy. A relevant increase in resistance was observed towards marbofloxacin (OR=15.72, $p<0.01$), sulfamethoxazole-trimethoprim (OR=7.30, $p<0.01$) and tetracycline (OR=5.82, $p<0.01$). Also, ESBL-producing *E. coli* increased during hospitalization (15.2%). These findings did not differ between birds of prey and synanthropic species. As expected, raptors were found in places with lower density of human activities, while synanthropic species were rescued in highly inhabited municipalities. However, antibiotic resistance levels of bacteria isolated were equivalent.

Our results suggest that wild birds could act as reservoirs of MDR bacteria, being potential sources for their spreading in the environment and to other species. Finally, to the best of authors' knowledge, this is the first investigation of the effects of hospitalization and antibiotic treatment on the development of antimicrobial resistance in *E. coli* isolated from wild birds.

[1] Guenther S., Grobbel M., Lu A., Becker B., Goedecke A., Friedrich N.D., Wieler L.H. Antimicrobial resistance profiles of *E. coli* from common European wild bird species, Vet Microbiol, 144:219-225, 2010.

[2] Giacopello C., Foti M., Mascetti A., Grosso F., Ricciardi D., Fisichella V., Lo Piccolo F. Antimicrobial resistance patterns of *Enterobacteriaceae* in European wild bird species admitted in a wildlife rescue centre, Vet Italiana, 52:139-144, 2016.

An overview on Wildlife Rescue Center "Federico II" activities in Naples, Italy

Adriano Minichino (1,2), Ludovico Dipineto (1), Ilaria Bongiorno (3), Giuseppina Mennonna (3), Pasquale Raia (2,3), Marina Pompameo (3), Vincenzo Caputo (3), Alessandro Fioretti (1,2)

(1) Università degli Studi di Napoli Federico II, Dipartimento di Medicina Veterinaria e Produzioni Animali (2) Centro di Recupero Animali Selvatici (CRAS) Federico II (3) Centro di Riferimento Regionale per l'Igiene Urbana Veterinaria, Asl Na1 centro

Corresponding author: Adriano Minichino (adrianominichino@gmail.com)

The wildlife rescue centre (CRAS) "Federico II" of Napoli was founded in 2010 with the aim to rehabilitate sick, injured, and orphaned native wildlife and releases healthy animals to their natural habitat. Through educational outreach, the CRAS provides environmental awareness, promotes a harmonious relationship with native wildlife, and encourages the community to protect our delicate ecosystems.

This study aimed to describe the main activities of the wildlife rescue centre "Federico II" of Napoli. centre "Federico II" of Napoli through a retrospective analysis performed on clinical records from 2018 to 2020. A total of 6162 animals were admitted at the centre and consisted of birds (n= 5654; 92%), reptiles (*n*=109; 2%), mammals (*n*=399; 6%). The main causes of admission were represented by *trauma* (*n*=1726; 28%) including fractures, luxations, and wounds; medical causes (*n*=1480; 24%) including shock, starvation, infections and poisoning; orphaned juveniles (*n*=1120; 18%); legal confiscation (*n*=1350; 22%), and other causes (*n*=486; 8%) as plumage injuries and metabolic diseases. A total of 6156 animals came from Campania region and, specifically, from Napoli (*n*=4444; 72%), Caserta (*n*=702; 11%), Avellino (*n*=283; 5%), Salerno (*n*=599; 10%), Benevento (*n*=128; 2%). In addition, 6 animals came from outside of the Campania region as Latina (*n*=1; 0.01%), Isernia (*n*=2; 0.03%) and Potenza (*n*=3; 0.05%). Animals were committed by "private citizen" (*n*=730; 38%), "Local health authorities" (*n*=265; 14%), "law enforcement" (*n*=699; 37%), "animals welfare association" (*n*=218; 11%). The average time of permanence is related to the cause of admission. In case of trauma, hospitalized animals remain from 7 to 60 days; the orphaned juveniles, from 15 to 50 days; medical causes, from 48 hours to 15 days; legal confiscation, from 48 hours to 180 days; other causes, from 48 hours to 180 days. The follow-up showed that *n*=3402 (55%) of the animals were released, and *n*=2761 (45%) died. The admissions were more frequent from May to August. This trend is due to a higher percentage of juvenile subject admitted, related to the reproduction and breeding season. According to the One Health concept, wild animals could be considered as a perfect environmental bioindicator. Moreover, in synergy with the Regional Centre for Urban Veterinary Hygiene (CRIUV) the CRAS also collect samples for epidemiological monitoring of zoonotic diseases in the Campania Region. Therefore, our results could represent a source of information that may lead to an improvement of the knowledge of the wildlife.

[1] Molina-Lopez R.A., Casal J., Darwich, L. Causes of morbidity in wild raptor populations admitted at a wildlife rehabilitation centre in Spain from 1995- 2007: A long term retrospective study, PLoS ONE, 6:e24603, 2011.

[2] Rodriguez B., Rodriguez A., Siverio F., Siverio M. Causes of raptor admissions to a wildlife rehabilitation center in Tenerife (Canary Islands), Journal of Raptor Research, 44:30-39, 2010.

[3] Grogan A, Kelly A. A review of RSPCA research into wildlife rehabilitation, Vet Rec, 172(8):211, 2013.

[4] Martinez J.A., Izquierdo A., Zuberogoitia I. Causes of admission of raptors in rescue centres of the East of Spain and proximate causes of mortality, Biota, 2:163–169, 2001.

Evaluation of a vaccination protocol using an inactivated gE-deleted marker vaccine against Bovine alphaherpesvirus 1 (BoHV-1) in water buffalo

Stefano Petrini (1), Alessandra Martucciello (2), Francesco Grandoni (3), Giovanna De Matteis (3), Giovanna Cappelli (2), Monica Giammarioli (1), Eleonora Scoccia (1), Carlo Grassi (2), Cecilia Righi (1), Giovanna Fusco (2), Giorgio Galiero (2), Michela Pela (1), Gian Mario De Mia (1), Esterina De Carlo (2)

(1) Centro di Referenza Nazionale per la Rinotracheite Infettiva del Bovino (IBR), Istituto Zooprofilattico Sperimentale dell'Umbria e delle Marche "Togo Rosati" (2) Centro di Referenza Nazionale per l'Igiene e la tecnologia dell'allevamento delle produzioni bufaline, Istituto Zooprofilattico Sperimentale del Mezzogiorno (3) Centro di Ricerca per la zootecnica e l'acquacoltura, Consiglio per la Ricerca in Agricoltura e l'analisi dell'economia (CREA)

Corresponding author: Stefano Petrini (s.petrini@izsum.it)

BoHV-1 can infect several animal species, including buffalo [1]. In this study, we evaluated a vaccination protocol using the DIVA strategy against BoHV-1 in this species. The Italian Ministry of Health approved the experiments under authorization number 859/2017-PR. For this purpose, ten buffaloes devoid of BoHV-1 neutralizing antibodies (NA) were used. The animals were divided into two groups (A, B) of 5 animals each. Group A was immunized with an inactivated gE-deleted marker vaccine. Two doses of the vaccine at 2 mL were administered to each animal at an interval of 30 days, starting at the age of 15 months. The vaccine was injected intramuscularly. Group B represented negative control. Sixty days after the first immunization, all buffaloes were challenged with a virulent BoHV-1 strain. Rectal temperature, serum and whole blood samples were taken a different experimental time. The vaccine did not induce any clinical signs or adverse reactions. A progressive increase in the NA titre was observed in group A with a mean titre of 1:15 and 1:74 on post-vaccination day 30 and 60, respectively. After the challenge, the only animals in group B showed clinical signs and shed the virus. Group A progressively increased the NA titre until the end of the experiment (59 PID) with a mean NA titre of 1:1,690. Differently, group B evidenced NA 15 PID with a mean NA titre of 1:27, and at 59 PID, this value increased to 1:64. In group A, a positive signal for gE was detected only on 59 PID. Otherwise, in group B, antibodies to gE were detected 15 PID, and the same value was maintained until 59 PID. In agreement with those published previously on cattle [2], the results of this study suggested that in buffalo, there is no risk of adverse reactions or clinical signs after the immunization. Moreover, in accordance with other reports [3], the immune response to vaccination was effective and protected the buffalos against the challenge. In conclusion, the protocol used in this study represents a valuable tool in protecting the buffalo species against BoHV-1 infection within the Regulation "Animal Health Law".

[1] Scicluna M.T., Caprioli A., Saralli G., Manna G., Barone A., Cersini A., Cardeti G., Condoleo R.U., Autorino G.L. Should the domestic buffalo (Bubalus Bubalis) be considered in the epidemiology of Bovine Herpesvirus 1 infection? Veterinary Microbiology, 143: 81-88, 2010.
[2] Petrini S., Righi C., Iscaro C., Viola G., Gobbi P., Scoccia E., Rossi E., Pellegrini C., De Mia G.M. Evaluation of passive immunity induced by immunisation using two inactivated gE-deleted marker vaccines against infectious bovine rhinotracheitis (IBR) in calves. Vaccines, 2020, 8(1).
[3] Castrucci G., Frigeri F., Salvatori D., Ferrari M., Sardonini Q., Cassai E., Lo Dico M., Rotola A., Angelini R. Vaccination of calves against bovine herpesvirus-1: assessment of the protective value of eight vaccines. Comparative Immunology, Microbiology and Infectious Diseases, 25: 29-41, 2002.

Streptococcus equi subsp. *zooepidemicus*: a relevant opportunistic equine pathogen

Francesca Paola Nocera (1), Monica Ambrosio (1), Elena D'Eletto (1), Filomena Fiorito (1), Luisa De Martino (1,2)

(1) Università degli Studi di Napoli "Federico II", Dipartimento di Medicina Veterinaria e Produzioni Animali (2) Università degli Studi di Napoli "Federico II", Task Force on Microbiome Studies (Napoli, Italia)

Corresponding author: Francesca Paola Nocera (francescapaola.nocera@unina.it)

Streptococcus equi subsp. *zooepidemicus* is a β-hemolytic streptococcus belonging to the Lancefiel group C; it is a rare human pathogen but is commonly isolated from bacterial infections in a wide range of animal species. Mostly in horses, it is frequently associated with endometritis. In this study, 196 uterine swabs were collected from mares with suspected bacterial endometriosis in the year 2018. Precisely, samples were plated on different types of solid culture agar media and incubated aerobically at 37°C for 24-48 h. Moreover, the same swabs were also inoculated in the broth-enrichment Brain Heart Infusion (BHI) and incubated aerobically at 37°C for 24 h. The day after, turbid BHI tubes were sub-cultured on the same agar plates. Once bacterial growth was detected, the isolated strains were first screened by using standard, rapid techniques: colony morphology, cellular morphology by Gram's staining method, catalase and oxidase tests. Then, the isolates were identified by matrix assisted laser desorption ionization time of flight mass spectrometry (MALDI-TOF-MS). The prevalence of positivity for *Streptococcus equi* subsp. *zooepidemicus* was 11.7% (23 strains). Only 4 (17.4%) samples gave bacterial growth on solid media without requiring enrichment, while the other isolates (19 strains, 82.6%) were obtained only after the broth enrichment step. The antibiotic resistance profiles were evaluated by the disk diffusion method on Mueller Hinton agar plates, according to the Clinical and Laboratory Standards Institute guidelines. High percentages of resistance to amikacin (95.6%) and other tested aminoglycosides, ampicillin (73.9%), tetracycline (69.6%) and colistin sulfate (82.6%) were observed. The determination of antibiotic susceptibility patterns revealed that only third generation cephalosporins, such as ceftiofur or ceftriaxone, were highly effective with 82.6% and 78.3% of the isolates inhibited, respectively. An alarming result was represented by the high prevalence of multidrug-resistant strains with 82.6% of the total isolates.

In conclusion, our results indicate that the rate of positivity for the detection of *Streptococcus equi* subsp. *zooepidemicus* in equine uterine swabs is significantly increased with the additional phase of broth-enrichment culture in comparison with direct-plating of samples.

The increasing spread of multidrug-resistant strains has become a relevant veterinary issue, highlighting the need of a continuous surveillance of this pathogen, in order to allow a rapid and effective antimicrobial treatment and, consequently, increase the pregnancy rate in mares.

In addition, *Streptococcus equi* subsp. *zooepidemicus* is not only a relevant veterinary pathogen, but has a zoonotic importance, with horses acting as reservoirs for humans, particularly horse personnel and veterinarians. Thus, further studies on risk factors on its zoonotic transmission are needed.

[1] Petersen M.R., Nielsen J.M., Lehn-Jensen H., Bojesen A.M. *Streptococcus equi* subsp. *zooepidemicus* resides deep in the chronically infected endometrium of mares, Clin. Theriogenol., 1: 393–409, 2009.

[2] Pisello L, Rampacci E, Stefanetti V, Beccati F, Hyatt DR, Coletti M, Passamonti F. Temporal efficacy of antimicrobials against aerobic bacteria isolated from equine endometritis: an Italian retrospective analysis (2010-2017), Vet. Rec., 185:598, 2019.

Results of a selective dry cow therapy approach in a commercial dairy farm of Lombardia

Laura Filippone Pavesi, Valentina Vailati, Clara Locatelli, Claudia Pollera, Giulia Sala, Valerio Bronzo

Università degli Studi di Milano, Dipartimento di Medicina Veterinaria

Corresponding author: Laura Filippone Pavesi (laura.filipponepavesi@studenti.unimi.it)

The animal health and welfare, associated with the decrease of drugs usage, have become one of the most important issues for farmers and consumers in the last decades. Antibiotics were widely used to prevent or treat bacterial infectious diseases. In the recent year, antibiotic drugs are administered more responsibly due to two main reasons: the consumer fears of residual drugs in food products and the possible development of microbial resistance (1). A relevant issue in dairy farms is related to the selective dry cow therapy approach, in order to reduce the use of antibiotics during dry period, in cows with low SCC and without intramammary infections (IMI) at dry-off (2). This study reports preliminary results regarding the use of selective dry cow therapy, in a commercial dairy farm in northern Italy. Selected herd is composed by 460 milking cows and is free from contagious udder pathogens. Selective dry therapy approach started in October 2020 with the aim to reduce antibiotics usage during dry period of 40%. Presented data regarding 27 enrolled cows, selected according to the following criteria: average SCC<200.000 cells/ml and no IMI from major udder pathogens. Cows were sampled at dry-off and 10 days after calving in order to asses SCC and IMI status, moreover they will be monitored for the first 100 days of lactation to record any cases of clinical mastitis. After sampling at dry-off cows were randomly assigned to the following treatment, only internal teat sealant (ITS) (16 cows) or intramammary antibiotic treatment and internal teat sealant (A+ITS) (11 cows). Results show no significant differences in IMI distribution at dry-off, between quarters dried only with teat sealant (20.3 %) and with antibiotic+teat sealant (36.4%); while SCC at dry-off was significantly higher in quarters dried with antibiotic+teat sealant (336,520 Vs 98,690 cells/ml). After calving were non-significant differences in IMI distribution between quarters belonging to two groups (ITS 25%; A+ITS 22.2%) and also in SCC values (ITS 50,790 cells/ml; A+ITS 226,920 cells/ml). IMI in quarters dried with only ITS showed a non-significant slight increase (22.5 Vs 25%) and a non-significant decrease in SCC values (98,690 Vs 50,790 cells/ml); while quarters dried with A+ITS showed a non-significant decrease in IMI (36.4 Vs 22.2 5 %) and a significant decrease in SCC values (336,520 Vs 226,920). No clinical mastitis was observed in both groups after calving. Despite the preliminary status of results, this study confirms that it is possible to apply selective dry therapy, without risk on new infection or SCC increase at calving, considering cows without IMI from major pathogens and SCC values <200.000 cells/ml in previous lactation period. The use of selective dry cow therapy is confirmed to be therefore a valuable method for reduction and more responsible usage of antibiotics in dairy farms in a one health perspective (3).

[1] Bronzo V., Lopreiato V., Riva F., Amadori M., Curone G., Addis M.F., Cremonesi P., Moroni P., Trevisi E., Castiglioni B. The Role of Innate Immune Response and Microbiome in Resilience of Dairy Cattle to Disease: The Mastitis Model, Animals (Basel), 10(8): 1397, 2020. Krömker V., Leimbach S. Mastitis Treatment-Reduction in antibiotic usage in dairy cows, Reprod. Domest. Anim. 52(Suppl 3): 21-29, 2017. Garcia S.N., Osburn B.I., Cullor J.S. A one health perspective on dairy production and dairy food safety, One Health, 7: 100086, 2019.

Methicillin-resistant staphylococci from ovine bulk-tank milk: identification and characterization

Barbara Turchi, Fabrizio Bertelloni, Luca Turini, Letizia Tartufoli, Domenico Cerri, Filippo Fratini

Università di Pisa, Dipartimento di Scienze Veterinarie

Corresponding author: Barbara Turchi (barbara.turchi@unipi.it)

Methicillin-resistant staphylococci represent one of the main causes of severe infection in humans and animals. They can easily spread through the dairy food chain, especially when involved in subclinical mastitis and released through milk without any clinical signs or milk alteration [1]. While literature on the presence methicillin-resistant *Staphylococcus aureus* (MRSA) in bovine milk is extensive [2], information on sheep milk is limited and even more scant when considering coagulase-negative species. The aim of this work was to assess the prevalence of methicillin-resistant staphylococci in samples of bulk-tank ovine milk collected in Tuscany and Latium (Italy). Ninety-seven samples, each of them from a different farm, were analysed. Methicillin-resistant staphylococci were recovered employing a selective pre-enrichment step, followed by sub-culture on selective agar medium (Mannitol Salt Agar, 4 mg/ml oxacillin). Gram positive and catalase positive cocci were subjected to DNA extraction and PCR for the detection of *mecA* and *mecC* genes associated with methicillin resistance. From 4 milk samples (4.1%), *mecA* positive staphylococci were isolated. One of them was coagulase-positive and 3 coagulase-negative. These isolates were subjected to phenotypic/genotypic identification and characterization (antibiotic susceptibility profile by disk diffusion method, slime production, presence of gene other than *mec* coding for antibiotic resistance, presence of gene coding for toxins, biofilm production and disinfectant resistance). Using the API-Staph system, one isolate was identified as *S. aureus*, while the other as *Staphylococcus sciuri* (n:2) and *Staphylococcus lentus* (n:1). The sequencing analysis of *rpoB* gene revealed the unreliability of phenotypic system for the identification of coagulase-negative staphylococci of animal origin. Indeed, only *S. aureus* identification was confirmed, while the other isolates were ascribed to *Staphylococcus fleurettii* species. They all were resistant to ampicillin and susceptible to gentamicin, trimethoprim-sulfamethoxazole, and chloramphenicol. *S. aureus* was also resistant to cephalothin, cefoxitin, cefotaxime, amoxicillin/clavulanic acid, and kanamycin, while only one out of the three *S. fleurettii* was resistant to tetracycline. As for *S. fleurettii* phenotypic resistance against cephalosporins, the employment of different criteria (EUCAST or CLSI) for results interpretation, might lead to a different isolate's categorization. Only *S. aureus* was positive for biofilm production. As for virulence-associated genes, only few of them were detected. In more details, 3/4 isolates harboured *tetK* (tetracycline resistance) and 1/4 isolates *blaZ* (penicillins resistance). In accordance with other authors, the prevalence of methicillin-resistant staphylococci in ovine bulk tank milk seems to be very low. However, it is important not to underestimate the risks associated with their presence within the farm and promote the correct use of antibiotics for mastitis treatment.

[1] Caruso et al. Methicillin-resistant *Staphylococcus aureus* (MRSA) in sheep and goat bulk tank milk from Southern Italy. Small Ruminant Research, 135:26-31, 2016.
[2] Lim et al. Transmission and persistence of methicillin-resistant *Staphylococcus aureus* in milk, environment, and workers in dairy cattle farms. Foodborne pathogens and disease, 10(8):731-736, 2013.

Exploring factors influencing passive transfer of colostral immunoglobulins in foals

Elisa Rampacci, Karen Mazzola, Francesca Beccati, Fabrizio Passamonti

Università degli Studi di Perugia, Dipartimento di Medicina Veterinaria

Corresponding author: Elisa Rampacci (elisa.rampacci@gmail.com)

The epitheliochorial structure of the equine placenta does not allow the transfer of immunoglobulins (IgG) to the fetus. As a result, foals are essentially considered agammaglobulinemic at birth and rely on the ingestion of colostrum to absorb IgG [1]. Failure of the foal to ingest or absorb IgG from colostrum resulting in serum IgG less than or equal to 400 mg/dl is defined as failure of passive transfer (FPT). A level of serum IgG of 400 to 800 mg/dl is considered partial failure of passive transfer (PFPT) [2]. These conditions predispose to septicaemia (or sepsis), which is a leading cause of illness and death in neonatal foals.

Against this background, we undertook a study aimed at analysing individual and management factors that potentially influence the passive transfer of IgG to foals.

Colostrum and serum samples were collected from 81 mares bred in Central Italy and their 81 foals, respectively, and analysed for IgG concentration by the gold standard method Single Radial Immunodiffusion (SRID), as recommended by the manufacturer (IDBiotech, France). Briefly, after diluting colostrum 1:600 and sera 1:150, 15 µL of each sample and standard was dispensed in SRID plate wells and incubated in a humid box at 37 °C for 22-24 h. At the end of the incubation period, the plates were bathed with 2% acetic acid solution for 1 min and rinsed twice with deionised water. Diameters of precipitates were recorded and the corresponding concentration of each sample was calculated based on the generated standard curve. Statistical tests were applied by JASP software to evaluate the influence of breeding farm management, breed, maternal age and parity, dystocia, timing of colostrum feeding, and early foal health on IgG passive transfer in the selected study population, as more appropriate.

IgG levels found in colostrum and sera were strongly correlated ($r=0.57$; $p<0.001$) by Pearson test. No statistically significant association was found between variables and colostrum quality. However, descriptive data suggested a potential contribution of breeding farm management, dystocia, and breed to colostrum quality, and breeding farm management, advanced material age, breed, dystocia, timing of colostrum feeding and colostrum quality assessed by stall-side Brix tests to adequate IgG passive transfer. Four out of the 81 foals (4.9%) had serum IgG less than 400 mg/dl (FPT), while 10/81 foals (12.3%) had circulating IgG comprised between 400 and 800 mg/dl (PFPT). Seven out of 14 foals (50%) showing inadequate IgG transfer suckled colostrum of poor quality (IgG less than 5000 mg/dl), mainly due to complicated lactation. A 28.6% of foals with severe or partial FPT developed respiratory infections within the first three months from birth but the survival rate was 100%.

IgG absorption is the first challenge for neonatal foals as they are at risk from birth until their immune system is mature enough to produce antibodies. Therefore, enhancing foaling management and preventive strategies is key to minimize FPT and neonatal infections.

[1] Dawson T et al. Current understanding of the equine immune response to *Rhodococcus equi*. An immunological review of *R. equi* pneumonia, Veterinary Immunology and Immunopathology, 135:1-11, 2010.

[2] Davis R, Giguère S. Evaluation of five commercially available assays and measurement of serum total protein concentration via refractometry for the diagnosis of failure of passive transfer of immunity in foals. Journal of the American Veterinary Medical Association, 227:1640-1645, 2005.

Coxiella burnetii seropositivity and associated risk factors evaluation among dairy cattle population in Campania region

Gianmarco Ferrara (1), Serena Montagnaro (1), Barbara Colitti (2), Ugo Pagnini (1), Sergio Rosati (2), Giuseppe Iovane (1)

(1) Università degli Studi di Napoli Federico II, Dipartimento di Medicina Veterinaria e Produzioni Animali (2) Università degli Studi di Torino, Dipartimento di Scienze Veterinarie

Corresponding author: Gianmarco Ferrara (gianmarco.ferrara@unina.it)

Coxiella burnetii, a widespread intracellular bacterium responsible for Q fever, affects multiple mammals including ruminants, the main reservoir and sources for human infection. This infection has an economic impact on livestock industry and is responsible of abortion and infertility disorders in ruminants [1].
This zoonotic pathogen has also been considered a potential bioterrorism agent by Center for Disease Control due to its very low infecting dose, its resistance in the environment and the ability to be spread by the wind. The Dutch outbreak, occurred between 2007 and 2010, was the largest ever reported with more than 4,000 notified cases, which fully revealed the impact on public health [1]. Currently, in Italy, information about Coxiellosis in ruminants is incomplete and based on reports of reproductive disorders in livestock farms. Furthermore, human outbreaks are periodic and mostly associated with direct or indirect exposure to infected flocks, particularly in Northern Italy.
The aim of the present study was to evaluate Q fever seroprevalence in dairy cows using an indirect commercial ELISA and an homemade recombinant based ELISA, analyzing the correlation between the main risk factors and positive animals. A total of 412 serum samples were randomly collected from dairy herds in Campania region and were assessed for the detection of specific IgG against *C. burnetii*.
Fifty-nine samples were positive to the commercial assay, confirming the extensive circulation of *C. burnetii* in Campania: Avellino 13%, Benevento 8%, Caserta 18.75%, Salerno 17%. Results obtained with equivalent approaches in other Italian regions found similar data in Piedmont and Sardinia among small ruminant population and, recently, among dairy cows in Sicily, indicating comparable epidemiological situations [2] [3] [4].
A questionnaire was administered to each owner in order to investigate on potential main risk factors (mostly concerning farm management) and their association with *C. burnetii* seropositivity. Statistical analysis performed on collected data indicated several variables significantly associated with higher prevalence: herd model, presence of other ruminant species, frequent animal introduction, anamnesis of reproductive disorders.
These results were in agreement with other studies described in literature which showed how different factors could affect the infection distribution. [4]. The overall seroprevalence found with the commercial kit was quite comparable to the one obtained using the prototype recombinant antigen ELISA: 54 out of 255 animals had antibodies against the recombinant-Ybgf protein. This discrepancy can be explained by the different nature and accuracy between the two tests involved (the commercial assay is based on phase I and phase II native antigens).
This study represents the first serosurvey for Q fever in Campania region and shows how dairy cattle are exposed to *C. burnetii* providing the basis for its prevention.

[1] Roest HI et al. The Q fever epidemic in The Netherlands: history, onset, response and reflection. Epidemiol. Infect. 139(1):1-12, 2011. [2] Galluzzo P et al. Seroprevalence of *Coxiella burnetii* in dairy cattle from Sicily. Vet. Ital. 55(3):247-252, 2019. [3] Masala G et al. Occurrence, distribution, and role in abortion of *Coxiella burnetii* in sheep and goats in Sardinia, Italy. Vet. Microbiol. 99(3-4):301-5, 2004. [4] Rizzo F et al. Q fever seroprevalence and risk factors in sheep and goats in northwest Italy. Prev. Vet. Med. 130:10-7, 2016.

Feeding pre-weaned calves with waste milk containing antibiotic residues is related to a higher incidence of diarrhea and alterations in the fecal microbiota

Martina Penati (1), Giulia Sala (1), Filippo Biscarini (2), Antonio Boccardo (1), Valerio Bronzo (1), Bianca Castiglioni (2), Paola Cremonesi (2), Paolo Moroni (1,3), Davide Pravettoni (1), Maria Filippa Addis (1)

(1) Università degli Studi di Milano, Dipartimento di Medicina Veterinaria (2) Consiglio Nazionale Delle Ricerche, Istituto di Biologia e Biotecnologia Agraria (3) Quality Milk Production Services, Animal Health Diagnostic Center, Cornell University

Corresponding author: Martina Penati (martina.penati@unimi.it)

The milk produced by cows receiving antibiotics for intramammary infection (IMI) is considered waste milk (WM). A convenient alternative for farmers is using it as calf food [1]. However, adding to the risk of selecting resistant bacteria, residual antibiotics might interfere with the gut microbiome development and influence gastrointestinal health [1]. We assessed the longitudinal effect of a standardized WM from 5 cows treated with intramammary cephalexin on the calf intestinal health and fecal microbiota in an 8-week trial. After 3 days of colostrum, 6 calves received WM, and 6 received bulk tank milk (BM) for 2 weeks. Then, for 6 weeks, all 12 calves received a weaning diet of milk substitute and starter feed. Every week for the first 2 weeks, and every 2 weeks for the remaining 6, we subjected all calves to clinical examination [2] and collected rectal swabs to investigate the fecal microbiota composition by 16S rRNA gene analysis [3]. Research protocols were approved by the University of Milan (protocol number 78_2018).

Almost all WM calves (5/6) developed diarrhea in the first 2 weeks (vs 1/6 BM calves). In the following 6 weeks, only 1 episode of diarrhea occurred in 1 WM calves. WM calves' body weight was significantly lower than BM calves along the trial. The 16S rRNA gene analysis indicated a sharp reduction in the fecal microbiota alpha-diversity of WM vs BM calves, most significant at Wk4 ($p<0.02$), two weeks after exposure to WM. Beta-diversity of the fecal microbiota evolved in all calves ($p=0.0069505$ difference between time-points). As for the alpha-diversity results, significant differences were observed between WM and BM calves at Wk4 ($p<0.05$).

Based on the normalized relative OTU levels, WM and BM calves showed significant differences at all timepoints. At the end of the trial, *Bacteroidetes*, *Firmicutes*, and *Saccharibacteria* decreased while *Chlamydiae* increased. Significant changes were also observed in 7 classes, 8 orders, 19 families, 47 genera. Among the most relevant findings was the general decrease of beneficial taxa, like *Faecalibacterium* [4], vs an increase in other taxa and potential pathogens, including *Campylobacter, Pseudomonas*, and *Chlamydophila* [5,6]. Lactobacillus and Lachnoclostridium increased, but since the first was present in the milk substitute, its higher abundance in WM calves might indicate a lower microbiome resilience.

In conclusion, adding to the risk of increasing antibiotic resistance, feeding pre-weaned calves with WM is related to a higher incidence of calf diarrhea and relevant changes in the fecal microbiota composition.

[1] Ricci et al. Risk for the development of Antimicrobial Resistance (AMR) due to feeding of calves with milk containing residues of antibiotics. EFSA J 15, 2017.

[2] Boccardo et al. Risk factors associated with case fatality in 225 diarrhoeic calves: A retrospective study. *Vet J* 228:38–40, 2017.

[3] Biscarini et al. Rumen microbiome in dairy calves fed copper and grape-pomace dietary supplementations: Composition and predicted functional profile. *PLoS One* 13:e0205670, 2018.

Prevalence and risk factors associated with nasal carriage of methicillin-resistant staphylococci in horses and their caregivers in North-Western Italy

Alessandro Bellato (1), Michela Bullone (1), Patrizia Robino (1), Patrizia Nebbia (1), Sara Morello (2), Daniela Marchis (2), Alberto Tarducci (1), Giuseppe Ru (2)

(1) Università degli Studi di Torino, Dipartimento di Scienze Veterinarie (2) Istituto Zooprofilattico Sperimentale del Piemonte, Liguria e Valle d'Aosta

Corresponding author: Alessandro Bellato (alessandro.bellato@unito.it)

Prevalence, characteristics, and risk factors for methicillin-resistant staphylococci (MRS) carriage in horses and their caregivers were studied in the North-Western Italian area. One hundred and ten healthy horses housed at 21 different barns and 34 persons in close contact with these horses were screened for the presence of MRS. Questionnaires were employed to collect data on barns, horses, and personnel. Nasal samples were taken from horses, caregivers and from the environment, processed for MRS detection and for investigating antimicrobial resistance (AMR) patterns against the most commonly employed molecules in equine clinical practice in our region. Only barns housing more than 6 horses were included in the study [1]. Horses had to be healthy based on history and clinical exam, and free from antimicrobials for at least 2 months at the time of sampling [2]. Personnel were included on a voluntary basis. Feed were analyzed for antimicrobial residues as a possible risk factor for AMR in isolated bacterial strains. The study protocol was approved by the local human and animal ethical committees (no. 63 - 26/06/2019 and n. 936 - 16/04/2019, respectively). Informed consent to collect the samples was obtained by the people directly involved in the study and by horse owners.

Methicillin-resistant staphylococci were isolated from 33 horses (30%), 11 humans (32.4%) and 3 environmental samples (14.2%). The isolates belonging to the Staphylococcus genus were all coagulase negative. *Staphylococcus sciuri* and *fleurettii* were predominant among equine MRS isolates, whereas *S. epidermidis* predominated in humans. The prevalence of MRS was greater in racehorses and their personnel compared to pleasure riding horses and show horses. Antimicrobial residues in feeds were unrewarding. The number of antimicrobial treatments administered at the barn during the last 12 months was the only risk factor associated with MRS carriage in horses. Based on the inclusion criteria we employed, this is significant as it shows that even when antimicrobial administration is implemented in a single animal, due to specific therapeutic needs, it has a long-term impact on the whole population the horse lives in. Moreover, we found that MRS carriage in caregivers was weakly associated with increased prevalence of MRS carriage in horses, supporting the existence of a significant interaction among bacterial community of horses and humans sharing the same environment [1], which deserves further attention.

[1] Weese J.S., Rousseau J., Traub-Dargatz J.L., Willey B.M., McGeer A.J., Low D.E. Community-associated methicillin-resistant *Staphylococcus aureus* in horses and humans who work with horses, J Am Vet Med Assoc 226, 580-583, 2005.
[2] Weese J.S., Lefebvre S.L. Risk factors for methicillin-resistant *Staphylococcus aureus* colonization in horses admitted to a veterinary teaching hospital, Can Vet J 48, 921-926, 2007.

Colistin resistant *Escherichia coli* and spread of ESBL *E. coli* strains in samples collected from organic, antibiotic-free and conventional broilers in farms and at slaughter

Laura Musa (1), Patrizia Casagrande Proietti (1), Maria Luisa Marenzoni(1), Tana Shtylla Kika (2), Valentina Stefanetti (1), David Ranucci (1), Maria Pia Franciosini (1)

(1) Università degli Studi di Perugia, Dipartimento di Medicina Veterinaria (2) Università Agricola di Tirana, Facoltà di Medicina Veterinaria

Corresponding author: Laura Musa (laura.musa@studenti.unipg.it)

The increase of antimicrobial resistance is a global concern for human and animal health. The use of antibiotics in poultry production increases the selection pressure for antibiotic-resistant bacteria [1], among these *E.coli* plays an important role for human health. In recent years extended-spectrum β-lactamase (ESBL)/AmpC *E. coli* has been frequently found as contaminant in broiler meat [2]. ESBL-producing *E. coli* are also often resistant to fluoroquinolones and aminoglycosides. Colistin also, which is an antimicrobial previously widely administered for prevention, treatment, metaphylaxis and growth promotion [3], has recently been reassessed as a critically important antimicrobial for human therapy [4]. We aim to highlight the susceptibility of *E.coli* towards colistin and the spread of ESBL *E.coli*, isolated in the different typologies of farming (conventional, antibiotic-free, organic) and in slaughterhouses located in Central Italy. A total of 174 *E. coli* strains were isolated in farms and at slaughter. To evaluate the antimicrobial susceptibility, all *E. coli* isolates were analyzed by the minimum inhibitory concentration (MIC) using the FRCOL Plates (0.12-128 µg/mL). The presence of ESBL producing *E.coli* was confirmed by the combination disc test, containing cefotaxime and ceftazidime alone, and in combination with clavulanic acid and for the microdilution method were used Sensititre extended-spectrum beta-lactamase plates. The results were interpreted according the EUCAST guidelines. Our results demonstrate the importance of the type of farming. The number of ESBL *E. coli* strains was higher in slaughterhouses (OR 2.72; IC95%: 1.23-6.02) than in farms and samples from conventional carcasses presented the highest percentage of ESBL *E. coli* (18.27%). No statistical differences were found between organic and antibiotic free sample's carcasses (4.30%; 9.67%). This could be supported by the large use of β lactams against *C. perfringens* infection in conventional farming, especially ampicillin. The highest number of colistin-resistant *E.coli* strains was found in the organic (OR 8.27; IC 95%: 3.26-20.96) and antibiotic-free (OR 4.73; IC 95%: 1.86-12.05) rearing systems. The vertical transmission associated with the absence of antibiotic treatment in these typologies of farming, in comparison to conventional one, may influence the increase of the resistant *E. coli* strains in an intestinal microbiota "*less pressured*" by antimicrobial use. The results emphasize that the therapeutic protocol in conventional management is based on the use of other antimicrobial classes being the use of colistin in derogation. Furthermore, the role of the environment contaminated also by antimicrobial resistant bacteria shed by wild animals should not be neglected in organic farms.

Ethical animal research
No experimental animals were used in this research.

[1] Diarra M. Antibiotics in Canadian poultry productions and anticipated alternatives. *Front. Microbiol.* 5:15, 2014. [2] Musa L. Antimicrobial Susceptibility of *Escherichia coli* and ESBL-Producing *Escherichia coli* Diffusion in Conventional, Organic and Antibiotic-Free Meat Chickens at Slaughter. Animals (Basel). 10:1215, 2020. [3] EMA. Updated advice on the use of colistin products in animals within the European Union: development of resistance and possible impact on human and animal health. 2016 [4] Kempf I. Colistin use and colistin resistance in bacteria from animals. Int. J. Antimicrob. Agents, 48, 2016.

The detection of *Anaplasma phagocytophilum* DNA in hair and spleen samples can improve the diagnosis of feline vector-borne diseases

Lorenza Urbani, Andrea Balboni, Maria Morini, Alessia Terrusi, Laura Gallina, Francesco Dondi, Mara Battilani

Alma Mater Studiorum – Università di Bologna, Dipartimento di Scienze Mediche Veterinarie

Corresponding author: Lorenza Urbani (lorenza.urbani2@unibo.it)

Feline Vector-Borne Diseases (FVBD) show increased global prevalence and some *Anaplasma* and *Ehrlichia* species infections have been reported in cats posing a risk to human health [1]. The diagnosis of *Anaplasma* and *Ehrlichia* species infection in cats is achieved by the combined use of different methods as cytologic examination evidencing intracytoplasmic morulae [1], serologic tests [1], and molecular assays [2]. The peripheral whole blood is considered the sample of choice for *Anaplasma* and *Ehrlichia* species DNA detection in cats, but false negative results are reported leading to underdiagnosed infections and underestimation of disease prevalence [3]. In order to have a more accurate assessment of the spread of FVBD, the presence of *Anaplasma* spp. and *Ehrlichia* spp. DNA in 37 owned and shelter-housed cats subjected to necropsy (April 2016 - May 2017) were prospectively investigated by testing spleen, bone marrow, blood clot and hair samples in end-point PCR [4]. The bacteria identified were genetically characterised. The assembled nucleotide sequences were analysed using the BLAST web interface. Multiple alignments between obtained and reference sequences available from the GenBank database were generated using BioEdit ver. 7.2.5 software. Phylogenetic relationships were evaluated using MEGA X ver. 10.1.7 software. Signalment and anamnestic data were retrieved from medical records. Data were evaluated using standard descriptive statistics and analysed using the Chi-squared test considering significant a P value <0.05. Spleen (one cat) and hair (two cats) samples from three shelter-housed cats (8.1%) tested positive for *A. phagocytophilum* DNA. All bone marrow and blood clot samples tested were negative. The sequences obtained showed a high nucleotide identity of 99.1-99.5% among samples and BLAST analysis allowed to align them with the reference sequences of *A. phagocytophilum*. In the phylogeny based on a fragment of the heat shock (groEL) gene nucleotide sequences, all the identified *A. phagocytophilum* clustered with bacteria infecting a wide range of hosts, including humans, and then showing a potential zoonotic role. Statistical association was found between positivity to *A. phagocytophilum* DNA and age, with all cats tested positive ranging from 6 to 24 months (P=0.003). From the results obtained, can be assumed that the use of unusual biological matrices, such as spleen or hair, could allow a more reliable detection of *A. phagocytophilum* DNA in cats with blood tested negative, minimising false negative results.

[1] Pennisi MG. *Anaplasma*, *Ehrlichia* and *Rickettsia* species infections in cats: European guidelines from the ABCD on prevention and management, Journal of Feline Medicine and Surgery, 19:542-548, 2017.
[2] Lappin MR. Update on flea and tick associated diseases of cats, Veterinary Parasitology, 254:26-29, 2018.
[3] Qurollo B. Feline Vector-Borne Diseases in North America, Veterinary Clinics of North America: Small Animal Practice, 49:687-702, 2019.
[4] Barber RM. Evaluation of brain tissue or cerebrospinal fluid with broadly reactive polymerase chain reaction for *Ehrlichia*, *Anaplasma*, spotted fever group *Rickettsia*, *Bartonella*, and *Borrelia* species in canine neurological diseases (109 cases), Journal of Veterinary Internal Medicine, 24:372-378, 2010.

Dioxin influences the replication of canine coronavirus

Claudia Cerracchio (1), Francesca Paola Nocera (1), Monica Ambrosio (1), Sara Lambiase (2), Luisa De Martino (1) and Filomena Fiorito (1)

(1) Università degli Studi di Napoli Federico II, Dipartimento di Medicina Veterinaria e Produzioni Animali (2) Istituto Zooprofilattico del Mezzogiorno (Portici, Napoli)

Corresponding author: Filomena Fiorito (filomena.fiorito@unina.it)

Exposure to 2,3,7,8-tetrachlorodibenzo-p-dioxin (TCDD), commonly known as dioxin, a highly toxic environmental contaminant, suppresses immune response and induces increased susceptibility to infectious agents. Indeed, TCDD may interfere with the replication of both human and animal viruses, as influenza A viruses, coxsackie virus B3, cytomegalovirus, herpes simplex II, and bovine herpesvirus 1 (1).

Canine coronavirus (CCoV) is an alphacoronavirus, including the two genotypes CCoV-I and CCoV-II. CCoV-II consists of two subtypes, the classical CCoV-IIa and the recombinant CCoV-IIb. They are generally responsible for self-limiting enteric infections, characterized by high morbidity and low mortality in dogs.

Recent studies report that emerging pantropic strains of CCoV may cause hypervirulent and multi-systemic fatal disease, in contrast to classical enteric coronavirus infections (2-3).

To elucidate cell effects due to enteric CCoV, permissive infection in a canine fibrosarcoma cell line (A-72) describes that CCoV-II causes cell death accompanied by apoptosis (4-5) and autophagy (6). Herein, following infection with the reference CCoV strain S378 in A-72 cell line, in the presence of very low doses of TCDD (0.01, 1 and 100 pg/mL), bio-screen *in vitro*, cytomorphological and virus yield analyses were carried out.

During CCoV infection, Trypan Blue exclusion test showed that TCDD decreased total cells number, whereas increased cell viability in uninfected cells. In addition, Giemsa staining revealed in infected cells morphological alterations, as development in syncytia of giant cells, detachment from culture plate, chromatin fragmentation and cytoplasmic vacuolization. These cell death features, as reported previously (4-6), were dramatically enhanced by the presence of TCDD. Determination of virus titers, assayed through $TCID_{50}$ method, indicated that CCoV-II efficiently replicated in A-72 (5), but virus yield was considerably increased by the presence of TCDD.

Taken together, our preliminary results support the concept that dioxin exposure could play a role as additional risk factor in promoting viral diseases.

Further investigations are needed to clarify the mechanism by which an environmental contaminant, like TCDD, is involved in the regulation of CCoV infection.

(1) Fiorito et al. 2,3,7,8-tetrachlorodibenzo-p-dioxin and the viral infection. Environ. Res. 153:27-34, 2017.
(2) Buonavoglia et al. Canine Coronavirus Highly Pathogenic for Dogs. Emerg. Infect. Dis. 12:492-494, 2006.
(3) Timurkan et al. Molecular characterization of canine coronaviruses: an enteric and pantropic approach. Arch. Virol. 166:35-42. 2021.
(4) Ruggieri et al. Canine coronavirus induces apoptosis in cultured cells. Vet. Microbiol. 121: 64–72. 2007.
(5) De Martino et al. Bid cleavage, cytochrome c release and caspase activation in canine coronavirus-induced apoptosis. Vet. Microbiol. 141:36-45. 2010.
(6) Fiorito et al. Canine Coronavirus (CCoV-II) induces autophagy, impairing the regulation of cellular iron homeostasis. Proceedings of 2nd International Conference of the European College of Veterinary Microbiology ICECVM 2020, 36.

Antimicrobial susceptibility patterns of *Pseudomonas aeruginosa* clinical isolates from canine otitis externa

Valentina Stefanetti (1), Luca Luciani (2), Elisa Rampacci (1), Raffaella Capuozzo (1), Fabrizio Passamonti (1)

(1) Università degli Studi di Perugia, Dipartimento di Medicina Veterinaria (2) Medico veterinario libero professionista

Corresponding author: Valentina Stefanetti (valentina.stefanetti@unipg.it)

Otitis externa (OE) is one of the most common disorders in dogs, accounting up to 18% of dogs presented to veterinary practice [1]. The management and the treatment of OE can be frustrating due to the multifactorial etiology of the disease. *Pseudomonas aeruginosa* is often isolated from OE and it represents a significant treatment challenge because of the severity of clinical signs and inherent and acquired antibiotic-resistance [2]. The goals of the present study were to analyze the antimicrobial susceptibility patterns of *P. aeruginosa* isolates from OE and correlate them with clinical findings. A total of 56 dogs of different age and sex presenting OE were enrolled in the study. The attending clinician divided these cases in erythematous-ceruminous or suppurative otitis based on the clinical signs and the type of exudate at presentation. Cytology was performed by collecting a small amount of exudate from the ear canals and a score was given according to a validated scale [3]. Auricular swab samples were collected for bacteriological examination and cultured aerobically at 37 °C for 24 hours. After the incubation, further analyses were carried on only on *P. aeruginosa* isolates. Antimicrobial susceptibility assay was performed according to CLSI [4]. A broth microdilution procedure was carried out to define the MIC values of antimicrobials commonly included in topical otic medication: enrofloxacin, gentamicin and polimixin B against all planktonic *P. aeruginosa* isolates (final concentration of 1.5×10^5 CFU/well) over a dose range of 32–0.125 µg/mL in serial two-fold dilutions. Overall 78 bacteria were isolated. 21/78 (27%) were identified as *P. aeruginosa* through MALDI-TOF. No statistical association between sex or age and *P. aeruginosa* isolation has been observed. All the *P. aeruginosa* strains were associated to a ertyroceruminous otitis and 18/21 (85.7%) were from chronic otitis. Additionally, all the *P. aeruginosa* were strongly associated with a cytological presence of rod bacteria. Interestingly, no strains showed resistance to gentamicin, 4/21 (19%) were resistant to polimixin B, 8/21 (38%) resulted resistance to enrofloxacin. The latter is according to the literature and it is probably due to the fact that fluorquinolons have been extensively used during the last 10 years for the management of canine otitis by *P. aeruginosa* [5]. Overall these data confirm the crucial role of *P. aeruginosa* in canine OE. All the *P. aeruginosa* strains were associated to a chronic disease and it could be due to its ability to produce biofilm. A challenge for the future research will be studying *P. aeruginosa* biofilm-forming ability and the minimal biofilm eradication concentration (MBEC) and minimal biofilm inhibitory concentration (MBIC) in order to assist clinicians in OE successful treatment.

[1] Hill et al. Survey of the prevalence, diagnosis and treatment of dermatological conditions in small animals in general practice, Vet Rec, 158(16):533-539,2006
[2] Pye. *Pseudomonas* otitis externa in dogs, Can Vet J, 59:1231-1234, 2018
[3] Budach SC. Reproducibility of a semiquantitative method to assess cutaneous cytology, Vet Derm 23: 426–e80, 2012
[4] Clinical and laboratory standard institute. Performance standards for antimicrobial susceptibility testing; Wayne, PA, USA: CLSI document M100-S22CLSI, 2018
[5] Seol et al. In vitro antimicrobial susceptibility of 183 *Pseudomonas aeruginosa* strains isolated from dogs to selected antipseudomonal agents, J Vet Med 49:188-192, 2002

Prevalence of methicillin-resistant staphylococci in stray cats in northern Italy

Gabriele Ratti, Angelica Stranieri, Martina Penati, Paola Scarpa, Camilla Luzzago, Alessia Gazzonis, Stefania Lauzi

Università degli Studi di Milano, Dipartimento di Medicina Veterinaria.

Corresponding author: G. Ratti (gabriele.ratti@unimi.it)

The prevalence of methicillin-resistant staphylococci (MRS) has increased in recent decades, posing a threat to human and animal health worldwide [1-3]. Pets may play a role in the spread of resistant clones. To date, information about carriage of MRS in stray cats is limited. The aim of this study was to determine the prevalence of MRS in stray cats from Northern Italy. Stray cats admitted to the Veterinary Teaching Hospital of Lodi for mandatory sterilization programs or for hospitalization were sampled between November 2019 and February 2021. Nasal and perianal swabs were collected from each cat. Enriched samples were characterized regarding their phenotypic resistance by disk diffusion using oxacillin (1 µg), according to the EUCAST guidelines [4] and were tested for the presence of the methicillin resistance determinants by real time PCR as previously described [5]. Species identification of methicillin-resistant single colonies was accomplished via matrix-assisted laser desorption/ionization-time-of-flight mass spectrometry (MALDI-TOF MS) using the direct transfer method, as previously described [6]. In total, 110 stray cats were analyzed and eight (7.3%) cats carried MRS in nasal and rectal mucosa. All the eight MRS isolates (3 *Staphylococcus pseudointermedius*, 2 *S. epidermidis*, 2 *S. haemolyticus* and 1 *S. sciuri*) harbored the *mecA* gene. Methicillin-resistant *S. aureus* was not detected. Statistical analysis showed that sex, age, hospitalization, season and geographical localization of the cat colonies were not correlated with MRS carriage. Our data indicate carriage of MRS in stray cats and the results are consistent with previously published prevalences in pets [1, 2, 7]. Considering that the presence of antimicrobial selection pressure is unlikely in cat colonies, our results also suggest that stray cats may be sentinels for MRS circulation. Further studies using whole genome sequencing and a One Health approach are required to define the molecular epidemiology and the public-health significance of MRS carriage in stray cats.

[1] Worthing et al. Methicillin-resistant staphylococci amongst veterinary personnel, personnel-owned pets, patients and the hospital environment of two small animal veterinary hospitals. Veterinary Microbiology, 223:79-85, 2018.
[2] Loeffler et al. Prevalence of methicillin-resistant *Staphylococcus aureus* among staff and pets in a small animal referral hospital in the UK. Journal of Antimicrobial Chemotherapy, 56:692-697, 2005.
[3] Epstein et al. Methicillin-resistant commensal staphylococci in healthy dogs as a potential zoonotic reservoir for community-acquired antibiotic resistance. Infection Genetics and Evolution, 9:283-285, 2009.
[4] The European Committee on Antimicrobial Susceptibility Testing. Breakpoint tables for interpretation of MICs and zone diameters, version 10.0, 2020.
[5] McDonald et al. Development of a triplex real-time PCR assay for detection of Panton-Valentine leukocidin toxin genes in clinical isolates of methicillin-resistant *Staphylococcus aureus*. Journal of Clinical Microbiology, 43:6147-6149, 2005.
[6] Singhal et al. MALDI-TOF mass spectrometry: an emerging technology for microbial identification and diagnosis. Frontiers in Microbiology, 6:791, 2015.
[7] Kizerwetter-Świda et al. High-level mupirocin resistance in methicillin-resistant staphylococci isolated from dogs and cats. BMC Veterinary Research, 15(1):238, 2019.

Management of two healthcare-associated infections outbreaks of *Serratia marcescens* and *Enterobacter cloacae* in a Veterinary University Hospital, and implementation of a surveillance system

Raffaele Scarpellini (1), Roberta Troia (1), Massimo Giunti (1), Elisabetta Mondo (1), Federica Giacometti (1), Federica Savini (1), Fabio Tumietto (2), Francesco Dondi (1), Ilaria Magagnoli (1), Luciano Pisoni (1), Veronica Cola (1), Andrea Balboni (1), Mara Battilani (1), Silvia Piva (1)

(1) Alma Mater Studiorum - Università di Bologna, Dipartimento di Scienze Mediche Veterinarie (2) Rete Interaziendale per Stewardship Antimicrobica e Controllo delle Infezioni Correlate all'Assistenza - Unità Operativa di Malattie Infettive IRCCS Policlinico di S. Orsola – Bologna

Corresponding author: Raffaele Scarpellini (raffaele.scarpellin2@unibo.it)

Healthcare-associated infections (HCAI) are considered an emerging problem in both human and veterinary medicine [1] [2], due to the multi-resistance pattern and the zoonotic potential of involved infectious agents. Veterinary University Hospitals (VUH) are high-risk places [3], and a surveillance plan is essential not only to control and restrict HCAI spread, but also to collect information about HCAI agents. This work aims at highlighting the effectiveness of a well-defined surveillance program by comparing two different nosocomial outbreaks at the VUH of Bologna. From April to September 2019, an unexpectedly high number of records of fever of unknown origin in small animals hospitalized at the VUH was associated with *Serratia marcescens* infection in twelve cases (4 isolates from blood culture, 3 from surgical implants, 2 from liver biopsies, 2 from urinary tract infection-UTI, 1 from surgical site infection-SSI). Three cases developed sepsis, and one case was euthanized due to progressive sepsis-induced organ dysfunction. A further environmental investigation was conducted, showing the presence of *S. marcescens* into soap dispensers and chlorhexidine dipped gauzes used for patient skin antisepsis. The VUH restricted admissions of dogs and cats for 7 days to stop the spread of infection and intervened with cleaning and a high-level of disinfection. Since November 2020, a surveillance program for bacterial HCAI has been developed in the VUH, focusing on two aspects: microbiological surveillance, that has been divided into passive and active surveillance, and tri-weekly reports to ensure an efficient information flow between microbiologists and hospital personnel. In February 2021, passive surveillance highlighted three cases of *Enterobacter cloacae* localized infections (2 from SSI, 1 from prostatic fine needle aspiration) in three dogs hospitalized in the VUH in the previous 15 days. Subsequent environmental sampling showed *E. cloacae* presence in a dog cage – both before and after routine cleaning - and in one staff member's hand. Three more cases of dogs hospitalized with *E. cloacae* infection (2 from SSI, 1 from UTI) were highlighted by passive surveillance in the following 11 days. This finding led to adopt restrictive measures for patient admissions for 3 days to control the outbreak and perform environmental disinfection. After that, the environmental sampling showed no *E. cloacae* detection in the UVH, and no more cases of *E. cloacae* infection were recorded in the following 4 weeks.

The two HCAI outbreaks reported in the present study suggest that a surveillance plan in UVHs could avoid more problematic consequences. Indeed, the second outbreak was better confined, and led to less severe clinical outcomes. Management of HCAI should be a priority for every hospital with high-level healthcare. A well-defined, written surveillance plan is one of the most useful and effective tools in terms of rapidity, proactivity and capacity to target efforts.

[1] Milton A.A.P. et al. Nosocomial infections and their surveillance in veterinary hospitals, Adv Anim Vet Sci., 3(2): 1-24, 2015.

Molecular and serological survey for SARS-CoV-2 in stray cats in northern Italy

Angelica Stranieri (1), Stefania Lauzi (1), Alessia Giordano (1), Luigi Galimberti (2), Gabriele Ratti (1), Nicola Decaro (3), Federica Brioschi (1), Davide Lelli (4), Silvia Gabba (5), Ndiana Linda Amarachi (3) Eleonora Lorusso (3), Ana Moreno Martin (4), Tiziana Trogu (4), Saverio Paltrinieri (1)

(1) Università degli Studi di Milano, Dipartimento di Medicina Veterinaria (2) Azienda di Tutela della Salute (ATS) città metropolitana di Milano Distretto Veterinario Alto Lodigiano (3) Università degli Studi di Bari, Dipartimento di Medicina Veterinaria (4) Istituto Zooprofilattico Sperimentale della Lombardia e dell'Emilia-Romagna, Brescia (5) Parco Tecnologico Padano Science Park Srl, Lodi

Corresponding author: Angelica Stranieri (angelica.stranieri@unimi.it)

In experimental settings, cat-to-cat transmission of SARS-CoV-2 has been demonstrated[1] and cases of SARS-CoV-2 positive or seropositive privately-owned cats have been reported worldwide[2,3]. Since seropositive stray cats have been detected in the proximity of farms of infected minks[4], the question whether stray cats may have an epidemiological role in the COVID-19 pandemic and may act as sentinel animals for the circulation of SARS-CoV-2 has been raised. The aim of the present study is to investigate if SARS-CoV-2 RNA and anti-SARS-CoV-2 antibodies are detected in free roaming cats belonging to feline colonies located in an area with a high rate of COVID-19 in human population and to correlate the results obtained in cats with the positivity rate in people. Interdigital, cutaneous, oropharyngeal, nasal, rectal swabs and blood samples, were collected from 99 cats living in feline colonies included in a public plan of demographic control of stray animals and admitted to the Veterinary Teaching Hospital of the University of Milan for castration. The study was approved by the Institutional Animal Care and Use Committee and by the Institutional Ethical Committee (approval numbers_31/20 and 43/20, respectively). Cats enrolled correspond to the 24.2% of the feline population living in the 25 sampled colonies and to the 5.6% of all the free-roaming registered cats. The presence of SARS-CoV-2 RNA in swabs was assessed by real time RT-PCR, whereas anti-SARS-CoV-2 antibodies were evaluated by commercially available ELISA Kits, being 90 cases confirmed by serum virus neutralization (SVN). Data obtained were compared with regards of size and location (urban vs rural) of the colonies, contacts between stray cats of the colony and other stray cats or people other than caretakers as well as the COVID-19 positivity of caretakers. Most of the colonies were in urban areas and resident cats had frequent contacts with external cats or people. In people of the same area, the SARS-CoV-2 RNA positivity ranged from 3.0% to 5.1% (mean rate: 4.1%) and the seropositive rate from 12.1% to 16.3% (mean rate: 14.2%). One COVID-19 positive caretaker was found. All the enrolled cats tested negative for SARS-CoV-2 RNA detection and were seronegative, although the negative results cannot exclude previous infections followed by serological negativization. Further studies on larger caseload, including information on concurrent infections, are warranted, also in the light of the emerging new viral variants, in order to confirm the results of this study that suggests that colony cats do not have an important epidemiological role in SARS-CoV-2 transmission.

[1] Halfmann et al. Transmission of SARS-CoV-2 in domestic cats. New England Journal of Medicine. 383(6), 592-594, 2020.
[2] Hosie, et al. 2021. Anthropogenic Infection of Cats during the 2020 COVID-19 Pandemic. Viruses 13(2), 185, 2021.
[3] Patterson et al. Evidence of exposure to SARS-CoV-2 in cats and dogs from households in Italy. Nature Communications 11(1), 1-5, 2020.
[4] Oreshkova et al. SARS-CoV-2 infection in farmed minks, the Netherlands, April and May 2020. Eurosurveillance, 25(23), 2001005, 2020.

Development and validation of a new protocol for a rapid identification of frozen or no more viable microorganisms by Bruker MALDI-TOF Mass Spectrometry system

Martina Linardi (1), Alessandro Buchicchio (2), Livio Galosi (1), Marcello Reale (1), Adolfo Maria Tambella (1), Vincenzo Cuteri (1), Anna-Rita Attili (1)

(1) Università degli Studi di Camerino, Scuola di Bioscienze e Medicina Veterinaria (2) Bruker Italia S.r.l., Daltonics Division.

Corresponding author: Martrina Linardi (marti.linardi@gmail.com)

Matrix-Assisted Laser Desorption Ionisation-Time of Flight Mass Spectrometry (MALDI-TOF/MS) became a promising and reliable tool for a fast and accurate microbial identification (ID). It is applied to fresh bacterial colonies and the use is expanding to direct analysis of clinical specimens [1]. The purposes of this study were: to develop and validate a new MALDI identification protocol, named "Wash Balls" extraction (WB), directly on frozen microorganisms, avoiding the microbial culture; to evaluate WB performance and applicability on no more viable frozen bacteria. A collection of 150 microorganisms, isolated from animal samples and stored at -20°C during the last 15 years, was cultured and tested following the Extraction Procedure Rev.4 as Gold standard (GS) using Bruker Microflex Lt® MALDI-TOF/MS. Each Cryobank™ (Mast Diagnostics, UK) or 20% glycerol-microbial suspension system was submitted to WB for validation. Briefly, 100 µL of 0.9% saline sterile solution was added to each freezing system, vortexed and the same amount transferred to an Eppendorf with 200 µL of sterile deionized water and 900 µL of ethanol. After centrifugation (14000 *rpm*) for 2 minutes, the supernatant was discarded. After drying at room temperature, 70% formic acid and pure acetonitrile were added to the pellet. Finally, 1µL of supernatant was pipetted onto a MALDI target and 1 µL of HCCA matrix solution was added. *Burkholderia cepacia* ATCC 25416, *Stenotrophomonas maltophilia* ATCC 13637, *Staphylococcus pseudintermedius* ATCC 49444 and *Escherichia coli* ATCC 25922 were used as control. The applicability of WB was tested on 64 no more viable frozen bacteria. Each strain was analyzed in double and twice by flexControl 3.4 software, and all spectra obtained were compared with the referent spectra of BDAL library using Biotyper 3.1 (Bruker Daltonics, Germany). Performance of WB was evaluated using SISA software, while differences between qualitative variables were analyzed by Chi-square test (STATA v. 13). Moreover, bioscore mean values were compared by Pearson's correlation (R^2). Among the 150 microorganisms, 94 different species were identified: 90 (60%) Gram positive, 55 (36.7%) Gram negative strains, and 5 (3.3%) yeasts. WB performance recorded a sensitivity of 98.6% and a specificity of 60%, with an accuracy of 96% and a substantial agreement (k=0.646). An ID correspondence of 92% was found between GS and WB. Identification at species level (bioscore >2.000) was obtained for 62.1% and for 77.8% (x=8.27, *P*=0.004) of microorganisms identified by GS and WB, respectively. In particular, WB showed a significant improvement in Gram positive ID (50% *vs* 78.4%; x=15.15, *P*=0.0001). A positive and strong correlation was recorded (r=0.97, R^2=0.95). Frozen and no more viable bacteria were identified by WB at genus (100%) and species (55%) levels. WB resulted in a valid, fast, cheap alternative to the GS. Moreover, it is a useful method to analyze and identify stored or no more viable microorganisms. The system and the time of freezing did not show spectrum differences and did not affect the ID. However, further studies will be carried out to evaluate the possible variations that the freezing process could cause to the microorganisms and their mass spectra.

[1] Tsuchida et al. Current status of Matrix-Assisted Laser Desorption/Ionization-Time-of-Flight Mass Spectrometry (MALDI-TOF MS) in clinical diagnostic microbiology, Molecules, 25:4775, 2020.

Canine parvovirus (CPV) infection and antimicrobial resistance (AMR) in bacteria isolated from dogs with parvovirosis

Giorgia Schirò, Delia Gambino, Francesco Mira, Maria Vitale, Annalisa Guercio, Giuseppa Purpari, Francesco Antoci, Francesca Licitra, Gabriele Chiaramonte, Maria La Giglia, Vincenzo Randazzo, Domenico Vicari

Istituto Zooprofilattico Sperimentale della Sicilia "A. Mirri"

Corresponding author: Francesco Mira (dottoremira@gmail.com)

Canine parvovirus type 2 (CPV-2) represents a major enteric viral threat for dogs, causing severe and often fatal epizootics of gastroenteritis worldwide [1]. Treatment is supportive and mainly based on rehydration, antiemetics and antimicrobial therapies, to limit the potential secondary bacterial infections [2,3]. Information on CPV and bacteria co-infection are limited but could be useful also considering the potential role of pets on the spread of antimicrobial resistant strains [4,5]. The aim of this study was the evaluation of antimicrobial susceptibility and multidrug resistance (MDR) profiles of bacterial species from tissue samples of dead dogs with canine parvovirus infection. Twenty-three dogs suspected of parvovirosis were subjected to necropsy for diagnostic purposes. Tissue samples were collected and analysed by the means of virological and bacteriological assays. Molecular assays for the detection and the characterization of the CPV strains and other enteric viral pathogens were performed. After bacterial isolation, antimicrobial susceptibility by the disk diffusion method and the determination of minimum inhibitory concentration were performed, testing critically important antimicrobials (CIAs); moreover, the detection of extended spectrum β-lactamase (ESBL), *ampC* β-lactamase genes, and genes for toxins of specific bacteria were performed. The CPV infection was confirmed in 23 dogs, showing both anatomopathological lesions referred to parvovirus infection and, in some dogs, lesions not specifically associated to the CPV infection. Thirty-one Gram-negative and twelve Gram-positive bacteria were isolated. All strains showed phenotypic resistances to the tested antibiotic, and seventeen multidrug resistant and high resistance to 3rd/4th generation cephalosporins and metronidazole bacteria were detected. Bacteria considered as potential agents of zoonoses or resistant to last generation antibiotics in human health were also observed. Furthermore, almost 50% of *Enterobacteriaceae* isolated were positive for at least one ESBL gene with the majority carrying also more genes simultaneously. In this study, resistant bacteria harbouring antibiotic resistance genes were isolated in dogs with confirmed parvovirosis. Despite the potential limited role of the bacteria in the clinical outcome, a high risk of intra- or cross-species transmission of multi resistant strains should be further considered for a rationale use of antibiotics to control antimicrobial resistance through animals according to the One Health approach.

[1] Decaro N, Buonavoglia C. Canine parvovirus--a review of epidemiological and diagnostic aspects, with emphasis on type 2c. Veterinary Microbiology, 24;155(1):1–12, 2012.
[2] Mohr et al. Effect of early enteral nutrition on intestinal permeability, intestinal protein loss, and outcome in dogs with severe parvoviral enteritis. Journal of Veterinary Internal Medicine, 17(6):791–8, 2003.
[3] Gerlach et al. [Therapy of canine parvovirus infection - review and current insights]. Tierarztl Prax Ausg K Kleintiere Heimtiere. 48(1):26–37, 2020.
[4] Botha et al. Prevalence of *Salmonella* in juvenile dogs affected with parvoviral enteritis. Journal of the South African Veterinary Association, 5;89(0):e1–6, 2018.
[5] Silva et al. *Clostridium perfringens* and *C. difficile* in parvovirus-positive dogs. Anaerobe. 48:66–9, 2017.

ORAL COMMUNICATIONS

ARNA

MAIN LECTURE

The implications of the new nutritional label

Claudio Truzzi

(1) GDO Quality Assurance Manager.

Corresponding author: C. Truzzi (claudio.truzzi@metro.it)

The alarmism about obesity has led to the development of the nutrition label. Reg 1169/11, regulates the implementation of the nutritional label which until now provided for a table difficult to interpret by the final consumer. This information has not slowed down the growth of overweight individuals or obese people who today exceed 25 million inhabitants in Italy.

Nutritional labels are always read by only 22% of the population, therefore an awareness campaign involving above all the elderly, children and the Ho.Re.Ca chanel. This situation has led to the birth of new nutritional labeling models such as the French nutri-score and the Italian nutrinform battery.

Both have advantages and disadvantages but with different alignments and political positions on the part of the EU countries. All this involves major problems both for the industry, which has to implement different nutritional labels according to the country of export, and for the final consumer who is even more confused. In conclusion, we can say that instead of focusing only on the choice of the type of nutritional label, it would be desirable to focus on the creation of healthier products, with low sales content, sugars, fats and additives and for a group of greater consumers.

Inflammatory status and metabolic changes at dry-off in high yield dairy cows

Matteo Mezzetti, Andrea Minuti, Fiorenzo Piccioli-Cappelli, Luca Cattaneo, Erminio Trevisi

Department of Animal Sciences, Food and Nutrition (DIANA), Facoltà di Scienze Agrarie, Alimentari e Ambientali, Università Cattolica del Sacro Cuore, Piacenza, Italy

Corresponding author: Andrea Minuti (andrea.minuti@unicatt.it)

At dry-off, increased udder pressure that follows milking interruption could induce severe pain triggering a raise of stress-related hormones [1]. These hormones concur with leukocytes activities during mammary gland involution in modifying the transcription of genes encoding for a variety of cytokines on immune cells. Altered cytokines production could dysregulate immune function up to early lactation, suggesting stress occurring at dry-off to be related with immune dysfunction affecting dairy cows during the peripartal period. Despite that, only recent concerns about animal welfare promoted a deeper investigation of dry-off as a cause of metabolic stress. Our study investigates metabolic changes occurring at dry-off and the contribution of milk yield (MY) in such alterations. The trial was carried out at Università Cattolica del Sacro Cuore research dairy barn (Experiment Station, San Bonico, Piacenza, Italy) in accordance with Italian laws on animal experimentation (DL n. 26, 04/03/2014) and ethics (Authorization of Italian Health Ministry N 1047/2015-PR). Thirteen Holstein dairy cows were dried off at 55 days from expected calving day (assumed as 0 days from dry-off, DFD) and divided in two groups according to their average daily MY in the last week of lactation, assuming a cut-off of 15 kg/d: low MY (7 cows) and high MY (6 cows). From -7 to 34 DFD dry matter intake (DMI) and rumination time were measured. Blood samples were collected at -7, 2, 7, 27 and 34 DFD to assess an haematological and metabolic profile and at -7, 7 and 34 DFD to test functions of circulating white blood cell (WBC) through *ex vivo* challenges. Data were included in a mixed model for repeated measures assuming MY at dry-off, time and their interaction as fixed effects. After dry-off, DMI was reduced and rumination time was increased in all the animals. High MY cows had greater DMI and rumination time than low MY cows. These outcomes suggest the lower energy content and the greater fiber amount in the dry ration as compared to the late lactation one to have affected the feeding behavior of the cows, especially in the group of cows with high MY at dry-off. In blood, WBC counts decreased at 7 DFD and increased the production of pro-inflammatory cytokines (interleukin 1-β and interleukin 6) at 7 and 34 DFD. These trends of WBC count and cytokines production were consistent with the activation of leukocytes and their migration from the blood into the mammary gland to contribute in the involution process [1]. Plasmatic concentrations of liver function indicators (γ-glutamyl transferase and bilirubin), positive acute phase proteins (APPs, i.e. ceruloplasmin and serum amyloid alpha); and nitrogen species (nitrate, nitrite, and nitric oxide) increased after dry-off. Conversely, negative APPs (retinol) and antioxidant species (thiol groups, tocopherol, and β-carotene) decreased. Those alterations were more marked in high MY animals (greater haptoglobin and nitrite concentrations). This study suggests that dry-off diverted liver function, triggered a systemic inflammation and depleted antioxidant systems, especially in the group of cows with high MY at dry-off.

A. K. Putman, J. L. Brown, J. C. Gandy, L. Wisnieski, and L. M. Sordillo, “Changes in biomarkers of nutrient metabolism, inflammation, and oxidative stress in dairy cows during the transition into the early dry period,” *J. Dairy Sci.*, vol. 101, no. 10, pp. 9350–9359, Oct. 2018.

Endocrine disruptors in buffalo milk production cycle

Ilaria Di Marco Pisciottano, Grazia Guadagnuolo, Tiziana Hauber, Mario Esposito, Pasquale Gallo

Istituto Zooprofilattico Sperimentale del Mezzogiorno, Dipartimento di Chimica

Corresponding author: Ilaria Di Marco Pisciottano (ilaria.dimarcopisciottano@izsmportici.it)

Bisphenol A (BPA) is a known Endocrine Disrupting Chemicals (EDCs), a wide panel of compounds that can interfere with physiological regulation of the endocrine system, thus causing health adverse effects. Because of its large use as plasticiser in the chemical and polymer industry, bisphenol A (BPA) has been worldwide studied, focusing on consumers' intake through diet due to migration from packaging into food. As a consequence, tolerable daily intake (TDI) [1] and specific migration limits [2] have been set, and other bisphenols have been used as BPA substitutes in plastic production, some of them showing similar disrupting activity. To the aim of studying human exposure to these compounds, in the frame of a research project funded by the Italian Ministry of Health, we investigated the contamination from many EDCs in the production cycle of buffalo milk. A novel method has been developed and validated for purification of 4-nonylphenol, 4-octylphenol and 17 bisphenols from drinking water, feed, feed additives, bulk milk and blood serum from buffaloes. The determination was carried out by liquid chromatography coupled to tandem mass spectrometry, that allowed us to detect the EDCs with high sensitivity, down to 0.003 ppb. The method was used to analyse 201 feed, 9 feed additives, 62 drinking water, 46 milk and 190 blood serum samples collected from 10 buffalo farms in Campania region, in 2019 and 2020. From each farm, at least four sample collections were performed in different periods and lactation phases, to evaluate EDC contamination through time. The results of our work showed no contamination of EDCs in drinking water samples, whereas in 43% of all the other samples at least one bisphenol was detected. In particular, the 64% of feed samples were contaminated by BPA in the range 1.0–174.7 ng/g, with only 3 samples higher than 50.0 ng/g. Bisphenol F, the second most abundant EDC, was detected only in 5% of feed samples at concentrations going from 10.0 to 142.2 ng/g. Among all the other EDCs, only bisphenol F diglycidyl ether, bisphenol S and bisphenol E were determined in six, five and one feed sample, respectively, at contamination levels between 1.2 and 40.3 ng/g. In 74% of bulk milk samples BPA and BPF were detected in the concentration range 0.5–8.7 ng/mL; only one sample contained bisphenol AF (BPAF) at 3.0 ng/mL. About feed additives, all the samples were contaminated by at least one among the same EDCs detected in the feed, at levels ranging from 4.1 to 436.5 ng/g. Blood serum samples were less contaminated (only 10% of samples) at low levels (0.045–6.4 ng/g), mainly by BPA. The results of our study show that bisphenols, in particular BPA, are present in the production cycle of buffalo milk. The feed and feed additives seem to be the main source of contamination, presumably because of packaging or plastics. A transfer of bisphenols to the milk is evident, supported by their presence in a significant percentage of buffalo sera. Although the contamination levels determined are low, we are studying a model to predict the transfer of BPA in buffalo mozzarella cheese and evaluate the possible intake for consumers. More insight is necessary about other bisphenols detected in milk and related risk for food safety.

[1] EFSA. Scientific opinion on the risks to public health related to the presence of bisphenol A (BPA) in foodstuffs. EFSA Journal. 13(1):3978, 2015.
[2] EU. Regulation n. 10/2011/EU on plastic materials and articles intended to come into contact with food. Off. J. Eur. Union, L12: 1-89, 2011.

Differentiation between Fresh and Thawed Cephalopods Using NIR Spectroscopy and Multivariate Data Analysis

Francesco Pennisi (1), Alessandro Giraudo (2), Nicola Cavallini (2), Giovanna Esposito (1), Francesco Geobaldo (2), Pier Luigi Acutis (1), Marzia Pezzolato (1), Francesco Savorani (2), Elena Bozzetta (1)

(1) Istituto Zooprofilattico Sperimentale del Piemonte, Liguria e Valle d'Aosta (2) Department of Applied Science and Technology, Politecnico di Torino

Corresponding author: Francesco Pennisi (francesco.pennisi@izsto.it)

The sale of frozen–thawed fish products, labelled as fresh, is currently one of the most common and insidious commercial food frauds [1]. For this reason, the development of reliable analytical techniques, able to detect the fraudulent substitution, attracts the industrial research interest. In this context different methods have been investigated over the past years, such as measurement of dielectric properties, erythrocyte and hematocrit evaluation, histology, protein extraction, proteomics, enzymatic essays [2, 3], and they have proved to be effective for their applicability. Conversely, all the above-mentioned techniques are rather time consuming and labour intensive, thus difficult to apply industrially.

Aim of the present study is indeed to set up an innovative and easy-to-use method to distinguish fresh from frozen-thawed cephalopod molluscs using NIR spectroscopy. Moreover, the use of handheld NIR instruments, which can quickly analyze a high number of samples directly on site, was considered. The present study was performed on two species, commonly sold in large-scale distribution: cuttlefish (*Sepia officinalis*) and musky octopus (*Eledone* spp.). For each species, fifty fresh specimens were analysed at refrigeration temperature (2±2°C), then frozen at –20°C in a domestic freezer for 10 days and finally thawed and analysed once again. The performance of three near-infrared (NIR) instruments in identifying storage conditions were compared: the benchtop NIR Multi Purpose Analyzer (MPA) by Bruker, the portable MicroNIR by VIAVI and the handheld NIR SCiO by Consumer Physics. All collected spectra were processed and analysed with chemometric methods (pre-processing and modeling in MATLAB environment, exploratory in PCA, classification with PLS-DA). The SCiO data were also analyzed using the analytical tools available in the online application (*The Lab*) provided by the manufacturer, to evaluate its performance. NIR spectroscopy, coupled with chemometrics, allowed discriminating between fresh and thawed samples with high accuracy: Cuttlefish between 82.3–94.1%, musky octopus between 91.2–97.1%, global model between 86.8–95.6%. It is important to underline that the best results were obtained using handheld and portable tools, which greatly reduces the instrumental complexity and simplifies the execution of the analysis, making it much more practical and suitable for screening directly on the production line. Concerning the MPA, the complexity of the spectral signal made it difficult to extract and elaborate the useful information, but still demonstrated remarkable prediction capability. Based on these findings, results show how frauds could be detected directly in the marketplace by consumers and companies through small portable devices with pre-set analyses, whereas official control laboratories would rely on benchtop analytical instruments, coupled with chemometric approaches, to develop accurate and validated methods, suitable for regulatory purposes.

1. Calosso et al. Global Footprint of Mislabelled Seafood on a Small Island Nation. Biological Conservation, 245, 108557, 2020.
2. Guglielmetti et al. Two-Dimensional Gel and Shotgun Proteomics Approaches to Distinguish Fresh and Frozen-Thawed Curled Octopus (*Eledone cirrhosa*). Journal of Proteomics, 186:1–7, 2018.
3. Bozzetta et al. Histology as a Valid and Reliable Tool to Differentiate Fresh from Frozen-Thawed Fish. Journal of Food Protection, 75:1536–1541, 2012.

ORAL COMMUNICATIONS

RNIV

MAIN LECTURE

‘One Health’ vaccinology to combat emerging viral zoonoses

Simon P. Graham
The Pirbright Institute, UK

Corresponding author: S. Graham (simon.graham@pirbright.ac.uk)

An expanding human population and the concomitant increase in demand for animal protein has led to the use of previously unused habitable land and disruption of ecosystems. Increased human-livestock-wildlife interactions has led to an increase in virus spillover events from wildlife reservoirs, which in turn has elevated the risk of epidemics of new and emerging zoonotic diseases. ‘One Health’ recognises that human, animal, and environmental health are tightly interconnected; an initiative that is righfully gaining more attention in the post-COVID-19 world. Vaccination is a powerful strategy to prevent and control viral outbreaks, as exemplified by the COVID-19 pandemic. Moreover, the vaccination of amplifying intermediate animal hosts provides an effective way to further protect human health. To support efforts to develop vaccines to combat emerging viral zoonoses, we have been using the pig as a pre-clinical model to support COVID-19 vaccine development and are developing a Nipah virus (NiV) vaccine for use in pigs, which would reduce the risk that NiV poses to the Asian pig industry, livestock keepers and public health. Pig-to-human transmission was responsible for the first and most severe NiV outbreak. This outbreak caused severe and lasting economic costs to the Malaysian pig industry. Despite the threat NiV poses to some of the most pig dense regions of the world, no vaccines are currently available. We have therefore evaluated the immunogenicity of recombinant NiV glycoprotein (G or F) based vaccine candidates delivered as protein subunits or by viral or mRNA vectors in pigs [1, 2]. Three vaccine candidates have been evaluated for efficacy and shown to confer a high degree of protection. These are now being evaluated for efficacy after a single immunisation and trialled under field conditions in Bangladesh. In addition to providing a platform for the further development of a NiV vaccine for pigs, we hope these studies will also benefit ongoing human vaccine development efforts. Since March 2020, we have been exploiting the utility of the pig as an outbred large animal model to evaluate the immungenicity COVID-19 vaccine candidates. We demonstrated that a single dose of the Unveristy of Oxford/Astra Zeneca ChAdOx1 nCoV-19 vaccine candidate induced antigen-specific antibody and T cell responses, but a booster immunisation enhanced these responses, with a significant increase in SARS-CoV-2 neutralising titres [3]. We showed that a low dose of a virus-like particle displaying the SARS-CoV-2 spike protein receptor-binding domain induced a strong neutralising antibody response that was superior to convalescent human sera [4]. Ongoing studies are evaluating second generation vaccine candidates designed to enhance protection against virus variants.

[1] R.K. McLean, S.P. Graham. Vaccine development for Nipah virus infection in pigs. Frontiers in Veterinary Science 6:16, 2019.
[2] Pedrera M. et al. Bovine herpesvirus-4-vectored delivery of Nipah virus glycoproteins enhances T cell immunogenicity in pigs. Vaccines 8:115, 2020.
[3] Graham S.P. et al. Evaluation of the immunogenicity of prime-boost vaccination with the replication-deficient viral vectored COVID-19 vaccine candidate ChAdOx1 nCoV-19. npj Vaccines 5:69, 2020.

Non-assembled ORF2 protein of Porcine Circovirus 2b does not confer protective immunity

Flavia Guarneri (1), Matteo Tonni (1), Giuseppe Sarli (2), Maria Beatrice Boniotti (1), Davide Lelli (1) , Ilaria Barbieri (1), Giulia D'Annunzio (2), Giovanni Loris Alborali (1), Barbara Bacci (2), and Massimo Amadori (1)

(1) Istituto Zooprofilattico Sperimentale della Lombardia e dell'Emilia Romagna (2) Università degli Studi di Bologna, DIMEVET

Corresponding author: Massimo Amadori (m_amadori@fastwebnet.it)

Porcine Circovirus 2 (PCV2) vaccines for pigs are based on either inactivated whole virion, or recombinant ORF2 capsid protein assembled into Virus-like Particles (VLPs). No data are available instead about the immunizing properties of free, non-assembled ORF2 protein. To investigate this issue, ORF2 of a reference PCV2b strain was expressed in a Baculovirus-based expression system without assembly into VLPs. The free purified protein was formulated into an oil vaccine at three distinct Ag payloads: 10.8 / 3.6 / 1.2 micrograms /dose. Each dose was injected intramuscularly into five, 37-day old piglets, carefully matched for the residual levels of maternally-derived antibody. Five control piglets were injected with mock vaccine (sterile PBS in oil adjuvant). Twenty-eight days later, all the pigs were challenged intranasally with 200,000 $TCID_{50}$ of PCV2b strain DV6503. After challenge infection, all the pigs remained in good health conditions with no sign of the Porcine Circovirus Associated Disease (PCVAD) complex. The recombinant vaccine did not induce significant antibody and PCV2-specific IFN-g responses. Cytokine ELISPOT and proliferation data confirmed a very poor induction of cell-mediated immunity. In terms of duration and height of post challenge viremia, there was no significant difference between vaccinated and control animals. The histological data indicated the absence of a detectable viral load and of PCVAD lesions in both vaccinated and control animals, as well as of histiocytes and multi-nucleated giant cells associated to an IFN-g response. We conclude that free, non-assembled ORF2 capsid protein does not induce protective immunity, in agreement with other models of immune response to non-enveloped animal viruses.

[1] Guarneri, F., Tresoldi, E.T., Sarli, G., Boniotti, M.B., Lelli, D., Barbieri, I., Bacci, B., D'Annunzio, G., Amadori, M. Protective immunity in swine induced by Porcine Circovirus 2b inactivated vaccines with different antigen payload. Vet. Microbiol. 252, 108887, 2021°.

[2] Guarneri F., Tresoldi E.T., Sarli, G., Boniotti, M.B., Lelli, D., Barbieri, I., Bacci, B., D'Annunzio, G., Amadori, M. Dataset of immune responses induced in swine by an inactivated Porcine Circovirus 2b vaccine. Data in Brief 35, 106906, 2021b.

[3] Segales, J. Best practice and future challenges for vaccination against porcine circovirus type 2. Expert Rev. Vaccines 14, 473-487, 2015.

Comparative functional and phenotypic analyses of the effects of IL-10 and TGF-b on porcine monocyte derived macrophages

Giulia Franzoni (1), Tania Carta* (1,2), Floriana Fruscione (3), Antonio Anfossi (2), Silvia Dei Giudici (1), Dionigia Meloni (1), Maria Luisa Sanna (1), Madrau Paola (1), Susanna Zinellu (1), Bernardo Chessa (2), Elisabetta Razzuoli (3), Annalisa Oggiano (1)*

(1) Istituto Zooprofilattico Sperimentale della Sardegna, Dipartimento di Sanità Animale (2) Università degli Studi di Sassari, Dipartimento di Scienze Veterinarie (3) Istituto Zooprofilattico Sperimentale del Piemonte, Liguria e Valle d'Aosta, Sezione di Genova con annesso CEROVEC
** both authors equally contributed*

Corresponding author: Giulia Franzoni (giulia.franzoni@izs-sardegna.it)

Macrophages are phagocytic cells involved in maintaining tissue homeostasis and defense against pathogens. Macrophages may be differentially activated, resulting in their polarization into different functionally specialized subsets, referred to as 'classically' (M1) and 'alternatively' (M2) activated macrophages [1]. More recently, M2 macrophages were further divided into three subsets: M2a (activated with IL-4 or IL-13), M2b (activated by exposure of immune complexes in combination with IL-1β or LPS) and M2c (stimulated with IL-10, TGF-β or glucocorticoids) [1]. Current knowledge on the porcine immune system present several gaps and few studies have focused on macrophages in different polarized states [2-4]. Porcine M2c macrophages were poorly characterized, thus we investigated the impact of two immunosuppressive cytokines (IL-10 and TGF-b) on porcine monocyte-derived macrophages (moMΦ). The phenotype and functionality of these cells was characterized though confocal microscopy, flow cytometry, ELISA, and RT-qPCR. Their impact on African Swine Fever Virus (ASFV) ability to replicate in moMΦ was also evaluated. Both cytokines induced CD14 and MHC II DR down-regulation and reduced IL-6, TNF-α, and CD14 expression, suggestive of an anti-inflammatory phenotype. Interestingly, neither IL-10 or TGF-β were able to trigger IL-10 induction or release by moMΦ. Differences between these cytokines were observed: stimulation with IL-10, but not TGF-□, induced up-regulation of both CD16 and CD163 on moMΦ. In addition, IL-10 down-regulated expression of IL-1α and IL-12p40 4h post-stimulation and induced a stronger impairment of moMΦ ability to respond to either TLR2 or TLR4 agonists. Finally, both cytokines did not impact ability of either virulent (26544/0G10) or attenuated (NH/P68) ASFV strains to replicate in moMF. Overall, our results provide an overview of porcine macrophage polarization by two immunosuppressive cytokines, revealing differences between IL-10 and TGF-β, and reporting some peculiarity of swine, such as absence of IL-10 induction or release, which should be considered in translational studies.

[1] Mosser DM. The many faces of macrophage activation. Journal of Leukocyte Biology, 73:209-212, 2003.
[2] Singleton H, Graham SP, Bodman-Smith KB, Frossard J, Steinbachet F. Establishing porcine monocyte-derived macrophage and dendritic cell systems for studying the interaction with PRRSV-1. Frontiers in Microbiology, 7:832, 2016.
[3] Garcia-Nicolas O, Baumann A, Vielle NJ, Gomez-Laguna J, Quereda JJ, Pallares FJ, Ramis G, Carrasco L, Summerfield A. Virulence and genotype-associated infectivity of interferon-treated macrophages by porcine reproductive and respiratory syndrome viruses. Virus Research, 179:204-211, 2014.
[4] Sautter CA, Auray G, Python S, Liniger M, Summerfield A. Phenotypic and functional modulations of porcine macrophages by interferons and interleukin-4. Development & Comparative Immunology, 84:181–192, 2018.

Effects of a mycoplasma-derived Pam2cys lipopeptide on porcine monocyte derived macrophages

Giulia Franzoni (1), Antonio Anfossi (2), Chiara Grazia De Ciucis (3), Tania Carta (1,2), Floriana Fruscione (3), Vito Guendalina (3), Dionigia Meloni (1), Silvia Dei Giudici (1), Simon P. Graham (4), Annalisa Oggiano (1), Bernardo Chessa (2), Elisabetta Razzuoli (3)

(1) Istituto Zooprofilattico Sperimentale della Sardegna, Dipartimento di Sanità Animale (2) Università degli Studi di Sassari, Dipartimento di Medicina Veterinaria (3) Istituto Zooprofilattico Sperimentale del Piemonte, Liguria e Valle d'Aosta, Sezione di Genova con annesso CEROVEC (4) The Pirbright Institute, PRRSV Immunology group

Corresponding author: Giulia Franzoni (giulia.franzoni@izs-sardegna.it)

Macrophages play a central role in innate immune response to both infectious and non-infectious stressors. They respond to different agonists modifying their phenotype and functions [1]. Macrophages polarized towards a M1 phenotype should enhance defenses to intracellular pathogens and promote tumor regression [1]; switching macrophages from a pro-tumor type to an anti-tumor state is a promising strategy in cancer immunotherapy [2]. Toll-like receptor (TLR)-2 agonists can be used as vaccine adjuvants or in cancer immunotherapy due to their immunomodulatory properties [2,3]. In the past, we observed that a lipopeptide derived from a surface lipoprotein of *Mycoplasma agalactiae* induced a strong pro-inflammatory cytokine response from porcine monocyte-derived macrophages (moMF) [4]. In this study, we aimed to further investigate the immunomodulatory properties of the mycoplasma-derived Pam2cys lipopeptide (Mag-Pam2cys), to better evaluate its potential use as immunomodulant. Impact of scalar doses of this TLR-2 agonist (10 and 100 ng/mL) on porcine moMF were investigated using confocal microscopy, flow cytometry, ELISA, and RT-qPCR. We observed that Mag-Pam2cys enhanced surface expression of MHCI I, MHC II DR, and CD25, and increased phagocytotic ability. It enhanced expression and release of IL-1b, IL-6, IL-12, TNF-α in a dose dependent manner. This TLR-2 agonist also enhanced expression of IL-10, CD14, p65 and down-regulated TLR-4, TLR-5, TLR-8 expression and had no impact on MYD88, BD1, MD2, Arg-1 expression. These data suggested that this Pam2cys lipopeptide polarized macrophages toward a M1-like phenotype, thus we investigated its impact on porcine moMF when administered simultaneously to an immunosuppressive cytokine: IL-10. Interestingly, Mag-Pam2cys did not alter IL-10 induced MHC II DR down-regulation or CD163 up-regulation at any of the concentration tested. Overall, our results provide an overview of porcine macrophage polarization induced by a mycoplasma-derived pam2cys lipopeptide, and this preliminary *in vitro* study will hopefully lay the foundation for the evaluation of this TLR-2 agonist as an immunomodulant in either cancer therapy or vaccine development.

[1] Mosser DM, Edwards JP. Exploring the full spectrum of macrophage activation, Nature Review Immunology, 8:958-969, 2008.
[2] A toll-like receptor agonist mimicking microbial signal to generate tumor-suppressive macrophages Feng Y, Mu R, Wang Z, Xing P, Zhang J, Dong L, Wang C. Nature Communications, 10:2272, 2019.
[3] Mifsud E, Tan ACL, Jackson DC. TLR agonists as modulators of the innate immune response and their potential as agents against infectious disease. Frontiers in Immunology, 5:79, 2014.
[4] Franzoni et al. Comparison of macrophage responses to African swine fever viruses reveals that the NH/P68 strain is associated with enhanced sensitivity to type I IFN and cytokine responses from classically associated macrophages. Pathogens, 9:209, 2020.

Leukocytes epigenetic modifications in sports horses during training: analysis of genome-wide methylation as an adaptation phenomenon

Katia Cappelli (1)(2), Samanta Mecocci (1), Gianpiero Marconi (3), Emidio Albertini (3), Stefano Capomaccio (1)(2)

(1) Università degli Studi di Perugia, Dipartimento di Scienze Veterinarie (2) Università degli Studi di Perugia, Centro di Ricerca sul Cavallo Sportivo (3) Università degli Studi di Perugia, Dipartimento di Scienze Agrarie, Alimentari e Ambientali

Corresponding author: Katia Cappelli (katia.cappelli@unipg.it)

The Thoroughbred horse is universally recognized as the sport equine breed - with the advantage from bicentennial selection - making it the perfect environment for studying the adaptation exercise and training. Training has a great impact on the physiology of an athlete and, like all stressful stimuli, can trigger an innate immune response and inflammation, which is part of a wider coping strategy of the host to restore homeostasis. Genomic and transcriptomic knowledge [1-4] are at state of the art in equine species while the epigenome and its modifications in response to environmental stimuli, such as training for example, is less studied. One of the major DNA epigenetic modifications is the 5' cytosine methylation, a biochemical modification essential in the mediation of biological processes and in shaping tissues phenotypic diversity. In addition, exercise has already been demonstrated to affect CpG islands methylation state, particularly in humans and mice.

In this work we highlighted, with a genome-wide analysis of methylation, how the adaptation to training in the Thoroughbred impacts on the leukocytes genome methylation patterns. The peripheral blood of twenty foals, subjected to the first workout season, under the same environmental conditions, were sampled at rest in a time course fashion, at the beginning of the training (T0), after 30 (T30) and 90 days (T90). The extracted DNA was analyzed with MCSeEd (Methylation content sensitive enzyme ddRAD), an innovative reduced representation technique for a genome-wide methylation context analysis [5]. A total of 1103 differently methylated genomic regions (DMRs) were found in the different pairwise comparisons, involving 424 genes in T90*vs*T0, 86 in T30*vs*T0 and 223 in T90*vs*T30. These CpG modifications are found in a large part of the genome and therefore referable to a physiological adaptation to training. DMRs were crossed with the latest available annotation and Gene Ontology as well as pathway analysis was performed on the resulting functional elements producing meaningful enriched terms and pathways correlated to exercise induced adaptation. Furthermore, we revealed different epigenetic signatures between the time course, suggesting that there are genes that modify this state early and others that instead require more persistent stimuli.

[1] McGivney, B.A., Hernandez, B., Katz, L.M., MacHugh, D.E., McGovern, S.P., Parnell, A.C., Wiencko, H.L. and Hill, E.W. (2019), A genomic prediction model for racecourse starts in the Thoroughbred horse. Anim Genet, 50: 347-357.

[2] McGivney, B.A., Han, H., Corduff, L.R. *et al.* Genomic inbreeding trends, influential sire lines and selection in the global Thoroughbred horse population. *Sci Rep* 10**,** 466 (2020). https://doi.org/10.1038/s41598-019-57389-5.

[3] Littiere TO, Castro GHF, Rodriguez MDPR, et al. Identification and Functional Annotation of Genes Related to Horses' Performance: From GWAS to Post-GWAS. *Animals (Basel).* 2020;10(7):1173. Published 2020 Jul 10. doi:10.3390/ani10071173.

RANKL Pathway Correlated to Inflammation and Wnt Signaling Activation in Equine Genital Squamous Cell Carcinoma Associated with EcPV2 Infection

Samanta Mecocci (1), Katia Cappelli (1,4), Ilaria Porcellato (1) , Federico Armando (2,3) Luca Mechelli (1), Chiara Brachelente (1), Marco Pepe (1,4) Rodolfo Gialletti (1,4), Benedetta Passeri (2), Paola Modesto (5), Alessandro Ghelardi (6) and Elisabetta Razzuoli (5)

(1) Università degli Studi di Perugia, Dipartimento di Medicina Veterinaria (2) Università degli Studi di Parma, Dipartimento di Medicina Veterinaria (3) University of Veterinary Medicine Hannover, Department of Pathology (4) Università degli Studi di Perugia, Centro di Ricerca sul Cavallo Sportivo (5) IZSLPV, Centro di Referenza di Oncologia Veterinaria e Comparata (CEROVEC) (6) Azienda Usl Toscana Nord-Ovest, UOC Ostetricia e Ginecologia, Ospedale Apuane

Corresponding author: Samanta Mecocci (samanta.mecocci@studenti.unipg.it)

Equine genital squamous cell carcinomas (egSCCs) are among the most common equine tumors after sarcoids, severely impairing animal health and welfare. *Equus caballus* papillomavirus type 2 (EcPV2) infection is often related to these tumors. EgSCCs associated with papillomavirus (PV) infection have been recently proposed as a model for human PV-induced SCC. In both species, PV mucosal infection often induces oropharyngeal, penile, anal, vaginal, and vulvar cancer (1). The aim of this study was to clarify the molecular mechanisms behind egSCCs associated with EcPV2 infection, investigating the receptor activator of nuclear factor-kappa B ligand (RANKL) signaling in NF-kB pathway, together with the Wnt and IL17 signaling pathways. To this propose 23 egSCCs were retrospectively selected. We analyzed the EcPV2 presence and the innate immune response through gene expression evaluation of key cytokines and transcription factors as previously described (2). Moreover, Ki67 index and Beta Catenin (BCAT) were assessed with immunohistochemistry (3). Nucleic acids were extracted and quantified by Qubit (Invitrogen, ThermoFisher Scientific, Waltham, MA, USA). EcPV2-*E6* DNA was checked, and viral presence was confirmed in 21 out to 23 cases (91%). Oncogenes expression was confirmed in 14 cases (60.8%) for *E6* and in 8 (34.7%) for *E2*. *RANKL*, nuclear factor kappa-light-chain-enhancer of activated B cells (*NFKB*)-*p50*, *NFKBp65*, interleukin *(IL)-6*, *IL17*, *IL23p19*, *IL8*, *IL12p35*, *IL12p40*, *β-catenin* (BCATN1), FOS like 1 (FOSL1), and lymphoid enhancer binding factor 1 (LEF1) were selected for gene expression evaluation and *β*2-microglobulin *B2M* was chosen as reference gene to normalize relative gene expression evaluation. RTq-PCR showed a significant up-regulation in tumor samples compared to healthy samples. IHC result showed BCATN1 protein presence in tumor samples. Overall, our results, in agreement with a previous study (2), describe an inflammatory environment characterized by the expression of inflammatory cytokines like *IL6* and *IL8* and the activation of *RANKL*/RANK and *IL17* and relative pathways. Moreover, the increase of *FOSL1*, *LEF1* and *BCATN1* gene and protein expression suggests an activation of Wnt signaling pathway that could be critical for Epithelial-Mesenchymal Transition (EMT) and tumor progression. In this respect, recent evidence in human SCC suggests RANKL/RANK involvement in EMT (4) and RANKL/RANK pathway as a feasible therapeutic target.

[1] Christensen et al., Recent Advances in Preclinical Model Systems for Papillomaviruses. Virus Res, 231:108–118, 2017.
[2] Porcellato et al. Equine Penile Squamous Cell Carcinomas as a Model for Human Disease: A Preliminary Investigation on Tumor Immune Microenvironment, Cells, 9:2364, 2020.
[3] Armando et al. Epithelial to Mesenchymal Transition (EMT) in a Laryngeal Squamous Cell Carcinoma of a Horse: Future Perspectives. Animals, 10: 2318, 2020.

The expression of PD1/PD-L1 and CTLA-4 in equine penile epithelial tumors

Ilaria Porcellato (1), Samanta Mecocci (1), Chiara Brachelente (1), Katia Cappelli (1,3), Alessia Tognoloni (1), Elisabetta Chiaradia (1), Valentina Stefanetti (1), Luca Mechelli (1), Marco Pepe (1,3), Rodolfo Gialletti (1,3), Alessandro Ghelardi (4), Benedetta Passeri (2) and Elisabetta Razzuoli (5)

(1) Università degli Studi di Perugia, Dipartimento di Medicina Veterinaria (2) Università degli Studi di Parma, Dipartimento di Medicina Veterinaria (3) Università degli Studi di Perugia, Centro di Ricerca sul Cavallo Sportivo (3) Azienda Usl Toscana Nord-Ovest, UOC Ostetricia e Ginecologia, Ospedale Apuane (4) IZSLPV, Centro di Referenza di Oncologia Veterinaria e Comparata (CEROVEC)

Corresponding author: Ilaria Porcellato (ilariaporcellatodvm@gmail.com)

During the last few years, cancer immunotherapy has shown promising results with different types of tumors. In particular, with the introduction of immune checkpoint inhibitor (ICI)-targeted immunotherapy, the immunotolerant tumor microenvironment can be overcome, reactivating T cells, enhancing anti-tumor immunity, and eliminating tumor cells more effectively. PD-1/PD-L1 pathway and CTLA-4 are two of the most common targets for immunotherapy [1].

Equine penile squamous cell carcinomas (SCCs) are one of the most common tumors of the external genitalia, with a reported incidence of 49-82.5%. These tumors, commonly arising on plaques or papillomas of older horses, are prone to local recurrence after surgical excision and distant metastases. Most of these tumors are now recognized to be caused by Equus caballus papillomavirus type 2 (EcPV2) [2].

Aim of this study is to evaluate the expression of PD-1/PD-L1 and CTLA-4 in the microenvironment of penile epithelial tumors of horses, in order to evaluate them as possible targets for future immunotherapy in this species.

Twenty cases of equine epithelial tumors were retrospectively selected from archive material. Nucleic acids were extracted to assess the presence of EcPV-2 DNA and to evaluate the gene expression of *PD-1* and *PD-L1*; immunohistochemistry was performed with antibodies against PD-L1 and CTLA-4. Both antibodies were previously validated by western blot on equine placenta and lymph node, respectively.

Results from qPCR revealed that 18/20 cases were positive for EcPV-2 L1 DNA. Fifteen out of 20 cases showed the expression of *PD-1* gene, whereas only 6/20 cases showed expression of *PD-L1* gene through RTqPCR approach. CTLA-4-positive cells were observed in all cases, disseminated through the tumor, but were few (Mdn=5; IQR=2,8-7,35 cells/HPF). Immunohistochemistry for PD-L1 showed positivity only in one case.

The results from our study indicate that tumor immunosuppression mediated by PD-1/PD-L1 pathway is not common in equine penile epithelial tumors. The role of CTLA-4 remains to be further explored, but the relatively low presence of lymphocytes expressing this protein seems to point at a low involvement of this pathway as well.

These preliminary results are in accordance with what reported for HPV-positive penile epithelial tumors in humans, for which the equine counterpart could represent a good comparative model [3].

[1] Lei, Y.; Li, X.; Huang, Q.; Zheng, X.; Liu, M. Progress and Challenges of Predictive Biomarkers for Immune Checkpoint Blockade. Front. Oncol. 2021, 11.
[2] Sykora, S.; Brandt, S. Papillomavirus infection and squamous cell carcinoma in horses. Vet. J. 2017, 223, 48–54

Pinus taeda hydrolyzed lignin role on bovine peripheral blood mononuclear cells response: an ex vivo study

Maria Giovanna Ciliberti (1), Marzia Albenzio (1), Pasquale De Palo (2), Antonella Santillo (1), della Malva Antonella (1), Marino Rosaria (1), Agostino Sevi (1), Mariangela Caroprese (1)

(1) Università degli Studi di Foggia, Dipartimento di Scienze Agrarie, Alimenti, Risorse Naturali e Ingegneria (2) Università degli Studi di Bari, Dipartimento di Medicina Veterinaria

Corresponding author: Maria Giovanna Ciliberti (maria.ciliberti@unifg.it)

Inflammation is a process originated by different types of stimuli that trigger a physiological response reestablishing homeostasis and controlling the internal constant milieu. The control of immune responses is challenging when an exposition to oxidative stress occurs, thus causing a condition of systemic inflammation, named oxinflammation. Phytochemicals extracted from plants and rich in natural antioxidants could help in restoring the antioxidant/oxidant balance and obtaining a modulation of immune response. Nutritional strategies based on lignin could represent a valuable resource of phenolic compounds with antioxidant effects. The aim of the present experiment was the evaluation of the dietary inclusion of Pinus taeda hydrolyzed lignin (PTHL) on the ex-vivo immune responses and oxidative stress biomarkers by peripheral blood mononuclear cells (PBMCs) isolated from beef steers. The animal experiment was performed with approval from Ethics Committee for animal testing–CESA (process number 2-X/17). Briefly, forty Limousine steers (6 months old), were randomly subdivided into two groups; the experimental group received the supplementation with PTHL (Oxyphenol®, I-Green, Padua, Italy); whereas, the control (CON) group did not receive supplementation. The experimental groups were fed ad libitum for 120 days, until 10 months of age (final weight of 521 kg for PTHL group versus 522 kg for CON). At 120 d of the experiment, blood samples from animals were collected from the jugular vein into sterile vacuum tubes containing EDTA. All experiments were performed using PBMC obtained from 10 beef steers (n=4 for PTHL group, and n=6 for CON group). PBMCs were isolated by Histopaque®-1077 density gradient. PBMCs from CON and PTHL groups were stimulated with concanavalin A (ConA, 5 µg/mL) and lipopolysaccharide (LPS, 1 µg/mL) according to Ciliberti et al. [1]. In order to test the effect during oxidative stress exposition, cells were challenged with 4 mM of hydrogen peroxide (H_2O_2) [2]. The proliferation test and the viability assay were carried out on cells, whereas, on supernatants, the cytokine profile (IL-10, IL-12, IL-8, IFN-γ, TNF-α) and the oxidative stress biomarkers (ROS/RNS production, Total Antioxidant Capacity, and Antioxidant/Oxidant Balance-AOB index) were evaluated. Data demonstrated that the dietary inclusion with PTHL had a cytoprotective role after H_2O_2 exposition, increasing the number of viable monocytes and decreasing the reactive oxygen/nitrogen species production in PBMCs supernatants. Cytokine profile revealed an immunomodulatory effect of PTHL, decreasing the secretion of TNF-α and increasing concomitantly the level of IL-8, both cytokines associated with monocyte activation and antioxidant response pathway. Results from the present experiment argued that PTHL may provide a modulation of oxidative stress and inflammatory response in an ex vivo study. Further in vivo studies are needed to support previous findings on PTHL as phytochemical in ruminant feed.

[1] Ciliberti et al. Extracts from Microalga *Chlorella sorokiniana* exert an anti-proliferative effect and modulate cytokines in sheep peripheral blood mononuclear cells, Animals, 9(2):45, 2019.
[2] Asiaei et al. Antioxidant and Protective Effects of Angipars™, a New Herbal Drug, on Hydrogen Peroxide-Induced Oxidative Stress in HepG 2 and A549 Cell Lines. Jundishapur Journal of Natural Pharmaceutical Products, 11(4), 2016.

OMIC characterization of cow, donkey and goat milk extracellular vesicles reveals their anti-inflammatory and immune-modulatory potential

Samanta Mecocci (1), Stefano Capomaccio (1), Daniele Pietrucci (2), Marco Milanesi (2), Giovanni Chillemi (2), Katia Cappelli (1)

(1) Università degli Studi di Perugia, Dipartimento di Scienze Veterinarie (2) Università della Tuscia, Dipartimento per la Innovazione nei sistemi biologici, agroalimentari e forestali

Corresponding author: Samanta Mecocci (samanta.mecocci@studenti.unipg.it)

Other than being a valuable nutrition source, milk represents a sophisticated signaling system that delivers maternal messages. This property seems to be mostly mediated by signaling molecules enclosed in micro/nano sized membrane-bound structures called Extracellular Vesicles (EVs) [1]. EVs act as signal mediators between distant cells and/or tissues, exerting biological effects as immune modulation, anti-inflammatory, anticancer, and pro-regenerative activity [2, 3]. Moreover, milk is a unique, scalable and reliable source of EVs. Our aim is to characterize the molecular content of cow, donkey and goat milk EVs (MEVs) through RNA and metabolites omic analysis in view of prospective applications as immunomodulatory and anti-inflammatory compound. After mass milk EVs isolation through differential centrifugations, an EDTA step for protein precipitation and a 200,000 x g ultracentrifugation for vesicles pelleting, total RNA was extracted and sequenced (mRNA and smallRNA libraries), highlighting over 10,000 transcripts and 2,000 smallRNAs in each species. Concerning smallRNAs, donkey was found to be the most differing species with 57% of the RPKM total amount referring to micro RNA (miRNA) and a conspicuous component of miscellaneous RNA (42%), mostly Y-RNA and Vault. For cow and goat, miRNA was the main represented type with over 99%; the remaining part enclosed protein-coding genes, snoRNAs, snRNAs, lngRNAs and scaRNAs. Among the 50 most expressed smallRNA, 11 were shared by the three species (all miRNAs), 18 between cow and goat only while donkey had 3 other common miRNAs with cow and 4 with goat. As regards mRNA, a comparison between MEV cargos was carried out selecting orthologous genes and ranking by relative expression level. Within the 10% of the most expressed orthologous genes in all three species (1223), 110 were shared. Donkey and goat were the most similar species with 335 shared genes while cow had only 170 genes in common with donkey and 155 with goat. Functional analysis on the 110 core genes revealed enriched GO terms related to translation and protein processing and potential involvement in innate and acquired immunity such as “IL12-mediated signaling pathway”. These terms were also confirmed in analysis on species pairwise shared genes with a further possible function on energy metabolism. Concerning the most abundant genes for each species, donkey and goat MEVs displayed additional terms relative to the immune system such as "innate immune response-activating signal transduction" and amino acids metabolism.

For the metabolomic analysis of MEVs of the three species mass spectroscopy (MS) coupled with Ultrahigh-performance liquid chromatography (UHPLC) was performed. Metabolites, both common or specific of a species, were identified and enriched metabolic pathways were investigated. Results are in particular accordance with our transcriptomic analysis and identify common pathways among the three species involving metabolites with immunomodulating effects such as arginine, asparagine, glutathione and lysine.

[1] Raposo, G. & Stoorvogel, W. Extracellular vesicles: Exosomes, microvesicles, and friends. *J Cell Biol* 200, 373–383 (2013).

The Role of Innate Immune Response and Microbiome in Resilience of Dairy Cattle to Disease: The Mastitis Model

Valerio Bronzo (1), Vincenzo Lopreiato (2), Federica Riva (1), Massimo Amadori (3), Giulio Curone (1), Maria Filippa Addis (1), Paola Cremonesi (4), Paolo Moroni (1, 5), Erminio Trevisi (2), Bianca Castiglioni (4)

(1) Università degli Studi di Milano, Dipartimento di Medicina Veterinaria (2) Università Cattolica del Sacro Cuore, Dipartimento di Scienze animali, Alimentazione e Nutrizione, Facoltà di Agraria, Scienze Alimentari e Ambientali (3) Rete Nazionale di Immunologia Veterinaria (4) Consiglio Nazionale delle Ricerche, Istituto di Biologia e Biotecnologia Agraria (5) Cornell University, Animal Health Diagnostic Center, Quality Milk Production Services

Corresponding author: Bianca Castiglioni (bianca.castiglioni@ibba.cnr.it)

A major concern for the development of livestock activities is represented by the gradual reduction of antibiotic usage in farm animals, which may disturb the fragile balance between animal health and production (1). Therefore, it is necessary to maintain the immunocompetence of farm animals within the structure of this new trend toward reduced drug usage. The immune system has evolved along with the phylogenetic evolution as a highly refined sensing and response system poised to react against diverse infectious and non-infectious stressors for better survival and adaptation (2). Metabolic priority for offspring survival is affected in dairy cattle by the levels of milk yield, often exceeding the potential of dry matter intake. Secondly, the subsequent negative energy balance gives rise to metabolic stress, e.g., a disequilibrium in the homeostasis of a living organism as a result of anomalous utilization of nutrients. It can be argued that high genetic merit for milk yield is correlated with a defective control of the inflammatory response underlying the occurrence of several production diseases (3). This is evident in the mastitis model where high-yielding dairy cows show high disease prevalence of the mammary gland with reduced effectiveness of the innate immune system and poor control over the inflammatory response to microbial agents (4). There is growing evidence of epigenetic effects on innate immunity genes underlying the response to common microbial agents (5). The aforementioned agents, along with other non-infectious stressors, can give rise to abnormal activation of the innate immune system, underlying serious disease conditions, and affecting milk yield (6). Furthermore, the microbiome also plays a role in shaping immune functions and disease resistance as a whole (7). Accordingly, proper modulation of the microbiome can be pivotal to successful disease control strategies. Effective monitoring tools, immunomodulators, and nutraceuticals should be combined with proper farm management and feeding regimes. Specific intervention protocols should be implemented in the first weeks after calving and at dry-off because the relevant stressors are pivotal to disease occurrence and early culling of high-yielding dairy cattle.

[1] Trevisi E. et al. Strategies for reduced antibiotic usage in dairy cattle farms. Res. Vet. Sci. 2014, 96, 229–233.
[2] Amadori M. The Innate Immune Response to Noninfectious Stressors: Human and Animal Models; Academic Press: London, UK, 2016.
[3] Bauman D.E.; Currie, W.B. Partitioning of nutrients during pregnancy and lactation: A review of mechanisms involving homeostasis homeorhesis. J. Dairy Sci. 1980, 63, 1514–1529.
[4] Sordillo L.M. et al. Immunobiology of the mammary gland J. Dairy Sci. 1997, 80, 1851–1865.
[5] Quintin J. et al. Innate immune memory: Towards a better understanding of host defense mechanisms. Curr. Opin. Immunol. 2014, 29, 1–7.
[6] Gunther J. et al. TLR ligands, but not modulators of histone modifiers, can induce the complex immune response pattern of endotoxin tolerance in mammary epithelial cells. Innate Immun. 2017, 23, 155–164.

Selective dry-cow therapy in cows with low somatic cell count: effects on immunometabolism and performance in the subsequent lactation

Luca Cattaneo (1), Fiorenzo Piccioli-Cappelli (1), Vincenzo Lopreiato (1), Andrea Minuti (1), Giorgia Lovotti (1), Norma Arrigoni (2), Erminio Trevisi (1,3)

(1) Università Cattolica del Sacro Cuore, Dipartimento di Scienze animali, della nutrizione e degli alimenti (DIANA), Piacenza (2) Istituto Zooprofilattico Sperimentale della Lombardia e dell'Emilia-Romagna, Piacenza (3) Centro di ricerca "Romeo e Enrica Invernizzi" per le produzioni lattiero-casearie sostenibili (CREI), Piacenza

Corresponding author: Luca Cattaneo (luca.cattaneo@unicatt.it)

Antimicrobial resistance represents a relevant concern for animal and human health and there is an increasing pressure to decrease antibiotic consumption in livestock. The control of udder health contributes in a significant way to the antimicrobials consumption of the dairy sector (1), and the dry-off could represent a phase to be improved. Nowadays, the average SCC is decreasing, many cows do not suffer from intramammary infections before dry-off. Therefore, there is the opportunity to avoid antibiotic therapy in those cows. Thus, the objective of this study was to evaluate the effects of selective dry-cow therapy in cows with low SCC at dry-off using only internal teat sealant or teat sealant paired with antibiotic therapy on udder health, milk production, metabolic and inflammatory conditions through the next lactation. The trial was performed following the Italian laws on animal experimentation and ethics (Prot. 7D5FE.9 in agreement with D. Lgs n. 116, 27/01/1992 and authorization 444/2019-PR in agreement with D. Lgs. n. 26, 04/03/2014). Fifteen Holstein dairy cows with negative bacteriological culture and somatic cells count (SCC) under 200,000 cells/mL the week before dry-off were enrolled in the study. Cows were abruptly dried-off and treated either with antibiotic plus teat sealant (AB, n = 7) or with internal teat sealant only (TS, n = 8). Foremilk and blood samples were collected on scheduled days from 10 days before dry-off to 28 days after calving. Milk composition and inflammatory and metabolic profile were assessed. Milk production and composition were recorded. Rumination time was monitored from 3 weeks before dry-off to 4 weeks after calving. Data were analyzed with PROC MIXED of SAS software.

Both rumination time and immunometabolic biomarkers revealed huge differences across the study period, as a result of dramatic changes happening at the turn of dry-off and calving. Besides, AB and TS cows had similar milk yield and reproduction performance both in the previous and subsequent lactation. Milk production during the first 120 days after calving was not affected by treatment, as well as milk composition in the first month of lactation. Despite similar SCC in the first 28 days in milk (36±23 vs. 73±22x10^3 cells/mL, for AB and TS respectively), TS had a numerically higher average SCC in the next lactation (55±104 vs. 212±98x10^3 cells/mL, for AB and TS respectively) and a slightly higher mastitis incidence in the first 100 days of lactation (14% vs 25%, for AB and TS respectively). Overall, rumination time, metabolic and inflammatory status were similar between AB and TS cows, with only punctual differences during the week after dry-off in calcium and GGT levels, and around calving in alkaline phosphatase concentrations. Results from this study highlighted the possibility, with a view to reducing antibiotic usage in dairy farms, of using internal teat sealant only in cows with low SCC, with little effects on udder health and performance in the subsequent lactation. The slight differences observed were limited to the udder and did not involve systemic reactions, as demonstrated by immunometabolic profile.

Acknowledgments
This study was funded by the "Romeo ed Enrica Invernizzi foundation", Milan, Italy.

(1) Kuipers A, Koops WJ, Wemmenhove H. Antibiotic use in dairy herds in the Netherlands from 2005 to 2012. Journal of Dairy Science, 99:1632–1648, 2016.

Preliminary evidence of endotoxin tolerance in dairy cows during the transition period

Alessia Inglesi (1), Flavia Guarneri (2), Susanna Draghi (1), Joel Filipe (1), Giulio Curone (1); Daniele Vigo (1), Massimo Amadori (3), Federica Riva (1)

(1) Università degli Studi di Milano, Dipartimento di Medicina Veterinaria (2) Istituto Zooprofilattico Sperimentale della Lombardia e dell'Emilia Romagna, Brescia, Italy (3) Rete Nazionale di Immunologia Veterinaria (RNIV), Brescia, Italy

Corresponding author: Alessia Inglesi (alessia.inglesi@unimi.it)

The blastogenic response of bovine Peripheral Blood Mononuclear Cells (PBMC) to lypopolysaccharides (LPS) has been investigated for a long time in our laboratories. In particular, a possible correlation between blastogenic response to LPS and disease resistance of dairy cows had been observed in previous studies [1]. In this study, we investigated the blastogenic response of PMBC sampled from cows at three different time points during the transition period (T0=15 days before calving; T1=7 days after calving; T2=21 days after calving). This study complied with Italian laws on animal experimentation and ethics (Italian Health Ministry authorization n. 628/2016-PR). Isolated PBMC from 8 cows were cultured in the presence or absence of LPS (20 µg or 1 µg/mL), and the blastogenic response was assayed after 72 h by a BrDU incorporation assay. Moreover, the gene expression of cytokines (IL-1, IL-6, TNF-α) and kynurenine pathway molecules (TDO2 and IDO1) was investigated by RT qPCR on both unstimulated and stimulated PBMC. Four of the cows under study developed diseases during the transition period (ketosis and retained placenta). The comparison between healthy and diseased cows suggested that healthy animals developed an advantageous endotoxin tolerance at a high concentration of LPS (20 µg/mL), as well as an efficacious inflammatory response at a lower one (1 µg/mL). On the contrary, diseased animals showed a much greater response at the high LPS concentration and sort of anergy at the lower one. Moreover, TNF-α gene expression was higher in PBMC of diseased cows at T0 and T1; IL-6 gene expression was higher in both LPS-stimulated and unstimulated samples at T2, with a decrease of TNF-α gene expression starting at T1. Based on the blastogenic response to LPS (20 µg/mL), cows were divided into High and Low responders. Unstimulated PBMC of Low responders showed higher levels of expression of the proinflammatory cytokines IL-1, IL-6 and TNF-α compared to High responders at all the 3 time points. PBMC of High responders seemed to better control LPS stimulation at high concentration (20 µg/mL) at all the time points in terms of cytokine gene expression. Finally, LPS-stimulated PBMC of High responders showed a tendency to express higher levels of TDO2 compared to Low responders. Our preliminary data suggest that during the peripartum period dairy cows can be grouped according to their blastogenic response into: A) High responders that seems to be more tolerant to endotoxins and could develop less inflammation in response to different stressors; B) Low responders that could be more prone to develop unwanted inflammatory conditions even in response to mild stimulations. The different response of the two groups is likely to involve the kynurenine pathway. Our preliminary study confirms our previous observation [2], but the endotoxin tolerance mechanism of dairy cow during transition period needs to be better characterized by further investigations.

[1] Catalani E, Amadori M, Vitali A, Lacetera N. Short communication: Lymphoproliferative response to lipopolysaccharide and incidence of infections in periparturient dairy cows. J Dairy Sci; 96 (11):7077–7081, 2013.
[2] Amadori M., Soares-Filipe JF, Riva F., Vitali A., Ruggeri J., Lacetera N. Characterization of the blastogenic response to LPS of bovine peripheral blood mononuclear cells. PlosOne, 13(10), 2018.

ORAL COMMUNICATIONS

SICLIMVET

Klebsiella pneumoniae in calves with neonatal diarrhea

Giulia Sala, Antonio Boccardo, Vincenzo Ferrulli, Angelo Belloli, Guido Grilli, Alessia Libera Gazzonis, Laura Filippone Pavesi, Davide Pravettoni

Università degli Studi di Milano, Dipartimento di Medicina Veterinaria

Corresponding author: Giulia Sala (giulia.sala@guest.unimi.it)

In cattle, *Klebsiella* spp. is considered a primary pathogen for mastitis and an opportunistic pathogen for respiratory diseases [1]. However, if widespread in the environment, it may cause neonatal diarrhea in calves [2].

The present study describes an outbreak of *Klebsiella pneumoniae* in a dairy cattle farm in northern Italy with an increased incidence of severe or fatal neonatal calf diarrhea (NCD) starting from October 2020. The calf mortality rate, due to NCD was 69% in October 2020 (new born calves: n=16; diseased calves: n=16, death calves: n=11). The farm was checked by our clinic between November 2020 and February 2021. Starting to November, in all new born calves (n=28), fecal samples were collected during the first week after calving, and analysed for rota- and corona- viruses, bacteria, and parasites including *Cryptosporidium parvum*. A blood sample of each calf was collected, and serum Total Protein (sTP) was measured to test the transfer of passive immunity. Moreover, transition milk of the dams, faeces of the dams and a sample of calving pen's bedding were collected and analysed microbiologically.

During the study period 9 calves developed NCD (7 cases in November, 1 in December, 1 in January). *K. pneumoniae* was identified in 5 of these 9 cases (56%). In 2 cases *K. pneumoniae* was the only pathogen isolated, while in the other 3 cases, the presence of *E. coli*, coronavirus, and rotavirus was demonstrated. *C. parvum* was never detected. Between the 19 new born calves that did not have NCD episodes during the study period, *K. pneumoniae* was identified in only 1 case (5%) and 1 calf died for polytrauma (crashed by the dam).

K. pneumoniae was not isolated from the feces and transition milk of dams and sample of calving pen's bedding.

The transfer of passive immunity was poor in the calves examined in November (average sTP = 46 g/L). After improvement of colostrum management and farm hygiene protocols, the transfer of passive immunity significantly increases (average sTP=60 g/L) and the mortality rate significantly decreases from 69% in October to 56% (10 of 18 new born calves) in November and to 17% (1 of 6 new born calves) and 22% (2 of 9 new born calves) in December and January, respectively. In February no case fatality was recorded.

K. pneumoniae resulted statistically correlated with NCD. The most relevant problem correlated to *K. pneumoniae* infection in calves was the mortality rate. As previously described [3], treatment of NCD, improvement of the farm hygiene and colostrum management, allowed to reduce NCD episodes, *K. pneumoniae* incidence in new born calves and mortality rate.

[1]Munoz M.A., Zadoks R. N. Patterns of fecal shedding of *Klebsiella* by dairy cows, Journal of dairy science, 90 (3):1220-1224, 2007.

[2]Haggag Y.N., Samaha H.A., Nossair M.A., Awad W.K., Epidemiological studies on diarrhea in some dairy cattle farms. Alexandria Journal of Veterinary Sciences, 51(2): 282-289, 2016.

[3]Boccardo A., Sala G., Ferrulli V., Pravettoni D., Cut-off values for predictors associated with outcome in dairy calves suffering from neonatal calf diarrhea. A retrospective study of 605 cases. Livestock Science, 245:104407, 2021.

Seroprevalence and clinical outcomes of *Neospora caninum*, *Toxoplasma gondii* and *Besnoitia besnoiti* infections in water buffaloes (Bubalus bubalis)

Maria Chiara Alterisio, Lavinia Ciuca, Antonio Bosco, Carlo Ferravante, Giuseppe Cringoli, Paolo Ciaramella, Maria Paola Maurelli, Antonio Di Loria, Laura Rinaldi, Jacopo Guccione

Università degli Studi di Napoli Federico II, Dipartimento di Medicina Veterinaria e Produzioni Animali

Corresponding author: Maria Chiara Alterisio (mariachiara.alterisio@unina.it)

Estimation under field conditions of clinical findings associated with parasitic infections is an interesting challenge in Mediterranean buffalo (MB) medicine, where knowledge regarding their consequence on the animal health and the dairy food chain is almost rare [1, 2]. Therefore, the present study aimed to investigate the seroprevalence and clinical findings associated with the presence of *N. caninum, T. gondii* and *B. besnoiti* in the dairy MB.

One hundred twenty-four water buffaloes characterized by poor reproductive performance [≥600 Days Open (DO)], but without any macroscopic reproductive disorders, were enrolled from 9 farms located in southern Italy. The historical information regarding the age and the number of DO as well as of events such as abortions, embryonic deaths, retained foetal membranes were collected for each animal. Blood sera samples were collected from the entire study population and analysed by 3 different indirect enzyme-linked immunosorbent assays (ELISAs). All the variables were analysed by standard descriptive statistics. Univariate statistical analysis and logistic regression models have instead verified risk factors and interaction between data observed. As of last, the odds ratio (OR) was used to quantify the association between each clinical parameter and the positive status for the parasites. Of 61/124 (49.2%) water buffaloes categorized as antibody positive for at least one of the aborting protozoa considered, 25/124 animals (20.2%) were seropositive only for *N. caninum,* while 17/124 (13.7%,) only for *T. gondii*. No buffalo showed specific antibodies for *B. besnoiti*. Nineteen of 124 animals (15.3%) resulted in seropositive to both protozoa (*T. gondii* and *N. caninum*). The mono-infection with *N. caninum* seems mainly associated with abortion and the presence of retained foetal membranes, while mono-infection with *T. gondii* has been associated with an increase of days open. Moreover, the co-infection by *N. caninum* and *T. gondii* strengthened the abortive effects (OR=7.330) and showed further negative effects on the parameter embryonic death (OR=2.607). The clinical-parasitological findings of the present investigation demonstrated the direct effects of *N. caninum* and *T. gondii* in the water buffalo as well as tested the absence of antibodies specific to *B. besnoti.* The study may be considered the starting point to build the essential basic knowledge promoting the awareness of the parasitological infection's relevance in this ruminant. The clinical screening of *N. caninum* and *T. gondii* might be suggested in the routine diagnosis of abortive agents in buffalo herds characterized by poor fertility performances or pregnancy losses.

[1] Gondim et al.. Importance of serological cross-reactivity among *Toxoplasma gondii, Hammondia* spp., *Neospora* spp., *Sarcocystis* spp. and *Besnoitia besnoiti*. Journal of Parasitology, 144: 851–868, 2017.

[2] Brasil et al. Risk factors for *Toxoplasma gondii* and *Neospora caninum* seropositivity in bubaloes in Paraiba State, Brazil. The Revista Brasileira de Parasitologia Veterinária, 24:459–463, 2015.

Metabolomics profile in healthy and hyperketonemic cows assessed by proton nuclear magnetic resonance spectroscopy (^{1}H-NMR)

Anastasia Lisuzzo (1), Luca Laghi (2), Chenglin Zhu (2), Vanessa Faillace (1), Massimo Morgante (1), Elisa Mazzotta (1), Matteo Gianesella (1), Enrico Fiore (1)

(1) University of Padova, Department of Animal Medicine, Production and Health (2) University of Bologna, Department of Agro-Food Science and Technology

Corresponding author: Anastasia Lisuzzo (anastasia.lisuzzo@phd.unipd.it)

In early lactation, dairy cows mobilize their energy reserves for milk production. The mobilization of adipose tissue leads to an increase in β-hydroxybutyrate (BHB). Ketosis is a metabolic disease that cause considerable economic issues and presents a BHB blood concentration ≥ 1.0 mmol/L [1]. Metabolomics is a new tool that may investigate the reactions to metabolic stress in dairy cows [2].

The aim of this study is to evaluate the serum metabolomics profile of healthy and hyperketonemic (HK) cows using ^{1}H-NMR and to assay potential biomarkers.

49 Holstein Friesian dairy cows were enrolled in this study (Ethics protocol number 91/2019, University of Padua). Blood samples were collected from the coccygeal vein and stored in tubes containing Clot Activator. Cows were divided into 3 groups according to BHB concentrations (mmol/L): G0 (BHB ≤ 0.5); G1 (0.5 < BHB < 1.0) and G2 (BHB ≥ 1.0). Significant metabolites were assessed by ANOVA (*p-value* <0.05) and ROC curves were calculated to develop potential biomarkers. 57 metabolites were identified in metabolomics profiling: 13 were significantly different between G0 and G2 groups. In G2, a higher concentrations of ketone bodies and 3-methylhistidine, a lower concentration of glycerol, creatine and creatinine were reported. The high concentration of 3-methylhistidine could be due to the increased muscle metabolism, conversely the decrease in creatine and creatinine may be related to the pauperization of total muscle body mass [3]. Reduction of glycerol could be justified by the fat mobilization for gluconeogenesis. The increase in anaerobic fermentation products (methanol, ethanol, 2,3-butanediol, acetate, propionate and 3-hydroxyisobutyrate) and the reduction in dimethylsulfone and formate, inversely related to methane production, suggested the increase in anaerobic fermentation in G2 [4]. The higher concentrations of trimethylamine N-oxide and methylsuccinate, the reduction in choline and isovalerate may explain impaired hepatic function and liver oxidative stress [5]. The reduction of glucogenic amino acids suggested their conversion into intermediates of Krebs cycle for gluconeogenesis. 10 metabolites were moderately accurate biomarkers (0.7 ≤ AUC ≤ 0.9) and 1 was highly accurate (BHB; AUC > 0.9) in predicting HK.

In conclusion, the results of this study showed a shift in metabolic status from healthy to HK cows and ten potential biomarkers were identified.

[1] Fiore et al. Identification of plasma fatty acids in four lipid classes to understand energy metabolism at different levels of ketonemia in dairy cows using thin layer chromatography and gas chromatographic techniques (TLC-GC), Animals, 10:1-20, 2020.

[2] Ametaj. A systems veterinary approach in understanding transition cow diseases: metabolomics, Proceedings of the 4th international symposium on dairy cow nutrition and milk quality, p.78-85, 2015.

[3] Megahed et al. Changes in skeletal muscle thickness and echogenicity and plasma creatinine concentration as indicators of protein and intramuscular fat mobilization in periparturient dairy cows, Journal of Dairy Science, 102:5550–5565, 2019.

[4] Yanibada et al. Inhibition of enteric methanogenesis in dairy cows induces changes in plasma metabolome highlighting metabolic shifts and potential markers of emission, Scientific Reports, 10:1–14, 2020.

[5] Wang et al. Pathway analysis of plasma different metabolites for dairy cow ketosis, Italian Journal of Animal Science, 15:545–551, 2016.

Serum metabolomics analysis identifies potential biomarkers for subclinical ketosis in ewes using proton nuclear magnetic resonance spectroscopy (^{1}H-NMR)

Anastasia Lisuzzo (1), Luca Laghi (2), Vanessa Faillace (1), Filippo Fiore (3), Nicoletta Spissu (3), Matteo Gianesella (1), Massimo Morgante (1), Chenglin Zhu (2), Livia Moscati (4), Elisa Mazzotta (1), Enrico Fiore (1)

(1) University of Padova, Department of Animal Medicine, Production and Health (2) University of Bologna, Department of Agro-Food Science and Technology (3) University of Sassari, Department of Veterinary Medicine (4) Experimental Zooprophylactic Institute of Umbria and Marche

Corresponding author: Anastasia Lisuzzo (anastasia.lisuzzo@phd.unipd.it)

A high energy demand, a reduction in dry matter intake and a consequent negative energy balance state (NEB) are common features in early lactation ruminants. Subclinical ketosis (SK) is a metabolic disease due to NEB and cause several health issues in dairy livestock. The β-hydroxybutyrate (BHB) is a biomarker used to identify SK with a cut-off ≥ 0.80 mmol/L in sheep [1]. Metabolomics is a powerful tool to investigate clinical conditions and to predict the development of specific clinical alterations [2].

The aim of this study is to evaluate serum of healthy sheep and ewes with SK using ^{1}H-NMR and to assay potential biomarkers for SK.

The study was carried out on 46 Sarda dairy sheep (Ethics protocol number 128469/2019, University of Sassari) selected within 10 days in milk. Blood sampling were collected from jugular vein and stocked into tubes containing Clot Activator. The ewes were divided in two groups according to BHB: < 0.80 and ≥ 0.80 mmol/L. The significant metabolites were assessed by t-test ($P \leq 0.05$), and ROC curves were performed to develop potential biomarkers. 54 metabolites were identified in the serum samples, and 14 were statistically significant. In sick ewes were reported a higher concentration of 3-methylhistidine and a lower concentration of alanine and tyrosine. These metabolites are indicators of impaired muscle metabolism [3]. Higher concentrations of ethanol, methanol, 2,3-butanediol, acetate and 3-hydroxyisobutyrate could suggest an increase in alcoholic fermentation and a possible variation in ruminal microbial populations. Trimethylamine N-Oxide marker of oxidative stress [4] was highly increased in SK ewes, clarifying the development of SK condition. Decrease in different glucogenic amino acids in SK ewes may be justified by their conversion into Krebs cycle substrates, purposed for gluconeogenesis [5]. High concentrations of succinate and lower concentrations of arginine and glutamine were reported in SK ewes, which could impair the Krebs cycle enzymatic functions [6] and the urea cycle, respectively. Eleven metabolites were identified as moderately accurate biomarkers (0.7< AUC <0.9) and two as highly accurate (AUC >0.9). The statistical analysis overall the most significant metabolites presented higher accuracy (AUC = 0.963) than acetone and BHB (AUC = 0.90 and 0.92) as early biomarker. In conclusion, a set of ten metabolites showed an excellent accuracy as biomarker, and metabolomics may represent a useful diagnostic tool to discriminate SK ewes.

[1] Doré et al. Definition of prepartum hyperketonemia in dairy goats, Journal of Dairy Science, 98:4535-4543, 2015. [2] Ametaj. A systems veterinary approach in understanding transition cow diseases: metabolomics, Proceedings of the 4th international symposium on dairy cow nutrition and milk quality, p.78-85, 2015. [3] Palma et al. The hepatic and skeletal muscle ovine metabolomes as affected by weight loss: A study in three sheep breeds using NMR-metabolomics, Scientific Reports, 6:1-11, 2016. [4] Xu et al. 1H-nuclear magnetic resonance-based plasma metabolic profiling of dairy cows with fatty liver, Asian-Australasian Journal of Animal Sciences, 29:219-229, 2016. [5]Sun et al. 1H-Nuclear magnetic resonance-based plasma metabolic profiling of dairy cows with clinical and subclinical ketosis, Journal of Dairy Science, 97:1552-1562, 2014. [6] Martínez-Reyes et al. Mitochondrial TCA cycle metabolites control physiology and disease, Nature Communications, 11:1-11,2020.

Diagnostic accuracy of a clearly defined increased bronchial sound and ultrasonography to detect lobar pneumonia (lung consolidation) in dairy calves affected by the bovine respiratory disease—a Bayesian approach

Antonio Boccardo (1), Giulia Sala (1), Salvatore Ferraro (2), Vincenzo Ferrulli (1), Davide Pravettoni (1), Angelo Belloli (1)

(1) Università degli Studi di Milano, Dipartimento di Medicina Veterinaria (2) Université de Montréal, Département de Sciences Cliniques

Corresponding author: Antonio Boccardo (antonio.boccardo@unimi.it)

Thoracic auscultation is an inexpensive tool used to diagnose pneumonia, and it is often the first diagnostic approach used by practitioners in the field. One of the major issues of thoracic auscultation in cattle is that there is no clear definition of lung sounds in the available literature. The lack of a clear definition of the lung' sounds is a cause of confusion in used terminology and interpretation of auscultated sounds. This study's objective was to evaluate the diagnostic accuracy of a thoracic auscultation system, characterized by a clear definition of lung sounds, to diagnose BRD in dairy calves compared to thoracic ultrasound (TUS) using the Bayesian latent class approach. We hypothesized that a clear definition of the increased bronchial sound increases thoracic auscultation's diagnostic accuracy in calves with lung consolidation.

A prospective diagnostic accuracy study was performed in 14 dairy farms regularly checked by our ambulatory clinic. Calves were observed for the detection of spontaneous cough. Coughing calves were caught and scored using the Wisconsin calf respiratory scoring chart. If one of the coughing calves reached a total respiratory score of 5 or more, it was considered a BRD case. All calves within the pen were then considered eligible for the study. One hundred and seventy-eight calves were submitted to thoracic auscultation by an experienced veterinarian using the stethoscope. Auscultation score ranged between 0 to 4 (0 = normal; 1 = increased breath sound; 2 = wheezes or crackles; 3 = increased bronchial sound; 4 = pleuritic friction rubs or decreased audibility or absence of breath sounds). The increased bronchial sound (score 3) was defined when the sound coming from inspiration was identical to expiration. A 6-level systematic thoracic ultrasonography (TUS) score was finally performed in all auscultated calves by an experienced veterinarian blinded to thoracic auscultation results. Data from thoracic auscultation has been dichotomized into two categories: score 3 (cases) and cumulated scores 0,1,2,4 (no cases). TUS data were dichotomized into calves with consolidated lungs (cases) and non-consolidated lungs (no cases). A Bayesian latent class model allowing for conditional dependence between tests was used to estimate tests' accuracy. The sensitivity of increased bronchial sounds was 48.1% (95% Bayesian credible interval [BCI]: 6.8–95.1%), specific was 73.3% (95% BCI: 24.1–98.1%). Sensitivity of TUS was 62.4% (95% BCI: 15.1–97.5%) and specificity was 61.4%(95% BCI: 11.5–95.6%).

The use of a clearly defined increased bronchial sound significantly improving specificity; in fact, increased bronchial sounds do not occur in unconsolidated calves. On the other hand, this observation may support the hypothesis that the low sensitivity of the increased bronchial sound is not linked only to the clinician's subjectivity and experience but also that this sound is not produced even in the face of obvious lung damages. Although this study's results raise intriguing questions about the use of the increased bronchial sounds in consolidated lungs, due to low sensitivity, thoracic auscultation cannot be used as an accurate tool in calves that do not show this adventitious sound. These results reinforce the rationale for using TUS as a tool to guide diagnosis and interventions to mitigate respiratory disease in dairy calves and confirm that the addition of TUS over auscultation shows a significant improvement in the accuracy of BRD diagnosis.

Evaluation of procalcitonin concentrations in plasma and milk in healthy cows

Chiara Orsetti (1,2), Micaela Sgorbini (1,2), Valentina Meucci (1), Marta Cresci (1), Francesca Bonelli (1,2)

(1) Università degli Studi di Pisa, Dipartimento di Scienze Veterinarie (2) Università degli Studi di Pisa, Centro di Ricerche Agro-Ambientali E. Avanzi

Corresponding author: Chiara Orsetti (chiara.orsetti@phd.unipi.it)

The diagnosis of mastitis is based on somatic cells count (SCC) in milk, a quick test with low sensitivity for bacterial infection [1]. Procalcitonin (PCT) has been investigated as plasmatic biomarker of bacterial infection in human [2] and veterinary medicine [3-6]. In humans, PCT was evaluated in milk in addition to plasma [7]. The aim of this study was to assess plasma and milk PCT concentrations in healthy cows with a quantitative ELISA kit for human use. This research was approved by the ethics committee for animal welfare of the University of Pisa (2825/14). The study involved 27 healthy Italian Friesian cattle for a total of 29 quarters. In order to be included, cows should be healthy based on clinical examination performed at the sampling time. Contextually, milk was evaluated and cows having a California Mastitis Test <1 and a SCC < 250.00 cells/ml were included [1]. Milk and blood samples were taken and analyzed with the ELISA kit (Sigma-Aldrich, USA). Validation of the ELISA kit was performed using 3 plasma and milk samples from septic cattle included in another research protocol (personnel data); sensitivity, limit of detection (LOD), precision, the intra-assay and inter-assay coefficient of variation (CV) (acceptance criteria was a CV <25%), parallelism and recovery (acceptance criteria were a recovery within 80% and 120%, respectively) were calculated. PCT concentrations were estimated as 1545.1±3100.0 pg/ml in plasma and 518.1±748.7 pg/ml in milk samples. The LOD has been calculated as 20 pg/ml. The method showed an inter- and intra-coefficient of variation (CV) for PCT in the three plasma samples of 11, 21, 12.6% and 20, 13.2% and 19.9%, respectively and an inter and intra-CV for PCT in the three milk samples of 65.3, 52.4, 60% and 45.2, 58, 65.2%, respectively. Parallelism, determined by serial two-fold dilutions of plasma samples with a high endogenous PCT level showed a recovery between 92% and 118%. Parallelism for the milk samples could not be calculated because the samples showed concentrations <LOD. This study supports the hypothesis that human PCT ELISA kit could be used to measure bovine PCT in plasma samples, with an intra-assay and inter-assay CV less than 20% and adequate recovery, but do not support the use of the same kit to detect PCT in milk samples.

[1] Jamali et al. Invited review: Incidence, risk factors, and effects of clinical mastitis recurrence in dairy cows, Journal of dairy science, 101(6):4729-46, 2018.
[2] Riedel S. Procalcitonin and the role of biomarkers in the diagnosis and management of sepsis, Diagnostic microbiology and infectious disease, 73:221-7, 2012.
[3] Bonelli et al. Plasma procalcitonin concentration in healthy horses and horses affected by systemic inflammatory response syndrome, Journal of veterinary internal medicine, 29(6):1689-91, 2015.
[4] Bonelli et al. Evaluation of plasma procalcitonin concentrations in healthy foals and foals affected by septic systemic inflammatory response syndrome, Journal of equine veterinary science, 35(8):645-9, 2015.
[5] Matur et al. Biology of procalcitonin and its potential role in veterinary medicine, Journal of Istanbul Veterinary Sciences, 1:16-27, 2017.
[6] Bonelli et al. Plasma procalcitonin concentration in healthy calves and those with septic systemic inflammatory response syndrome, The veterinary journal, 234:61-5, 2018.
[7] Struck et al. High concentrations of procalcitonin but not mature calcitonin in normal human milk, Hormone and metabolic research, 34:460-5, 2002.

Hematological and biochemical parameters in recovered or deceased roe deer

Maria Irene Pacini, Francesca Bonelli, Irene Nocera, George Lubas, Micaela Sgorbini

Università di Pisa, Dipartimento di Scienze Veterinarie

Corresponding author: Micaela Sgorbini (micaela.sgorbini@unipi.it)

Clinical pathology data are useful in the overall clinical assessment. The study assessed haematological and biochemical parameters in roe deer admitted to the Veterinary Teaching Hospital during 2010-21 to verify differences between recovered and deceased animals.

The study was done on a cohort of 69 roe deer rescued in the Pisa district. At admission, animals underwent a clinical examination and blood were collected as soon as possible. If animals were difficult to handle, blood collection was performed under sedation or general anaesthesia. The blood was collected in both K3-EDTA and serum tubes. The EDTA samples were used to perform a complete blood count using a laser cell counter (ProCyte Dx, IDEXX). Samples containing clots or grossly haemolysed were discarded from the analysis. Serum was analysed using a spectrophotometric and immunoturbidimetric analyser (Liasys, Analyzer Medical System) and the following parameters were evaluated: creatinine, urea, total and direct bilirubin, aspartate transaminase (AST), gamma-glutamyl-transferase (GGT), creatine kinase (CK), alkaline phosphatase (ALP) and total protein.

The animals were retrospectively divided into 2 groups according to the outcome: recovered (R) (n=16) or deceased (D) (n=53). The data distribution was evaluated using the Kolmogorov-Smirnov test and results were expressed as median, minimum and maximum values. The Mann-Withney test for unpaired data was applied to assess differences between deceased vs. survived for each parameter analysed.

The recovered group included 5/16 <3 mo and 6/16 4-24 mo and 5/16 >24 mo and the deceased group included 6/53 <3 mo, 29/53 4-24 mo, 18/53 >24 mo, respectively. Roe deers <3 mo were victims of an ill-advised rescues or injured by a combine harvester/bitten by domestic dogs, while the young and adult ones were injured in car accidents or trapped in nets or fences. RBC values were 9.1 (6.8-11.8 $x10^9$/L) and 10.6 $x10^9$/L (4.1-14.1 $x10^9$/L), CK activity was 601 (70-42,660 UI/L) and 8,933 U/L (106-166,960 U/L), total bilirubin concentration was 0.5 mg/dL (0.2-0.8 mg/dL) and 0.8 (0.1-12.8 mg/dL) in the recovered and deceased group, respectively. RBC (p=0.046), CK (p=0.006) and total bilirubin (p=0.002) showed higher values in the deceased group vs. the recovered one.

The values for RBC and total bilirubin obtained in the deceased group might reflect severe blood loss and/or hematoma due to trauma caused by road accident that is considered the principal cause of rescue in wildlife ungulates in Tuscany (1,2). The increased CK activity might be associated with rhabdomyolysis due to trauma or muscle exhaustion due to incorrect transport practices (2).

[1] Masciarelli L. Fauna guida sicura, fauna selvatica e sicurezza stradale. Assessorato alla difesa alla fauna, Provincia Pisa, Italia: Press, 2009.
[2] Pacini MI et al. Wildlife ungulate rescue and emergency services in the Pisa area (Tuscany, Italy): evaluation of a 9-years period (2010-2018), Front Vet Sci, 7:626.

Ultrasound liver monitoring as precision farming technique for the assessment of cystic echinococcosis in sheep

Antonio Di Loria, Jacopo Guccione, Giuliano Borriello, Antonio Bosco, Paola Pepe, Francesco Prisco, Giuseppe Cringoli, Orlando Paciello, Laura Rinaldi, Paolo Ciaramella

University of Napoli Federico II, Department of Veterinary Medicine and Animal Productions

Corresponding author: Antonio Di Loria (adiloria@unina.it)

Echinococcus granulosus (*E. granulosus*) is a cestode causing cystic echinococcosis (CE) in intermediate hosts (human and livestock) and dwelling in the small intestines of the definitive hosts (canids) in its adult form. CE is a widespread zoonotic parasitic disease having a negative effect on human-animal health and livestock production [1]. Early in vivo diagnosis, control and prevention of the infection of *E. granulosus* in sheep are crucial steps to reduce its diffusion. Currently, liver ultrasonography is one of the most reliable diagnostic techniques for CE assessment in the intermediate hosts [2,3]. So, the aims of the present study were: (i) to evaluate the sensibility and specificity of a new fast ultrasonographic method in different sheep's body weight; (ii) to compare the latter with the protocol developed on the Italian Sarda sheep, based on a single hypochondrial acoustic window (HYP) [2], as well as (iii) to try to define a new and fast-focused technique for CE detection under field conditions. One-hundred-seventy-two female sheep of different breeds were submitted to a complete liver ultrasound examination (cUS) starting from the right hypochondrium to the 5th intercostal space (IS) by a single expert operator. The evaluated scan area was divided in Zone 1 (Z1, from the right hypochondrium to the 11th IS), Zone 2 (Z2, from the 10th IS to the 8th IS) and Zone 3 (Z3, from the 7th IS to 5th IS). Moreover, also the HYP technique was performed. Each zone (ZONAL scan) was analysed individually along with the contiguous ones (HYP+ Z1; Z1+Z2, Z2+Z3). During each scanning, the hydatid lesions detected were localized in the corresponding zones. After the clinical procedures, the animals were slaughtered, and necropsy's results were recorded (gold standard). All US techniques were compared using sensitivity and specificity as well as the number and percentage of positive zone detected. Because of the non-homogenous weight distribution, the sample population was later divided into Group 1 (G1, weight ≤50 kg: 22/172 - 13%), Group 2 (G2, 51≤ weight ≤ 75: 69/172 – 40%), Group 3 (G3, weight ≥76: 81/172 – 47%). cUs showed the highest level of sensitivity and specificity, as well as the highest number of positive zones when compared to all the other techniques (p≤0.01). cUS, resulted the best technique also during the comparison by weight distribution; HYP and HYP+Z1 showed performance similar in lighter sheep (Group 1). The present investigation confirmed that ultrasonography could be considered a reliable intra-vitam technique for CE assessment. Indeed, scanning the entire organ (from the hypochondrium to the 5th IS) is recommended under field conditions to optimize the diagnostic performance. However, the time needed for the exam execution can represent a limit especially for screening in large flocks; further strategies to reduce the time consuming under field conditions should be evaluated to improve the widespread of ultrasound use for CE diagnosis in sheep flock.

1. Craig et al, Prevention and control of cystic echinococcosis. *Lancet Infect. Dis.* 2007, *7(6)*,
2. Dore et al., Ultrasound as a monitoring tool for cystic echinococcosis in sheep. *Vet Par.* 2014, *203*, 59–64.
3. Hussein et al., Ultrasonographic features of the liver with cystic echinococcosis in sheep. *Vet. Rec. Open* 2014, 1(1):e000004.

Automatic assessment of feeding, ruminating and locomotion behaviours in dairy cows naturally affected by diseases during peripartum period

Jacopo Guccione (1), Fadul Mahmoud (2), Maria Chiara Alterisio (1), Maher Alsaaod (2), Daniele Furio (1), Carlo Ferravante (1), Antonio Di Loria (1), Adrian Steiner (2), Paolo Ciaramella (1)

(1) Università degli Studi di Napoli Federico II, Dipartimento di Medicina Veterinaria e Produzioni Animali (2) Universität Bern, Vetsuisse Fakultät, Wiederkäuerklinik

Corresponding author: Jacopo Guccione (jacopo.guccione@unina.it)

The goal of the study was to assess and compare the feeding, ruminating and locomotion behaviour between cows naturally affected by diseases and healthy cows in the first week after calving. Forty-two, free-stall-housed, pluriparous Holstein x Friesian cows were enrolled from 14 days (d) before up to 1 week (wk) after calving. Based on the output of a 3-dimensional accelerometer placed either in a nose-band sensor or a pedometer [RumiWatch®, ITIN+HOCH GmbH, Fütterungstechnik, Liestal, Switzerland], different feeding, rumination and locomotion behaviours were continuously recorded along this period. *Eating time, ruminating time, ruminating boluses, eating chews, ruminating chews, other activity time*, *lying time, standing time, walking time, lie down, stand up, lying bouts, standing bouts, walking bouts and number of strides* were the parameters considered [1, 2]. Cows' health status was continuously monitored through general clinical examination and weekly complete blood analysis. Animals affected by ≥1 disease were considered sick. RumiWatch® data were converted into 24-hour summaries, and days around calving (d -1, d 0 and d +1) were excluded from the analysis. The mean values of wk -2, wk -1 and wk +1 relative to calving were calculated and compared. Moreover, activities registered on the day the disease was first clinically diagnosed (dd0), one and two days before disease diagnosis were also described (dd -1 and dd -2). Lastly, differences between dd0 vs. dd-1 (ΔD1), dd0 vs. wk -1 (ΔD2), and wk +1 vs. wk -1 (Δweeks) were assessed. At the end of the clinical monitoring phase, cows were divided into group-S (n=24 sick cow; all of them diagnosed in wk +1) and group-H (n=18 healthy cows). In group-S, eating and ruminating parameters were significantly decreased in wk +1 compared to wk -1, while no difference was detected in group-H, for the same time period. In groupsS and H, s*tanding* and *walking time* as well as the *number of strides* were significantly increased in wk +1 compared to wk 1. *Lying time* was instead significantly decreased in wk +1 compared to wk -1, in both groups. At wk +1 and dd0, *eating* and *ruminating time*, *eating* and *ruminating chews*, as well as *ruminating boluses*, were significantly lower in group-S compared to group-H, while other activity time was significantly higher. For ΔD2 and Δweeks, the difference between *eating* and *ruminating time*, *eating* and *ruminating chews* was significantly lower in group-S compared to group-H. Regarding the locomotion behaviours, at wk +1 and dd-2, the *lying time* in group-S was significantly higher compared to group-H, while the *standing time* was significantly lower. Besides, the *number of strides* was significantly lower in group-S compared to group-H, at wk +1. The results of the study show that novel precision dairy farming technologies may provide essential support for early disease detection, allowing to improve animals' health and well-being as well as the overall farm efficiency.

[1] Alsaaod et al. Development and validation of a novel pedometer algorithm to quantify extended characteristics of the locomotor behavior of dairy cows, Journal of Dairy Research, 98:6236–6242, 2015. [2] Zehner et al. System specification and validation of a noseband pressure sensor for measurement of ruminating and eating behavior in stable-fed cows, Computers and Electronics in Agriculture, 136:31-41, 2017.

Evaluation of pulmonary artery stiffness in asthma affected horses

Elena Alberti (1), Luca Stucchi (1), Chiara Maria Lo Feudo (1), Giovanni Stancari (1), Bianca Conturba (2), Francesco Ferrucci (1), Enrica Zucca (1)

(1) Equine Sports Medicine Laboratory "Franco Tradati", Department of Veterinary Medicine, Università degli Studi di Milano (2) Veterinary Teaching Hospital, Equine Medicine Unit, Università degli Studi di Milano

Corresponding author: Elena Alberti (elena.alberti@unimi.it)

Pulmonary artery stiffness (PAS), an index of pulmonary artery elasticity, increases in people with pulmonary diseases, such as asthma, and could be an early predictor of pulmonary hypertension (PH) [1,2]. The right ventricular systolic time intervals (STIs) are pulsed-wave Doppler parameters that decrease in humans and dogs with PH [3,4].

Equine asthma is a chronic lower airways inflammatory disease in which, as in humans, recurrent episodes of hypoxemia, hypercapnia and mediators associated with inflammation induce thickening of the pulmonary wall vessels over time, leading to progressive increase of pulmonary vascular resistance and consequently PH [5].

In literature, there are no studies regarding PAS in horses and only a case-report measured STIs in a pony with severe asthma [6]. The aims of this study are to evaluate PAS and STIs in horses affected by mild (MEA) and severe equine asthma (SEA) and to assess their repeatability and reproducibility. The study was approved by the Institutional Animal Care Committee (OPBA_27_2020).

According to clinical examination and cytology of the bronchoalvelolar lavage fluid, horses were divided into MEA (4 horses) and SEA (7 horses) groups.

STIs and PAS were measured from the Pulsed-wave Doppler waveform of the pulmonary valve, obtained from right parasternal short axis view at the level of pulmonary artery, with settings arranged to acquire maximal frequency shift (MFS). The acceleration time (AT) was measured from the onset of Doppler waveform to the beginning of the maximum velocity plateau. The ejection time (ET) was recorded from the onset of Doppler waveform to its end. PAS was calculated as MFS/AT ratio. Moreover, AT/ET ratio was calculated.

Bland-Altman test and linear regression analysis showed a good intra-observer and inter-observer agreement for all parameters. Mann-Whitney test revealed a significantly low AT and high PAS in SEA patients.

In conclusion, our preliminary results suggest that PAS and STIs can be assessed consistently in horses and that differences between SEA and MEA affected horses may be detected.

[1] Baysal SS and Has M. Evaluation of pulmonary artery stiffness in newly diagnosed adult patients with asthma, Echocardiography, 36:870–6, 2019.
[2] Altiparmak IH et al. Evaluation of pulmonary artery stiffness in patients with obstructive sleep apnea syndrome, Echocardiography, 33:362–71, 2016.
[3] Görgulü S et al. A new echocardiographic approach in assessing pulmonary vascular bed in patients with congenital heart disease: Pulmonary Artery Stiffnes, Anadolu Kardiyol Derg, 3:92–7, 2003.
[4] Schober KE and Baade H. Doppler echocardiographic prediction of pulmonary hypertension in West Highland white terriers with chronic pulmonary disease, J Vet Intern Med, 20:912–20, 2006.
[5] Decloedt A et al. A. Right ventricular function during acute exacerbation of severe equine asthma, Equine Vet J, 49:603–8, 2017.
[6] Lightowler C et al. Doppler-derived evidence of severe pulmonary arterial hypertension in a pony with recurrent airway obstruction, Vet Rec, 164:340–1, 2009.

A study on epithelial alterations in severe equine asthma and their reversibility

Michela Bullone (1), Amandine Vargas (2), Roxane Boivin (2), Mathilde Leclère (2), Jean-Pierre Lavoie (2)

(1) Università degli Studi di Torino, Dipartimento di Scienze Veterinarie (2) Université de Montréal, Faculté de Médecine Vétérinaire

Corresponding author: Michela Bullone (michela.bullone@unito.it)

Airway remodeling is a feature of severe equine asthma. Epithelial alterations are described in asthmatic horses based on the available literature, but studies employing a systematic approach are still scarce. Due to the central role that the airway epithelium is likely to play in disease pathophysiology and especially in orchestrating the inflammatory response, the alterations at this level deserve attention. The present study hypothesized that structural alterations of the airway epithelium occur in large conducting bronchi and are related to IL-13, a Th-2 type cytokine with a central role in asthma pathophysiology. We also hypothesized that epithelial remodeling is reversible with anti-inflammatory (corticosteroid) treatment.

We first compared 5 control horses and 6 asthmatic horses, all exposed to a dusty environment for 4 weeks. Then, we studied 12 asthmatic horses in exacerbation of the disease and after 4 weeks of inhalation therapy with either fluticasone (2.5 mg BID, n=6 horses) or salmeterol (0.25 mg TID, n=6). Lung function (impulse oscillometry system) and endobronchial biopsies (EBB) were performed at each time point. The biopsies were processed for histology. Epithelial remodeling (epithelial thickness, goblet cell density, epithelial cell density per mm of basal membrane, epithelial proliferation) was evaluated on Movat pentachrome-stained tissue sections. Epithelial proliferation was studied by immunohistochemistry (PCNA+ cells). The mRNA expression of IL-13, IL-4, IL-8, IL-6 and IL-17 was quantified by qRT-PCR. Data from asthmatic and control animals were compared using Mann-Whitney tests. The effect of time and treatments were evaluated with 2-way ANOVA with Sidak post-tests. Correlations were performed with Spearman tests. The procedures were approved by the local Animal Ethical Committee.

Asthmatic horses showed increased epithelial cell density and a tendency to increased epithelial cell proliferation and thickness compared to control horses maintained in the same environmental conditions, while no difference was observed for goblet cell counts. IL-13 positively correlated with goblet cell density in control horses, while the correlation was negative in asthmatic horses. During the second study, horses were exposed to a dusty environment using the same protocol used in the first study. Epithelial remodeling was much more pronounced, however. Fluticasone significantly reduced epithelial thickness, goblet cell density and epithelial proliferation to values previously found in control animals. Salmeterol effects were mostly unrewarding. Data from this second cohort of horses confirmed the negative relationship between goblet cell density and IL-13 and IL-6 expression. Our data suggest a large variability in epithelial remodeling in equine asthma cases. Epithelial density was the most reliable marker for epithelial remodeling in equine asthma, and it was not affected by any of the treatments tested, suggesting it is irreversible.

Measurement of lung function by means of forced oscillation in Mild-Moderate Equine Asthma patients

Luca Stucchi (1), Sabrina Ferla (1), Chiara Maria Lo Feudo (1), Bianca Conturba (2) Elena Alberti (1), Enrica Zucca (1), Francesco Ferrucci (1)

(1) Equine Sports Medicine Laboratory "Franco Tradati", Department of Veterinary Medicine, Università degli Studi di Milano (2) Veterinary Teaching Hospital, Equine Medicine Unit, Università degli Studi di Milano

Corresponding author: Luca Stucchi (luca.stucchi@unimi.it)

Horses can spontaneously develop "Equine Asthma", a non-infectious chronic lower airway disorder sharing several similarities with human asthma (1). In its milder form, Mild-Moderate Equine Asthma (MEA), the clinical signs are characterized by coughing, mucus accumulation within the airways and poor performance (2). In horses with MEA the airway inflammation is often subtle, and the conventional method of lung function measurement is not able to detect pulmonary dysfunction (3). Forced oscillation technique (FOT), a non-invasive method to measure lung function, showed promising results (4), but its use in clinical practice is still limited, due to the complexity of the results and the cost of the equipment. However, in human medicine the technology of FOT has grown in popularity and currently there are devices available on the market with low-cost technology and relying on largely automatic data processing algorithms.

Aim of the study was to test the application of a novel FOT device, especially assembled for equine patients, based on this new technology, and to evaluate its sensitivity in the detection of lung dysfunction in horses affected by MEA.

To perform the study, 10 MEA horses and 4 healthy controls were selected, age matched. All cases were selected from clinical patients admitted to the Equine Unit of the Veterinary Teaching Hospital of the University of Milan. The study was approved by the University of Milan Animal Welfare Organization (OPBA 48/19) and all horse owners signed informed consent. All horses underwent FOT measurement at frequencies from 1 to 6 Hz, during 30 seconds for each frequency. Data of flux and pressure obtained were collected and the whole breath, inspiratory and expiratory resistance (R) and reactance (X) of the respiratory system were calculated. The comparison between the two groups was performed by means of Mann Whitney test. Statistical significance was set at $p<0.05$.

Results showed no differences between cases and controls for all the parameters measured at every frequency except for expiratory X at 3Hz ($p=0.024$).

The results of this study confirmed the difficulty in identifying pulmonary dysfunction in horses affected by MEA. Nevertheless, FOT device seems to be sensitive in detecting a difference between MEA and control horses, suggesting to focus future researches on expiratory reactance measured at 3Hz.

[1] Lange Consiglio A. et al. Insights into animal models for cell-based therapies in translational studies of lung diseases: Is the horse with naturally occurring asthma the right choice? Cytotherapy 21(5):525-534, 2019.

[2] Couetil L. et al. Inflammatory airway disease of horses-revised consensus statement. J Vet Intern Med 30:503-515, 2016.

[3] Couetil L. et al. Clinical signs, evaluation of bronchoalveolar lavage fluid, and assessment of pulmonary function in horses with inflammatory respiratory disease. Am J Vet Res 62:538-546, 2001.

[4] Hoffman A.M. and Mazan M.R. Program of lung function testing horses suspected with small airway disease. Equine vet Educ 11(6);322-328, 1999

Efficacy of a nutraceutical supplement in the management of Equine Squamous Gastric Disease in endurance horses

Chiara Maria Lo Feudo, Luca Stucchi, Bianca Conturba, Elena Alberti, Enrica Zucca, Francesco Ferrucci

Università degli Studi di Milano, Equine Sports Medicine Laboratory "Franco Tradati", Department of Veterinary Medicine

Corresponding author: Chiara Maria Lo Feudo (chiara.lofeudo@unimi.it)

Equine Gastric Ulcer Syndrome is a common condition of the equine patient, representing a major cause of poor performance in sport horses [1]. The highest prevalence of gastric ulceration is observed in racehorses, while in endurance horses it varies from 67% to 93%, depending on their competing level [2, 3]. In adult horses 75-80% of ulcers are found in the squamous portion of the stomach [4]: this condition is defined as Equine Squamous Gastric Disease (ESGD). Although mucosal ulcers may heal spontaneously, this is uncommon in horses in training, and medical treatment is often required: omeprazole is the drug of choice for prevention and treatment of ESGD [1]. However, it is expensive and requires prolonged administrations; therefore, interest in nutraceutical supplements with anti-ulcerogenic properties has increased. This study aims to investigate the efficacy of a feed supplement containing pectin, soy lecithin, zinc oxide and chestnut extract, for the treatment of ESGD in endurance horses. Among a population of patients referred to the Equine Unit of the Veterinary Teaching Hospital of the University of Milan, 15 endurance horses from three different stables were selected on the basis on their gastroscopic examinations. All procedures performed on horses were approved by the University of Milan Animal Welfare Organization (Protocol Number OPBA_156_2019) and included informed owner consent. Scores were assigned to the lesions of the squamous mucosa according to the Equine Gastric Ulcer Council scoring system [5]; horses not considered to require medical therapy (grade 1-2 of 4 without ESGD clinical signs) were enrolled and randomly assigned to treatment or control group. Treatment group received the feed supplement for 30 days associated with management changes (increase of pasture turnout, constant access to hay and reduction of nonstructural carbohydrate intake), while control group was only subjected to the same management modifications. After treatment time, gastroscopy was repeated and scores re-assigned. Moreover, all horses were weighed at the beginning and at the end of the study. ESGD grades before and after treatment time were compared within groups using a Wilcoxon paired test, while a paired *t* test was used to evaluate weight variations within groups over time. Statistical significance was set at $P<0.05$. After the treatment period, a significant decrease in ESGD grade was observed in the treatment group ($P=0.0078$), while there was no change over time in ESGD grade in the control group ($P>0.9999$). No significant weight change was observed over time in neither group. The results of this study suggest that this supplement, when administered for 30 days, may be effective at promoting healing of the squamous mucosa in endurance horses affected by ESGD.

[1] Sykes et al. European College of Equine Internal Medicine Consensus Statement-Equine Gastric Ulcer Syndrome in Adult Horses, J Vet Intern Med, 29(5):1288–99, 2015. [2] Nieto et al. Prevalence of gastric ulcers in endurance horses - A preliminary report, Vet J, 167(1):33–7, 2004. [3] Tamzali et al. Prevalence of gastric ulcer syndrome in high-level endurance horses, Equine Vet J, 43(2):141–4, 2011. [4] Bezdekova et al. Pathomorphological study on gastroduodenal ulceration in horses: localisation of lesions, Acta Vet Hung, 55(2):262-72, 2007. [5] Andrews et al. Recommendations for the diagnosis and treatment of equine gastric ulcer syndrome (EGUS), Equine Vet Educ, 11(5):262-72, 1999.

Monitoring of biochemical profile and Serum Amyloid A in lactating jennies

Marilena Bazzano (1), Laura Bonfili (1), Carmela Scollo (2), Beniamino Tesei (1), Fulvio Laus (1)

(1) Università degli Studi di Camerino, Scuola di Bioscienze e Medicina Veterinaria (2) Libero Professionista

Corresponding author: Marilena Bazzano (marilena.bazzano@unicam.it)

In the last years, the assessment of specific acute phase proteins has been added to routine blood work (such as hematological and biochemical analysis) to support clinicians in the early recognition and differentiation of acute infection and inflammation from other more benign clinical disease. In horses, serum amyloid A (SAA) is an acute phase protein of the family apolipoproteins, mainly produced by the liver, that rapidly increases in response to inflammation [1]. Despite the increasing interest in donkeys reared for milk production and the high potential of SAA for the early diagnosis of inflammatory diseases in equids, no study investigated SAA concentrations in dairy donkeys. For the present study, 20 clinically healthy Ragusana jennies were divided into two groups: group A included jennies that foaled within 48 hours while group B included jennies at 30 days of lactation. On blood samples, a biochemical profile was performed including Potassium (K), Sodium (Na), Chloride (Cl), Calcium (Ca), Phosphorus (P), Calcium/Phosphorus ratio (Ca:P) blood urea nitrogen (BUN), γ-glutamiltrasferasi (GGT), glucose (Glu), creatinine (Cre), glutamic oxaloacetic transaminase (GOT), serum glutamic pyruvic transaminase (GPT), lactate dehydrogenase (LDH), Alkaline phosphatase (ALP), cholesterol (Chol), triglyceride (Trig), creatine kinase (CK), total bilirubin (tBil), direct bilirubin (dBil), indirect bilirubin (iBil), total protein (TP), albumin (Alb), globulins (G) Albumin/Globulin ratio (Alb:G). SAA levels have also been assessed by a solid phase sandwich enzyme-linked immunosorbent assay (ELISA). Student's T-test was performed to evaluate significant differences in biochemical profile and SAA between Group A and B. All jennies delivered at term (mean gestation length 355±19 days), by spontaneous eutocic parturition, healthy viable foals. Statistical analysis revealed significant higher values of SAA ($p<0.001$), Chol ($p=0.035$), tBil ($p=0.001$), dBil ($p=0.002$) and iBil ($p=0.001$), and lower Alp ($p=0.011$) in group A compared with group B. The modifications of biochemical profile observed in jennies during the early post-partum are similar to changes observed in periparturient mares [2,3]. The evidence of higher SAA in jennies after foaling expressed the stress associated with delivery, event that might lead to disturbances in the systemic homeostasis of mare and the occurrence of perinatal disorders [3]. Several studies confirm that regular assessment of serum SAA level carried out within the broadly understood prophylaxis can be a sensitive index of disturbances in the internal homeostasis of the body, especially in the absence of clinical symptoms. This is of particular importance in dairy jennies as the early recognition of pathological conditions during the first post-partum and the early lactation period can have direct effects both on milk production and on donkey foal survival. Based on obtained results, future studies about possible correlation between CBC and SAA during peripartum in jennies could be of interest.

[1] Long A, Nolen-Walston R. Equine Inflammatory Markers in the Twenty-First Century: A Focus on Serum Amyloid A, Vet Clin Equine 36:147–160, 2020.
[2] Bazzano et al. Metabolic Profile of Broodmares During Late Pregnancy and Early Post-Partum, Reprod Dom Anim, doi: 10.1111/rda.12411, 2014.
[3] Krakovski et al. The Level of Prolactin, Serum Amyloid A, and Selected Biochemical Markers in Mares Before and After Parturition and Foal Heat, J Equine Vet Sci, 84:102854, 2020.

Maternal and neonatal evaluation of derivated reactive oxygen metabolites and biological antioxidant potential in donkeys

Micaela Sgorbini (1), Francesca Bonelli (1), Benedetta Aliboni (1), Giulia Percacini (2), Anna Pasquini (1), Alessandra Rota (1)

(1) Università di Pisa, Dipartimento di Scienze Veterinarie (2) Libero professionista, Genova, Italy

Corresponding author: Micaela Sgorbini (micaela.sgorbini@unipi.it)

Oxidative status has been studied at delivery in horses (1), but not in donkeys. The aim was to dose donkey mares' and foals' concentrations of Reactive Oxygen Metabolites-derivatives (d-ROMs) and Biological Antioxidant Potential (BAP) to evaluate maternal and neonatal oxidative status at delivery.

Fifteen Amiata jennies and 17 foals (2 from twin foaling) were included (OPBA 2825/14). Inclusion criteria: pregnancy length >353 days; eutocic unassisted delivery. Foals were evaluated for APGAR score and physical parameters (2). Immediately after delivery, maternal and foal venous blood samples were drawn by the jugular vein and from one of the two umbilical arteries. Blood collection was performed only once in jennies involved in twin foaling. Plasma lactate (Accutrend Lactate, MI) was immediately evaluated, d-ROMs and BAP (Diacron srl, Italy) were assessed in a single batch. The Mann-Whitney test for unpaired data were applied to verify differences for d-ROMs and BAP values between jennies vs. their foals and umbilical cord. Results obtained from twins were only reported and not statistically evaluated. Significance was set at $p<0.05$.

Twelve/17 foals were fillies (1/12 second-born twin), 5/17 colts (1/5 second-born twin). All foals survived until the end of the observation. APGAR score was 7-8/8 in not-twin foals and 6-7/8 in twins. d-ROMs and BAP values was significantly higher in jennies vs. their foals or vs. umbilical artery blood and between jennies vs. their foals, respectively. Lactatemia was significantly higher and glycemia lower in foals vs. dams.

The d-ROMs and BAP concentrations were higher if compared to horses, but with a similar trend (1), supporting that: a) placenta may be a protective factor for the fetus (4); the donkey foals' antioxidant system at birth may not be effective due to the poor reserve of endogenous antioxidants and the inability to operate an up regulation of the defense mechanisms in response to the increase of ROS production as reported in newborn babies (3) equine foals (1). Although the number of twins did not allow a statistical analysis, the d-ROMs, BAP and lactate values in these neonates seemed to be similar to what found in the not-twin donkey foals. Moreover, both second born twins showed a higher APGAR value compared to the first ones. We might speculate that the delivery of twins does not seem to cause them an oxidative imbalance, and that the longest wait for the second foal before birth does not seem to reduce viability.

(1) Sgorbini et al. Maternal and neonatal evaluation of derivated reactive oxygen metabolites (d-ROMs) and biological antioxidant potential in the horse. Theriogenol, 83:48-51, 2015.
(2) Bonelli et al. Relation between Apgar scoring and physical parameters in 44 newborn Amiata donkey foals at birth. Theriogenol, 142: 310-314, 2020.
(3) Qanungo et al. Antioxidant status and lipid peroxidation in human feto-placental unit. Clinica chimica acta, 285:1-12, 1999.

Fluorescence light energy in the management of multidrug resistant canine pyoderma cases: a prospective exploratory study

Andrea Marchegiani, Alessandro Fruganti, Matteo Cerquetella, Andrea Spaterna

Università degli Studi di Camerino, Scuola di Bioscienze e Medicina Veterinaria

Corresponding author: Andrea Marchegiani (andrea.marchegiani@unicam.it)

The increasing in prevalence of staphylococcal antimicrobial resistance has been also associated with pyoderma in dogs [1,2]. In addition, prolonged antibiotic treatment, as often needed in severe cases of pyoderma, has been related to influencing possible development of multidrug resistance (MDR) [3]. Phovia™ System (Vetoquinol) is a fluorescence light energy device, which consists of a topical photoconverter gel and an LED lamp. A previous work has shown the ability of Phovia to improve pyoderma lesions as adjunct therapy to systemic antibiotics [4]. The aim of the present study was to evaluate the effect of the Phovia on clinical manifestations of multidrug resistant canine deep pyoderma (CDP) and canine pododermatitis (CP) when administered as solely management. The study protocol was successfully submitted to the Italian Ministry of Health (approval number 0004931-P-27/02/2017) and dog owners signed an informed consent prior to inclusion. Sixteen client-owned dogs affected by CP (5 dogs) and CDP (11 dogs) were scored using a dedicated scoring system [4] and received a single Phovia applications twice weekly, until clinical resolution, intended as total disappearance of the lesions, was achieved. A roughly two-millimeter layer of photoconverter gel was applied on the lesions and illuminated with a LED lamp for two minutes at approximately five centimeters distance. Mean time to achieve complete resolution was 5.2±3.6 weeks (median 3 weeks) for CP cases and 4.2±1.5 weeks (median 4 weeks) for CDP ones. Phovia shows promise as an aid to managing clinical signs while reducing reliance on antibiotics for MDR pyoderma. In this study, Phovia was responsible for the decrease of lesion scores and resolution of MDR pyoderma infection without any adjunct therapy, having a potential useful role to play in the management of such conditions, promoting complete clinical resolution of lesions.

[1] Loeffler A, Linek M, Moodley A, Guardabassi L, Sung JML, Winkler M, et al. First report of multiresistant, mecA-positive *Staphylococcus intermedius* in Europe: 12 Cases from a veterinary dermatology referral clinic in Germany. Veterinary Dermatology 18:412–421, 2017.
[2] Cain CL, Morris DO, O'Shea K, Rankin SC. Genotypic relatedness and phenotypic characterization of *Staphylococcus schleiferi* subspecies in clinical samples from dogs. American Journal of Veterinary Research 72:96–102, 2011.
[3] Summers JF, Brodbelt DC, Forsythe PJ, Loeffler A, Hendricks A. The effectiveness of systemic antimicrobial treatment in canine superficial and deep pyoderma: A systematic review. Veterinary Dermatology 23:305–330, 2012.
[4] 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. Veterinary Dermatology 30:371–e109, 2019.

ILE (Intravenous Lipid Emulsion) in the management of SSRIS (Selective Serotonin Re-Uptake Inhibitors) intoxication, in a dog

Ceccherini G.[1], Tulone F.[1,2], Romanò A., Marchetti V.[2]

[1]San Concordio Private Veterinary 24h Hospital [2]Department of Veterinary Science, University of Pisa

Corresponding author: G. Ceccherini (gianilaceccherini@virgilio.it)

The SSRIs are used in treatment of major depression in humans with less significant side effects. SSRIs act specifically on synaptic serotonin concentrations by blocking its reuptake and increasing levels in the presynaptic membrane. Clinical signs of SSRI overdose result from excessive amounts of serotonin in the central nervous system. These signs include nausea, vomiting, mydriasis, hypersalivation, and hyperthermia[1].

We describe the successful management of SSRIs intoxication in a dog by using ILE as an adjunctive therapy.

A healthy, one-year-old Jack Russel Terrier, presented in emergency setting for vomiting, ataxia, weakness, tremors. Owners supposed the ingestion of some tranquilizers pills of PAROXETINE®, a SSRIs inhibitor. Time of ingestion was unknown. Major body system was first evaluated: dog showed depression, rectal temperature was 39°C, mucosal colour membrane was pale, refilling time was 2 secs, heart rate was 120 bmp and electrocardiogram showed a sinus rhythm, systolic pressure was 190 mmHg; mild abdominal pain was assessed; AFAST and TFAST didn't evidence any pleural or abdominal effusion or other abnormalities. During clinical examination dog started to have seizures and a IV bolus of midazolam at 0.3mg/kg was administered. A venous blood gas revealed a mild metabolic acidosis (pH 7.3, HCO3- 18 mmol/L, BE-ecf -7.2 mmol/L, Lac 2.3 mmol/L). A complete chemical blood work was performed and resulted within normality. Knowing the log P of Paroxetine (3.2), ILE was administered considering a Log P>1 for the lipophilicity of a substance. ILE was administered at first as a bolus of 5 ml/kg in 30 minutes, then as a CRI at rate of 0.25ml/kg/min for six hours. During ILE administration animal was monitored and any adverse reaction was registered. No other bolus of midazolam was required. After six hours from the beginning of the infusion, ILE was stopped with a complete resolution of neurological symptoms.

Intravenous lipid emulsion (ILE) is an emerging treatment for certain lipophilic drugs [2]. As paroxetine shows lipophilic properties, ILE was supposed to be a potential adjunctive treatment.

To the Author's knowledge this is the first case of SSRIs successfully treated with ILE.

[1] Roberto Mandrioli, Michele Protti, Laura Mercolini New-Generation, Non-SSRI Antidepressants: Therapeutic Drug Monitoring and Pharmacological Interactions. Part 1: SNRIs, SMSs, SARIs, Curr Med Chem, 25(7):772-792, 2018.

[2] Fernandez AL, Lee JA, Rahilly L, Hovda L, Brutlag AG, Engebretsen K. The use of intravenous lipid emulsion as an antidote in veterinary toxicology. J Vet Emerg Crit Care; 21 (4): 309-320. 2011.

ILE (Intravenous Lipid Emulsion) in the management of SSRIS (Selective Serotonin Re-Uptake Inhibitors) intoxication, in a dog

Ceccherini G.[1], Tulone F.[1,2], Romanò A., Marchetti V.[2]

[1]San Concordio Private Veterinary 24h Hospital [2]Department of Veterinary Science, University of Pisa

Corresponding author: G. Ceccherini (gianilaceccherini@virgilio.it)

The SSRIs are used in treatment of major depression in humans with less significant side effects. SSRIs act specifically on synaptic serotonin concentrations by blocking its reuptake and increasing levels in the presynaptic membrane. Clinical signs of SSRI overdose result from excessive amounts of serotonin in the central nervous system. These signs include nausea, vomiting, mydriasis, hypersalivation, and hyperthermia[1].

We describe the successful management of SSRIs intoxication in a dog by using ILE as an adjunctive therapy.

A healthy, one-year-old Jack Russel Terrier, presented in emergency setting for vomiting, ataxia, weakness, tremors. Owners supposed the ingestion of some tranquilizers pills of PAROXETINE®, a SSRIs inhibitor. Time of ingestion was unknown. Major body system was first evaluated: dog showed depression, rectal temperature was 39°C, mucosal colour membrane was pale, refilling time was 2 secs, heart rate was 120 bmp and electrocardiogram showed a sinus rhythm, systolic pressure was 190 mmHg; mild abdominal pain was assessed; AFAST and TFAST didn't evidence any pleural or abdominal effusion or other abnormalities. During clinical examination dog started to have seizures and a IV bolus of midazolam at 0.3mg/kg was administered. A venous blood gas revealed a mild metabolic acidosis (pH 7.3, HCO3- 18 mmol/L, BE-ecf -7.2 mmol/L, Lac 2.3 mmol/L). A complete chemical blood work was performed and resulted within normality. Knowing the log P of Paroxetine (3.2), ILE was administered considering a Log P>1 for the lipophilicity of a substance. ILE was administered at first as a bolus of 5 ml/kg in 30 minutes, then as a CRI at rate of 0.25ml/kg/min for six hours. During ILE administration animal was monitored and any adverse reaction was registered. No other bolus of midazolam was required. After six hours from the beginning of the infusion, ILE was stopped with a complete resolution of neurological symptoms.

Intravenous lipid emulsion (ILE) is an emerging treatment for certain lipophilic drugs[2]. As paroxetine shows lipophilic properties, ILE was supposed to be a potential adjunctive treatment.

To the Author's knowledge this is the first case of SSRIs successfully treated with ILE.

[1] Roberto Mandrioli, Michele Protti, Laura Mercolini New-Generation, Non-SSRI Antidepressants: Therapeutic Drug Monitoring and Pharmacological Interactions. Part 1: SNRIs, SMSs, SARIs, Curr Med Chem, 25(7):772-792, 2018.

[2] Fernandez AL, Lee JA, Rahilly L, Hovda L, Brutlag AG, Engebretsen K. The use of intravenous lipid emulsion as an antidote in veterinary toxicology. J Vet Emerg Crit Care; 21 (4): 309-320. 2011.

Classification of septic shock phenotypes by hypotension and hyperlactatemia in cats

Roberta Troia, Francesca Buzzurra, Elena Ciuffoli, Giulia Mascalzoni, Armando Foglia, Ilaria Magagnoli, Francesco Dondi, Massimo Giunti

Alma Mater Studiorum - Università di Bologna, Dipartimento di Scienze Veterinarie

Corresponding author: Francesca Buzzurra (francesca.buzzurra@gmail.com)

Sepsis, the life-threatening organ dysfunction caused by a dysregulated host response to infection, is a global health burden affecting critically human and veterinary patients [1, 2]. Three different phenotypes of shock, according to blood pressure and blood lactate, are recognized in humans with sepsis [3]. Dysoxic shock, representing the combination of fluid-refractory hypotension and hyperlactatemia, is characterized by greater disease severity and mortality compared to cryptic shock (hyperlactatemia alone) and vasoplegic shock (hypotension with normal blood lactate) [1, 3, 4]. Septic shock is poorly described in cats [2], and the prevalence and the prognostic impact of these phenotypes are not known. The aim of this study was to analyse the characteristics and prognostic implications of three septic shock phenotypes in a population of cats with sepsis. The project was approved by the local Institutional Animal Care and Use Committee.

Cats with sepsis and septic shock hospitalized at the veterinary teaching hospital of the University of Bologna were prospectively included. Cats with septic shock were defined by the presence of hypotension (systolic blood pressure <90 mmHg) requiring vasopressor support, hyperlactatemia (>2 mmol/L), or both, and subgrouped in 3 classes according to the cited human classification. Clinical and clinicopathological variables including the Acute Patient Physiologic and Laboratory Evaluation (APPLE) and the Sequential Organ Assessment (SOFA) scores, occurrence of multi-organ dysfunction syndrome (MODS) and outcome were compared among groups. Odds ratios for mortality were calculated using logistic regression analysis. Significance was set at $P < 0.05$.

The study enrolled 41 cats with uncomplicated sepsis and 49 cats with septic shock (dysoxic shock n=22; cryptic shock n=17; vasoplegic shock n=8). Cats with dysoxic shock had significantly higher APPLE scores compared to cases from the other subgroups, while cats with cryptic shock had significantly lower SOFA score than cats with dysoxic or vasoplegic shock. Mortality rates were not significantly different among cryptic (59%), dysoxic (68%) and vasoplegic shock (100%), while MODS occurrence was significantly lower in cats with cryptic shock (46.2%) compared to cases affected by dysoxic (95.5%) and vasoplegic (100%) shock. Cats with septic shock had higher frequency of MODS and greater mortality rate than cats with uncomplicated sepsis. This is the first study that proposes a novel septic shock classification in cats. Our results highlight that not all septic patients are equal, and that the characterization of septic shock cats based on hypotension and hyperlactatemia could improve patient assessment and allow better cases stratification in research studies.

[1] Singer et al. The third international consensus definitions for sepsis and septic shock (Sepsis-3), JAMA, 315(8):801–810, 2016.
[2] Troia et al. Multiorgan dysfunction syndrome in feline sepsis: prevalence and prognostic implication, J Feline Med and Surg, 21:559-565, 2019.
[3] Swenson et al. Evaluation of a novel 5-group classification system of sepsis by vasopressor use and initial serum lactate in the emergency department, Intern Emerg Med, 13:257-268, 2018.
[4] Hernandez et al. Persistent sepsis-induced hypotension without hyperlactatemia: is it really septic shock? J Crit Care, 26:435, 2011.

Two-dimensional echocardiographic estimates of left atrial volumes obtained from two different views in dogs are similar but not interchangeable

Domenico Caivano (1), Mark Rishniw (2), Francesco Birettoni (1), Noemi Nisini (1), Francesco Porciello (1)

(1) Università degli Studi di Perugia, Dipartimento di Medicina Veterinaria (2) Cornell University NY USA, Department of Clinical Science, College of Veterinary Medicine

Corresponding author: Domenico Caivano (domenico.caivano@unipg.it)

Echocardiographic left atrial (LA) volume estimates can help clinicians to quantify LA dimension and function in dogs.[1,2] Little information currently exists regarding the interchangeability of LA volume estimates using a monoplane Simpson's Method of Discs (SMOD) on images obtained from the left apical four-chamber (LA4C) and right parasternal long axis four-chamber (RPLA) views.[3] Therefore, we sought to examine the agreement between the two methods of obtaining LA volumes in a heterogenous population of healthy dogs and dogs with various cardiac diseases affecting the left heart. Additionally, we compared the LA volumes obtained by SMOD with estimates obtained from cube or sphere volume formulas using linear dimensions.

Archived echocardiographic examinations were retrieved and, where both RPLA and LA4C views were recorded, included in the study. We obtained measurements from 130 dogs that were either apparently healthy (n=32) or had various left-sided cardiac chambers diseases (n=98). Of the dogs with left-sided cardiac chambers diseases, 2 dogs had patent ductus arteriosus, 2 dogs had mitral dysplasia, 2 dogs had subaortic stenosis and 92 dogs had myxomatous mitral valve disease of varying severity. The LA volume of each dog was measured using a monoplane SMOD, from both views, in systole and diastole. All measurements were performed by one experienced examiner (D.C.). Estimates of LA volume based on the RPLA-derived LA diameters (cube or sphere volume) were also calculated. We then used Limits of Agreement analysis to determine agreement between the estimates obtained with each view, and those calculated from linear dimensions.

The two methods obtained by SMOD provided similar estimates for both systolic and diastolic volumes but did not agree sufficiently to be interchangeable (absolute differences were mostly <10ml). The LA4C method tended to slightly underestimate (small LA sizes) and overestimate (large LA sizes) LA volume compared to RPLA method, with increasing disagreement as the LA size increased, for both systolic and diastolic volumes. Estimates based on cube method overestimated volumes compared to SMOD methods, for both systolic and diastolic volumes. The unidimensional volume estimates using the sphere method showed agreement with the SMOD estimates, in systole and diastole, similar to that obtained when comparing the two SMOD estimates.

Our study suggests that SMOD estimates of the LA volume from the two echocardiographic views are similar but not interchangeable. Clinicians might consider using LA4C-derived LA diameters to estimate LA volume by sphere volume formula.

[1] Wesselowski S. et al. Discrepancies in identification of left atrial enlargement using left atrial volume versus left atrial-to-aortic root ratio in dogs, Journal of Veterinary Internal Medicine, 28:1527-33, 2014.
[2] Höllmer M. et al. Left atrial volume and function in dogs with naturally occurring myxomatous mitral valve disease, Journal of Veterinary Cardiology, 19:24-34, 2017.
[3] LeBlanc N., Scollan K., Sisson D. Quantitative evaluation of left atrial volume and function by one-dimensional, two-dimensional, and three-dimensional echocardiography in a population of normal dogs, Journal of Veterinary Cardiology, 18:336-49, 2016.

Medical futility, therapeutic obstinacy and euthanasia in canine chronic kidney disease end-stage. When to euthanize and when to keep going?

Maura Albanese (1), Michela Pugliese (2), Giordana Merola (1), Annamaria Passantino (2)

(1) Libero professionista (2) Università degli Studi di Messina, Dipartimento di Scienze Veterinarie

Corresponding author: Maura Pugliese (mpugliese@unime.it)

In veterinary practice, it is common to have to assist "patients" in the end stages of the disease and to assess the need to undertake, continue or suspend life-sustaining treatments. The problem could also become particularly relevant in veterinary nephrology, given the chronic renal failure (CKD) is the final stage of most diseases [1]. If from the moral point of view mitigating the suffering of an animal in the end-stage of a disease can be right, ethically there is the risk of incurring euthanasia only for the selfishness of an owner who does not want to subject the animal too long and expensive therapeutic cycles or vice versa in the therapeutic obstinacy for the great love.

The issue of euthanasia's lawfulness in owned pets is a complex issue, which is at the crossroads of ethical, animal health, and legal questions because it goes beyond the simple protection and safeguard of the life of the animals themselves [1]. Because there are no specific rules, deciding when to end a pet's life involves both the owner and the veterinarian. The veterinarian must play the role of guarantor of biodiversity [2] and should assess - according to "*scientia et coscientia*"- if continue or withdraw life-support treatments [3]. He/she should be the promoter and defender of animal rights even when he/she choices to carry out euthanasia [4].

The rationale for this last choice in the case of CKD is based on the assessment of the nature of the disease, the prognosis, and potential quality of life after treatment, the availability and likelihood of success of treatment, the animal's age, and the ability of the owner to pay the treatment [5]. Therefore, to adequately modulate medical intervention without therapeutic obstinacy in pets with end-stage of CKD [5], represents one of the most difficult decision-making moments in veterinary clinical practice. Based on these considerations, the study provides a medico-legal and ethical analysis of medical futility, therapeutic obstinacy and, euthanasia disputes in canine CKD, turning from a descriptive approach analyzing different scenarios to a more normative approach. A particular emphasis is placed on the role that the veterinarian plays in these disputes. Finally, how clinician veterinarians should respond to requests for CKD interventions that they deem medically or ethically inappropriate will be outlined.

[1] Bartges JW. Chronic kidney disease in dogs and cats. Vet Clin North Am Small Anim Pract 42(4):669-692, 2012.
[2] Nogueira Borden LJ, Adams CL, Bonnett BN, Ribble CS, Shaw JR. Comparison of veterinarian and standardized client perceptions of communication during euthanasia discussions. J Am Vet Med Assoc, 254(9):1073-1085, 2019.
[3] Reading RP, Kenny DE, Fitzgerald KT. The crucial contribution of veterinarians to conservation biology. Top Companion Anim Med., 28(4):131-4, 2013.
[4] Proctor HS, Carder G, Cornish AR. Searching for Animal Sentience: A Systematic Review of the Scientific Literature. Animals, 3, 882-906, 2013.
[5] Hernandez E, Fawcett A, Brouwer E, Rau J, Turner PV. Speaking Up: Veterinary Ethical Responsibilities and Animal Welfare Issues in Everyday Practice. Animals, 8:15, 2018.
[6] Brown S, Elliott J, Francey T, Polzin D, Vaden S. Consensus recommendations for standard therapy of glomerular disease in dogs. IRIS Canine GN Study Group Standard Therapy Subgroup. J Vet Intern Med., 27(1): S27-43, 2013.

Monitoring changes in body surface temperature in dogs affected by spinal cord injuries during physiotherapy exercise in water treadmill using infrared thermography (IRT)

Elisa Mazzotta (1), Alessandro Lazzarini (2), Elena Tognato (2), Silvia Meggiolaro (2), Enrico Fiore (1)

(1) Department of Animal Medicine, Production and Health, University of Padova (2) Veterinary free practitioners

Corresponding author: Elisa Mazzotta (elisa.mazzotta@unipd.it)

Infrared Thermography (IRT) is a rapid, non-invasive and non-contact heat detecting technology [1, 2]. The aim of the present study is to evaluate the sensitivity of thermographic technique in detecting body surface temperature (BST) variations in healthy (H) and spinal cord injured dogs (SCD, spinal cord disease), and to outline the differences in temperature at rest, during and after water-treadmill physiotherapy sessions in SCD dogs. Sixty-seven dogs of different sex, breed, body weight and age were enrolled: 53 dogs affected by disc pathologies examined three weeks after neurosurgery (44 dogs with thoracolumbar disc herniation, 3 dogs with vertebral luxation, 6 dogs with post traumatic spinal cord injuries) and 14 healthy dogs. The IRT T420 (FLIR®System, Wilsonville, Oregon, USA) was used to collect thermographic images per each dog assessing the total image (IMAGE) and the column area from *T1* to *L7* vertebrae (AR01). In SCD dogs, the surgery wound region (AR02) was evaluated before, during and after the water-treadmill (Hydro Phisyo™ HP 300) physiotherapy session (T0-T1-T2). The data were processed through ThermaCAM® Researcher Basic 2.8c software and analysed through SAS 9.4 (SAS Institute, Cary, USA) software. The statistical analysis reported a significant BST difference ($P < 0.05$) between H and SCD dogs about T°max and T°max-min values in IMAGE ($P<0.05$). In SCD group, AR01 evaluations were reported significant ($P <0.0001$) in T°min, T°max and T mean. In AR02 temperature gradients showed a significant effect ($P <0.0001$) in T°max and in T°max-min, thus in T°mean ($P <0.001$). A significant effect of water-treadmill exercise in all BST investigated areas was found (T0-T1-T2). In SCD dogs T°min in AR01 showed significant variations between T0 and T2 ($P<0.001$) and between T1 and T2 ($P<0.0001$). In addition, T°max in AR01 reported significant differences between T0 and T1 ($P<0.05$) and T1 and T2 ($P<0.001$), therefore T°max-min values were significant different between T0 and T2 ($P<0.001$) and between T1 and T2 ($P<0.001$). In AR01, T°mean measurements were significantly variated between T0 and T1 ($P<0.05$) and T1 and T2 ($P<0.001$). In AR02 significant effects were reported in T°max between T0 and T1 ($P<0.001$), and in T°mean between T1 and T2 ($P<0.05$). IRT measurements were not affected by race, sex, size and age ($P>0.05$). A significant difference in BST was reported between healthy and pathological dogs, characterized by the inflammation of the injured spinal cord regions. IRT showed high sensitivity both in detecting and localizing the BST differences according to the severity of the disc pathology and surgical wound inflammation areas. IRT showed the water-treadmill exercise effects in the different injured spinal cord regions, reliable on the increase in blood flow and muscle activity. The IRT lack of specificity cannot replace most sensitive imaging techniques such as radiology, magnetic resonance, computed tomography and scintigraphy. Although IRT could be a viable non-invasive and rapid method to support both the clinical examination and the assessment of the medical treatment effectiveness in SCD dogs.

[1] Stelletta C, Gianesella M, Vencato J, Fiore E, Morgante M. Thermographic Applications in Veterinary Medicine. In: *Infrared Thermography*. InTech; 2012.
[2] Grossbard BP, Loughin CA, Marino DJ, et al. Medical infrared imaging (Thermography) of Type I thoracolumbar disk disease in chondrodystrophic dogs. *Vet Surg*. 2014.

Anaemia in dogs at different stages of chronic kidney disease

Ilaria Lippi, Francesca Perondi, George Lubas, Francesca Romoli, Eleonora Gori, Alessio Pierini, Veronica Marchetti

Università degli Studi di Pisa, Dipartimento di Scienze Veterinarie

Corresponding author: Ilaria Lippi (ilaria.lippi@unipi.it)

Anaemia is considered a common finding in dogs affected by chronic kidney disease (CKD), which typically occurs as a normochromic, normocytic, and non-regenerative [1,2]. However, no information regarding the characteristics of anaemia according to the severity of kidney disease is currently available in dogs.

The aim of the present study was to retrospectively evaluate frequency and severity of anaemia in CKD dogs at different stages of the International Renal Interest Society (IRIS).

Medical records of CKD dogs, presented to the Veterinary Teaching Hospital of Pisa University, between January 2014 and December 2020, were retrospectively evaluated. Inclusion criteria for CKD included complete case-log with history, laboratory and ultrasonographic findings consistent with CKD. Specifically, diagnosis of CKD was based on chronic history of azotaemia (more than 3-4 months), progressive weight loss, poor appetite, polyuria-polydipsia (PU/PD), and renal ultrasound findings of irregular shape, reduced cortico-medullary distinction, hyperechoic cortices. Based on the severity, anaemia was classified as mild (haematocrit, HCT 30-40%), moderate (HCT 20-30%), or severe (HCT <20%). Regeneration rate was considered poor (reticulocytes <60,000/µL), mild (reticulocytes 60,000-150,000/µL), or moderate (reticulocyte >150,000/µL) based on the number of reticulocytes. Among the initial 3,648 dogs examined 482 animals were enrolled. Among these enrolled dogs, 231 dogs (47.9%) were in CKD IRIS stage 2, 109 dogs (22.6%) were in IRIS stage 3, and 142 dogs (29.5%) were in IRIS stage 4. Anaemia was present in 302/482 dogs (63%). The frequency of anaemia was 47% (108/231) in IRIS 2, 71% (77/109) in IRIS 3, and 82% (117/142) in IRIS 4. Normochromic and normocytic anaemia was the most frequent type of anaemia, which was found in 208/302 (69%) dogs. The remaining 31% of anaemic dogs showed normochromic and microcytic anaemia (19%), hyperchromic and microcytic anaemia (8%), hypochromic and macrocytic anaemia (3%), hypochromic and microcytic anaemia (0.6%), hypochromic and normocytic anaemia (0.4%). Non-regenerative anaemia was found in 239/302 dogs (79%), and its frequency increased significantly with the IRIS stage ($p=0.0001$; Phi=1.03). A statistically association between frequency ($p=0.0001$; Phi=0.327) and severity ($p<0.001$; Phi=1.04) of anaemia, and progression of the IRIS stage was found. The frequency of moderate and severe forms of anaemia increased significantly in dogs at IRIS stage 3 and 4, compared to IRIS stage 2. A moderate degree of negative linear correlation was found between HCT and serum creatinine ($p<0.0001$; r-0.34). In our study anaemia was a very frequent disorder, which affected approximately 63% of the CKD canine population. Although anaemia may occur at any stage of CKD, its frequency increases significantly with the progression of the IRIS stage, affecting 71% of dogs at IRIS 3, and 82% of dogs at IRIS 4. Similarly, to human medicine [3], the association between severity of anaemia and poor regeneration rate, and proportion of lost kidney function, may suggest a more severe condition of reduced bone marrow activity in dogs at advanced stages of CKD.

[1] Polzin DJ. In Nephrology and Urology of Small Animals. ed. Wiley-Blackwell, 2011
[2] Fiocchi et al. The use of darbepoetin to stimulate erythropoiesis in the treatment of anemia of chronic kidney disease in dogs, Journal of Veterinary Internal Medicine, 31: 476-485, 2017.
[3] Cases et al. Anemia of chronic kidney disease: protocol of study, management, and referral to nephrology, Nefrologia, 38: 8-12, 2018

Assessment of stress and acute pain in postoperative patients in continuous infusion at low doses of dexmedetomidine

Roberta Di Prinzio, Andrea Paolini, Chiara Alocci, Giovanni Aste

Università degli Studi di Teramo, Facoltà di Medicina Veterinaria

Corresponding author: Roberta di Prinzio (rdiprinzio@unite.it)

Hospitalization in intensive care unit (ICU) is often associated with pain and stressful conditions for patients. In human medicine it has been demonstrated how the activation of the sympathetic-adrenal-medullary system has negative effects on the recovery of the hospitalized patient. Multimodal pain management could improve overall patient comfort and prevent establishment of chronic pain pathways [1]. Injectable dexmedetomidine is widely used for sedation, restraint, anxiolysis, and analgesia in veterinary medicine.

The aim of the double blinded and placebo-controlled pilot study is to evaluate the effect of low dose of continuous rate infusion (CRI) of dexmedetomidine on the management of stress and pain in association with analgesic therapy in dogs hospitalized after orthopedic surgery. Clinical evaluation of patients was performed assessing stress score (SS) [2] and Glasgow Pain Scale (GCSp).

Were enrolled 20 dogs admitted to the University Veterinary Teaching Hospital of Teramo to perform elective orthopedic surgery. Inclusion criteria are ASA1 or 2before surgery patients received meloxicam 0.2 mg/kg.

All dogs received epidural block for hind limb and axillary brachial plexus block for forelimb surgery; in case of pain, analgesia rescue was fentanyl bolus of 2 mcg/kg. After surgery patients are divided in 2 groups (n =10). Analgesic therapy was methadone 0.3 mg/kg. Group D received Dexmedetomidine at 0.5µg/kg/h with a 1µg/kg loading bolus; control group R received lactated Ringer's solution CRI. SS and GPSp assessment were performed at different times: T0 (after surgery, corresponding to start of the CRI); T1 (30' after CRI started); T2, T4, T6 and T8 (every 2h up to 8h from admission in ICU). The median value of SS for any time point was 0.5, 4.0, 5.0, 4.0, 7.5, 8.0 in group D; 0.0, 4.0, 5.0, 7.0, 7.0, 8.0 in group R. The median value of SS for any time point was 2.0, 3.0, 3.0, 3.0, 4.0, 4.0 in group D; 2.0, 3.0, 3.0, 4.0, 4.0, 4.0 in group R.

Statistical analysis was carried out using *Jamovi (version 1.6, 2021)*: a weak correlation was highlighted between the scores assigned to stress and pain both in group D (rho 0.44, $p < 0.001$) and group R (rho 0.374, $p<0.001$). Comparing the average of the stress score data, significant differences are highlighted between D and R groups when considered all over measurements ($p=0.017$), but no differences in any time point. Comparing the average GCSp data of the two groups measured in the pre-established times and in all over measurements, no significant differences are highlighted in any time point.

Results suggest that pain management has been equally effective in both groups and the use of low dose dexmedetomidine could be an effective and safe practice to improved stress status and quality of recovery in IU, but at this dosage, results suggest that does not affect pain even if it cannot be excluded that it improves pain management through the opioid-sparing properties. Further studies are needed to extend the number of cases and the power of statistical data. However, in this study the drug was used at the lowest dosage reported in the literature and it could be interesting to evaluate its effect of pain and stress control by gradually increasing the dose up to 3 µg/kg/h.

[1] Chohan. Anesthetic considerations in orthopedic patients with or without trauma, Top Companion Anim Med, 25:107-19, 2010.

[2] Gomart et al.. Accuracy of different temperature reading techniques and associated stress response in hospitalized dogs, J Vet Emerg Crit Care, 24:279-85,2014.

Thrombocytopenia and platelet indices: a retrospective study on 534 dogs

Carola Sofia Selva Coddè, Kateryna Vasylyeva, Michele Tumbarello, Francesco Dondi, Chiara Agnoli

Alma Mater Studiorum - Università di Bologna, Dipartimento di Scienze Mediche Veterinarie

Corresponding author: Chiara Agnoli (chiara.agnoli2@unibo.it)

Thrombocytopenia is a common haemostatic disorder in dogs (6.7%) and can be detected during various diseases [1]. In addition, microscopic evaluation, platelet (PLT) count and PLT indices, obtained by automated laser-based haematology analysers, could provide information on PLT morphology, activation, and regeneration. In human medicine, PLT indices differentiate the mechanisms of thrombocytopenia and indicate the haemostatic capability of platelets [2]. In veterinary medicine, data about the diagnostic usefulness of PLT indices during diseases associated to thrombocytopenia are lacking [1].

The aim of this study was to retrospectively evaluate PLT count and indices including mean PLT volume (MPV), platelecrit (PCT), mean PLT content (MPC), mean PLT mass (MPM), PLT distribution width (PDW), PLT content distribution width (PCDW), and PLT mass distribution width (PMDW), in diseases associated with thrombocytopenia, focusing on primary immune-mediated thrombocytopenia (pITP) in dogs. Medical records of the Veterinary university hospital of the University of Bologna were reviewed (January 2015-June 2020), to identify dogs with a complete blood count (CBC) performed by ADVIA 2120 (Siemens Healthcare Diagnostics), thrombocytopenia (defined as a PLT count <150000 PLT/µl) and absence of PLT clumps at the microscopic blood smear evaluation. Dogs in which a diagnosis could not be clearly identified were excluded. A healthy control group of 105 dogs was selected for comparison. Variables were compared among groups using non-parametric statistics; risk factor analysis was performed using univariate logistic regression. Significant results were analysed with Receiver Operating Characteristics (ROC) curve analysis to define area under the ROC curve (AUC) and the best sensitivity (Se) and specificity (Sp). $P<0.05$ was considered significant. Overall, 534 thrombocytopenic dogs were enrolled and divided into six groups based on the underlying diagnosis: pITP (n=32), primary immune-mediated haemolytic anaemia (n=23), infectious/inflammatory diseases (n=227), neoplastic diseases (n=116), trauma (n=23) and miscellanea (n=113). The miscellanea group was excluded from the analysis because of the extreme variability of the diseases included in the group. Dogs with pITP had a significantly different results if compared to controls and other groups. In particular, a lower PLT count (6000/µl, 1000-109000; $P<0.001$), PCT (0%, 0-0.2; $P<0.001$), MPC (19.3 mg/dL, 9.5-28.1; $P<0.001$), and a higher MPV (16.8 fL, 4.4-28.5; $P<0.001$), PCDW (8 mg/dL, 2-11.2; $P<0.001$) and PMDW (1.3 pg, 0.5-1.7; $P<0.001$) were detected in pITP dogs. A platelet count ≤26000/µl was strongly associated with pITP diagnosis (AUC=0.954; Se=90.6%, Sp=92.3%; $P<0.001$) and it was associated with the presence of spontaneous bleedings (AUC=1; Se=100%, Sp=100%; $P<0.001$).

In conclusion, platelet indices results seemed to have a characteristic pattern in dogs with pITP and these variables could be a useful tool in the diagnosis and management of these patients.

[1] Bommer et al. Platelet distribution width and mean platelet volume in the interpretation of thrombocytopenia in dogs, Journal of Small Animal Practice, 49(10):518-524, 2008.
[2] Vinholt et al. An overview of platelet indices and methods for evaluating platelet function in thrombocytopenic patients, European Journal of Haematology, 92(5):367-376, 2014.

Preliminary evaluation of the erythrocyte membrane lipidome in feline chronic enteropathy

Paolo Emidio Crisi (1), Maria Veronica Giordano (1), Francesca De Santis (1), Paraskevi Prasinou (1), Alessia Luciani (1), Alessandro Gramenzi (1), Carla Ferreri (2), Andrea Boari (1)

(1) Università degli Studi di Teramo, Facoltà di Medicina Veterinaria (2) Consiglio Nazionale delle Ricerche, Istituto per la sintesi organica e la fotoreattività (ISOF)

Corresponding author: Paolo Emidio Crisi (pecrisi@unite.it)

Feline chronic enteropathy (CE) is a common gastrointestinal disorder in cats and comprises food-responsive enteropathy (FRE), inflammatory bowel disease (IBD) and alimentary lymphoma (AL).

The analysis of erythrocyte membrane lipidome represents a powerful tool for assessing the quantity and quality of fatty acids (FA) in humans [1], and recently it was evaluated also in healthy dogs [2] and in dogs affected by different forms of CE [3].

The aim of this study was to compare the FA membrane profile of healthy cats (Control, n=17) with 11 cats affected by CE.

Erythrocyte membranes were isolated from EDTA-treated blood and a cluster of 10 FA, including saturated FA (SFA: stearic, palmitic), mono-unsaturated FA (MUFA: palmitoleic, oleic, vaccenic), polyunsaturated FA ω-6 (PUFA-ω6: linoleic, dihomo-gamma-linolenic [DGLA], arachidonic) and PUFA ω-3 (eicosapentaenoic [EPA], docosahexaenoic [DHA]) was determined by Gas-Chromatography. Relevant lipid parameters (SFA/MUFA, SFA/PUFA, ω6/ω3, PUFA balance, unsaturation and peroxidation indexes) were also calculated.

The project has been approved by the Health Ministry and the Committee on Animal Research and Ethics of the Universities of Chieti-Pescara, Teramo and Experimental Zooprophylactic Institute of AeM (CEISA), Protocol UNICHD12 n. 1168.

Cats affected by CE were diagnosed with FRE (n=3), IBD (n=6) or AL (n=2).

Control cats were 7 were males (4 castrated) and 10 females (7 sterilized) with a mean age of 51 months (standard deviation [sd]±44), while cats with CE were 3 males (1 castrated) and 8 females (4 sterilized) with a mean age of 89 months (sd±61). The mean bodyweight was 3.8 (sd±1.2) in control cats and 3.4 (sd±0.9) in CE cats. No differences were observed between Control and CE in regards of age (p=0.07), bodyweight (p=0.35) and sex (p=0.69). Compared to the Control group, the erythrocytes membranes of cats affected by CE had a lower content of palmitic acid (p=0.03) and linoleic acid (p=0.02). These results are similar to those obtained in dogs affected by CE [3] and gain further importance if one considers that domestic cats could not convert linoleic acid to arachidonate [4] and that the linoleic acid transformation to gamma linolenic and DGLA acids is the main anti-inflammatory control in this species. Moreover, in healthy cats, SFAs and the ω-6 PUFAs constitute approximatively 90% of the total erythrocytes FAs suggesting that significant changes of palmitic and linoleic acids levels, found in CE cats, may mirror a relevant metabolic derangement. Given its non-invasiveness the analysis of erythrocyte membrane lipidome may become a useful tool to gather information potentially leading to personalized therapeutic intervention targeted to decrease inflammation and to increase protective components in cats affected by CE.

[1] Ferreri C, Chatgilialoglu C. Role of fatty acid-based functional lipidomics in the development of molecular diagnostic tools. Expert Rev Mol Diagn, 12:767–780, 2012.[2] Prasinou et al. The Erythrocyte Membrane Lipidome of Healthy Dogs: Creating a Benchmark of Fatty Acid Distribution and Interval Values. Front Vet Sci, 7:502, 2020. [3] Crisi et al. The erythrocyte membrane lipidome in dogs with chronic enteropathy. In: Research Communications of the 29th ECVIM-CA Congress. J Vet Intern Med, 34(1):339-530, 2020. [4] Rivers et al. Inability of the cat to desaturate essential fatty acids. Nature, 258(5531):171-173, 1975.

ORAL COMMUNICATIONS

SICV

Efficacy and safety of dexmedetomidine as adjuvant in femoral-sciatic nerve blocks in dogs undergoing tibial plateau levelling osteotomy (TPLO)

Caterina Di Bella, Luca Pennasilico, Riccardo Botto, Angela Palumbo Piccionello

Università di Camerino, Scuola di Bioscienze e Medicina Veterinaria

Corresponding author: Caterina Di Bella (caterina.dibella@unicam.it)

The aim of the study has been to evaluate the efficacy and safety of dexmedetomidine combined with bupivacaine in femoral-sciatic nerve blocks (F-S blocks) in dogs undergoing TPLO. Our hypothesis is that dexmedetomidine, administered locally, improving peri-operative analgesia and prolonging the duration of peripheral block. Thirty dogs were selected for this prospective, clinical study and randomly assigned to received bupivacaine 0,5 % (0,1 mL/kg) plus dexmedetomidine (0,5 µg/kg) locally (BDloc group = 10), bupivacaine 0,5% (0,1 ml/kg) locally plus dexmedetomidine (0,5 µg/kg) administered IM (BDsys group = 10) and local bupivacaine 0,5% (0,1 ml/kg) plus NaCl 0,9%, IM (Bupi group = 10). All patients were premedicated with acepromazine (10 µg/kg) and induced with propofol (5 mg/kg). General anesthesia was maintained with isoflurane in pure oxygen. The F-S blocks were performed with a specific peripheral nerve stimulator (Stimuplex HNS 12). The main hemodynamic and respiratory parameters were registered every 5 minutes until the end of surgery. Intraoperative nociception was assumed if heart rate (HR) or mean blood pressure (MAP) increased by >20% from baseline, in which case fentanyl (1 µg/kg) was administered. Following recovery from anesthesia, signs of postoperative pain were assessed 2, 4, 6, 8, 10, 15, 18, 20 and 24 hours from the F-S blocks using the Glasgow Composite Pain Scale (GCPS). Patients with scores >5 (scale 0 to 20) received methadone (0.3 mg/kg) IM and were then withdrawn from further pain scoring. At the same times we assessed pain on the wound touch, footstep capacity and skin sensitivity of the femoral and sciatic nerves. The monitored parameters were compared at each time of the study with the one-way ANOVA for repeated measures and the Fisher's test ($P<0.05$). No subject required intraoperative rescue analgesia. In BDloc group, the GCPS score was < 4 for everyone at all times of the study. Differently, in Bupi and BDsys groups, the 100% of subjects achieved score ≥5 between 8 and 10 hours. Furthermore, in BDloc, 70% of dogs did not need systemic analgesia until 24 hours from the block, instead, in Bupi and BDsys, 100% of subjects showed skin sensitivity and required a rescue analgesia within 10 hours. At T18, 50% of BDloc subjects still did not show skin sensitivity. Our results prove that the addition of dexmedetomidine as adjuvant for the F-S blocks may prolong the sensory block, ensured sufficient analgesia for up to 24 hours. The exact mechanism whereby alpha-2 agonists prolong motor and sensory blockade by local anaesthetics is not definitively known, however, previously performed studies described different mechanisms which justify the local action of dexmedetomidine such as the vasoconstrictive effect that allow the local anesthetic to remain in situ for a longer time. Furthermore, dexmedetomidine is likely to augment local anesthetic effects by hyperpolarizing nerve tissues at presynaptic C-fibers and postsynaptic dorsal horn neurons [1,2]. In conclusion, the combination of bupivacaine-dexmedetomidine may provide a better recovery, good analgesia for up to 24 hours and significant reduction in systemic opioid administration.

[1] O O. et Smith LJ. A comparison of epidural analgesia provided by bupivacaine alone, bupivacaine + morphine, or bupivacaine + dexmedetomidine for pelvic orthopedic surgery in dogs. Vet Anaesth Analg, 40:527–536, 2013.

[2] Bartel et al. Comparison of bupivacaine and dexmedetomidine femoral and sciatic nerve blocks with bupivacaine and buprenorphine epidural injection for stifle arthroplasty in dogs. Vet Anaesth Analg, 43:435–443, 2016.

Constant rate infusion of lidocaine, tumescent anesthesia and their combination in dogs undergoing unilateral mastectomy

Cecilia Vullo (1), Adolfo Maria Tambella (2), Annastella Falcone (3), Salvatore Monti (3), Giuseppe Catone (3)

(1) Department of BioChimFarAm, University of Messina (2) School of Biosciences and Veterinary Medicine, University of Camerino (3) Department of Veterinary Sciences, University of Messina

Corresponding author: Cecilia Vullo (cvullo@unime.it)

Tumescent anaesthesia (TUM) is a technique for regional anaesthesia of the skin using direct infiltration of large volumes of diluted local anaesthetic combined with a vasoconstrictor. This technique has been applied in bitches and cats who underwent a unilateral mastectomy, demonstrating it facilitated the surgery procedure and assured satisfactory postoperative analgesia [1,2,3]. The aim of this study was to evaluate if the addition of TUM to lidocaine CRI modified intraoperative cardiopulmonary function in dogs undergoing unilateral mastectomy and provided adequate early postoperative analgesia. The study was approved by the Bioethics Committee of Messina. Twenty-four mixed-breed neutered dogs presented for unilateral mastectomy were included in the study. Dogs were premedicated with dexmedetomidine (3 μg/kg) and methadone (0.2 mg/kg) intravenously. Induction of anaesthesia was produced by administration of propofol as required to enable endotracheal intubation. The animals were connected to a breathing circuit and isoflurane in 100% oxygen was delivered for maintenance of anaesthesia. Then, the dogs were randomly assigned to one of the three following groups: Group LID (n=8): an IV loading dose of lidocaine (2 mg/kg, Lidocaine 2%) followed by a CRI of 100μg/kg/min; Group TUM (n=8): an IV loading dose of lactated Ringer's solution followed by a CRI of Ringer's solution in addition to local TUM applied immediately before mastectomy; Group LID/TUM (n=8): an IV loading dose of lidocaine (2 mg/kg followed by a CRI of 100 μg/kg/min) in addition to local TUM. Group LID received an equivalent volume of lactated Ringer's solution instead of local TUM. The ECG, invasive SAP, DAP and MAP, HR/min, RR/min, SpO2, T°C and EtCO2 mmHg were continuously recorded. Arterial blood pH, PaO2 and PaCO2 and HCO3- were recorded immediately after the introduction of the arterial catheter (T0), immediately after the start of the surgery (T1), and at 15 (T2), 30 (T3), 40 (T4) minutes following the start of the surgery. Subjective postoperative pain scores were evaluated using the Italian version of the Glasgow Composite Pain Scale-Short Form (ICMPS-SF). The scale was applied once the dogs had fully recovered from the sedative effects of the anaesthetic drugs (RT0), and following 15 (RT1), 30 (RT2), 45 (RT3), and 60 minutes (RT4). In the same time frame, considering postoperative pain scores exceeding level 6/24 as clinical decision-point for the requirement of rescue analgesia, IV administration of 0.2 mg/kg methadone was provided.

For all dogs in the LID group (five at T0, two at T2 and one at T3) and five dogs in the TUM group (three at T0 and two at T1) rescue analgesia was required (LID vs TUM, p=0.2000). No dog in the LID/TUM group reached the threshold for rescue analgesia, hence showing a significant difference compared to both LID group (p=0.0002) and TUM group (p=0.0256).

[1] Credie L et al. Perioperative evaluation of tumescent anaesthesia technique in bitches submitted to unilateral mastectomy. *BMC Vet Res* 178, 2013.
[2] Abimussi CJ et al. Tumescent local anesthesia with ropivacaine in different concentrations in bitches undergoing mastectomy: plasma concentration and post-operative analgesia. *Vet Anaesth Analg* 2014, 41, 516-525.
[3] Moreira CM et al. Evaluation of tumescent local anesthesia in cats undergoing unilateral mastectomy. *Vet Anaesth Analg* 2021, 48, 134-141.

Use of the SpO_2/FiO_2 diagram to assess gas exchange in horses under general anesthesia

Scardia A. (1), Pimenta E.L.M. (2), Ross-Russell R.I. (3), Jones J.G. (4) Staffieri F. (1)

(1) Department of Emergencies and Organ Transplantation, Section of Veterinary Clinics and Animal Production, University of Bari, Bari, Italy (2) Escola de Veterinária, Universidade Federal de Minas Gerais (UFMG), Belo Horizonte, Brasil (3) Department of Paediatrics, Cambridge University Hospitals, Cambridge, UK (4) Dept of Anaesthesia, Addenbrookes Hospital, University of Cambridge, UK

Corresponding author: A. Scardia (annalaura.scardia@uniba.it)

Oxygenation impairment relates not only to pulmonary atelectasis (true shunt) but also to poorly aerated regions (low V/Q) of the lung, which contributes to the total venous admixture. Invasive techniques for measuring the different components of the venous admixture have been used in the horse, e.g. multiple inert gas elimination technique [1,2] and pulmonary artery catheterization [3,4]. Sapsford and Jones presented a noninvasive method to discern true shunt from low V/Q regions in humans: the SpO_2/FiO_2 curve [5]. The aim of this study was to test the feasibility of this diagram to assess gas exchange function in horses during general anesthesia. Ten horses were included in the study. The study protocol was approved by the Ethical Committee of the Federal University of Minas Gerais (number 10/2020). Horses were anesthetized using detomidine, ketamine and diazepam and anesthesia was maintained with isoflurane in O_2. All patients were ventilated mechanically. Once anesthetic plane was stable, FiO_2 was progressively reduced with the following steps: 100%, 60%, 40%, 30% and 21% (FiO_2 TRIAL). Each step was maintained for 10 minutes and SpO_2 was recorded at the end of each step. An arterial blood sample was collected at the phases of 100% and 21% FiO_2 in order to calculate intrapulmonary shunt with "Fshunt" formula [6] The Fshunt value calculated at 21% FiO_2 was defined as "venous admixture", the one calculated at 100% FiO_2 as "true shunt". The FiO_2 *vs* SpO_2 data points were analysed using a computer algorithm which estimates a shunt value (S-Shunt) fitting the obtained data with an ideal SpO_2/FiO_2 curve. Mean values and standard deviation were calculated for all the data. Physiological data were compared with a two-way repeated test ANOVA. Correlation between shunt, venous admixture and S-Shunt was determined using the Spearman Rank Correlation Coefficient test, the analysis of the regression curve and the coefficient of determination (r^2). Values of $P<0.05$ were considered statistically significant. Results showed that Fshunt value was higher at 21% compared to 100% FiO_2. A significant and strong correlation ($P=0.0069$; $r=0.787$; $r^2=0.5934$) and a significant and moderate correlation ($P=0.0489$; $r=0.634$; $r^2=0.1996$). was found between S-Shunt and true shunt and venous admixture, respectively.

SpO_2/FiO_2 diagram proved to be a noninvasive tool to characterize gas exchange in horses under general anesthesia, providing a reliable estimation of true shunt and venous admixture.

[1] Nyman G. and Hedenstierna G. Ventilation perfusion relationships in the anaesthetized horse, Equine vet. J., 21 (4): 274-281, 1989. [2] Marntell S. et al. High inspired oxygen concentrations increase intrapulmonary shunt in anaesthetized horses, Vet Anaesth Analg., 22(6):338-47, 2005. [3] Hubbell J.A.E. et al. Effect of 50% and maximal inspired oxygen concentrations on respiratory variables in isoflurane-anesthetized horses, BMC Vet Res, 7:23, 2011. [4] Nyman G. et al. Pulsed delivery of inhaled nitric oxide counteracts hypoxaemia during 2.5 hours of inhalation anaesthesia in dorsally recumbent horses, Vet Anaesth Analg., 39(5):408-7, 2012. [5] Sapsford D.J. and Jones J.G. The PIO2 vs. SpO2 diagram: a non-invasive measure of73 (12) pulmonary oxygen exchange, Eur J Anaesthiol., 12(4):375-86, 1995. [6] Araos J. et al. Use of the oxygen content-based index, Fshunt, as an indicator of pulmonary venous admixture at various inspired oxygen fractions in anesthetized sheep, Am J Vet Res, 73 (12):2013-20, 2012.

Assessment of pain-related emotions in dogs by three observer groups using a qualitative method and a pain scale

Francesca Zanusso (1), Barbara Contiero (1), Flaviana Gottardo (1), Simona Normando (2), Giulia Maria De Benedictis (1)

(1) Università degli Studi di Padova, Dipartimento di Medicina Animale, Produzioni e Salute (2) Università degli Studi di Padova, Dipartimento di Biomedicina Comparata e Alimentazione

Corresponding author: Francesca Zanusso (francesca.zanusso@unipd.it)

Pain is an unpleasant sensory and emotional experience. In contrast with humans, who can usually communicate their emotional states, animals do not have any verbal means of communication [1]. Thus, the capability of human observers to appreciate animal emotions is essential. Free Choice Profiling (FCP) methodology was used in qualitative behavioral assessment (QBA) in many species [2, 3, 4, 5, 6, 7]. QBA investigates the emotional status of the animals and it does not rely on what an animal does, but on how it does what it does. FCP approach allows observers to generate their own vocabulary to describe emotional expression of observed animals, enhancing the active interpretation of animals rather than providing them with a fixed list of terms [2]. To our knowledge, FCP methodology has been never applied to pain assessment. This study aims to investigate the ability of three observer groups to blindly recognize pain-related emotions in 20 dogs (10 "in pain" dogs and 10 "healthy" dogs) using FCP methodology. The observers (10 dog owners, 10 veterinary medical students and 10 veterinarians) applied FCP watching twice the footages of the dogs. In video session 3, students and veterinarians assessed dogs' pain using Glasgow Composite Pain Scale-Short Form (GCPS-SF) [8]. FCP data were analyzed using Generalized Procrustes Analysis (GPA): a good agreement was achieved by each observer group, confirming the applicability of this methodology in dogs [5]. "Healthy" dogs were mainly described as *tranquillo* ("quiet") and *vivace* ("lively"), while the majority of "in pain" dogs were considered *dolorante* ("sore/in pain") and *sofferente* ("suffering"). Pain-related dimension of the consensus profile was different among groups as well as the capability to differentiate between "healthy" and "in pain" dogs. Thus, observers' cultural background and personal experience could affect QBA in suffering dogs. Moreover, a high correlation was found between FCP data and GCPS-SF scores. The results of this study show that qualitative methods such as FCP could be used in association with semi-quantitative methods to investigate the effect of pain on animal emotional expression.

[1] Flecknell PA. Refinement of animal use-assessment and alleviation of pain and distress, Laboratory animals 28:222-231, 1994.
[2] Wemelsfelder et al. Assessing the 'whole animal': a free choice profiling approach, Animal Behaviour 62:209-220, 2001.
[3] Rousing T, Wemelsfelder F. Qualitative assessment of social behaviour of dairy cows housed in loose housing systems, Applied Animal Behaviour Science 101:40-53, 2006.
[4] Napolitano et al. The qualitative assessment of responsiveness to environmental challenge in horses and ponies, Applied Animal Behaviour Science 109:342-354, 2008.
[5] Walker et al. The assessment of emotional expression in dogs using a Free Choice Profiling methodology. Animal Welfare 19:75-84, 2010.
[6] Napolitano et al. Qualitative behaviour assessment of dairy buffaloes (*Bubalus bubalis*), Applied Animal Behaviour Science 141:91-100, 2012.
[7] Patel et al. Using Qualitative Behaviour Assessment to investigate human-animal relationship in zoo-housed giraffes (*Giraffa camelopardalis*), Animals 9:381, 2019.

Evaluation of three different constant rate infusions of dexmedetomidine in cats undergoing elective surgery

Claudia Acquafredda (1), Marzia Stabile (1), Rossella Samarelli(2), Deborah Errico (1), Annarita Imperante (1), Rodrigo Trisciuzzi (1), Alberto Crovace (3), Francesco Staffieri (1)

(1) Università degli Studi di Bari, Dipartimento dell'emergenza, dei trapianti di organo e terapie cellulari (D.E.T.O) (2) Università degli Studi di Bari, Dipartimento di Medicina Veterinaria (3) Università degli Studi di Bari, Dipartimento di Scienze Mediche di Base, Neuroscienze e Organi di Senso

Corresponding author: Claudia Acquafredda (claudiaacquafredda19@gmail.com)

The aim of the study was to compare three constant rate infusions (CRI) of dexmedetomidine (DEX) in cats undergoing elective ovariectomy. Our hypothesis was that DEX would have a dose dependent effect on the quality of anesthesia, recovery and intraoperative analgesia. Sixty cats were randomly allocated to four groups which received a different CRI during anaesthesia: 1µg/kg/h (DEX1), 2µg/kg/h (DEX2) and 3µg/kg/h (DEX3) of DEX and 0.9% of saline solution (CTR) (1). Premedication was performed intramuscularly with Dexmedetomidine (5µg/kg), Alfaxalone (1mg/kg) and Buprenorphine (10µg/kg). General anaesthesia was induced with Alfaxalone administered to effect and cats were orotracheally intubated and connected to a non-rebreathing Bain system and was maintained with isoflurane in pure oxygen. Before the start of the CRIs (T0), Heart rate (HR), Doppler blood pressure (DBP), Respiratory rate (RR), Oxygen Saturation (SpO2), End-Tidal concentration of CO2 (EtCo2) and Isoflurane (EtIso), Temperature, and Tidal volume were recorded. The CRI was discontinued after 30 minutes (T30). Data were recorded every 15 minutes, up to 45 minutes when the administration of isoflurane was discontinued, and cats were recovered. Intraoperative rescue analgesia (2µg/kg of fentanyl) was administered when an increase of HR, DBP and/or RR over 20% of the values recorded just before starting surgery, was observed. The number of doses of fentanyl received during surgery was recorded for each case. During recovery the following times were recorded: extubation, first head movements, sternal recumbency and standing. All cats were observed for 60 minutes and the quality of recovery was scored every 15 minutes using a validated scale (2). The occurrence of agitation was recorded in each case. Normal distribution was evaluated by Shapiro-Wilk test. Linear mixed models fit by REML were performed in order to assess overall changes over time of the study variables between the four groups. Statistical analyses were performed using R software, with statistical significance set at $p<0.05$. At T15 and T30 DBP was higher in DEX3 (140±34.7 and 148.5±30 mmHg) than D1 (104±29 and 105±29 mmHg) and CTR (108±21.7 and 110.5±28.8 mmHg). At T30, EtIso was lower in D3 (1.38±0.33%) as compared to D1 (1.5±0.39%) and CTR (1.8±0.56 mmHg). The average doses of fentanyl were lower in D3 (0.46 ± 0.83) group as compared to D1 (1.4 ± 0.98) and CTR (1.5±0.96) groups. Time to get the standing position was longer in D3 (73.4±10.6 min) compared to D1 (33,6±22.3 min), D2 (37.4±20.8 min) and CTR groups (33.4±21.8 min). Agitation was observed less in the DEX2 (2/15, 13.3%) and DEX3 (0/15, 0%) groups compared to DEX1 (6/15, 40%) and CTR (13/15, 86.6%). The results show that at 2 and 3 µg/kg/h DEX provides an effective and similar intraoperative analgesic effect compared to lower dosages with a lower incidence of agitation during recovery. The infusion at 3 µg/kg/h causes higher intraoperative DBP, reduces the need of isoflurane and extends the recovery time compared to 2 µg/kg/h.

[1] B.T. Simon et al. The clinical effects of a low dose dexmedetomidine constant rate infusion in isoflurane anesthetized cats, The Veterinary Journal 24(2018) 55-60.
[2] MA Gieseg, et al. (2014) A comparison of anaesthetic recoveries in cats following induction with either alfaxalone or ketamine and diazepam, New Zealand Veterinary Journal, 62:3 103-109.

Outcomes of surgical treatments for brachycephalic obstructive airway syndrome in dogs

Fabio Leonardi (1), Simone Di Sclafani (2), Jessica Rosa (1), Silvia Paviolo (1), Manuel Dall'Aglio (1), Giovanna Lucrezia Costa (3)

(1) Università di Parma, Dipartimento di Scienze Medico Veterinarie (2) Libero professionista, Parma (3) Università di Messina, Dipartimento di Scienze Veterinarie

Corresponding author: Fabio Leonardi (fabio.leonardi@unipr.it)

Brachycephalic obstructive airway syndrome (BOAS) in dogs is characterised by various anatomical abnormalities causing different clinical signs which vary in intensity. This variability makes difficult the assessment of the surgical outcome after upper airway surgery. The present study aims to investigate the outcome after surgical treatment of BOAS in dogs using respiratory and digestive clinical score grading systems. Dogs requiring surgical treatment of BOAS were included. The exclusion criteria were as follows: previous surgical treatment of BOAS, hospitalization for the management of BOAS before surgery, and medical treatment of BOAS in the previous 60 days. We enrolled 11 dogs (10 English bulldogs and 1 Pug) requiring surgical treatment of BOAS. The following symptoms were recorded: snoring (11), exercise intolerance (10), coughing (9), stertor/stridor (9), vomiting/regurgitation (8), dyspnoea (8), apnoea (5), syncope (4), and diarrhoea (3). The "respiratory clinical score" (range 0-4) and "digestive clinical score" (range 0-4) were used to evaluate the severity of BOAS. The mean preoperative respiratory and digestive scores were 3.5±0.7 and 2.3±1.6, respectively. All dogs underwent surgical treatment of the soft palate. Furthermore, the following surgical procedures were performed: tonsillectomy (5), alaplasty (2), and sacculectomy (1). Postoperative complications were recorded as follows: bleeding (2), dysphoria (1), and peripharingeal swelling (1). Dogs were re-evaluated at 1 and 3 months after surgery, and the "respiratory" and "digestive scores" were recorded. ANOVA analysis was performed to evaluate the data ($p \leq 0.05$). At 1 month and 3 months after surgery, the mean respiratory (2.2±1.1) and digestive (0.7±0.9) scores were significantly reduced ($p=0.002$ and $p=0.005$, respectively) compared to scores before surgery. No significant difference was recorded between scores at 1 month and 3 months. All owners reported a remarkable improvement of canine welfare after surgery. Our data underscore that clinical signs related to BOAS reduce in both intensity and frequency at 1 month after surgery, and the clinical improvement lasts for three months. No further improvements are recorded at 3 months postoperatively. The use of respiratory and digestive clinical score grading systems make easy and objective postoperative evaluation in dogs underwent surgical treatment for BOAS.

[1] Broux O et al. Effects of manipulations to detect sliding hiatal hernia in dogs with brachycephalic airway obstructive syndrome, Veterinary Surgery, 47:243-251, 2018.
[2] Meola SD. Brachycephalic airway syndrome, Topics in Companion Animal Medicine, 28:91-96, 2013.
[3] Riecks W, Birchard SJ, Stephens JA. Surgical correction of brachycephalic syndrome in dogs: 62 cases (1991-2004), Journal of the American Veterinary Medical Association, 230:1324-1328, 2007.

Factors associated with short- and long-term outcome in 59 foals with hematogenous musculoskeletal infection (1996-2020)

Nicola Scilimati, Marco Pepe, Elena Contu, Alice Bertoletti, Sara Nannarone, Rodolfo Gialletti, Francesca Beccati

Università degli Studi di Perugia, Dipartimento di Scienze Veterinarie, Sezione di Chirurgia e Radiodiagnostica

Corresponding author: Nicola Scilimati (nicola.scilimati@yahoo.it)

Orthopaedic infection is common in young foals and may limit athletic performance [1]. Several studies investigated survival; however, few studies evaluated the long-term outcome [2,3]. The aim of this study is to investigate factors associated with survival and return to function in foals with hematogenous orthopaedic infection.

Clinical records of <1 year-old foals with a diagnosis of musculoskeletal infection (MSI), referred to the VTH of Perugia between 1996 and 2020, were reviewed. Foals with infection secondary to penetrating wounds or surgery were excluded. The following information was retrieved: age, sex, breed, aptitude, cause of admission, presence of pyrexia, white blood cell (WBC) count, plasma serum amyloid A (SAA) and fibrinogen concentrations, type of hematogenous articular infection, septic process localisation, use of CT examination, synovial fluid total nucleated cell count (TNCC) and protein concentration, antibiotic therapy before admission, bacteriologic culture results from synovial fluid or septic lesion samples, antimicrobial therapy based on sensitivity test, concurrent pathologies, type of surgical treatment. For statistical purposes, the outcomes considered were: foals that were discharged from the hospital (short-term survival), and foals that returned to function. Return to function was defined as foal that participated in its intended discipline post-injury.

Fifty-nine foals were included; mean age was 61 days. There were 35 colts and 24 fillies. The most represented breeds were Thoroughbreds, Warmbloods and Trotter. WBC count was available in 34 foals, 24 of which had leukocytosis. Blood analysis showed increased SAA in 14/15 foals and fibrinogen in 17/21. At the time of admission, temperature was 38.5°C-39°C in 29 foals, >39.0°C in 26 foals, no pyrexia in 3. Most common affected sites were stifle and hock. Multiple joint involvement was detected in 8 foals; concurrent diseases were found in 32 foals. Radiographic lesions were classified as type S/T in 36 foals, type E/P in 9 foals, complex in 7 and other in 7. Culture from synovial fluid/lesions were negative in 14/48 foals. Most represented bacteria were *Streptococcus* spp. and *Staphylococcus* spp. The most used systemic antimicrobial therapy was aminoglycoside combined with β-lactam. Intravenous regional perfusions were performed in 26 foals with (n=11) or without arthroscopic lavage. Abscess incision and drainage was performed in 5. 74.5% foals were discharged from hospital and 83,8% returned to athletic function. A negative association was detected between WBC count and survival. The majority of bacterial isolates were gram-positive and the isolation of gram-negative organisms has been linked with reduced survival and athletic outcome [1,4]. Foals treated for MSI at the VTH of Perugia had a good prognosis for survival and return to function.

[1] Steel et al. Factors associated with prognosis for survival and athletic use in foals with septic arthritis: 93 cases (1987–1994). JAVMA,215:973–7,1999.
[2] O'Brien et al. Factors associated with survival and racing performance of 114 Thoroughbred foals with septic arthritis compared to maternal siblings (2009-2015). EVJ, early view.

[3] Wright et al. Haematogenous septic arthritis in foals: Short- and long-term outcome and analysis of factors affecting prognosis. EVJ,29:328-336,2017.

[4] Vos, Ducharme. Analysis of factors influencing prognosis in foals with septic arthritis. Ir Vet J,61:102,2008.

Comparison between image-guided transbronchial cryobiopsies and thoracoscopic lung biopsies in canine cadaver. A pilot study

Ilaria Falerno (1), Roberto Tamburro (1), Leonardo Della Salda (1), Francesco Collivignarelli (1), Andrea Paolini (1), *Francesco Simeoni (1), Luigi Navas (2), Massimo Vignoli* (1)

(1) Università degli Studi di Teramo, Facoltà di Medicina Veterinaria (2) Università degli Studi di Napoli Federico II, Dipartimento di Medicina Veterinaria e Produzioni Animali

Corresponding author: Ilaria Falerno (i.falerno@unite.it)

Interstitial lung diseases (ILDs) are heterogeneous group of non-neoplastic and non-infectious diseases, characterized of inflammation and fibrosis of the lung parenchyma. History, clinical findings, laboratory tests and diagnostic imaging can lead to a suspicion diagnosis. However, the definitive diagnosis is histological but often obtained post-mortem. To date, the only method of sampling lung tissue with a high diagnostic yield is represented by surgical lung biopsies (SLB), highly invasive and with high risk/benefit ratio. In human medicine, recently have been introduced transbronchial lung cryobiopsies (TBLC). Those are described to be less invasive, with manageable side effects and able to significantly increase diagnostic confidence in most patients with ILDs.

The aim of this pilot study is to evaluate the feasibility and diagnostic yield of TBLC compared to SLB. This is the first step of a study on cryobiopsies in veterinary medicine. It was approved from Ethical Animal Care and Use Committee of the University of Naples Federico II (n. PG/2020/0079565).

Two fresh dog cadavers underwent lung biopsy procedure by performing both CT-fluoroscopy-guided TBLC and video-assisted thoracoscopic surgery (VATS) lung biopsies. 15 pulmonary cryobiopsies and 15 VATS lung biopsies were collected. TBLC were performed first. A flexible cryoprobe measuring 115 cm in length and 1,7 mm in diameter was used (ERBE, Germany). The probe was cooled with carbon dioxide. The cryoprobe was inserted by flexible bronchoscope under CT-fluoroscopic guidance. Reached the sampling site, the cryoprobe was cooled for a mean time of 8 seconds and the frozen sample was extracted attached to the probe's tip. Then, VATS lung biopsy was performed using an endoscopic cutting device. A first macroscopic evaluation of the samples size was carried out. Then, tissue specimens were fixed in formalin for histological examination. At first macroscopic evaluation, the mean area of the cryobiopsy samples was 28 mm^2 (ranged 12-60 mm^2). The mean area of the surgical biopsies was 50 mm^2 (ranged 18-108 mm^2). To histological examination, cryobiopsy was smaller than VATS biopsies (main 11.34 mm^2 vs 17.36 mm^2) but large enough to reach a specific diagnosis or to allow pattern recognition. Morphological features on TBLC and SLB were concordant in all cases.

Seven cases of VATS biopsies and one case of cryobiopsy were non-diagnostic/inadequate. Cryobiopsy samples showed fewer artifacts and a higher percentage of alveolar tissue than VATS samples. The main artifacts and non-pulmonary tissues found in cryobiopsies were parenchymal collapse, the presence of parietal pleura/chest wall, and cryobiopsy consisting solely or mainly of the bronchial wall or medium-sized vessels. Our results showed that TBLC is a feasibility and useful technique and that more than 4 samples need to be collected for proper diagnostic evaluation.

[1] Reinero C. Interstitial Lung Diseases in Dogs and Cats Part I: The Idiopathic Interstitial Pneumonias, Veterinary Journal, 243:48-54, 2019. [2] Hetzel J, Wells AU, Costabel U, et al. Transbronchial cryobiopsy increases diagnostic confidence in interstitial lung disease: a prospective multicenter trial, European Respiratory Journal, 10;56(6), 2020. [3] Raghu G, Remy-Jardin M, Myers JL, et al. Diagnosis of Idiopathic Pulmonary Fibrosis. An Official ATS/ERS/JRS/ ALAT Clinical Practice Guideline. American Journal of Respiratory and Critical Care Medicine, 198:e44-68, 2018.

Minimal Invasive Piezoelectric Craniotomy in a large animal model

Alberto Maria Crovace [1], Luca Lacitignola [2], Laura Fracassi [2], Annarita Imperante [2], Rodrigo Trisciuzzi [2], Claudia Acquafredda [2], Sabino Luzzi[3] and Francesco Staffieri[2]

(1) Dipartimento di Scienze mediche di Base, Neuroscienze e Organi di Senso, Università degli Studi di Bari Aldo Moro (2) DETO Sez. Cliniche Veterinarie e P.A., Università degli Studi di Bari Aldo Moro (3) Neurosurgery Unit, Department of Clinical-surgical, Diagnostic and Pediatric sciences, Università degli Studi di Pavia, Fondazione IRCCS Policlinico San Matteo

Corresponding author: Alberto Maria Crovace (alberto.crovace@uniba.it)

Craniotomy is a surgery that presents lots of critical points, such as the localization, the protection of brain and the instrumentation required to perform it [1]. Conventional techniques require instruments that perform bone cutting flawlessly without damaging the nervous tissue underneath. In this study we evaluated the performances of Mectron Piezosurgery® Touch bone scalpel to perform craniotomy in a sheep model. Piezosurgery works using the indirect piezoelectric effect so it's possible to perform an osteotomy without damaging softer tissues. The frequencies used are automatically set to 22-30 Hz that are ideal to cut the bone tissue spearing soft and nervous tissues, the other important feature of the instrument is an irrigation system that allows a perfect cooling of the cutting area, crucial to avoid thermic damage [2]. The performances of the instrument were evaluated in a matter of efficiency, duration, blood loss and safety by the surgeon who performed the craniotomy. The tips, who comes in different shapes and lengths, were selected to perform a 3x2.8 cm oval cut using a guide on the parietal bone to access the brain underneath removing also the dura madre as part of the procedure. Particularly the tip selected for this study is the new OT12, which has a saw-like edge and allows to perform cuts in different shapes with ease since its conformation allows a better propagation of ultrasounds and the possibility to cut up to 10 mm of depth. After the approval of the ethical committee (approval n° 654/2020-PR) 16 sheep were recruited for a study in which craniotomy was required, the cut was performed with the Piezosurgery in all the animals, the bone portion was removed, replaced with a custom-made plate and put in formalin for histological and SEM evaluations. The surgery was bloodless, clear-cut and precise in all the subjects without complications, the total duration of the surgery was of ± 45 minutes in all the patients. The bone gussets were collected after the cut using a periosteal elevator and it was evaluated the integrity of the membranes underneath, in only two animals there were complication related to bleeding of spongious bone vessels solved using bone wax; all the samples collected had smooth edges without signs of thermic damage. The results are comparable with the ones in small animals highlighted in literature [3] although the different thickness of the skull may be an issue to take in consideration, moreover the OT12 tips has shown excellent performances in terms of efficiency and handling. Concluding, Mectron Piezosurgery Touch can be considered a solid alternative to traditional instruments to perform craniotomy in large animals and set the conditions to perform ostectomy on long bone metaphysis and also in large dogs cervical ventral slots.

[1] Crovace AM, Luzzi S, Lacitignola L, Fatone G, Giotta Lucifero A, Vercellotti T, Crovace A. Minimal Invasive Piezoelectric Osteotomy in Neurosurgery: Technic, Applications, and Clinical Outcomes of a Retrospective Case Series. Vet Science 7(2):68; 2020.

[2] Bengazi F, Lang NP, Canciani E, Viganò P, Velez JU, Botticelli D. Osseointegration of implants with dendrimers surface characteristics installed conventionally or with Piezosurgery®. A comparative study in the dog. Clin Oral Implants Research;25(1):10-5; 2014

[3] Vercellotti T., Palermo A., Molfetta L. The Piezoelectric Osteotomy in Orthopaedics: Clinical and Histological Evaluations (Pilot Study in Animals) Mediterr. J. Surg. Med. 2001; 9:89–95.

Humero-anconeal elbow incongruity; a new proposed aetiopathogenesis for humeral intracondylar fissure in spaniel breed dogs

Alan Danielski (1,3), Russell Yeadon (2), Gerardo Fatone (3)

(1) The Ralph Veterinary Referral Centre, UK (2) Lumbry Park, UK (3) Università degli Studi di Napoli "Federico II", Dipartimento di Medicina Veterinaria e Produzioni Animali

Corresponding author: Alan Danielski (alan.danielski@gmail.com)

Humeral intracondylar fissure (HIF) is a common cause of thoracic limb lameness in Spaniel breed dogs. This disease was first described in 1994 and it was thought to be due to a failure to fuse of the two centers of ossification of the humeral condyle. In 2011 this theory was challenged by a study that reported the development of a condylar fissure in the humerus of a 5-year old Cocker Spaniel that 2 years previously had a completely normal elbow. A stress fracture hypothesis was postulated as a cause for late onset development of HIF but ongoing uncertainty about the aetiopathogenesis of this condition persists.

In our study we describe for the first time a cartilaginous lesion present on the caudal aspect of the medial humeral condyle of 14 dogs with HIF that we believe to develop because of impingement between anconeal process and humeral condyle. Our hypothesis is that this lesion will be present in dogs with HIF and that underlying dynamic humero-anconeal incongruity is implicated in development of HIF in middle-age Spaniels.

Spaniels with thoracic limb lameness and HIF diagnosed by CT scan were included in the study. All these cases underwent arthroscopic inspection of the joint by use of a caudo-medial camera portal. Direct viewing of the cartilaginous lesion was achieved and it was followed by extension/flexion of the limb to assess for dynamic impingement between the tip of the anconeal process and the humeral condyle at level of the before noted cartilaginous lesion. The control group was provided by dogs of a different breeds (with no signs of HIF on CT examination) that required elbow arthroscopy for treatment of elbow dysplasia. Fourteen Spaniels (21 elbows) met the inclusion criteria and were included in the study. These dogs (9 English Springer Spaniels and 5 Cocker Spaniels) had a median age of 6y 1m (range 9-101 months). All dogs showed a focal cartilaginous lesion (4-6mm in diameter) on the caudal aspect of the humeral condyle approximately 0.5-2mm medial to the isthmus of the humeral condyle (or, when visible, medial to the HIF line). This lesion varied from being an indentation into the humeral cartilage (n=3), a lesion with cartilage fibrillation (modified Outerbridge grade II, n=4), to an obvious partial/complete thickness focal lesion (grade III, n=14). In all these cases, when the elbow was extended to 120-150°, the tip of the anconeal process perfectly matched the lesion. In such a circumstances, the joint space between ulna and humerus was absent at the level of the tip of the anconeal process whilst it appeared wider further distally along the ulnar trochlear notch. Twenty dogs (31 elbows) were included in the control group. Median age was 5y 8m (range 9-141 months). None of them showed the cartilaginous lesion on the caudal aspect of the medial humeral condyle. A previously unreported cartilage lesion at the caudal aspect of the medial humeral condyle was identified in dogs with HIF and was not present in a control group of unaffected dogs. This lesion appears to be associated with impingement between the medial humeral condyle and anconeal process ("humero-anconeal incongruity"). We propose that this may be the cause for the intracondylar instability associated with HIF development.

[1] Marcellin-Little et al. Incomplete ossification of the humeral condyle in spaniels. Veterinary Surgery, 23:475-87, 1994.
[2] Farrell et al. Computed tomographic documentation of the natural progression of humeral intracondylar fissure in a cocker spaniel. Veterinary Surgery, 40:966-71, 2011

Ultrasonography evaluation of umbilical structures in clinically healthy donkey foals during the first week of life

Irene Nocera (1,2), Valentina Vitale (1,2), Micaela Sgorbini (1,2), Benedetta Aliboni (1,2), Fulvio Laus (3), Aurora Mannini (1), Marilena Bazzano (3)

(1) Università di Pisa, Dipartimento di Scienze Veterinarie (2) Università di Pisa, Ospedale Didattico Veterinario (3) Università di Camerino, Scuola di Bioscienze e Medicina Veterinaria

Corresponding author: Irene Nocera (irene.nocera@vet.unipi.it)

The umbilicus has been recognized as a potential access for pathogens in equine foal, causing umbilical infections and potentially life threating (1). Early diagnosis based on ultrasonographic appearance and measurement of internal anatomic structures is crucial to avoid severe complications and promptly implement appropriate therapy (2). The aim of the present study was to evaluate ultrasonographically the umbilical remnant in donkey foals, in the first week of life, and to compare with equine and bovine species.

Fifteen healthy donkey foals were included in the study. The ultrasound evaluation of the umbilical remnant was performed at 24 hours (T0), and then at 3 (T1) and 7 (T2) days of life. Ultrasound was performed using a real-time B mode scan using a portable ultrasound machine (MyLab30Gold, Esaote, Italy) and a multifrequency 5-7.5 MHz frequency linear probe transducer (3). The Kruskal-Wallis test and the Dunn's multiple comparisons test were applied to verify differences in relation to time for all the umbilical remnant structures measured. Statistical significance was set at $p<0.05$.

No statistical differences were observed in relation to time, regarding umbilical remnant measurements. Correlation was found between donkey foals' body weight and left artery at T0.

The ranges of umbilical measurements, including urachus, vein and arteries, reported for horses and calves can be considered valid also for donkeys (1,4), showing no evident correlation with body weight and umbilical cord length (2,5). The regression of the structures is probably slower compared with equine foals, but it is comparable to what has been reported for calves (2,4). Thus, the different regression timing might be taken into account when evaluating foals with possible umbilical disease, within the first week of life.

[1] Magri M. Ultrasonography of the umbilical remnant in foals. In practice, 40:301-305, 2018.
[2] McCoy et al. Normal regression of the internal umbilical remnant structures in Standardbred foals. Equine Vet J, 52:876-883, 2020.
[3] Magri M. Ultrasonography of umbilical structures. In Atlas of equine ultrasonography. Eds J.A.Kidd, K.G.Lu, M.L.Frazer. WileyBlackwell. pp 483-493, 2014.
[4] Guerri et al. Ultrasonographic evaluation of umbilical structures in Holstein calves: A comparison between healthy calves and calves affected by umbilical disorders. Journal of dairy science, 103:2578-2590, 2020.
[5] Turini et al. Evaluation of jennies' colostrum: IgG concentrations and absorption in the donkey foals. A preliminary study. Heliyon, 6: e04598, 2020.

Shear wave elastography of the lens in horses: preliminary results

Adriana Palozzo, Paola Straticò, Giulia Guerri, Maria Cristina Pincelli, Lucio Petrizzi

Unit of Equine Medicine and Surgery, Veterinary 26 Teaching Hospital, Faculty of Veterinary Medicine of the University of Teramo

Corresponding author: Giulia Guerri (guerri.giu@gmail.com)

Congenital Cataracts are the largest group of developmental lens opacities in horses. Acquired cataracts are common sequelae of uveitis and trauma [1]. These changes in the nature of the lens proteins result in alteration in its hardness [2] that can be measured through elastography.

Elastography is a non-invasive ultrasonographic technique that measures the elasticity of tissues, according to its degree of its deformation to mechanical compression [3].

The aim of this preliminary study was to determine feasibility, reproducibility and repeatability of two-dimensional Shear wave elastography (2D-SWE), to establish quantitative and qualitative reference values for lens stiffness in healthy horses.

After a complete clinical and ophthalmological examination, a trans-palpebral B-mode US and 2D-SWEof the two eyes were performed by two experienced operators using a high frequency linear probe (10 MHz) connected to an ultrasound system (Logiq S8, GE Healthcare) under sedation with detomidine (0.01mg/kg)

Quantitative analysis of lens stiffness was performed by manually drawing a region of interest (ROI) of 3,10 cm in circumference over each lens. Mean SW velocity (m/s) and Young's Modulus (kPa) at each selected ROI were calculated using the 2D-SWE software. Statistical analysis was performed with SPSS V. 27. Qualitative analysis was obtained by the real-time visualization of a color qualitative elastogram superimposed on B-mode image. Eight horses were included (mean age group 4 years). Mean stiffness expressed in m/s and kPa was, respectively, 4.6±0.6 and 64.5±19.2 in the left lens, and 4.9±0.9 and 73.9±29.2 in the right lens. Significant differences ($P<0.05$) were detected between the right and left lens, but not between nor within observers ($P>0.05$). Qualitative analysis of the elastograms showed that the lens appeared like a mosaic of colors with a greater presence of blue than red.

2-D SWE is a non-invasive procedure feasible for examination of the lens in horses. The establishment of reference values of elasticity of these structures in sound horses will serve as comparison for horses with lens disorders.

[1] Gilger B.C., Equine ophthalmology, 3rd ed. (ed. Wiley Blackwell) 2018.
[2] Tabandeh et al. Hardness and ultrasonic characteristics of the human crystalline lens. Journal of Cataract & Refractive Surgery, 2000, 26.6: 838-841.
[3] Sigrist RMS. Et al. Ultrasound Elastography: Review of Techniques and Clinical Applications Theranostics, 7(5):1303-1329, 2017.

Radiographic, ultrasonographic and elastosonographic evaluations of patellar ligament after tibial plateau leveling osteotomy (TPLO) in dogs

Luca Pennasilico (1), Caterina Di Bella (1), Antonella Volta (2), Michela Altamura (1), Riccardo Botto (1), Angela Palumbo Piccionello (1)

(1) Università degli Studi di Camerino, Scuola di Bioscienze e Medicina Veterinaria; (2) Università degli Studi di Parma, Dipartimento di Scienze Mediche Veterinarie

Correspondig author: Luca Pennasilico (luca.pennasilico@unicam.it)

The aim of the study was to evaluate the onset and progression of structural and functional changes of the tibial patellar ligament in ten dogs underwent TPLO. Preoperatively (T0) and then one (T30), two (T60) and six months (T180) after surgery, they underwent clinical evaluation, radiographic and ultrasound examination of the patellar ligament. Besides, elastosonographic evaluation was performed at T0 and T30. The thickness of patellar tendon was measured one centimeter distal to the base of the patella, one centimeter proximal to the tibial tuberosity and halfway between the first two points, on mediolateral stifle x-ray view. Longitudinal scans were acquired for ultrasonographic and elastosonographic examinations, patients were placed in lateral recumbency with the affect limb up. The ultrasonographic assessment of patellar ligament was performed using a score ranged from a minimum of zero (ligament apparently devoid of pathological changes) and a maximum of three (extremely damaged ligament). Elastosonography assessed the tissue elasticity through the elasticity color map (red: soft; green: intermediate; blue: hard). [1] Elasticity was measured calculating the percentage of tissue softness with a grading scale from 1 to 5 (soft, mostly soft, intermediate, mostly hard and hard). Shapiro–Wilk test and Wilkinson test was used for the analysis of data. A P value < 0.05 was considered statistically significant. A significant increase in radiographic thickness of the patellar ligament was highlighted at T30 and T60 in each measured portion (proximal, middle and distal) of the anatomical structure. At T180 the thickness of proximal ligament portion returned to a value similar to T0 while the thickness of middle and distal portion remained significantly higher than T0 values. The most important increase, after surgery, was recorded at the distal portion of the patellar ligament at all times. At T0, only 2/10 patients showed ultrasound changes (score = 1). At T30, instead, 5/10 patients received a score of 2, 3/10 patients of 1 and 2/10 patients of 0. At T60, only 2/10 patients had score of 2, 6/10 patients had a score of 1, and 2/10 had a score of 0. At T180, just 3/10 patients had a score of 1, the other 7/10 had a score of 0. At T0 elastosonographic investigation showed that the patellar ligament was substantially soft, with an average percentage of softness of 76.9% (+/- 25.6) and an average percentage of hardness of 24.1% (+/-19.2). At T30, hardness significantly increased (51.8 +/- 20 %) and softness was reduced (47.1% +/- 26.9 %). According with literature [1], the significant distal thickening of the patellar ligament, highlighted by radiographic measurements, which persists even 180 days after the TPLO, suggests an increase in stress on the tibial insertion of the ligament. In our study, the apex of tendinopathy is achieved 30 days after surgery but, over time, patellar tendinosis gradually decreases. The ultrasonographic results are in agreement with the radiographic ones, but didn't show a severe pathologic finding. Contextually, for the first time, the elastosonography demonstrated a significantly increase of hardness in the patellar ligament at 30 days (T30) post TPLO. The onset of patellar tendinopathy is attributable to the change in the distribution of the forces on the tibia after TPLO. We can affirm that there are radiographic, ultrasonographic and elastosonographic changes of patellar ligament after TPLO.

1. Mattern, K. L et al. Radiographic and ultrasonographic evaluation of the patellar ligament following tibial plateau leveling osteotomy. Veterinary Radiology & Ultrasound, 47(2), 185-191, 2006.

Computed tomography features of divisional bile ducts in healthy Labrador Retrievers

Mirko Mattolini (1,2), Simonetta Citi (2), Federica Rossi (1)

(1) Clinica Veterinaria dell'Orologio (BO) (2) Università di Pisa, Dipartimento di Scienze Veterinarie

Corresponding author: Mirko Mattolini (mirko.mattolini@phd.unipi.it)

Gallbladder and bile ducts pathology are common in dogs and typically investigated with ultrasonography. Abdominal computed tomography (CT) is becoming more and more popular especially in large dogs or with challenging diagnoses. The first step in interpreting CT findings is the knowledge of the cross-sectional anatomy of the involved structures. Detailed bile ducts anatomy is reported in canine cadavers [1][2], however detailed studies describing some CT biliary tract features are lacking in the veterinary literature, especially the divisional ducts anatomy draining the individual divisions of the liver in to the common bile duct. In dogs, the most common pattern includes 3 (2 central and 1 right or left) or 4 (2 central + 1 left and 1 right) divisional ducts. The aim of this retrospective descriptive study was to evaluate the visibility, size and pattern of the divisional ducts in contrast-enhanced CT (CECT) sequences in a group of adult Labrador Retriever without evidence of hepatobiliary diseases. Moreover, the correlation with the visceral fat area (VFA%) was evaluated [3]. Inclusion criteria for the study were a complete abdominal CECT study, absence of tomographic signs of hepatobiliary pathologies and normal blood tests; forty dogs met inclusion criteria. In CT, the bile divisional ducts were visible when surrounded by peritoneal fat. A single right divisional duct (RDD) was visualized in 4/40 dogs (10%), a single left divisional duct (LDD) was detected in 9/40 dogs (22.5%), and in 17/40 dogs (42.5%), both RDD and LDD were visualized. In 10/40 (25%) RDD and LDD were not highlighted. When visible, the RDD has a mean diameter of 0.23 cm (range 0.3 to 0.16 cm) and a mean length of 1.01 cm (range 0.49 to 2.95 cm). The LDD has a mean diameter of 0.24 cm (range 0.13 to 0.33 cm) and a mean length of 2.67 cm (range 1.42 to 3.97 cm). The two central divisional ducts were not identified in any of the included patients because always surrounded by hepatic parenchyma. No significant correlation with the VFA% was found (p=0.91). In conclusion, this is the first study describing the divisional bile duct normal CT anatomy. This provides a base for the correct identification of these normal structures and could be useful for further studies in patients with hepatobiliary diseases.

[1] Sleight, D.R. and Thomford, N.R. Gross anatomy of the blood supply and biliary drainage of the canine liver, Anat. Rec. 166: 153–160, 1970.
[2] Imagawa T. et al., Anatomical Variations of the Extrahepatic Ducts in Dogs: Knowledge for Surgical Procedures, J. Vet. Med. Sci. 72(3): 339–341, 2010.
[3] Nagao I. et al., Evaluation of visceral fat mass in dogs by computed tomography, J. Vet. Med. Sci. 81(11): 1552–1557, 2019.

Evaluation of an in-plane ultrasound-guided retrobulbar block in the dog

Chiara Di Franco, Umberto Rosati, Aida Fages Carcéles, Samanta Nardi, Giovanni Barsotti, Martina Nuti, Angela Briganti

Università degli Studi di Pisa, Dipartimento di Scienze Veterinarie

Corresponding author: Chiara Di Franco (chiara.difranco@phd.unipi.it)

Locoregional anaesthesia techniques in ophthalmic veterinary surgery have been the subject of active study especially in recent years [1,2,3,4]. The aim of this study was to describe an in-plane ultrasound-guided approach for the retrobulbar block anesthesia (RBA) in dogs. The study was divided in 3 phases. Phase 1: an ultrasound image (UI) has been sought on a dolichocephalic dog skull, with a water immersion ultrasound study in order to visualize specific landmarks for the development of the RBA technique. Phase 2: eleven cadavers of dogs of different breeds and weights were used in order to assess the applicability of the proposed technique. Once the retrobulbar cone and the orbital fissure were identified, with a micro convex probe, an injection was performed with an *in-plane* technique, using a volume of 0.05 mL/kg of methylene blue solution 2%. This technique allowed the execution of a satisfactory injection in 18 out of 21 cases. Anatomical dissections also confirmed the absence of spread of the dye in the brain in all cases. In the 3rd phase the technique of RBA was applied on clinical patients undergoing mono or bilateral enucleation (approval of OPBA n. 25/2020). Six patients were enrolled in this preliminary phase, two of which underwent bilateral enucleation for a total of 8 blocks performed. All dogs were premedicated intramuscularly with dexmedetomidine 3 mcg/kg and methadone 0.2 mg/kg. Subsequently, the patients were induced with propofol and maintained with isoflurane in oxygen (FiO_2 60%). The retrobulbar block was performed with a volume of 0.05 mL/kg with 0.5% ropivacaine. Standard clinical parameters were monitored every 5 minutes starting from Tbase (presurgical time); in case of increase of mean arterial pressure more than 20% respect to the Tbase parameters a rescue analgesia with fentanyl 2 mcg/kg was programmed until the restoring of the parameters. During anaesthesia no rescue analgesia was required. At the end of the procedure, an NSAID was dispensed. Postoperatively, a Glasgow pain scale was performed every hour from the patient's awakening: the median pain score recorded was 2 (1-4) and no animal required postoperative rescue analgesia. The study demonstrated the applicability of a new ultrasound-guided retrobulbar injection technique with an *in-plane* approach which allows to increase the safety and precision of retrobulbar injections through a single injection site with a volume of 0.05 mL/kg of anaesthetic solution. Visualizing the needle throughout the procedure makes this technique safer, more effective and ensured good intra- and post-operative analgesia.

[1] Shilo-Benjamini Y et al. Retrobulbar vs peribulbar regional anesthesia techniques using bupivacaine in dogs. Vet Ophthalmol. 2018;00:1–9.
[2] Shilo-Benjamini Y, A review of ophthalmic local and regional anesthesia in dogs and cats, Veterinary Anaesthesia and Analgesia, .2018.10.004.
[3] Viscasillas J, et al. Ultrasound-guided posterior extraconal block in the dog: anatomical study in cadavers. Veterinary Anaesthesia and Analgesia 2019, 46, 246-250.
[4] Viscasillas J, Ter Haar G (2017) Ultrasound guided trigeminal nerve block as local anaesthetic technique for exenteration and excision of the zygomatic arch with partial caudal maxillectomy in a dog. Vet Anaesth Analg 3, 688-690.

The use of the virtual-problem-based learning in veterinary education during SARS-CoV-2 (COVID-19) pandemic emergency: a resource or a disadvantage?

Fabiana Micieli, Chiara Del Prete, Giovanni Della Valle, Paolo Ciaramella, Jacopo Guccione

University of Napoli Federico II, Department of Veterinary Medicine and Animal Productions

Corresponding author: Fabiana Micieli (fabiana.micieli@unina.it)

The COVID-19 global pandemic emergency is forcing Veterinary College all over the world to modify deeply the traditional teaching approach employed. Due to the nationwide lockdown, the social distancing and the restrictions adopted in our country to reduce the virus spread, the Department of Veterinary Medicine of Napoli decided to replace the "traditional and in presence" hours dedicated to the clinical, pre-graduating, veterinary medical training with multiple sessions of clinical virtual-problem-based learning (v-PBL). In veterinary education, problem-based learning (PBL) represents one of the most popular and flexible teaching systems able to replicate and simulate real-life experiences [1]. This prospective cross-sectional case-control study aimed to evaluate the students' perception of the v-PBLs compared to the traditional veterinary clinically training (t-VCT).

All the fifth-year students who completed the t-VCT (76 students) or the clinical v-PBL (46 students) represented the study population. The t-VCT consisted of supervised management of clinical cases admitted at the Veterinary Teaching Hospital or performed in the field under academic-staff supervision. The v-PBL consisted of genuine clinical case shared by tutors throughout an online platform. For each case, in the v-PBL group the time was approximately divided as follow: 1h of case-introduction by the tutor; 8h of self-learning and problem-solving activities (consisting of 15 to 20 progressive questions and activities related) and 4h of virtual group-discussion activity between students and tutor. To evaluate the student perception of the teaching methods, a survey with 18-Likert and two open-end questions was delivered to all the students in the study population. The latter was divided into five sections: "demographic data", "satisfaction", "clinical skills", "supervision" and "student's perceptions of the training".

The survey was completed by 49% of the students (36.8% and 69.6% for t-VCT and v-PBL, respectively). Cronbach's alpha coefficient for internal consistency for the questionnaire was 0.9 (acceptable). Overall, the students' satisfactions degree regarding the training experiences was high in both groups. Nevertheless, the v-PBL group overall felt less satisfying as compared to the t-VCT group. The students of v-PBL group perceived that they could not improve their practical clinical skills through online sessions. All students of the v-PBL group had not suggestions to improve the v-PBL although they emphasized how it could be employed as support of traditional practical activities, in the future. All the students considered the trainings correctly focused on relevant learning objectives and the task clearly explained. Moreover, despite the social distancing imposed, the students in both groups express an overall satisfaction for the supervision activities performed by the academic staff.

Stimulating the integration of knowledge and lifelong learning skills replicating life experiences the v-PBLs represented an attractive curricular alternative for veterinary education in a period in which the conventional didactic systems have been deeply changed by the COVID-19 pandemic emergency. Moreover, it should be considering to integrate the traditional practical teaching approach with PBL to increase motivation and physiological arousal in students.

[1] Lane EA. Problem-based learning in veterinary education. J Vet Med Educ. 35(4):631-6, 2008.

Animal welfare science and law in UniSS courses: knowledge and opinion of students

Varoni M.V.[1,4], Serra P.A.[2,4], Sanna Passino E.[1,3,4]

[1] Department of Veterinary Medicine, University of Sassari, Italy [2] Department of Clinical, Surgical and Experimental Medicine, University of Sassari, Italy [3] Comparative Surgery Research Laboratory, University of Sassari, Italy [4] Animal Welfare and Ethics Committee (AWEC), University of Sassari, Italy

Animal welfare is a very complex and multidisciplinary concept with scientific, ethical and legal dimensions studied in some universities courses. In this paper we describe the results of an online survey consisting of twenty-three questions created to evaluate the knowledge and interest in animal welfare of students attending scientific, medical (including veterinary) and biomedical courses at the same University (UniSS, University of Sassari, Italy). Students attended fifteen different Scientific Courses. The survey, divided in four sessions, collected advice about students' basic information (gender, age, year of course attended, educational program), level of knowledge on animal welfare, level of knowledge about 3Rs and the students' personal beliefs on animal experimentation. We represent also the wordcloud based on the terms most frequently used by students in their opinions. The study addresses these issues in all study courses in which the use of animals for experimentation is potentially envisaged. Interpretation of animal welfare and its translation into the management of students' courses are both influenced by context and by cultural and societal values.

Quality of sedation and early cardiovascular and respiratory variables after medetomidine and midazolam with ketamine or alfaxalone administration in swine

Luca Bellini, Giulia Maria De Benedictis

Università degli Studi di Padova, Dipartimento di Medicina Animale, Produzioni e Salute

Corresponding author: Luca Bellini (luca.bellini@unipd.it)

Swine involved in biomedical research often require chemical restraint to perform minor procedures or before general anaesthesia. In this species, intramuscular (IM) route is preferred for chemical immobilization because the approachable veins are limited due to anatomical conformation and temperament. Ketamine (10-20 mg/kg) or alfaxalone (5 mg/kg) by IM administration were used for immobilization in swine, but they were associated with cardiovascular effects and seldom muscle rigidity or twitches [1, 2]. Co-administration of benzodiazepine or alpha-2 agonist improves hypnosis and mitigates side effects with a decrease in dose. The aim of this study was to compare the quality of sedation and the early cardiovascular and respiratory effects of two IM protocols using midazolam, medetomidine and low doses of ketamine or alfaxalone, in swine. The ethical committee of the University of Padova and the Italian Ministry of Health (28/2016) approved the study. Sixteen swine were randomly divided in two groups and received medetomidine 7 µg/kg and midazolam 0.4 mg/kg mixed with ketamine 7 mg/kg (group MMK) or alfaxalone 2 mg/kg (group MMA) into the epaxial muscle of the neck. A semiquantitative scale scored sedation at 5 (Rest5) and 10 (Rest10) minutes after IM injection. The descriptors were: posture from 0 (normal) to 5 (not moving when stimulated); resistance to being rolled in dorsal recumbency from 0 (normal resistance) to 3 (no resistance); palpebral reflex from 0 (normal) to 2 (absent); jaw muscle tone 0 (normal) or 1 (absent). Time from IM injection to loss of righting reflex (LRR) was noted as any other side effects. After collecting measurements at Rest10, swine were moved into a pre-surgical suite: heart and respiratory rate (HR and RR), mean arterial blood pressure (MAP) and haemoglobin oxygen saturation (SpO2) were recorder and an intravenous catheter placement was attempted. Mann-Whitney or Student's T test were used to compare the variables between groups in non-normally and normally distributed variables, respectively. All swine resulted sedated within 10 minutes, and it was possible to insert the IV line. Only in group MMA, 7 swine had twitches within 10 minutes after administration. No statistical differences were observed between groups at Rest5 ($p=0.670$) and Rest10 ($p=0.429$). However, at Rest5 the score was statistically lower than at Rest10 with a median (min-max) of 9 (3-10) and 10 (8-11) in group MMA ($p=0.038$), and 9 (5-10) and 10 (9-10) in group MMK ($p<0.01$). The time of LRR was similar between groups being at 198±64 and 174±50 seconds ($p=0.419$) in group MMA and MMK, respectively. Cardiovascular and respiratory variables remained within clinically acceptable limits with no difference between groups (HR 111-146 beat/min; RR 21-64 breaths/min; MAP 66-107 mmHg; SpO2>94%). In conclusion, low dose of ketamine or alfaxalone associated with medetomidine and midazolam produced a satisfactory immobilization, adequate to allow the placement of an intravenous catheter while maintaining physiologic variables within clinical acceptable limits.

[1] Santos et al. Effects of intramuscular dexmedetomidine in combination with ketamine or alfaxalone in swine, Veterinary Anaesthesia and Analgesia, 43:81-85, 2016.
[2] Santos et al. Effects of intramuscular alfaxalone alone or in combination with diazepam in swine, Veterinary Anaesthesia and Analgesia, 40:399-402, 2013.

Comparison of intrarectal and intramuscolar effects of ketamine, dexmedetomidine and midazolam in cats

Andrea Paolini (1), Ilaria Cerasoli (2), Massimo Vignoli (1), Ilaria Falerno (1), Amanda Bianchi (1), Francesco Collivignarelli (1), Roberto Tamburro (1)

(1) Università degli Studi di Teramo, Dipartimento di Scienze Veterinarie (2) Clinica Veterinaria Borghesiana, Roma

Corresponding author: Andrea Paolini (apaolini@unite.it)

Correct treatment of anxiety and pain in cats can help veterinarians to carry out clinical and routine diagnostic procedures. Drugs administration by intrarectal route (IR) is widely used in human medicine. IR is easy to access for the sedation of pediatric not cooperative patients with safety margins.
The aim of this study is to test the clinical efficacy of the intrarectal administration of ketamine, dexmedetomidine and midazolam mixture compared to the intramuscolar route (IM) in the feline species.
Twenty client-owned cats were involved in this prospective, blinded and randomized clinical trial. Project was approved by CEISA (Committee on Animal Research and Ethics of the Universities of Chieti-Pescara, Teramo, L'Aquila and of the Experimental Zooprophylactic Institute of Abruzzo-Molise) protocol N 8/2020. Heart rate (HR), respiratory rate (RR), pulse rate (PR), peripheral oxygen saturation (SpO_2), systolic, mean and diastolic non invasive blood pressure values and rectal temperature (°C) were recorded. Inclusion criteria: patients ASA I or II, body condition score >3/9 or <7/9, no previous medical history of adverse reaction of drugs. Cats were included in the study and assigned randomly in two groups. Protocol used in the intrarectal group (IRG) was dexmedetomidine 0.003mg/kg, ketamine 4mg/kg and midazolam 0.4mg/kg while in intramuscular group (IMG) was dexmedetomidine 0.003mg/kg, ketamine 2mg/kg and midazolam 0.2mg/kg. Cardiorespiratory values were recorded prior to drugs administration and after 2 and every 5 minutes until the end of the procedure up to the recovery. Depth of sedation and recovery time was registered. Data were reported on Excel and One-way ANOVA and Welch's test were used for statistical analysis. Drugs administration by two different routes seems to be better tolerated in the IRG. No statistical difference was found for cardiovascular, respiratory and temperature parameters in both groups. In IMG mean sedation level increased rapidly, reaching a value of 5.1 (SD±1.66) 10 minutes after drug administration, up to a maximum of 6.6 (SD±1.6) at minute 25. Gradual decrease in the level of sedation was detected, up to the mean value of 3.3 (SD ± 1.8) at 40 minutes post treatment. In IRG maximum level of sedation was reached 15 minutes after drugs administration with a mean value of 3.5 (SD±1.7) then decreased to a mean value of 1.0 (SD±1.3) at 40 minutes post treatment. Recovery of the quadrupedal station was 57 minutes (SD±9.88) IMG, 44.9 minutes (SD±5.79) in IRG. Flow by oxygen was required in 70% of the IMG cats ($Sp0_2$<95%), in contrast none of the patients in the IRG needed it. There are no significant changes in rectal temperature. This preliminary study suggests that IR sedation could be an effective and safe practice to perform minimally invasive clinical and diagnostic procedures in healthy cats.

[1] Hua et al. Physiological and Pharmaceutical Considerations for Rectal Drug Formulations, Frontiers in pharmacology, 10:1196, 2019.
[2] Lam et al. Systematic Review: Rectal Administration of Medications for Pediatric Procedural Sedation, The J of emergency medicine, 55 (1), 51-63, 2018.
[3] Schroers et al.Pharmacokinetics of low-dose and high-dose buprenorphine in cats after rectal administration of different formulations, 2019.
[4] Borchard et al. Pharmacokinetics of ketamine HC1 and metabolite I in the cat: a comparison of i.v., i.m., and rectal administration, Journal of Veterinary Pharmacology and Therapeutics, 11(1), 84–93, 1988.

Pseudomembranous cystitis: an uncommon ultrasound appearance of cystitis in cats and dogs

Caterina Puccinelli, Ilaria Lippi, Camilla Madarena, Tina Pelligra, Tommaso Mannucci, Francesca Perondi, Mirko Mattolini, Simonetta Citi

Università degli Studi di Pisa, Dipartimento di Scienze Veterinarie

Corresponding author: Caterina Puccinelli (caterina.puccinelli@phd.unipi.it)

In veterinary medicine, the pseudomembranous cystitis (PC) is a rare condition described only in the cat. This pathology is characterized by a peculiar ultrasound feature of the urinary bladder, represented by multiple hyperechoic luminal septa and/or strips resembling membranes. The ultrasound appearance of PC was observed also in the dog, but there are no published studies describing this pathology in dogs. Purposes of this retrospective study were to describe ultrasound features of PC in both cats and dogs, possible predisposing factors, comorbidities and outcome. Cats and dogs with an ultrasonographic diagnosis of PC during the period from 2015 to 2020, were included in the study. Ultrasound findings recorded were: pseudomembranes' characteristics (type of adhesion to the bladder wall and presence of associated acoustic shadowing), abnormalities of bladder's wall and content, anomalies of the pericystic space and of kidneys and/or ureters. Medical records for included patients were reviewed for signalment, history, reason of presentation, urological findings, urinalysis findings, urine culture when available, therapy and outcome. A total of 10 cats and 4 dogs met inclusion criteria. Four pseudomembranes' adhesion patterns were described: type 1 "complete adhesion", type 2 "partial adhesion with compartmentalization", type 3 "partial adhesion without compartmentalization", type 4 "mixed partial adhesion". All the four patterns were present in the feline group, on the other hand the type 1 was not observed in the canine group. The presence of pseudomembranes' acoustic shadowing was observed in the 60% of cats and not detected in any dog. The 80% of cats included in our study were presented for urethral obstruction (UO) and/or have at least one episode of UO in the previous two months. In two cats an ultrasound exam was performed also before the diagnosis of PC and the ultrasound images showed findings suggestive of chronic cystitis. Thirteen patients on 14 received only medical therapy and all of them recovered successfully. In the present study, PC was confirmed to be a rare disorder in cats and also in dogs and we observed some ultrasonographic differences between the two species. Although the exact pathogenesis remains unknown, the elevated frequency of UO in cats with PC may suggest a potential role of ulcerative feline idiopathic cystitis, or relapsing chronic cystitis in the development of PC. Finally, in our cohort of patients, PC had a benign prognosis and based on our findings the medical approach can be a non-invasive, conservative and effective approach for PC. In conclusion, in patients affected by PC ultrasound may be a very useful tool to assess the evolution of PC over time, and to monitor associated complications, such as chronic cystitis and UO.

[1] Le Boedec et al. Pseudomembranous cystitis, an unusual condition associated with feline urine outflow obstruction: Four cases. J Feline Med Surg, 13(8):588-93, 2011.
[2] Sutherland-Smith J. Bladder and Uretra. In Atlas of Small Animal Ultrasonography. II ed. JohnWiley & Sons, Inc.: Oxford, UK, pp. 363-386, 2015.
[3] Vila et al. Successful medical management of pseudomembranous cystitis in three cats with lower urinary tract obstruction. Aust Vet J, 96(1-2):33-38, 2018.

Comparison of two preoperative radiographic methods for tibial tuberosity advancement in dogs by use modified Maquet technique: a cadaveric study

Federica Aragosa (1), Giovanni Della Valle (1), Chiara Caterino (1), Sara Buonocore (1), Francesco Lamagna (1), Alfonso Piscitelli (2), Alan Danielski (1,3), Gerardo Fatone (1)

(1) Università degli Studi di Napoli Federico II, Dipartimento di Medicina Veterinaria e Produzioni Animali (2) Università degli Studi di Napoli Federico II, Dipartimento di Agraria (3) The Ralph Veterinary Referral Centre, UK

Corresponding author: Federica Aragosa (federica.aragosa@unina.it)

Several surgical techniques have been described in the veterinary literature to address cranial cruciate ligament rupture (CCLR), which is considered the most common cause of pelvic limb lameness in dogs (1). Tibial tuberosity advancement (TTA) and its subsequent adaptation as Modified Maquet Procedure (MMP) are widely used to neutralize cranial tibial thrust in dogs with CCLR (2). Preoperative planning for tibial tuberosity advancement is a mandatory step of MMP (2). Those measurement can also be influenced by several factors such as the method used to measure the tibial plateau angle (TPA), limb positioning, anatomical factors and presence of periarticular osteophytosis that can make identification of anatomical landmarks more difficult (3). The discrepancy between values measured on radiographs and values obtained in vivo has been reported in the literature by several authors. This study aims to evaluate the validity of two preoperative radiographic planning; Tibial Anatomy-based Method (TAM) and Conventional Method (CM) were used to select the correct wedge to achieve a final TPA of 90° in dogs using the MMP. The sample consisted of 20 stifle joints harvested from 10 adult canine specimen of mesomorphic breeds. The sample was randomly assigned to the two measurement techniques, 10 stifles for TAM (group A) and 10 stifles for CM (group B) respectively. Medio-lateral radiographic projections of the stifle joint positioned at 135° were obtained and used to select the correct wedge size to advance the tibial tuberosity. The MMP was then used to achieve the correct advancement according to CM and TAM methods. Preoperative and postoperative TPA, size and correct wedge position were recorded. The position of the wedge relative to the Maquet hole was found to be correct in both groups with an average value of 4.6 mm. The distance between the proximal edge of the wedge and the insertion of the patellar tendon was on average 12 mm. Mean pre and postoperative TPA was 95.9° (±4.1) and 89.7° (±4.1) for group A; 97.2° (± .1) and 89.1° (±4.5) for group B respectively. The mean wedge size was 9.18 (±1.2) for group A and 8.8 mm (±2.18) for group B. Both groups exhibited statistically different TPA between pre- and post-operative measurements (A: $p<0,03$; B: $p<0,017$). Both radiographic methods led to a postoperative TPA consistent with 90 (±5) when MMP was used but failed to yield the true advancement of the intended 90° correctly. In our opinion, an intraoperative new measurement method is needed to confirm intraoperatively achievement of the desired 90° TPA.

1. Vaughan LC: The history of canine cruciate ligament surgery from 1952–2005. Vet Comp Orthop Traumatol 2010;23:379–384.
2. Ness MG. The Modified Maquet Procedure (MMP) in Dogs: technical development and initial clinical experience. J Am Anim Hosp Assoc 2016;52(04):242–250.
3. Paul Pillard, Veronique Livet, Quentin Cabon, Camille Bismuth, Juliette Sonet, Didier Fau, Cla ude Caroz-zo, Eric Viguier, Thibaut Cachon. (2017) Evaluation of a new method to determine the tibial tuberosity advancement distance required to reduce the patellar tendon-tibial plateau angle to 90° with the modified Maquet technique in dogs. *American Journal of Veterinary Research* 78:4, 517-528.

An MR relaxometry based tool for identifying brain lesions: an experimental study on a rabbit model

Francesca Del Signore (1), Massimo Vignoli (1), Leonardo Della Salda (1), Roberto Tamburro (1), Ilaria Cerasoli (2), Andrea Paolini (1), Matteo Chincarini (1), Emanuela Rossi (3), Nicola Ferri (3), Mariarita Romanucci (1), Tonino Talone (1), Francesco de Pasquale (1)

(1) Università degli Studi di Teramo, Dipartimento di Scienze Veterinarie (2) Clinica Veterinaria Borghesiana, Roma (3) Istituto Zooprofilattico Sperimentale dell'Abruzzo e del Molise Giuseppe Caporale, Teramo

Corresponding author: Francesca Del Signore (fdesignore@unite.it)

Magnetic Resonance Relaxometry is a quantitative MRI-based technique able to estimate tissue relaxation times T1 and T2 [1]. This approach allows increasing the MRI diagnostic accuracy mostly in case of brain neoplasia or neurodegenerative disorders in human medicine [2,3]. However, few reports are available on the application of this technique in the clinical field of veterinary medicine, especially with low-field scanners [4,5] that are widely used in veterinary practice [6]. Thus, in this work we developed a multivariate T1 and T2 based relaxometry approach to assess the feasibility of this technique to improve the detection of subtle brain lesions in companion animals. Sixteen New Zeland White rabbits have been studied. On each subject under general anesthesia, a minimal amount of autologous blood has been manually injected on the left hemisphere (all the steps of the study have been carried out in strict accordance with the recommendation from national committee for animal welfare (protocol N° 726/2019-PR)). Then, after T1 and T2 maps estimation, a hierarchical clustering procedure was run. Specifically, this was driven by the T1 signals from a set of regions of interest selected on the T2 map. This allowed the comparison of the signal between the suspected lesion and the healthy parenchyma. To validate the proposed technique, the scanned brains underwent histopathological analyses to estimate the performance of the proposed classifier in terms of Receiver Operator Curve analyses. The results showed that, in terms of identification of the lesion and its contours, the proposed approach resulted accurate outperforming the standard T1/T2w techniques. The ROC analysis placed the performance of the proposed classification procedure close to the edge of “highly accurate”. The proposed protocol, in terms of the adopted scanner, sequences, and analysis tools, is suitable for the clinical practice, and thus it can be potentially used on large-scale multi-center clinical studies for the routine veterinary practice to improve lesions visualization in case of brain disorders of companion animals.

[1] Cheng H-LM et al. Practical medical applications of quantitative MR relaxometry. Journal of Magnetic Resonance Imaging., 36(4):805–24, 2012. [2] Fathi Kazerooni A, et al. Characterization of active and infiltrative tumorous subregions from normal tissue in brain gliomas using multiparametric MRI. Journal of Magnetic Resonance Imaging, 48(4):938–50, 2018. [3] Lönnfors-Weitzel T, et al. T2-relaxometry predicts outcome of DBS in idiopathic Parkinson’s disease. NeuroImage: Clinical,12:832–7, 2016.[4] Lorincz BA, et al. Novel approach to magnetic resonance imaging of epileptic dogs - T2 relaxometry of the brain with emphasised hippocampus. Acta Veterinaria Hungarica, 65(2):185–97, 2017. [5] Del Signore F, al. The potential role of magnetic resonance brain relaxometry in veterinary medicine: a preliminary study. Journal of Biological Regulators and Homeostatic Agents, 33(6):1725–36, 2019.[6] Konar M, Lang J. Pros and cons of low-field magnetic resonance imaging in veterinary practice. Veterinary Radiology & Ultrasound, 52(1 Suppl 1):S5–14, 2011.

Laparoscopic salpingectomy in *Papio hamadryas*

Annarita Imperante (3), Pietro Laricchiuta (2), Claudia Acquafredda (3), Marzia Stabile (3), Francesco Staffieri (1), Luca Lacitignola (1)

(1) Dipartimento Dell'Emergenze e Trapianti di Organo, sez. Cliniche Veterinarie e P.A., Università degli Studi di Bari (2) Zoosafari di Fasano, Brindisi- Italy (3) Dottorato di Ricerca in "Trapianti di Tessuti ed Organi e Terapie Cellulari", Università degli studi di Bari

Corresponding author: Annarita Imperante (annarita.imperante@uniba.it)

The aim of this study was to assess the feasibility, safeness, and effectiveness of laparoscopic salpingectomy as irreversible contraceptive surgical therapy for extensive control birth program in captive baboons (*Papio hamadryas*) in a Zoo in South Italy. The clinical activity was authorized with written informed consent by the Zoo's property (Lion 3000 S.p.A.) and obtained the favorable opinion of the ethics committee of DETO (05/2020). Our hypothesis was that laparoscopic salpingectomy can be performed in baboon species in a reasonable surgical time with minor complication rates during the sterilization campaign.

The surgical procedures were performed with three portals technique with 5mm instruments and telescope, placed at the umbilical and hypogastric regions. The dissection of the salpinx was performed with a radiofrequency bipolar vessel sealing device from the fimbriae to the uterine attachments. We evaluated the surgical times, the learning curve, and intra- and post-operative complications occurrence. These included fifteen baboons (n=15), eight adults and six sub-adults with a mean weight of 9.32 kg (range, 4-14.2 kg; SD 3.09 kg). The total duration of surgery was 28.75 min (range 16-50 min; SD 9.60 min). The installation phase was completed in a mean of 7.68 min (range 3-15 min; SD 3.43 min), and the time to complete the salpingectomy of both salpinges was 9.68 min (range 4-20min; SD 3.97 min). The linear regression analysis of the time to complete the salpingectomy versus the order of procedures showed a negative correlation (r^2=0.61% $p<0.05$). No complications were recorded. laparoscopic salpingectomy in *Papio hamydryas* showed significant advantages in terms of surgical time, low invasiveness and intra and post-operative complications. The laparoscopic salpingectomy could be employed as an optimal surgical choice in birth control programs for non-human primates in captivity.

[1] Yu PH, Weng CC, Kuo HC, Chi CH: Evaluation of endoscopic salpingectomy for sterilization of female Formosan macaques (*Macaca cyclopis*). Am J Primatol 77:359-367, 2015.
[2] Kumar V: Clinical evaluation of Laparoscopic sterilization techniques in female Rhesus Macaques (*Macaca mulatta*). Archives of Veterinary Science:20-26, 2012.
[3] Bauer C: The baboon (*Papio* sp.) as a model for female reproduction studies. Contraception 92:120-123, 2015.

Crisci's method for canine L-PRF preparation: protocol standardization, macroscopic and histologic evaluations

Chiara Caterino (1), Giovanni Della Valle (1), Federica Aragosa(1), Davide De Biase (1) Alessandro Crisci(2), Federica Cardillo(3), Francesco Lamagna (1), Gerardo Fatone (1)

(1) Università degli Studi di Napoli Federico II, Dipartimento di Medicina Veterinaria e Produzioni Animali (2) Università degli Studi di Salerno, Dipartimento di Medicina, Chirurgia e Odontoiatria "Scuola Medica Salernitana" (3) Medico Veterinario Libero Professionista

Corresponding author: Chiara Caterino(chiara.caterino@unina.it)

Leukocites-Platelets Rich Fibrin (L-PRF) is a new generation of platelet concentrates; it was widespreadly used, as an aoutologous platelet-based wound sealant and hemostatic agent in surgical wound regeneration (1,2). L-PRF clot or membrane is a solid fibrin-based biomaterial, with a specific 3D distribution of the leukocytes and platelet aggregates and stem cells (3). This biological scaffold releases growth factors (i.e., TGFbeta1, PDGF-AB, VEGF) and matrix proteins (fibronectin, vitronectin and thrombospondin-1) during healing process after the application. To the Authors' knowledge both in human and veterinary medicine a unique standardized protocol was not reported. The aim of this prospective study was to apply the Crisci's L-PRF protocol in canine specie and evaluate macroscopically and histologically the L-PRF membranes obtained by using Wound Box® (5) to standardize the L-PRF protocol in dog. Eighty-six dogs in good general condition with no history of recent NSAIDs intake (15 days of washout) and/or any medication either disease related to coagulation process were enrolled. During the routine clinical examination, a whole blood sample of 10 ml was taken and divided in two rates 1 ml for CBC and 9 ml for L-PRF clot production. A dedicated device, L-PRF Wound Box®, was then used to obtain L-PRF membranes. The weight and size of each membrane was registered with a goldsmith digital scale and electronic caliper and then collected in a sterile Eppendorf and preserved in 10% neutral buffered formalin. Mean and standard deviation of membranes length, width and weight was calculated and compared with mean human and horse values using Student's T-Test ($p<0.05$). Mean and standard deviation of CBC value was calculated and compared with range in dog. All dogs showed a CBC in normal range. The Crisci's protocol was reproducible and let us obtain 86 L-PRF clots on 86 samples. The Wound Box® gave a membrane of mean (±SD) length (cm), width (cm) and weigh (g) of 1.97 (±0.89), 0.95 (±0.36), 0.46 (±0.20) respectively. Histology analysis confirmed a well-defined histoarchitecture with 5 layers reproducing density and distribution of blood cells in this biomaterial. The fibrin network was clearly distinguished with the near absence of color and the scarce presence of cells. Since the L-PRF parameters found in dogs are clearly like those of humans and horse, our data confirms the effectiveness of Crisci's protocol and of Wound Box to produce L-PRF membranes in dogs.

1. Dohan, D.M.; Choukroun, J.; Diss, A.; Dohan, S.L.; Dohan, A.J.; Mouhyi, J.; Gogly, B. Platelet-rich fibrin (PRF): a second- generation platelet concentrate. Part I: technological concepts and evolution. *Oral Surg. Oral Med. Oral Pathol. Oral Radiol. Endod.*, 2006, *101*(3) e37-44.
2. Everts, P.A.; Devilee, R.J.; Brown Mahoney, C.; van Erp, A.; Oosterbos, C.J.; Stellenboom, M.; Knape, J.T.; van Zundert, A. Exogenous application of platelet-leukocyte gel during open subacromial decompression contributes to improved patient outcome. A prospective randomized double-blind study. *Eur. Surg. Res.*, 2008, *40*(2), 203-210.
3. Dohan Ehrenfest, D.M.; Del Corso, M.; Diss, A.; Mouhyi, J.; Charrier, J.B. Three-dimensional architecture and cell composition of a choukroun's platelet-rich fibrin clot and membrane. *J. Periodon- tol.*, 2010, *81*(4) 546-555.

Efficacy of Curcuvet® and Boswellic acid combined with conventional nutraceutical product in treating canine osteoarthritis: a pilot randomized clinical trial

Chiara Caterino (1), Federica. Aragosa (1), Giovanni Della Valle (1), Dario Costanza (2), Francesco Lamagna (1), Alfonso Piscitelli (3), Annalisa Nieddu (4), Giuseppina Vinciguerra (1), Gerardo Fatone (1)

(1) Università degli Studi di Napoli Federico II, Dipartimento di Medicina Veterinaria e Produzioni Animali (2) Università degli Studi di Napoli Federico II, Centro Interdipartimentale di Radiologia Veterinaria (3) Università degli Studi di Napoli Federico II, Dipartimento di Agraria (4) Manager Medico, Divisione Veterinaria, Aurora Biofarma

Corresponding author: Giovanni Della Valle (giovanni.dellavalle@unina.it)

Osteoarthritis is a progressive degenerative joint disease which is high prevalent in dogs. In the late stage of the disease, it determines chronic neuropathic pain which leads to reduced quality-of-life in affected patients(1). To date it has not yet been identified a specific treatment, but it has been proved that nutraceutical and dietary supplements may play an important role in controlling inflammation and pain(2,3).

The aim of this study was to evaluate, by the use of force plate gait analysis, the clinical efficacy of Boswellia and Curcuvet® combined with conventional nutraceutical therapy compared with conventional nutraceutical alone in dogs affected by osteoarthritis.

Client-owned dogs, over 12 months old and 20 kg of body-weight, with a confirmed diagnosis of Osteoarthritis, were included in this randomized, double-blinded study. The dogs were randomly divided into two groups: the first group (A) received a conventional nutraceutical with a combination of acid boswellic and Curcuvet®, while the second group (B) received a conventional nutraceutical. All the enrolled dogs underwent a washout period before starting the treatment with nutraceuticals products which were the only admitted treatment over the study period. A full orthopaedic and neurologic examination, and force plate gait analysis were performed before starting the treatment, at 45, 90, and 60 days post-treatment. Ground reaction forces were recorded and analyzed.

Twenty dogs were enrolled in the study. In both groups there was an increasing values of ground reaction forces. These results might indicate that both nutraceutical products determined a better condition in terms of pain feeling but that effect is much more visible after 60 days from the end of the administration in treated group. In conclusion Curcuvet® in combination with boswellic acid could be considered a valid aid in a multimodal treatment for canine osteoarthritis.

[1] Henrotin Y, Sanchez C, Balligand M. Pharmaceutical and nutraceutical management of canine osteoarthritis: present and future perspectives. Vet J 2005;170:113–123.
[2] Comblain F, Serisier S, Barthelemy N, et al. Review of dietary supplements for the management of osteoarthritis in dogs in studies from 2004 to 2014. J Vet Pharmacol Ther 2016; 39:1–15.
[3] Johnson, K. A., Lee, A. H. & Swanson, K. S. Nutrition and nutraceuticals in the changing management of osteoarthritis for dogs and cats. J. Am. Vet. Med. Assoc.; 2020; (256): 1335–1341.

Lameness evaluation by pressure mat analysis in dogs affected by Cranial Cruciate ligament and treated with Porous Tibial Tuberosity Advancement (TTA)

Laura Fracassi (1), Marzia Stabile (1), Rodrigo Trisciuzzi (1), Alberto Maria Crovace (2), Donato Monopoli Forleo (3), Francesco Staffieri (1)

(1) Department of Emergencies and Organ Transplantation, Section of Veterinary clinics and Animals Production, University of Bari (2) Department of Basic medical sciences, neurosciences and sense organs, University of Bari (3) Istituto Tecnologico de Canaria (ITC)

Corresponding author: Laura Fracassi (laura.fracassi@uniba.it)

Cranial cruciate ligament (CrCL) rupture is one of the most common orthopedic conditions of the stifle joint in dogs (1).

A thorough clinical evaluation, particularly focus on the gait impairment, plays a key role in the investigation of these patients. Considering that clinical grading could be affected by subjective bias, different lameness scoring scales have been recommended for a standardized grading of lameness in clinical practice (2). Quantitative gait analysis systems have become a useful tool in monitoring gait and grading lameness, also for the assessment of procedures and treatments efficacy (3).

Porous Tibial Tuberosity Advancement (TTA) is one of the most frequently used techniques to resolve cranial cruciate rupture in dogs, proved effective in the quickly improvement of the patient's quality of life (4,5).

The aim of our study was to compare the lameness caused by CrCL in dogs, before and after surgical stabilization with porous TTA, using a quantitative gait analysis system (GAIT4 Dog R walkway, CIR Systems Inc., Sparta, NJ). For the purpose of the study, Gait4 lameness Score (GLS), Total pressure Index % (TPI %) and Stance Time % (ST%) for the affected limb were evaluated.

Eleven dogs of various common breed (weight mean 34±12) with CrCL rupture were enrolled in the study. After a clinical evaluation, dogs walked on the pressure sensing walkway system, keeping a constant velocity, before and one month after the surgery. GLS, TPI% and ST % were collected and statistically evaluated with R statistical software (R:A Language and Environment for Statistical Computing, R Core Team, Vienna, Austria, 2020, https://www.R-project.org).

Normality was assessed by Shapiro-Wilk test. The data were analyzed using Wilcoxon test for non-normally distributed data and paired t-student test for normally distributed data. The results showed that the GLS and TPI% of the affected limb were significant improved after surgery (p-value=0.00006 for each other); on the contrary the ST % results didn’t show statistically significant value.

The data show that TTA improves dog's mobility impairment and the quality of life, already after one month after surgery, and that the use of gait analysis system helps to give an objective lameness evaluation and grading.

(1) Kyllar M, Čížek P. Cranial cruciate ligament structure in relation to the tibial plateau slope and intercondylar notch width in dogs. J Vet Sci. 2018;19(5):699-707.
(2) Brønniche Møller Nielsen M, Pedersen T, Mouritzen A, Vitger AD, Nielsen LN, Poulsen HH, et al. (2020) Kinetic gait analysis in healthy dogs and dogs with osteoarthritis: An evaluation of precision and overlap performance of a pressure-sensitive walkway and the use of symmetry indices. PLoS ONE 15(12): e0243819.

Treating elongated soft palates in Brachycephalic dog breeds: an alternative surgical technique

Samanta Nardi (1), Stefano Patroncini (1,2), Giovanni Barsotti (1)

(1) Ospedale Didattico Veterinario "Mario Modenato", Dipartimento di Scienze Veterinarie- Università di Pisa (2) Private practitioner

Corresponding author: Samanta Nardi (samanta.nardi@phd.unipi.it)

In dogs, the anatomical and pathological changes associated with brachycephalic skull shape cause several concomitant sequelae, including airway obstructive syndrome and correlated and/or concomitant disorders. Brachycephalic airway obstructive syndrome (BAOS) represents the main problem for these dogs. BAOS is characterized by an enlarged soft palate, stenotic nares, abnormal turbinate growth, macroglossia, hypoplastic trachea, and redundant pharyngeal folds. These anatomical abnormalities cause airway resistance and airflow turbulence. This results in mucosal inflammation and secondary changes, such as tonsillar hyperplasia, everted laryngeal saccules, laryngeal collapse, and bronchial collapse. The presence of a thickened and abnormally long soft palate is a key morphological alteration contributing to the pathogenesis of the BAOS and it has been observed in 85–100% of affected dogs.

In the veterinary literature, several techniques have been reported to resection the soft palate, but none has been proved to be significantly more effective than the others.

The aim of our case series was to describe a modified technique of staphylectomy which combines the reduction of the length of the soft palate with its reduction in thickness. The technique consists in executing the staphylectomy with an ultrasound-based Harmonic Focus scalpel, followed by the internal emptying of the soft palate with the same device. Subsequently, three monofilament absorbable sutures (two on the sides and one centrally) were applied, in order to juxtapose the mucosa of the oropharynx and nasopharynx, so as to ensure a better healing of the tissues.

Eight brachycephalic dogs aged 18.5±13.6 months (range: 8 to 44 months) underwent our technique. Respiratory grading scores (from 1 to 3) before and 30 days after surgery were used to record the severity of the disease and the improvement in symptoms, respectively. The average time of our procedure was 11±2.5 minutes (range: 8 to 15 min). Rhinoplasty was also performed in 7/8 dogs; only one dog received concurrent tonsillectomy. No complications were observed in the intra-operative and post-operative period, and there was an improvement in symptoms in all the dogs treated.

In conclusion, the technique is quick to perform, and seems to be a safe and effective in dogs affected by BAOS.

(1) Ekenstedt et al. Canine Brachycephaly: Anatomy, Pathology, Genetics and Welfare. Journal of comparative pathology, 176:109-115, 2020.
(2) Findji et al. Folded flap palatoplasty for treatment of elongated soft palates in 55 dogs. Eur J Companion Anim Pract, 19: 125-32, 2009.
(3) Kirsch et al. Comparison of carbon dioxide laser versus bipolar vessel device for staphylectomy for the treatment of brachycephalic obstructive airway syndrome. The Canadian Veterinary Journal, 60: 160, 2019.
(4) Lindsay et al. Brachycephalic airway syndrome: management of post-operative respiratory complications in 248 dogs. Australian veterinary journal, 98:173-180, 2020.
(5) Pichetto et al. The anatomy of the dog soft palate. III. Histological evaluation of the caudal soft palate in brachycephalic neonates. The Anatomical Record, 298:618-623, 2015.
(6) Tamburro et al. Short-term surgical outcomes and histomorphological evaluation of thermal injury following palatoplasty performed with diode laser or air plasma device in dogs with brachycephalic airway obstructive syndrome. The Veterinary Journal, 253:105-391, 2019.

Sentinel lymph node mapping with preoperative lymphoscintigraphy in canine mammary tumors: preliminary results

Manfredi Martina (1,2), Ferrari Roberta (1,2), De Zani Donatella (2), Spediacci Carlotta (1,2), Pecile Alessandro (1,2), Bassi Jessica (1), Chiti Lavinia Elena (1,2), Stefanello Damiano (1,2), Longo Maurizio (1), Grieco Valeria (1,2), Di Giancamillo Mauro (1,2), Zani Davide Danilo (1,2)

(1) Università degli Studi di Milano, Dipartimento di Medicina Veterinaria (2) Università degli Studi di Milano, Centro Clinico-Veterinario e Zootecnico-Sperimentale

Corresponding author: Martina Manfredi (martina.manfredi@unimi.it)

Clinical staging of canine mammary tumors includes the evaluation of lymph nodes (LN) involvement to define prognosis and treatment. The choice of which LN should be assessed can be challenging because of individual variations along with the formation of atypical lymphatic pathways by tumor-induced lymphangiogenesis [1]. The presence of mammary neoplasia in dogs may change the regional lymphatic pattern, recruiting different LN compared to healthy glands [2]. Therefore, sentinel lymph node (SLN) mapping is crucial for the assessment of tumor lymphatic spread. This is a case series of SLN mapping with preoperative lymphoscintigraphy in 5 dogs with mammary tumors. Each patient presented for mammary tumor surgical resection with cytologically confirmed neoplasia on the 4th (3 cases) and on the 5th (2 cases) mammary gland. Preoperative planar lymphoscintigraphy was performed with a peritumoral injection of 99m-Technetium labeled nanosized human serum albumin. Orthogonal static images were acquired until the SLN was clearly identified. The same day or at day 1 after lymphoscintigraphy, dogs were admitted to surgery for unilateral radical mastectomy and SLN extirpation. Prior to surgery, a peritumoral injection of sterile methylene blue was performed in 4 dogs. The SLN extirpation was guided by a handheld gamma probe. Any LN presenting blue stain or radioactive counts double than the background was extirpated [3]. All extirpated LN were negative for metastasis at histopathology. Preoperative lymphoscintigraphy identified the tributary LN in all cases. Differences between pre- and intra-operative techniques in SLN detection have been found in 2 dogs with tumor located at the 4th gland, which is most frequently affected by neoplasia and presents the greater variability in lymphatic draining patterns [4]. In one case, the intraoperative techniques found an additional SLN probably missed on planar images due to superimposition. In the second case, lymphoscintigraphy identified 3 LN while intraoperative gamma probe found radioactivity only in one. This was the only patient that underwent surgery the day after lymphoscintigraphy. In authors' previous experience, performing intraoperative mapping the day after radiopharmaceutical injection never affected the intraoperative LN identification rate compared to preoperative lymphoscintigraphy, although further studies are warranted. In conclusion, considering the unpredictability of lymphatic drainage patterns, preliminary results highlight the importance of including SLN mapping in the staging of dogs with malignant mammary tumors. The combination of preoperative and intraoperative techniques is recommended to increase SLN detection rate.

[1] Tuohy et al. A review of sentinel lymph node evaluation and the need for its incorporation into veterinary oncology. Vet Comp Oncol, 7(2): 81-91, 2009.
[2] Pereira et al. Lymphatic drainage on healthy and neoplasic mammary glands in female dogs: can it really be altered? Anat, Histol, Embryol, 32(5):282-290, 2003.
[3] Ferrari et al. Biopsy of sentinel lymph nodes after injection of methylene blue and lymphoscintigraphic guidance in 30 dogs with mast cell tumors. Vet Surg, 49:1099-1108, 2020.

Quantitative contrast-enhanced ultrasound (CEUS) of urinary bladder lesions in dogs

Spediacci Carlotta (1), Manfredi Martina (1), Luti Tiziana (3), Longo Maurizio (1), Israeliantz Nicolas (3), De Zani Donatella (2), Zani Davide (1), Di Giancamillo Mauro (1)

(1) Università degli Studi di Milano, Dipartimento di Medicina Veterinaria (2) Università degli Studi di Milano, Centro Clinico-Veterinario e Zootecnico-Sperimentale (3) Royal Dick School of Veterinary Studies, University of Edinburgh

Corresponding author: Carlotta Spediacci (carlotta.spediacci@unimi.it)

Ultrasonography is the most widely used technique for evaluation of lower urinary tract [1-2]. In human literature, contrast enhanced ultrasound (CEUS) is useful for the diagnosis of urinary bladder neoplasia with quantitative evaluation providing indication of malignancy based on time-intensity curves (TICs) [4]. The most important parameters are "time to peak" (TTP) "echo power "(PE), "rise time" (RT), "fall time" (FT), "mean transit time" (mTT) and "contrast arrival time" (CAT) [1]. CEUS of the lower urinary tract is poorly described in veterinary medicine and there are no studies investigating CEUS differences between neoplastic and non-neoplastic urinary bladder lesions [1]. The aim of this study is to quantitatively characterize CEUS pattern of neoplastic and non-neoplastic urinary bladder lesions in dogs. This was a prospective multicentric ethically approved study (VERC 131.17). All canine patient referred for investigation of lower urinary tract symptoms in a period of two years were enrolled. Inclusion criteria were clinical signs related to lower urinary tract and achievement of diagnosis based on cyto-histopathology. Based on pathology results, dogs were divided in neoplastic (NP) and non-neoplastic (N-NP) groups. Standard B-mode US exam of the bladder (Esaote MyLab6, IT) followed by CEUS was performed, with injection of 0.04ml/kg of sulphur-hexafluoride contrast agent (SonoVue®, Bracco, IT). Once selected the ROI, the TICs were elaborated, and all parameters registered. Nine dogs of different signalment were enrolled. Histological and cytological analysis diagnosed five neoplastic lesions (100% urothelial carcinoma), and four inflammatory diseases (25% epithelial cell dysplasia, 50% polypoid cystitis, 25% bacterial cystitis). Preliminary descriptive statistical analysis of quantitative CEUS of NP reveals RT 3.99+/-1.25; TTP 5.6+/-1.99; FT 9.2+/-2.7; mTT 47.6+/-32.3; PE 21.6%. N-NP showed the following parameters: RT 14.3+/-3.4; TTP 26.7+/- 16.5; FT 31.6+/-16.3; mTT 26.7+/-16.5; PE 14%. CAT values were highly variable among individuals, ranging between 2-29.5 s. This data shows that NP and N-NP are characterized by different TICs. NP have higher PE, shorter RT and FT phases than inflammatory lesions. Greater variability between groups was found for TTP and mTT. For each parameter, few values deviated significantly from the average. This occurred when diffuse thickening of the bladder wall was visible. It is likely that the selection of the ROIs was challenging due to the flattened shape of the lesions. The software used did not take into account the CAT. As this parameter is highly variable, we believe that removing CAT from the TICs might improve the accuracy of CEUS. In conclusion, this preliminary result shows that quantitative CEUS may represent a valid tool for non-invasive detection of urinary bladder neoplasia, especially if crescent lesions are present.

1. Caruso et al. Bladder tumor staging: comparison of contrast-enhanced and gray-scale ultrasound, Am J Roentgenol 194:151–156, 2010.
2. Hanazono et al. Ultrasonographic findings related to prognosis in canine transitional cell carcinoma, Vet Rad Ultrasound, 55(1) 79-84, 2014.
3. Drudi et al. CEUS in the study of bladder, method, administration and evaluation, a technical note, J Ultrasound, 17(1)57-63, 2010.
4. Macrì et al. Quantitative evaluation of canine urinary bladder

The Heart to Average Vertebra index (HAV): preliminary results of a new method for the assessment of cardiac size in dogs

Dario Costanza (1), Adelaide Greco (1), Diego Piantedosi (2), Maria Pia Pasolini (2), Dario Bruzzese (3), Pierpaolo Coluccia (1), Leonardo Meomartino (1)

(1) Centro Interdipartimentale di Radiologia Veterinaria, Università degli Studi di Napoli "Federico II" (2) Dipartimento di Medicina Veterinaria e Produzioni Animali, Università degli Studi di Napoli "Federico II" (3) Dipartimento di Sanità Pubblica, Università degli Studi di Napoli "Federico II"

Corresponding author: Dario Costanza (dario.costanza@unina.it)

The Vertebral Heart Size (VHS) index, described by *Buchanan and Bücheler* in 1995 [1] for the quantitative radiographic evaluation of the cardiac silhouette size in dogs, nowadays is widely used in clinical practice. Over the years, many studies established normal ranges for different breeds [2,3] and validated the method using operators with different experience levels [4]. A key limitation of the VHS is the impossibility to measure it on dogs with thoracic spine alterations, such as hemivertebrae, butterfly vertebrae, severe spondylosis, and reduced intervertebral disc spaces. This study aims to evaluate a new method, the Heart to Average Vertebra (HAV) index to assess the heart size, overcoming the aforementioned VHS's limitations. To obtain the HAV index, the sum of the long axis (LA) and the short axis (SA) of the cardiac silhouette were divided to a single vertebral body length. All the radiographic examinations of the thorax performed at the Interdepartmental Center of Veterinary Radiology of Naples in the period between September 2018 and January 2021 were retrospectively evaluated. Exclusion criteria were the presence of alterations of the thoracic spine and the inability to clearly visualize the cardiac silhouette; the exclusion from the study was evaluated by one of the authors not involved in performing the measurements. All the right lateral radiographs were analyzed by three blind independent operators with different levels of experience. On each radiograph the length of each single vertebra from T4 to T8, including the caudal intervertebral space, and the VHS were determined. The sum of the two cardiac axes was subsequently divided by the length of every single vertebra of the T4-T8 tract. Eighty dogs (23 females, 26 neutered females, 23 males and 8 neutered males), ranging from 8 months to 16 years and weighting between 1.2 and 30.5 kg (mean 12.15±SD 6.8 kg) were included in the final sample. The two methods were compared using the concordance correlation coefficient (CCC) and the Bland-Altman plot. The data analysis showed a strong correlation between VHS and AVH, particularly when considering T6 as "average vertebra" (CCC=0.91, 95% confidence interval 0.87–0.94,), and a mean absolute error (MAE) equal to 0.33 (95% confidence interval 0.28–0.39). These preliminary results show that the HAV index could be a valuable alternative to the VHS. In the Authors' opinion, this new method, in addition to being less time-consuming and more intuitive than VHS, can be used when VHS is not applicable.

[1] Buchanan, J.W.; Bücheler, J. Vertebral scale system to measure canine heart size in radiographs. J. Am. Vet. Med. Assoc, 206:194–9, 1995.
[2] Bavegems, V.; Van Caelenberg, A.; Duchateau, L.; Sys, SU; Van Bree, H.; De Rick, A. Vertebral heart size ranges specific for whippets. Vet. Radiol. Ultrasound, *46*:400–403, 2005.
[3] Jepsen-Grant, K.; Pollard, R.E.; Johnson, L.R. Vertebral heart scores in eight dog breeds. Vet. Radiol. Ultrasound, *54*:3–8, 2013.
[4] Hansson, K.; Häggström, J.; Kvart, C.; Lord, P. Interobserver variability of vertebral heart size measurements in dogs with normal and enlarged hearts. Vet. Radiol. Ultrasound, *46*:122–130, 2005.

Ultrasonography of the vas deferens in dogs: normal and pathologic appearances

Tina Pelligra, Simonetta Citi, Caterina Puccinelli, Matteo Tesi, Alessandra Rota

Università degli Studi di Pisa, Dipartimento di Scienze Veterinarie

Corresponding author: Tina Pelligra (pelligratina@gmail.com)

Ultrasonography is the imaging method of choice for evaluating the male reproductive system of dogs. Although the normal sonographic appearance of testis and epididymis is documented in the literature, the sonographic appearance and size of the vas deferens have rarely been described. The aim of this study is to determine the reliability of sonographic visualization of the vas deferens and to describe its ultrasound findings. In our study were included 30 dogs and the identification of the vas deferens was attempted bilaterally in the extra-abdominal and intra-abdominal segments in all patients; when possible, the total thickness and lumen size were measured; furthermore, lumen content and reactivity of surrounding tissues have been evaluated. The subjects were divided into two groups: healthy subjects (14), used as a control group and pathological subjects (16). In the first group, the vas deferens appeared as an anechoic tubular structure that was noncompressible, did not contain blood flow and, in some cases, had two parallel linear reflectors representing the internal walls of the lumen surrounded by hypoechoic muscular wall and a thin external hyperechoic line in longitudinal plane. In transversal plane the vas deferens had a “target-like” apparence. In the second group, the following anomalies have been diagnosed: 11 dogs with benign prostatic hyperplasia, 3 dogs with benign prostatic hyperplasia and testicular neoplasia, 1 dog with acute prostatitis and 1 dog with acute epididymitis and prostatitis. In these subjects the vas deferens appeared partially or diffusely dilated with anechoic or hypoechoic intraluminal contents and increased in thickness. In healthy subjects the total thickness of the vas ranged from 0.8 mm to 1.5 mm (median 1.1 mm), in agreement with what is reported in anatomy. The lumen of the normal vas deferens ranged from 0.2 mm to 0.3 mm (median 0.2 mm). In pathological subjects the total thickness of the vas deferens was ranged from 1.0 mm to 4.0 mm (median 1.7 mm). The lumen of the pathologic vas deferens ranged from 0.2 mm to 1.0 mm (median 0.3 mm). The results of the present study therefore indicate the efficacy of the ultrasound examination for the evaluation of the vas deferens in dogs and suggest a correlation between urogenital pathologies and anomalies of the vas deferens.

[1] Li et al. Ultrasonography in diagnosis of congenital absence of the vas deference, Med Sci Monit, 22:2643-2647, 2016.

[2] Middleton et al. High-resolution sonography of the normal extrapelvic vas deference, J Ultrasound Med. 28(7):839-46, 2009.

[3] Guerin et al. Transitional cell carcinoma involving the ductus deference in a dog, J Am Vet Med Assoc. 240:446-49, 2012.

Doppler Ultrasonography of lateral palmar digital arteries in healthy and chronic laminitic horses: preliminary data

Pierpaolo Coluccia (1), Dario Costanza (1), Leonardo Meomartino (1), Luigi Auletta (3), Anna Maselli (2), Fabiana Micieli (2), Maria Pia Pasolini (2), Adelaide Greco (1)

(1) Centro Interdipartimentale di Radiologia Veterinaria, Università degli Studi di Napoli "Federico II" (2) Dipartimento di Medicina Veterinaria e Produzioni Animali, Università degli Studi di Napoli "Federico II"
(3) Institute of Biostructure and Bioimaging, National Research Council (IBB CNR), Napoli, Italy

Corresponding author: Pierpaolo Coluccia (pierpaolo.coluccia@unina.it)

Doppler Ultrasonography (US) is a sensitive, noninvasive and not so expensive method useful for the routine examination of blood flow in vessels of standing non sedated horses [2, 3]. Several diseases may disturb digital peripheral blood flow. Laminitis is one of the most severe diseases that may be associated with endotoxemia and bearing weight overload [1]. Palpation of digital pulses provides an insensitive measurement of digital blood flow and it is a subjective and highly operator-dependent technique not reliable to quantify slight differences in blood supply to the equine digit. The purpose of this study was to describe in detail the technique of the Doppler US of the lateral digital palmar arteries (LPDA) in the thoracic limb of healthy and laminitic Italian Standardbred (IS) horses, evaluating morphology and features of the digital blood flow. The LPDA of both forelimbs was examined in 12 IS non-sedated mares, 2 of which laminitic, aged between 4 and 17 years and 453 to 639 kg of weight. The artery was first localized through B-mode US, and then visualized through Colour Doppler. The arterial flow was submitted to Pulsed Wave (PW) Doppler and the spectral waves obtained were classified in resistive and non-resistive [2]. For each forelimb, the following parameters were recorded: Heart rate (HR), Systolic Peak Velocity (SPV cm/s), End-Diastolic Velocity (EDV cm/s) and Resistive Index (RI). The variability of the findings between subjects was assessed using a linear mixed model, considering the presence/absence of laminitis, weight, pregnancy, and age as fixed components of the variance (main effects). The subject, the side (right vs left) and the side within the subject were considered as random components. The comparison between healthy and pathological subjects for the Doppler US indices was carried out with Student's t-test (RI). The technique was well tolerated by horses and took, on average, 21.6 minutes. All the Doppler waves obtained, except for one mare, were resistive. The HRs clinically measured and those obtained by US did not significantly differ. No significant variability was observed in the RI values, considering both the standard and random components, except for bodyweight, that affected the variability of SPV and EDV. The Spearman r test showed that only SPV was significantly and negatively correlated to weight. The RI and VTD significantly differed between healthy and laminitic horses. The study confirmed that Doppler US of the LPDA could be a valuable diagnostic tool to monitor the changes in blood flow to the foot both in physiologic and in pathologic conditions. However, as for other ultrasonographic techniques, it is essential to standardize the procedure to minimize the patient's intrinsic and extrinsic variables. Further studies are needed to evaluate the use of Doppler US of the digital flow in monitoring the effects of bodyweight change during the pregnancy, especially in laminitic mares.

[1] Aguirre et al. Usefulness of Doppler ultrasonography to assess digital vascular dynamics in horses with systemic inflammatory response syndrome or laminitis. J Am Vet Med Assoc 15;243(12):1756-61, 2013.
[2] Cochard et al. Doppler ultrasonographic features of thoracic limb arteries in clinically normal horses. Am J Vet Res, 61:183–190, 2000.
[3] Pietra et al. Influence of weight bearing and hoof position on Doppler evaluation of lateral palmar digital arteries in healthy horses. Am J Vet Res, 65(9):1211-5, 2004.

Comparative metabolomic profiles of normal and osteoarthritic canine synovial fluids in a spontaneous animal model

Marzia Stabile (1), Chiara R. Girelli (2), Luca Lacitignola (1), Rossella Samarelli (1), Antonio Crovace (1), Francesco P. Fanizzi (2), Francesco Staffieri (1)

(1) Sezione di Cliniche Veterinarie e Produzioni Animali, Dipartimento dell'Emergenza e dei Trapianti d'Organo, Università degli Studi di Bari (2) Dipartimento di Scienze e Tecnologie Biologiche ed Ambientali, Università del Salento

Corresponding author: Marzia Stabile (stabilemarzia@gmail.com)

Osteoarthritis (OA) is a common degenerative musculoskeletal disease of synovial joints, characterized by a metabolic imbalance resulting in inflammation and cartilage degradation (1).

Nuclear magnetic resonance (NMR) spectroscopy was proposed as valid analysis to characterize the metabolomes of biofluids, able to detect changes associated with osteoarthritis condition. Particularly, synovial fluid provides a unique source of chemical information and holds great promise for biomarker discovery (2).

The aim of the study was to evaluate the metabolic profile of dogs with natural occurring OA and compare them to healthy dogs, to establish the representative metabolites, associated with both scenarios. For this purpose, after the approval of the ethical committee (DETO/223/III/13/2018) and owners consent, dogs with naturally occurring OA referred for orthopedic consultation and dogs OA–free referred for routine control, were enrolled. Seventeen synovial fluid samples were collected from affected joints of OA dogs (OA group, n=8) and from OA-free joints of healthy dogs (H group, n=9). The samples collection was performed under anesthesia and according to the anti-septic technique. After the procedure, synovial fluids (SF) were stored at a temperature of -20°C, until the NMR measurements. All measurements were performed on a Bruker Avance III 600 Ascend NMR spectrometer, operating at 600.13 MHz for ^{1}H observation. The characterization of the metabolites was determined by the analysis of two-dimensional homo and heteronuclear NMR and by comparison with the literature data (3,4).

For data analysis, Principal Components Analysis (PCA), and Orthogonal Partial Least Squares Discriminant Analysis (OPLS-DA) were performed.

Representative NMR spectra for different metabolites were identified in both groups. OPLS-DA analysis performed by comparing H group and OA group gave a good predicted model (1+1+0 components gave R2X=0.508, R2Y=0.979, Q2=0.899), with a clear separation observed between two groups. Particularly, SFs of OA group were characterized by a higher relative content of lipids, β-hydroxybutyrate, glutamine, alanine, trimethylamine-N-oxide and histidine, while the H group showed a higher relative content of lactate. Our results show that significant biochemical differences exist between normal and osteoarthritic synovial fluid in a natural model of OA in dogs. Our data prove that the fatty acid metabolism plays a key role as source of energy in the arthritic joint. Lactate metabolite cannot be considered a marker of pathological condition, because it is present in high concentration also in healthy joint.

[1] Woodell-May JE, Sommerfeld SD. Role of Inflammation and the Immune System in the Progression of Osteoarthritis. J Orthop Res. 2020;38(2):253-7.
[2] Jaggard MKJ, Bhattacharya R, Williams HRT, Lindon JC, Gupte CM. A systematic review of the small molecule studies of osteoarthritis using nuclear magnetic resonance and mass spectroscopy. Osteoarthritis Cartilage. 2019 Apr;27(4):560-570.
[3] Lacitignola L, Fanizzi FP, Francioso E, Crovace A. 1H NMR investigation of normal and osteo-arthritic

New technologies and medical devices for the reconstruction of skin losses with a mini-invasive approach

Rodrigo Trisciuzzi (1), Rossella Elia (2), Claudia Acquafredda (1), Alberto Maria Crovace (3), Laura Fracassi (1), Annarita Imperante (1), Marzia Stabile (1), Antonio D'Amati (4), Luca Lacitignola (1)

(1) Università degli Studi di Bari 'Aldo Moro', Department of Emergency and Organ Transplantation, DEOT (2) Section of Plastic and Reconstructive Surgery, Department of Emergency and Organ Transplantation, University of Bari, Bari, Italy (3) Department of Basic Medical Sciences, Neurosciences and Sensory Organs (4) Department of Basic Medical Sciences, Neurosciences and Sensory Organs, Human Anatomy and Histology Unit, University of Bari, School of Medicine, Bari, Italy

Corresponding author: Rodrigo Trisciuzzi (rodrigotrisciuzzi@gmail.com)

Wound healing is a well-organized and harmonic process consisting in a series of interactions events between cells and other cells and ECM (extra cellular matrix) with the objective of restoring the integrity of the skin after injury [1]. Severe tissue lost represents a hard challenge for plastic surgeons and negatively influence patient's quality of life, especially for veterinary patients due to their scarce behavioural compliance [2]. The objective was to evaluate the effects of different topical treatments for second intention wound healing on surgical wound model in sheep. Micrografts, atelocollagen and topical disinfectants (sodium hypochlorite and chlorhexidine 0.05% solutions) were applied. After the approval of ethical committee, were produced six circular 4 cm diameter lesions by the protocol established by Broeckx et al. [3], on the back of six sheep. Control lesions were treated topically with physiological fluid. Daily photography samples were collected to go under blind review by various observers. Scoring system developed by Hadley et al. [4] were applied to standardize the clinical evaluation. Statistical analysis from photography scoring assessment showed a statistically significant wound healing process on all lesions from all treatments. Reepithelization percentage was significantly earlier on atelocollagen treated lesion; latest epithelized lesion was represented by sodium hypochlorite treated lesions. No suppuration, micotic infection or complication were detected during all 42 days of lesions monitoring cycle. The global animal wound care market is projected to reach USD 1.5 billion by 2025 from USD 1.0 billion in 2020, at a Compound Annual Growth Rate (CAGR) of 7.4% during the forecast period. This represents a very challenging aspect of veterinary wound care process all over the world [5] and our study contributes to deepen the topic of severe skin injury management. Common disinfectants represent a necessary alternative for clinical practice but micrografts and medical devices should be included to improve the healing process.

1. Ozgok Kangal MK, Regan JP. Wound Healing. 2020 Jul 10. In: StatPearls [Internet]. Treasure Island (FL): StatPearls Publishing; 2021 Jan–. PMID: 30571027.
2. The Micrograft Concept for Wound Healing: Strategies and Applications Atanu Biswas, M.D.,1 Manish Bharara, Ph.D.,2, Journal of Diabetes Science and Technology Volume 4, Issue 4, July 2010). (Development of a histomorphologic scale to quantify cutaneous scars after burns.
3. Broeckx SY, Borena BM, Van Hecke L, Chiers K, Maes S, Guest DJ, Meyer E, Duchateau L, Martens A, Spaas JH. Comparison of autologous versus allogeneic epithelial-like stem cell treatment in an in vivo equine skin wound model. Cytotherapy. 2015;17(10):1434–46.
4. Hadley HS, Stanley BJ, Fritz MC, Hauptman JG, Steficek BA. Effects of a crosslinked hyaluronic acid based gel in the healing of open wounds in dogs. Vet Surg. 2013; 42:161–9.
5. https://www.marketsandmarkets.com/Market-Reports/animal-wound-care-market-253831778.html, Animal Wound Care Market by Product (Surgical (Suture, Sealant, Glue) Advanced (Film, Foam, Hydrogel, Hydrocolloid dressing) Traditional (Dressing, Bandage, Absorbent, Tape) & NPWT Device) Animal (Companion, Livestock) & End User - Global Forecast to 2025.

Factors correlated to sentinel lymph node metastasis in canine mast cell tumors: when surgeons could avoid lymphadenectomy?

Lavinia Elena Chiti (1,2), Patrizia Boracchi (3), Roberta Ferrari (1,2), Chiara Giudice 1(1,2), Valeria Grieco (1,2), Davide Danilo Zani (1,2), Martina Manfredi (1,2), Damiano Stefanello (1,2)

(1) Università degli Studi di Milano, Dipartimento di Medicina Veterinaria (2) Università degli Studi di Milano, Centro Clinico-Veterinario e Zootecnico-Sperimentale (3) Università degli Studi di Milano, Dipartimento di Scienze Cliniche e di Comunità

Corresponding author: Roberta Ferrari (roberta.ferrari@unimi.it)

The present study aims to identify variables of cutaneous and subcutaneous canine mast cell tumor (MCT) associated and not associated with the sentinel lymph node (SLN) metastasis. This could supports surgeons in avoiding SLN mapping and biopsy in some dogs.

Dogs undergoing surgery of cutaneous/subcutaneous MCTs and biopsy of SLNs[1] were prospectively included. If multiple SLNs were detected, the higher histological node evaluation (HN)[2] was assigned to MCT. The strength of association between HN and variables was measured using the prevalence ratio of HN[3]. Two separate analyses were performed: HN0-1 vs. HN2-3, HN0-1-2 vs. HN3. Also, subjects were grouped in clusters with similar clinical characteristics.[4] The ability of the variables/cluster to discriminate between HN was evaluated by area under the ROC curve (AUC)[5].

Fifty Kiupel-low-grade and sixteen subcutaneous MCT were included. Dimension of MCT and SNL number were associated with HN2-3 with a low discrimination ability (AUC<0.64). Dimension remained significantly correlate to HN3 when analyzed alone with a strong discriminate ability (AUC>0.71). Subcutaneous MCTs were also associated with HN3 but with a moderate discriminate ability (AUC=0.68). Four cluster profiles were identified. The ability of profiles to discriminate between HN0-1 and HN2-3 was moderate (AUC=0.67). The ability of profiles to distinguish between HN 0-1-2 and HN3 was strong (AUC=0.80).

Based on these results, nowadays, surgeons should suggest SLN mapping and biopsy in all Kiupel-low-grade MCT, especially if tumor ≥3 cm, and in subcutaneous MCT. Considering the low number of HN3 (7/66), further study should confirm these results.

[1] Ferrari et al. Biopsy of sentinel lymph nodes after injection of methylene blue and lymphoscintigraphic guidance in 30 dogs with mast cell tumors. Veterinary Surgery, 49:1099-1108, 2020.
[2] Weishaar et al. Correlation of nodal mast cells with clinical outcome in dogs with mast cell tumour and a proposed classification system for the evaluation of node metastasis. Journal of Comparative Pathology, 151:329-338, 2014.
[3] McNutt et al. Estimating the relative risk in cohort studies and clinical trials of common outcomes. American journal of epidemiology, 157:940-943, 2003.
[4] Husson et al. Exploratory multivariate analysis by example using R. Chapman and Hall/CRC, 2017.
[5] Keller et al. Evaluating the association of the new National Surgical Quality Improvement Program modified 5-factor frailty index with outcomes in elective colorectal surgery. *Colorectal Disease*, *22*:1396-1405, 2020.

Assessment of urethral mucosa perfusion in a porcine experimental model of hyper- and hypotension with a novel photoplethysmography device

Martina Cardinali (1, 2), Mathieu Magnin (2), Jeanne-Marie Bonnet-Garin (2), Christian Paquet (2), Jean-Ives Ayoub (2), Bernard Allaouchiche (3), Stephane Junot (2)

(1) Università degli Studi di Padova, Dipartimento di Medicina Animale, Produzioni e Salute (2) Université de Lyon, VetAgro Sup (3) Université Claude Bernard Lyon, Hospices Civils de Lyon

Corresponding author: Martina Cardinali (martina.cardinali@phd.unipd.it)

Assessment of tissue perfusion is a key goal for the anesthetist, as alterations of microcirculation are thought to lead to an anaerobic cell metabolism and to the development of multiple organ failure in the critically ill patient. Recently, the concept of "hemodynamic coherence" guided resuscitation has been used to show that while resuscitation procedures are based on correcting hemodynamic variables, this approach does not necessarily lead to an improvement of microperfusion.

The study aims to evaluate the urethral mucosa perfusion in pigs in an experimental model of pharmacologically induced hyper- and hypotension, using an instrument specifically designed for this purpose. Using a dedicated photoplethysmography sensor placed in a foley catheter, and in direct contact with the urethral mucosa, it is possible to continuously obtain information of the ratio of the pulsatile blood flow to the non-pulsatile static blood in the urethra, the so-called urethral perfusion index (uPI). This index is considered a reliable method for evaluating local perfusion.

The study was conducted at the Institute Claude Bourgelat after the approval of the local ethical board for animal research and care (Vet Agro Sup, Marcy l'Etoile, France, authorization number: 1819).

Twelve female piglets (31-50 kg bodyweight) were anesthetized, intubated and mechanically ventilated. Physiological parameters recorded included heart rate, invasive arterial blood pressure, cardiac output, cardiac index, stroke volume index, systemic vascular resistance index and uPI. After 30 minutes of stable condition, hyper- or hypotension were induced at specific timepoints with an overdose of sevoflurane and excessive doses of norepinephrine, respectively. Friedman tests with a posteriori multiple comparison were performed and a generalized linear mixed model (GLMM) was used to assess the relationship between uPI and mean arterial pressure (MAP). The urethral perfusion was correctly traced by the photoplethysmography device at all-time points. There was a positive correlation between MAP and uPI until a certain point, called dissociation threshold (DT), beyond which they became negatively correlated. The DT was specific for each piglet and consistently indicative of an inversion of the relationship between uPI and MAP, and it was around 63±10 mmHg of MAP. This result highlights that blood pressure does not always guarantee adequate tissue perfusion and in extreme conditions, such as during resuscitation, tissue perfusion should be closely monitored. The assessment of urethral mucosa perfusion using the photoplethysmography proved successful to detect local alterations in microcirculation. Moreover, the use of a photoplethysmography device on the urethral mucosa is advantageous because it is not invasive, easy to place with a manual maneuver, and concomitant with the urethral catheterization routinely performed to quantify urine output in the critical patient, in contrast to previous studies evaluating intestinal perfusion (2). Further studies are needed to appraise the feasibility of this method in a clinical setting.

[1] Ince, C. Hemodynamic coherence and the rationale for monitoring the microcirculation. Crit Care 19, S8 (2015).

Comparison of time of entry and complication rate with Veress needle technique and modified Hasson technique in Veterinary Laparoscopic surgery

Amanda Bianchi, Francesco Collivignarelli, Lorenzo Scaletta, Ilaria Falerno, Massimo Vignoli, Andrea Paolini, Roberto Tamburro

Università degli Studi di Teramo, Dipartimento di Scienze Veterinarie

Corresponding author: Roberto Tamburro (rtamburro@unite.it)

Minimally invasive surgery is increasingly being used in veterinary medicine. Laparoscopic procedures have several advantages compared with open surgery: magnification of images, reduced post-surgical pain, stress, infection rate and hospitalization time.

The establishment of pneumoperitoneum is a critical step, the procedure could be time-consuming and most of complication described in laparoscopic surgery are related with insertion of devices into the abdominal cavity to insufflate gas.

Two main techniques have been employed to achieve pneumoperitoneum: the closed entry method, with Veress needle, and the open Hasson technique. First port is necessary to start insufflation and then to realize operative channel to introduce laparoscopic instruments in the abdomen. Many authors have compared the time necessary to create the first portal with different techniques in human medicine but no studies in veterinary medicine have analyzed this aspect. In veterinary medicine literature, complications associated with creation of pneumoperitoneum included spleen, bowel or bladder injury, pneumothorax and subcutaneous emphysema. A fatal air embolism has been reported.

The aim of the present prospective study was to compare time related to create first portal and intraoperative complications using the Verres Needle (VN) technique and the open modified Hasson technique (HT).

The sample population was constituted by 30 entire female dogs. Dogs were organized into 2 groups and two different entry techniques were used: Veress Needle (VN=group A) and modified Hasson technique (HT=group B).

In Veress needle technique (VN) the instrument was inserted 1 cm right to the umbilicus through the abdominal wall after a small skin incision made with a scalpel blade.

Modified Hasson technique (HT) was achieved performing a 5mm skin incision 5mm caudally to umbilicus. Subcutaneous fat was resected until exposure of linea alba; abdominal wall was lifted with mosquito Klemmer and penetrated with #11 scalpel blade. A 6mm trocar-cannula was introduced. For both techniques, time from skin incision to instruments placement and establishment of pneumoperitoneum was measured. Complications related with abdominal entry were classified as major in case of organ perforation, minor in case of subcutaneous emphysema and gas leakage.

The VN and HT required respectively 374 seconds and 242.85 seconds for first portal creation ($p<0.05$). Intraoperative complications occurred in 10/30 dogs in VN group (33.3%) and in 20/30 in HT group (66.6%). VN and HT major complications rates were 20% and 0% respectively ($p<0.05$), minor were 20% and 35% respectively ($p<0.05$). None surgery requested conversion to laparotomy.

According to study's results, Hasson technique appeared associated with a lower complication rate and required less time to create the first portal compared to Veress needle technique.

[1] Anderson SJ, Fransson BA. Complications related to entry techniques for laparoscopy in 159 dogs and cats. *Vet Surg.*;48(5):707-714, 2019.

Endoscopic and surgical removal of gastrointestinal foreign bodies in dogs: an analysis of 72 cases

Cristina Di Palma (1), Maria Pia Pasolini (1), Luigi Navas (1), Andrea Campanile (2), Francesco Lamagna (1), Gerardo Fatone (1), Fabiana Micieli (1), Valeria Uccello (3) Daniela Donnarumma (4), Ciro Esposito (4), Barbara Lamagna (1)

(1) Dipartimento di Medicina Veterinaria e Produzioni Animali, Università degli Studi di Napoli "Federico II" (2) Endovet Professional Association, Roma, Italia (3) A.S.L. Napoli 1 Centro, Napoli, Italia (4) Libero professionista, Napoli, Italia

Corresponding author: Cristina Di Palma (cristina.dipalma@unina.it)

In veterinary emergency practice gastrointestinal foreign bodies' (GFB) removal is a common procedure that is performed with different techniques such as endoscopy or surgery [1]. The aims of this retrospective, multi-centric, clinical study were to report the most common locations and types of objects recovered and to investigate clinical factors and outcome in dogs undergone surgical or endoscopic treatment for GFB removal.

Records of dogs with GFB diagnosis referred to the Teaching Veterinary Hospital of the University of Naples "Federico II" or treated in different veterinary hospitals of the urban area of Naples from September 2017 and September 2019 were examined. Data obtained from each case included breed, age, clinical signs at presentation, duration of clinical signs (length of time from known ingestion or from the beginning of clinical signs), type and location of GFB, methods of management (endoscopy, gastrotomy, enterotomy or enterectomy), length of hospitalization and outcome.

Seventy-two dogs were enrolled in the study including 42 dogs of 22 different pure breeds and 30 mixed breed dogs. There were 42 males (58%) and 30 females (42%). The median age was 36 months (range: 5 months to 8 years). Dogs aged ≤12 months accounted for 19% of the cases, 56% aged from 12 to 48 months, and 25% were >48 months. Vomiting was the most common clinical sign (82%); the time from initial presentation of vomiting to the diagnosis of GFB was: <12 hours in15%, 12-24 hours in 54%, 24-36 hours in 17% and >36 hours in 14% of cases. The type of GFB detected varied greatly: kids' toy (14%), metallic object/coin (13%), cloth (13%), sock (8%), ball (8%), plastic material (8%), peach stone (7%), fishhook (6%), sewing needle (4%), hair tie (4%), pacifier (3%), plant materials (3%) and others (9%). In this study 68% of GFB were localized in the stomach, 25% in the intestinal tract (50% duodenum, 28% jejunum, 22% ileum), while 7% of cases GFBs were both in the stomach and the small intestine. Endoscopic retrieval was successfully performed in 56% of GFB (localized in stomach or duodenum), whereas the 44% of dogs underwent surgery (gastrotomy 46%, enterotomy 42%, enterectomy 3%, gastrotomy/enterotomy 3%, gastrotomy/enterectomy 3% and enterotomy/enterectomy 3%). The length of hospitalization was: ≤ 1 day in the 42% (patients treated by endoscopy), 2-3 days in 55% (33% endoscopy, 67% surgery), >3 days in 3% (50% endoscopy, 50% surgery). Dogs' survival rate was 100% in cases treated by gastric endoscopic or surgical removal, 94% in cases treated with enterotomy and 33% in cases in which enterectomy was required.

In the present study the main location of canine GFB was the stomach, maybe due to early presentation by the owners [1]. Cloths, socks and fishhooks were most frequently detected in the stomach, whereas kids' toys, balls, and peach stones were lodged equally in stomach and small intestine. Furthermore, the results suggest that endoscopic and surgical procedures may achieve high rates of success in the management of foreign bodies in the upper gastrointestinal tract; especially endoscopy is associated with short time of hospitalization. In surgical procedures a poor prognosis seems to be correlated with multiple surgical accesses and enterectomy.

ORAL COMMUNICATIONS

SIFTVET

Main Lecture

WILDLIFE POISONING: THE VETERINARY TOXICOLOGY UNIT OF CÁCERES, IN SPAIN

Marcos Pérez-López

Toxicology Unit, Veterinary School of Caceres. Avda de la Universidad s/n, 10003 Cáceres (Spain)

Corresponding author: Marcos Pérez-López (marcospl@unex.es)

Poisonings have a very prominent place within the pathologies that most commonly affect wildlife, whether they are associated with accidental causes or criminal actions. The specialists in charge of looking after these animals and the whole environment must have the necessary tools to be able to make an accurate clinical diagnosis in the event of a suspected poisoning. However, to establish a clear link between a certain substance and a specific symptom is quite difficult. This fact, together with the great diversity of xenobiotics that can occur, makes the diagnosis a very complicated process.

The use of all laboratory tools that allow the diagnosis of those toxicological processes is essential. In fact, even if this diagnosis comes long after the clinical process that had to be solved, it can help to create a registry of cases that can preserve the lives of later patients who are affected by the same poison. In addition, when legal procedures have to be faced, the existence of these detailed analytics will be a priceless tool.

The choice of the sample to be sent is vital, in order to carry out an adequate detection of poisons. Experience indicates that gastric content is the most useful sample, but sometimes it is not sufficiently representative, and other samples, such as blood, Central Nervous System or portions of the liver must be sent. Special relevance should be given to the remission of possible poisoned baits that are located in the vicinity, since with no doubt these constitute the most conclusive samples in any poisoning process.

According to the experience of the Toxicology Unit of Cáceres, among the most common agents that cause poisoning in wildlife, the highest percentage is undoubtedly occupied by pesticides. Within this broad family of chemical agents, carbamate insecticides have long been the most implicated in poisonings (specially related to both carbofuran and aldicarb), although, associated with restrictive legislation initiated by the EU, in recent years their importance has declined as new agents (rodenticides, herbicides ...) have increased their impact.

With these considerations, the consolidation of veterinary toxicology groups in charge of carrying out analyzes on samples of wild fauna is one of the best tools to detect possible cases of poisoning. In this sense, it is of enormous interest to promote collaboration between these groups within the EU, to share experiences and methodologies that allow to fight more effectively against the problem of poisons in European wild fauna.

Heavy metals concentration in *Mugil cephalus* and *Diplodus annularis* from Campania Region: preliminary observation

Andrea Ariano (1), Marcello Scivicco (1), Francesco Esposito (2), Elvira De Rosa (2), Aniello Anastasio (1), Lorella Severino (1)

(1) Università degli Studi di Napoli Federico II, Dipartimento di Medicina Veterinaria e Produzioni Animali (2) Università degli Studi di Napoli Federico II, Dipartimento di Sanità Pubblica

Corresponding author: Andrea Ariano (andrea.ariano@unina.it)

Over the last few decades, the marine environment has been contaminated by persistent pollutants of anthropogenic origin. Heavy metals contamination has been identified as a concern in the coastal environment due to discharges from industrial wastes, agricultural and urban sewage. Marine organisms can accumulate heavy metals through various pathways, including respiration, adsorption and ingestion [1]. Fish is a fundamental component of human nutrition. It is an essential source of proteins, polyunsaturated fatty acids and micronutrients. However, through the consumption of fish products, people may be exposed to heavy metals. *Mugil cephalus* (mullet), belonging to the *Mugilidae* family, and *Diplodus annularis* (seabream), belonging to the *Sparidae* family, are two fish species widespread in the coastal waters of the Mediterranean and are frequently used as human food in Italy.

This study set out to quantify the total concentrations of heavy metals (Cd, Pb, Cr, As) in muscle samples of *Mugil cephalus* and *Diplodus annularis* in three different areas of the coast of the Campania region (Italy) and evaluate the health risk of people, related to the consumption of these two fishes. Samples of *Mugil cephalus* (n=15) and *Diplodus annularis* (n=15) were caught along the southern Tyrrhenian Sea coast between July and December 2020, in three sites of Campania region: Domitia coast, Flegrea area and an area near the Sarno River's mouth. After capture, samples were weighed, and total lengths measured. The abdominal muscle of each fish was collected and homogenized using a laboratory mixer. Aliquots of each sample were digested in ultrapure HNO_3 and H_2O_2 in a microwave digestion system. The final volume was obtained by adding ultrapure milliQ water. Metal concentrations in the digested samples were determined using a Thermo ScientificTM ICAPTM RQ inductively coupled plasma mass spectrometer (Q-ICP-MS). All samples were analyzed in duplicate and each sample was measured in triplicate by Q-ICP-MS detection.

The results highlighted a statistically significant difference between the Sarno River and the other two areas, as regards As levels in *Mugil cephalus* (One-way ANOVA with posthoc Tukey HSD Test, $p<0.05$). Comparing the two species, As levels were statistically higher in *Diplodus annularis* than *Mugil cephalus* (median values: 31.93 and 7.82 µg/kg) and this finding could be due to the different dietary habits of the two species. The other metals showed concentration in the range 0.05–8.59, 0.24–0.89 and 0.12–0.89 µg/kg, respectively for Cd, Cr and Pb, with no statistically significant difference among areas and species. The results obtained in the current study show low levels of Cd and Pb in all samples and well below the maximum levels set by the European Commission for fishes and were indicative of low risk for human consumption.

[1] Çoğun et al. Metal concentrations in fish species from the northeast Mediterranean Sea. Environmental Monitoring and Assessment 121.1 (2006): 431-438.

Levels of Pb, Cd, Cr and As in bees, honey and pollen collected from Campania Region: preliminary results

Marcello Scivicco (1), Andrea Ariano (1), Francesco Esposito (2), Fabiana Di Duca (2), Luigi Esposito (1), Lorella Severino (1)

(1) Università degli Studi di Napoli Federico II, Dipartimento di Medicina Veterinaria e Produzioni Animali (2) Università degli Studi di Napoli Federico II, Dipartimento di Sanità Pubblica

Corresponding author: Marcello Scivicco (marcello.scivicco@unina.it)

Bees (*Apis mellifera*) are pollinating insects belonging to the hymenoptera family. They are considered to be excellent biological indicators thanks to their ethological and behavioral characteristics which allow constant contact with the surrounding environment. Bees report the chemical damage of the environment in which they live both through high mortality, in the case of pesticides, and through residues that can be found in their bodies, or in the hive products, in the case of pollutants such as heavy metals [1]. Bees and hive products have been the subject of numerous environmental monitoring studies for heavy metals and trace elements.

The present study aimed to determine the concentration of heavy metals (Cd, Pb, Cr, As) in bees, honey, and pollen collected in eight sites of the Campania region in southern Italy.

Samples (n=32) of bees (*Apis mellifera*), pollen and honey were taken during spring 2020 from eight apiaries in the Campania region. Five apiaries were located in the Vesuvian area, in the province of Naples and three apiaries were located in the province of Caserta. The samples were placed in individual containers and frozen at -20°C. The samples were homogenized individually by a laboratory mixer. Aliquots of each sample were digested inultrapure HNO_3 and H_2O_2 in a microwave digestion system. The final volume was obtained by adding ultrapure milliQ water. Metal concentrations were determined using a Thermo Scientific TM ICAPTM RQ inductively coupled plasma mass spectrometer (Q-ICP-MS). All samples were analyzed in duplicate and each sample was measured in triplicate by Q-ICP-MS detection.

Regardless of the sampling site, bees, and pollen showed comparable levels for each metal, whereas the concentration of these contaminants in honey samples was statistically lower than the other related matrices (One-way ANOVA with posthoc Tukey HSD Test, $p<0.05$). Pb, Cr, and As levels in all samples lay in the ranges 0.28–19.67, 0.37–2.79, and 0.05–1.22 µg/kg, respectively. With regard to Cd, its levels varied from 9.79 to 77.91 µg/kg, and the highest concentrations that occurred in bees, pollen, and honey were 77.91, 55.99, and 28.36 µg/kg. These data are consistent with Giglio et al. (2017), who performed a study on concentration in honey bees as an environmental indicator, and taken together, our results do not seem to highlight a concerning scenario [2]. However, even though no regulation limit has been set for Cd in honey so far, its occurrence suggests that the dietary exposure among younger consumers might deserve further investigation.

[1] Goretti et al. Heavy metal bioaccumulation in honey bee matrix, an indicator to assess the contamination level in terrestrial environments, Environmental Pollution, 256: 113388, 2020.
[2] Giglio et al. *Apis mellifera ligustica*, Spinola 1806 as bioindicator for detecting environmental contamination: a preliminary study of heavy metal pollution in Trieste, Italy, Environmental Science and Pollution Research, 24:659–665, 2017.

Levels of organochlorine compounds in mediterranean trout (*Salmo trutta)* from Calabria region, Italy

Anna Monnolo, Filomena Del Piano, Raffaelina, Maria Carmela Ferrante

Università degli Studi di Napoli, Dipartimento di Medicina Veterinaria e Produzioni Animali

Corresponding author: Maria Carmela Ferrante (ferrante@unina.it)

Polychlorinated biphenyls (PCBs) and organochlorine pesticides (OCPs) are lipophilic organochlorine compounds (OCs) that bioaccumulate in lipid-rich tissues and biomagnify along the food chains. Chronic exposure to OCs induces damage to several body systems among which the immune system of animals and humans [1]. The daily human exposure to OCs is for the most due to the consumption of foods of animal origin, particularly dairy and seafood products [2]. Brown trout (*Salmo trutta*) is widespread in freshwater and coastal marine environments in Europe. It is a predatory fish at the top of the trophic food chain, normally part of human diet [3]. Our research aimed at investigating the occurrence of PCBs and OCPs in Mediterranean brown trout muscle, to evaluate the toxicological risk for consumer. The modulation of OC pollutants on the intestinal parasitic infection degree was also considered. Eleven male and twelve female specimens (two-thirds of the total sexually immature) were collected from Sila National Park (Calabria region, Italy). The concentrations relative to the following pollutants were determined: 5 OCPs namely *p,p'*-DDT, *p,p'*-DDE and *p,p'*-DDD (overall indicated as ΣDDT), HCB and Dieldrin; 15NDL-PCBs (where NDL stands for Not Dioxin Like) including the six indicator congeners denoted as Σ6NDL-PCBs; 3 non-ortho DL-PCBs (77, 126, 169) denoted as Σ3DL-PCBs, and 5 mono-ortho DL-PCBs (105, 118, 156, 157 and 167) reported as Σ5DL-PCBs. The 8 DL-PCBs analysed are a subset of the 12 DL-PCBs for which, together with 17 dioxins, the EU legislation set maximum levels in a range of foodstuffs. Sample units underwent a liquid/liquid manual extraction. The cleaned extracts were analysed using a HRGC/LRMS equipment. The mass spectrometer operated in EI mode and a SIM program was constructed for acquisition and quantification. Data were expressed as ng g^{-1} on lipid weight (lw) basis. Helminths were collected, identified, and counted as described by krone [4]. It was observed the preponderance of NDL-PCB concentrations (mainly determined by Σ6NDL-PCBs) followed, in decreasing order, by ΣDDT, Σ5DL-PCBs, Σ3DL-PCBs, HCB, and Dieldrin. The mean value of Σ6NDL-PCBs amounted to 201.9 ng g^{-1} lw while ΣDDT was 100.2 ng g^{-1} with the major contribution of DDT metabolite *p,p'*-DDE detected in all sample units (97.6 ng g^{-1} on average). Regarding the DL-PCBs, the average concentrations were 11.3 and 35.4 ng g^{-1} lw for Σ3DL-PCBs and Σ5DL-PCBs, respectively. About the toxicological risk evaluation, the OC concentrations are always below the current European Maximum Residue Limits and do not pose a risk to consumer health. Controlling for sex and reproduction stage (through multiple regression analysis), a higher OCs concentration tends to reduce the probability of parasite infestation. The relationship is statistically significant ($p<0.05$) for Σ6NDL-PCBs, Σ15NDL-PCBs, and Σ5DL-PCBs.

[1] Masset et al. Science of the Total Environment, 15 (674): 472-481, 2019.
[2] Weber et al., Environmental Sciences Europe, 30 (1): 1-42, 2018.
[3] Dewailly et al. Food and Chemical Toxicology, 45 (8):1343-8, 2007.
[4] Krone. In Raptor, Research and Management Techniques. Bird, Bildstein (Eds.) 2007.

Bioaccumulation of Cadmium and Mercury in blood and serum of Athletic Horse from Messina, Italy

Alessio Filippo Peritore (1), Enrico Gugliandolo (2), Rosalia Crupi (2), Francesco Fazio (2), Patrizia Licata (2)

(1) Department of Chemical, Biological, Pharmaceutical and Environmental Science, University of Messina, Italy (2) Department of Veterinary Science, University of Messina, Viale Annunziata, 98168, Messina, Italy

Corresponding author: Alessio Filippo Peritore (aperitore@unime.it)

The objective of this study was to evaluate the levels and the potential bioaccumulation of cadmium (Cd) and mercury (Hg) in horse from the industrial risk area of Sicily (Italy) as a bioindicator of environmental pollution. Samples of whole blood and serum collected from twenty clinically healthy Italian Saddle horses, geldings aged 8–11 years and with an average body weight of 460 kg, were processed by means of Thermo Scientific iCAP-Q ICP–MS spectrometer and by DMA-80 for determination of Cd and Hg concentrations, respectively. One-way analysis of variance (ANOVA) was applied to show the differences in various trace elements in the biological substrates. Pearson's test was applied to evaluate the correlation of mineral concentrations between whole blood and serum. Pearson correlation for Cd element concentrations and haematology markers were also performed. Results showed a significant correlation between blood concentrations and serum (r= r2=0.99). Furthermore, a significant correlation was also showed for Hg between blood concentrations and serum (r= r2=0.23).
The concentrations of Cd and Hg in serum and whole blood samples did not show correlation between these elements. For Cd and Hg a different distribution is observed in the biological substrates (blood and serum) studied. In particular, as can be seen from the histogram, blood appears to be the substrate that is best prepared for the dosage of Hg compared to Cd. This suggests that Cd and Hg may be a potential means to investigate suspected exposure to excessive levels of trace minerals, take as single biomarker. In this study, the concentrations of Hg observed in the different biological components showed levels below the respective benchmarks, and therefore, we can say that at present, there are no toxicological.

Effects of natural antioxidants on aflatoxin B1 hepatotoxicity in cattle: targeted *in vitro* post-transcriptional studies

Marianna Pauletto, Mery Giantin, Irene Bassan, Mauro Dacasto

Università degli Studi di Padova, Dipartimento di Biomedicina Comparata e Alimentazione

Corresponding author: Marianna Pauletto (marianna.pauletto@unipd.it)

Aflatoxin B1 (AFB1) is a natural feed and food contaminant from *Aspergillus flavus* and *A. parasiticus*. AFB1 is a group I carcinogen for humans, but most of food-producing species are differently susceptible. In dairy industry, AFB1 and its derivative "AFM1" are concerns for food safety for the related economic losses and their possible presence in milk and dairy food-products [1]. Nevertheless, a full understanding of AFB1 molecular toxicity in cattle is still lacking. AFB1 is mostly hepatotoxic, but it causes also oxidative stress and the modulation of several other biological pathways. In the past decade, the dietary supplementation with natural antioxidants (AOs), or food by-products that contain a fair amount of them, has been considered among the strategies to mitigate the presence and toxicity of mycotoxins [2]. Therefore, studies aiming at clarifying the AFB1 molecular mechanisms of toxicity in cattle, and the potential protective role of natural AOs, should be conducted. Recently, the protective role of four natural AOs, *i.e.* curcumin (CUR), curcuminoids (CUM), resveratrol (RES), and quercetin (QUE) has been investigated in a foetal bovine hepatocyte cell line (BFH12), exposed to AFB1, by measuring cytotoxicity and transcriptional changes. Overall, these AOs reversed the AFB1-dependent toxicity, albeit to a variable degree. Most relevant transcriptional effects were observed in molecular pathways associated with antioxidant and anti-inflammatory response, cancer, and drug metabolism [3,4]. In the present study, confirmatory-targeted post-transcriptional studies were executed, using BFH12 and the same experimental settings described elsewhere [3,4]. Briefly, cells were pre-incubated for 24 hrs with the cytochrome P450 (CYP) inducer PCB126 and, then, for 16 hrs with CUR and CUM (10 µM), QUE and RES (30 µM). Lastly, cells were incubated for further 48h with 3.6 µM AFB1, either alone or in combination with aforementioned AOs concentrations. We measured the amount of malondialdehyde (MDA, a marker of oxidative stress), and the catalytic activities of the antioxidant enzyme diaphorase (NQO1) and CYP3A. Two commercial colorimetric kits were used for MDA and NQO1 evaluation, while a commercial fluorescence kit that allows the direct measurement of the enzyme activity on cell monolayers was selected to for CYP3A assessment. The obtained results corroborated former transcriptional data, highlighting a significant protective role of these AOs. Additionally, RES was confirmed as the most effective AO, followed by CUM, CUR, and QUE. Present results confirm AOs might represent potentially useful dietary supplements to protect cattle against aflatoxicosis.

Funding: research supported by the Ministry of Education, University and Research (2015NL8JWS_003) and University of Padua (DOR1917008) to M.D. and M.P. respectively.

[1] Becker-Algeri et al. Mycotoxins in bovine milk and dairy products: a review. Journal of Food Science, 81:R544-552, 2016.

[2] Mavrommatis et a. Impact of mycotoxins on animals' oxidative status. Antioxidants (Basel), 10:214, 2021.

[3] Pauletto et al. Natural antioxidants attenuate aflatoxin B1 transcriptional effects in a cattle fetal liver cell line (BFH12). Proceedings of 73nd Convegno Sisvet, Olbia, June 19th-22nd, 2019, 203.

[4] Pauletto et al. Curcumin mitigates AFB1-induced hepatic toxicity by triggering cattle antioxidant and anti-inflammatory pathways: a whole transcriptomic in vitro study. Antioxidants (Basel), 9:1059, 2020.

Food contaminants and animal health: a whole-transcriptomic *in vitro* approach to unveil aflatoxin B1 toxic effects in cattle

Silvia Iori, Irene Bassan, Marianna Pauletto, Mauro Dacasto, Mery Giantin

Università degli Studi di Padova, Dipartimento di Biomedicina Comparata e Alimentazione

Corresponding author: Mery Giantin (mery.giantin@unipd.it)

Aflatoxin B1 (AFB1) is a widespread human carcinogenic food contaminant. Many food-producing species, including ruminants, are differently susceptible to such mycotoxin. The metabolism of AFB1 occurs mostly in the liver where it undergoes bioactivation, with the production of a carcinogenic and genotoxic epoxide derivative, and other metabolites, like the hydroxylated carcinogenic derivative aflatoxin M1 (AFM1) and aflatoxicol [1]. Dairy cows fed with AFB1-contaminated feedstuffs may excrete AFM1 into the milk, thus representing a public health concern. Despite the numerous studies about the presence of AFB1 and AFM1 in dairy milk and food-products, little information is available on the overall toxic effects of AFB1 in bovine liver. A recent *in vitro* study on a bovine foetal hepatocyte cell line (BFH12), pre-incubated with a known CYP1A inducer (PCB126), described for the first time the hepatic transcriptional perturbations elicited by AFB1 [1]. To better characterise the molecular mechanisms involved in AFB1 hepatotoxicity, we exposed BFH12 cells for 48 hrs to increasing AFB1 concentrations (0.9, 1.8, 3.6 μM). Whole-transcriptomic changes were measured by RNA-*seq*. The enrichment analysis identified pathways linked to stress signals and inflammatory response, oxidative stress, cancer, apoptosis, cellular reorganization, and drug metabolism. In particular, genes coding for antioxidant (i.e., NQO1) and phase II enzymes (i.e., UGT1A1 and GSTs) were downregulated in a dose-dependent manner. A similar behaviour was observed for NRF2, a master regulator of the antioxidant response. However, the BTB Domain and CNC Homolog 1 (BACH1) gene, a repressor of genes involved in the oxidative stress response, was upregulated at the highest AFB1 concentration. A similar trend was noticed for the small MAF BZIP Transcription Factors (sMAFs). Both NRF2 and BACH1 form heterodimers with sMAFs; once bound to DNA response elements, they activate (NRF2) or repress (BACH1) the transcription of cytoprotective genes. Moreover, sMAFs may form homodimers and lead to downregulation of target antioxidant genes [2]. Interestingly, TLR2 (a pattern recognition receptor) was dose-dependently upregulated by AFB1; this might be suggestive of a role in AFB1-related inflammatory response. Indeed, TLR2 might trigger an intracellular signalling cascade involving a kinase (p38 MAPK), which allows the nuclear translocation of the activator protein-1 (AP-1) and NF-κB, with the consequent release of pro-inflammatory cytokines (e.g., IL-6). IL-6, in its turn, could affect the metabolism inhibiting CYP expression [3]. In the present study IL-6 and CYP2B6 showed a dose-dependent opposite behaviour (induced the first, inhibited the second). Further studies (e.g., flow cytometry, immunoblotting, AFB1 metabolite profiling) are envisaged to better decipher the molecular mechanisms involved in cattle liver response to AFB1.

Funding: research supported by grants (BIRD207109) to M.G. and a PhD fellowship to S.I. (University of Padua).

[1] Pauletto et al. Insights into aflatoxin B1 toxicity in cattle: an in vitro whole-transcriptomic approach. Toxins (Basel), 12:429, 2020.
[2] Ma Q. Role of Nrf2 in oxidative stress and toxicity. Annual Reviews in Pharmacology and Toxicology, 53:401–426, 2013.
[3] Dickmann et al. Effects of interleukin-6 (IL-6) and an anti-IL-6 monoclonal antibody on drug-metabolizing enzymes in human hepatocyte culture. Drug Metabolism and Disposition, 39:1415–1422, 2011.

Occurrence of Ochratoxin A in bulk dry dog food

Federica Battaglia, Luigi Intorre, Lucia Casini, Valentina Meucci

Università di Pisa, Dipartimento di Scienze Veterinarie

Corresponding author: Federica Battaglia (federica.battaglia@phd.unipi.it)

Aflatoxins, Ochratoxin A (OTA), and *Fusarium* mycotoxins have been found in both raw ingredients and final products of feed and their presence in pet food is a potential health threat to companion animals [1]. The use of bulk feed delivery systems is increasingly widespread in the pet food market. Several stores offer dry pet foods in bulk without packaging to lower prices while increasing sales and margins. Bulk pet food is kept in transparent and non-hermetic plastic dispensers, with exposure to light and humidity, which can favor the development of fungi and the production of mycotoxins, the entry of insects and dust which can alter the product with consequent loss of nutritional value [1].

The aim of this study was as to determine the presence OTA in 48 samples of bulk dry dog pet food by using a validated HPLC-FLD method. All samples were collected from stores located in Tuscany. The sample weight was 400 g. All samples were grounded using a blender, mixed thoroughly and stored in sealed plastic bags at – 20°C until analysis. The chromatographic system consisted of a Perkin Elmer (Waltham, USA) Series 200 binary pump and a Jasco FT-1520 fluorescence detector (Jasco, Tokyo, Japan). The excitation wavelength was set at 380 nm and emission wavelength at 420 nm. Totalchrom Navigator® software was used for data processing. Ten grams of ground sample was fortified with internal standard (Ochratoxin B, OTB) and extracted according to Meucci et al. [2]. Recovery of the analytical method was higher than 85%. Intra- and inter-day repeatability expressed as relative standard deviation were less than 15%. The limit of detection (LOD) and limit of quantification (LOQ) were 0.001 and 0.002 µg/kg, respectively.

OTA was found in 16 out of 48 samples corresponding to 33% of the total, with concentrations ranging from 0.11 ppb to 1.21 ppb. All concentrations fall within the reference values of the EU Recommendation 2016/1319. The highest concentration of OTA (1,21 ppb) was found in a sample having a totally vegetable composition.

Given the present results and considering the high percentage of cereals present in the analyzed feeds, it is recommended to control more thoroughly the storage of bulk feeds in order to avoid the proliferation of unwanted fungi and molds while maintaining the nutritional and health characteristics unaltered.

[1] Leung et al. Mycotoxins in Pet Food: A Review on Worldwide Prevalence and Preventative Strategies, Journal of Agricultural and Food Chemistry, 54: 9623−9635, 2006.
[2] Meucci et al. Natural occurrence of ochratoxin A (OTA) in edible and not edible tissue of farmed gilthead seabream (*Sparus aurata*) and European seabass (*Dicentrarchus labrax*) sold on the Italian market, Food Control, 120, 2021.

Effects of Red Orange and Lemon Extract on oxidative stress and inflammatory status in kid lambs

Sara Damiano (1), Consiglia Longobardi (2), Livia D'Angelo (1), Roberta Matera (1), Alessio Cotticelli (1), Paolo De Girolamo (1), Roberto Ciarcia (1)

(1) Università degli Studi di Napoli Federico II, Dipartimento di Medicina Veterinaria e Produzioni Animali (2) Università degli Studi della Campania "Luigi Vanvitelli", Dipartimento di Salute Mentale, Fisica e Preventiva

Corresponding author: Sara Damiano (sara.damiano@unina.it)

Oxidative stress causes several pathological conditions in farm animals that induce a reduction in animal welfare and production with major economic losses for livestock. To reduce the free radical increase, antioxidant protective systems are developed to modulate oxidative stress-induced cell injuries by scavenging free radicals [1]. Polyphenols, the most extensive groups of chemicals in fruits, vegetables, green and black teas, are extensively researched for their potential beneficial effects, such as anticancer, anti-inflammatory and anti-degenerative properties [2,3]. However, polyphenols positive effect intake and *in vivo* antioxidant defenses are not clearly demonstrated.

The present study aimed to assess the efficacy of a red orange and lemon extract (RLE), rich in polyphenols, to counteract the oxidative stress processes in lambs. A significant increase of oxidative stress in the newborn period, with a reduction of the antioxidant defense system, has been already demonstrated. We have investigated whether lamb treatment for 40 days (n=60 for each group) with RLE (90 mg/kg by gavage) was able to prevent the alteration of antioxidant and anti-inflammatory mechanisms, typically in newborn period. The trial was authorized by the Animal Welfare Body of the University of Naples Federico II (PG / 2019/0028161 of 03/19/2019). At the end of treatment, plasma redox status was assessed by oxidative stress markers: malondialdehyde (MDA), superoxide dismutase (SOD), catalase (CAT) activities and glutathione (GSH) content were checked through spectrophotometric methods . Moreover, 8-hydroxy-2'-deoxyguanosine (8-OHdG) in urine was measured as a biomarker of DNA damage and, finally, the markers of inflammatory status (IL-1B and IL-6) were measured in plasma by ELISA assays.

Results showed that RLE improved the antioxidant defence system in lambs by increasing SOD and CAT activities, as well as GSH content. Furthermore, a significant reduction in oxidative stress-induced damage was evidenced through MDA and 8-OHdG measurements in the treated group respect to control one. Moreover, RLE is also able to prevent inflammatory status alterations. These data demonstrate the potential beneficial effects of RLE in lambs and open new perspectives in the use of such natural compound as an alternative feeding resource to ameliorate farm animal health.

[1] Carocho, M. et al. A review on antioxidants, pro oxidants and related controversy: natural and synthetic compounds, screening and analysis methodologies and future perspectives. Food Chem Toxicol. 2013;51:15–25.
[2] Surai, P.F. Plyphenol compounds in chicken/animal diet: from the past to the future. Animal Physiology and Animal Nutrition 2014, 98:19-31.
[3] Damiano, S. et al. Effects of a Red Orange and Lemon Extract in Obese Diabetic Zucker Rats: Role of Nicotinamide Adenine Dinucleotide Phosphate Oxidase. J Clin Med. 2020, 25;9(5):1600.

Effects of the microbiota depletion on kidney function

Consiglia Longobardi (1)(2), Sara Damiano (3), Mariavittoria D'Acierno (1)(2), Anna Iervolino (2), Giovambattista Capasso (2)(4), Roberto Ciarcia (3)

(1) Università degli Studi della Campania "Luigi Vanvitelli", Dipartimento di Salute Mentale, Fisica e Preventiva (2) Biogem Istituto di Ricerche Genetiche "Gaetano Salvatore" (3) Università degli Studi di Napoli "Federico II", Dipartimento di Medicina Veterinaria e Produzioni Animali (4) Università degli Studi della Campania "Luigi Vanvitelli", Dipartimento di Medicina Traslazionale

Corresponding author: Mariavittoria D'Acierno (vittoria.dacierno@gmail.com)

The interconnection between gut microbiota and kidney is one of the most today's avant-garde research area [1]. Most of the studies in literature based on the interaction between microbiota and its host makes use of germ-free animals (GF), i.e. difficult to maintain from the time of birth [2].

In the present project, we aimed to generate a gnotobiotic model with antibiotic treatments that induce the reduction in the intestinal microbiota preserving, at the same time, the renal physiology.

For this purpose, 5-6 weeks old C57/BL6 male mice (n=25) were randomly divided into 5 groups and treated with 4 broad-spectrum antibiotics mix at various concentrations by using different ways of administration as oral gavage, drinking water (DW) and combination of both (authorization number 820/2020-PR).

In particular, Group 1 (n=5) mice were treated with cocktail 1 antibiotics (ampicillin 1mg/mL, gentamicin 1mg/mL, metronidazole 1mg/mL, neomycin 1mg/mL and vancomycin 0.5 mg/mL) administered completely by oral gavage for ten days; Group 2 (n=5) provided the administration of cocktail 2 (ampicillin 1mg/mL, neomycin 1mg/mL, metronidazole 1mg/mL and vancomycin 0,5mg/mL) by DW; Group 3 (n=5) was administered with cocktail 3 consisting of 2mg/kg amphotericin-B for 3 days, followed by for 14 days of vancomycin (100mg/kg), neomycin (200mg/kg), metronidazole (200mg/kg) and amphotericin-B (2mg/kg) mix by oral gavage, while ampicillin (1mg/mL) was provided in DW; Group 4 (n=5) was treated with a combination of ampicillin, neomycin, metronidazole and vancomycin (40mg/mL each) both by oral gavage and DW; Control Group was treated with milliQ water only.

Alteration of serum creatinine, associated with oliguria, was presented by mice treated with cocktail 2, probably due to the persistent administration by DW. Also, the mice treated with cocktail 3 showed aforementioned symptoms, as clear signs of renal failure. Interestingly, no renal impairment or dehydration was observed in mice administered with cocktails 1 and 4, that may hold promise for generation of this gnotobiotic model.

Further studies are ongoing to establish which cocktail, between 1 and 4, is the best for depleting microbiota thought the 16S sequencing in fecal samples. Moreover, the GFR (Glomerular filtration rate) will be measured to confirm the preservation of renal function.

This mouse model can be useful to dissect the gut-kidney axis and to investigate the role of fecal microbiota in the development of kidney diseases.

[1] Stavropoulou E, Kantartzi K, Tsigalou C, Konstantinidis T, Romanidou G, Voidarou C, Bezirtzoglou E. Focus on the Gut-Kidney Axis in Health and Disease. Front Med (Lausanne). 2021 Jan 21;7:620102. doi: 10.3389/fmed.2020.620102. PMID: 33553216; PMCID: PMC7859267.
[2] Al-Asmakh M, Zadjali F. Use of Germ-Free Animal Models in Microbiota-Related Research. J Microbiol Biotechnol. 2015 Oct;25(10):1583-8. doi: 10.4014/jmb.1501.01039. PMID: 26032361.

Interstitial cystitis/Bladder Pain Syndrome (IC/BPS): role of N-palmitoyl-D-glucosamine-hesperidin

Enrico Gugliandolo (1), Rosalia Crupi (1), Alessio Filippo Peritore (2), Patrizia Licata (1), Cristian Piras (3) ,Salvatore Cuzzocrea (2), Domenico Britti (3)

(1) Università degli Studi di Messina, Dipartimento di Scienze Veterinarie (2) Università degli Studi di Messina, Dipartimento di CHIBIOFARAM (3) Università Magna Graecia Catanzaro, Dipartimento Scienze della Salute

Corresponding author: Enrico Gugliandolo (egugliandolo@unime.it)

Interstitial cystitis/Bladder Pain Syndrome (IC/BPS) is a chronic inflammatory disease characterized by visceral pain. Feline interstitial cystitis (FIC), an idiopathic and painful condition frequently occurring in cats, reproduces many features of IC/BPS. Recently, a rat model of chronic cyclophosphamide (CYP)-induced cystitis has been developed and validated. It was shown to share strong similarity with IC/BPS (and FIC), i.e., the development of persistent painful behaviour, bladder edema and focal urothelial damage. Aliamides are pain relieving and anti-inflammatory lipid compounds whose parent molecule is palmitoylethanolamide (PEA) (1). Although much attention has been paid so far to PEA, some interesting evidence on the benefits of the aliamide N-palmitoyl-D-glucosamine (PGA) is currently being gathered (1,2). The supplementation with PGAm and hesperidin could be a useful pharmacological strategies for IC/BPS, in fact PGAm combines the multi targets action of PEA as analgesic and anti-inflammatory with the cytoprotective proprieties of glucosamine an important precursor of glycosaminoglycans (GAG) (3), natural constituents of the protective layer of the urothelium, the association with hesperidin counteract also the oxidative stress induced in IC/BPS. Thus the aim of this study was to evaluate the effects of supplementing micronized PGA (PGAm) together with the antioxidant hesperidin to rats with chronic CYP-induced cystitis (PR n°549\2018). Cystitis was induced by repetitive intraperitoneal injections of CYP (40mg/kg every three days from day 0 to day 6). Daily oral supplementation with PGAm-hesperidin (3:1 ratio) was started 3 days before CYP and maintained to the end of the experiment (day 10). CYP instillation caused macroscopic and histological bladder inflammatory changes, increased lipid peroxidation and lowered the pain threshold. PGAm-hesperidin decreased CYP-induced bladder inflammation and oxidative stress (measured through MPO(Myeloperoxidase) and MDA(Malondialdehyde) levels, respectively), decrease the number of mast cells and relieved visceral pain. Based on these findings and the known safety profile, PGAm-hesperidin may be a useful adjunct in the management of human IC/BPS and the related feline lower urinary tract disease FIC.

[1] Gugliandolo et al. Dietary Supplementation with Palmitoyl-Glucosamine Co-Micronized with Curcumin Relieves Osteoarthritis Pain and Benefits Joint Mobility, Animals, 10(10):1827, 2020.
[2] Gugliandolo et al. Palmitoylethanolamide and Related ALIAmides: Prohomeostatic Lipid Compounds for Animal Health and Wellbeing, Vet Sci, 7(2):78, 2020.
[3] Ostardo et al. Adelmidrol + sodium hyaluronate in IC/BPS or conditions associated to chronic urothelial inflammation. A translational study, Pharmacol res134:16-30, 2018

Presentation results of the 2017-2019 histological monitoring plan

Marzia Pezzolato, Elisa Baioni, Cristiana Maurella, Alessandro Benedetto, Elena Biasibetti, Elena Bozzetta

CIBA, Istituto Zooprofilattico Sperimentale del Piemonte, Liguria e Valle d'Aosta, Turin

Corresponding author: Marzia Pezzolato (marzia.pezzolato@izsto.it)

The use of growth promoting agents in food producing animals is forbidden within the European Union to preserve consumer health. The abuse of these substances is monitored through National Residue Control Plans, whose results are resumed in the annual technical report of the European Food Safety Authority on drug residues analysis. As a strategy of monitoring of growth promoters abuse in cattle, the Italian Ministry of Health introduced in 2008 the Histological Monitoring Control Plan (HMCP).
The HMCP applied in Italy from 2008 to 2016 was drawn in order to disclose whether at the national level the batches sent to the slaughterhouse exceed a predefined prevalence threshold level for corticosteroids and sexual steroid (P=15%). To achieve such objective, histological analyses were performed on a statistically significant number of slaughtered animals by the net of the the Experimental Zooprophylactic Institutes (IIZZSS) official laboratories. The histopathological approach is able to detect lesions induced by sexual hormones and glucocorticoids in bovine target organs (i.e. sexual accessory glands and thymus) [1][2]. Results were reviewed in order to obtain further information regarding high-risk categories to be accounted for in the sampling design of the new National Monitoring Plan of the present study.
The objective of the new monitoring plan was to verify whether at the national level the batches sent to the slaughterhouse exceed a predefined prevalence threshold level (as expected from the previous monitoring plans) for each illicit treatment subject to surveillance, separately for beef and calves (P=13% for beef, P=10% for veal).
According to results of 2008-2016 plans a new HMCP was designed and a sample size of 147 batches was calculated in order to increase the performance of the surveillance system.
The total number of beef batches sampled for corticosteroids was 141 in 2017, 127 in 2018 and 123 in 2019 for a total of 340, 325 and 304 beef analyzed each year, respectively.
The total number of calves batches sampled for sex steroid was 129 in 2017, 130 in 2018 and 137 in 2019 for a total of 426, 386 and 390 calves analyzed each year, respectively.
The overall prevalence of corticosteroid suspect treatment was 11.3% (95% CI 6.6-17.8) in 2017; 10.2% (95% CI 6.6-16.9) in 2018 and 8.9% (95% CI 4.6-15.4) in 2019.
The overall prevalence of sex steroid suspect treatment is 2.3% (95% CI 0.5 -6.6) in 2017; 6.2% (95% CI 2.7-11.8) in 2018 and 12.4% (95% CI 7.4-19.1) in 2019, but the difference in the temporal trend resulted not significant.
Results achieved by the monitoring histological plan applied from 2017 to 2019, are in contrast with those reported by the Italian NRMP and demonstrate that the phenomenon of illicit treatments is far to be overwhelmed [3]. Even if histology is not able to identify and quantify single molecules illegally used, the results obtained by the monitoring plan can better address official controls.

[1] Groot MJ et al., Vet Med Sci, 54:238-246, 2007.
[2] Bozzetta E et al., Food Addit Contam Part A Chem Anal Control Expo Risk Assess 28:1187-92, 2011.
[3] EFSA, 2020.Technical report. Report for 2018 on the results from the monitoring of veterinary medicinal product residues and other substances in live animals and animal products. EFSA Supporting Publications 17:3.

Oxidative stress and immune response to vaccination against *Mycoplasma gallisepticum* in backyard chickens

Tiziano Iemmi, Alessandro Menozzi, Giuseppina Basini, Francesca Grasselli, Paolo Serventi, Simone Bertini

Università di Parma, Dipartimento di Scienze Medico-Veterinarie

Corresponding author: Alessandro Menozzi (alessandro.menozzi@unipr.it)

The level of oxidative stress (OS) of an organism depends on the balance between pro- and anti-oxidant factors; when the concentration of reactive oxygen species (ROS) overwhelms the protective ability of the antioxidant system, OS occurs [1].

The immune response is one of the main endogenous sources of ROS, released from white blood cells as metabolic waste and as a weapon against pathogens [2,3].

In our study OS was evaluated in 12 backyard chickens following vaccination for *Mycoplasma gallisepticum* (MG) by conjunctival route with vaccine strain MG 6/85 (10^3-10^4 CFU). A blood sample was collected from each chicken from the right ulnar vein during routine health monitoring activity, immediately after vaccination (T0) and after 14 (T1) and 21 days (T2) for evaluation of antibody levels against MG (sample-to-positive [S/P] ratio), blood cell counts and measurement of OS biomarkers. S/P was assessed with a commercial ELISA kit (ID Screen® MG Indirect); blood cell count was carried out with a haemocytometer (Natt & Herricks staining), and on blood smear (Diff-Quick staining) for differential count. OS was evaluated by measuring malondialdehyde (MDA) levels as an index of lipid peroxidation, determinable reactive oxygen metabolites (d-ROMs), which are considered a reliable measure of the concentration of plasmatic ROS, superoxide anion (by WST-1 test), and nitric oxide (NO). Enzymatic and scavenging non-enzymatic antioxidant capacity were evaluated by means of superoxide dismutase (SOD) analysis, and ferric reducing antioxidant power (FRAP) assay, respectively. Mann-Whitney U-test and Pearson correlation index were used for statistical analysis; $p<0.05$ was considered statistically significant.

Significant increases in WBC ($p=0.036$), heterophiles ($p=0.031$), lymphocytes ($p=0.042$) and S/P ratio ($p=0.018$) were observed at T2 with respect to T0, suggesting an effective immune activation following vaccination. Monocyte number at T1 was significantly lower than at T0 ($p=0.006$), followed by an increase from T1 to T2 ($p=0.035$), up to a level close to initial value.

MDA was significantly higher at T2 with respect to T0 ($p=0.001$), while both NO and superoxide anion decreased from T1 to T2 ($p=0.003$ and 0.05). SOD significantly increased from T1 to T2 ($p=0.024$), whereas FRAP was lower at T2 with respect to T1 ($p=0.021$). The increase in lipid peroxidation levels suggests that immune activation due to MG vaccination may induce OS [4]. S/P ratio at T2 resulted negatively correlated with NO and d-ROMs levels at T0 ($r=-0.30$ and -0.57, respectively).

The data of this study confirm the presence of an important relationship between OS and immune response, and suggest that higher levels of d-ROMs and NO may be predictive of a lower immune response to vaccination against MG in chickens.

[1] Birben E. et al. Oxidative stress and antioxidant defense. World Allergy Organization Journal, 5: 9-19, 2012.

[2] Costantini D. & Møller A.P. Does immune response cause oxidative stress in birds? A meta-analysis. Comparative Biochemistry and Physiology Part A: Molecular & Integrative Physiology, 153: 339-344, 2009.

[3] Costantini, D. Oxidative stress in ecology and evolution: lessons from avian studies. Ecology letters, 11: 1238-1251, 2008.

[4] Weining K.C. et al. Biological properties of recombinant chicken interferon-g. European Journal of Immunology, 26: 2440-2447, 1996.

Personalised medicine in veterinary practice: the paradigmatic example of canine ABCB1

Mery Giantin, Rosa Maria Lopparelli, Alessandra Feltracco, Mauro Dacasto

Università degli Studi di Padova, Dipartimento di Biomedicina Comparata e Alimentazione

Corresponding author: Mery Giantin (mery.giantin@unipd.it)

Pharmacogenetics (PG) is the study of variations in DNA sequence as related to drug response. Close relationships between DNA variants and ADME or drug target genes exist, and may be actionable in the clinic, ultimately resulting in drug phenotype predictions in patients (personalised medicine concept). Variations in drug response (unexpected toxicity or therapeutic failure) occurs also in veterinary species, and most of the available knowledge refers to dogs. However, canine PG lags behind human one, for a number of scientific and economic reasons. Most commonly described canine pharmacogenes are cytochromes P450, drug transporters (e.g., *ABCB1*), and intended or unintended drug targets (e.g., β1 adrenergic receptor and *KIT* protooncogene) [1-2]. In the present study, we developed an in-house protocol for canine *ABCB1* mutational analysis; in particular, the *ABCB1-1Δ* four base-pair deletion, resulting in a premature stop codon and non-functional *P*-glycoprotein (*P*-gp) [2]. The protocol, an adaptation of a previously published one [3], essentially consists in a quantitative Real time PCR assay and the use of TaqMan™ probes. Genomic DNA from whole blood was isolated using the DNeasy Blood & Tissue kit (Qiagen). For the correct interpretation of genotyping data, 15 reference samples (5 *ABCB1* WT/WT, 5 WT/mut, and 5 mut/mut), kindly provided by Prof. Joachim Geyer (Justus Liebig University of Giessen, Germany), were used. Moreover, we cloned and sequenced *ABCB1* and *ABCB1-1Δ* from two dogs with a known genotype, in order to obtain the reference sequences for bioinformatic analysis. Finally, the assay was tested in a cohort of 130 blood samples from different dog breeds and coming from different clinical practices. We never observed the *ABCB1-1Δ* mutation in Border Collie dogs. However, *ABCB1* mut/mut (2/4, 50%) and *ABCB1* WT/mut (1/4, 25%) genotypes were identified in Rough Collies. In Australian Sheperd dogs, only the heterozygous *ABCB1* WT/mut genotype (7/17, 41.18%) was observed. Cross-bred and mixed Collie dogs resulted negative. Interestingly, a case report occurred during the trial. Specifically, a Rough Collie healthy dog, given selamectin spot-on for parasites control, was IM administered acepromazine before induction of inhalatory anesthesia for tartar removal. The animal showed increased depth and duration of sedation. According to the observed clinical symptoms and breed-predisposition, the dog was genotyped and found *ABCB1-1Δ* mut/mut positive. In conclusion, a PG assay to detect *ABCB1-1Δ* mutation was set up, validated and proved reliable, rapid, and cheap. At present, we are increasingly using this assay to help the clinician to adopt the best therapeutic approach whenever the use of *P*-gp substrate drugs is envisaged. On a wider scenario, future advances in canine and veterinary PG will improve the information on drug individual susceptibility (i.e., efficacy or toxicity), further improving animal health and well-being.

[1] Marconato et al. Concordance of c-kit mutational status in matched primary and metastatic cutaneous canine mast cell tumors at baseline. Journal of Veterinary Internal Medicine, 28:547-53, 2014.
[2] Mealey et al. Personalized medicine: going to the dogs? Human Genetics, 138:467-481, 2019.
[3] Klintzsch et al. Detection of the nt230[del4] MDR1 mutation in dogs by a fluorogenic 5' nuclease TaqMan allelic discrimination method. Veterinary Journal, 185:272-277, 2010.

Pharmacokinetics of dexmedetomidine intravenous continuous rate infusion and repeated subcutaneous administration in isoflurane anaesthetised horses

Federica Di Cesare (1), Giuliano Ravasio (2), Vanessa Rabbogliatti (3), Federica Alessandra Brioschi (2), Martina Amari (3), Viviana Negro (2), Roberto Villa (1), Petra Cagnardi (2)

(1) Università degli Studi di Milano, Dipartimento di Scienze Veterinarie per la Salute, la produzione Animale e la Sicurezza Alimentare (2) Università degli Studi di Milano, Dipartimento di Medicina Veterinaria (3) Università degli Studi di Milano, Dipartimento di Medicina Veterinaria, Centro Clinico e Zootecnico Sperimentale

Corresponding author: Federica Di Cesare (federica.dicesare@unimi.it)

In horses, the combination of an inhalant anaesthetic with a sedative such as dexmedetomidine (DEX) is a valid approach to reduce inhalant requirement and to improve recovery quality when achieving balanced anaesthesia [1, 2].

The study aimed to validate a new robust method for DEX determination in equine serum using HPLC-HRMS Orbitrap analysis and then to determine DEX pharmacokinetic profile during continuous rate infusion (CRI) or repeated subcutaneous (SC) injection in horses anaesthetised due to diagnostic procedures.

In a parallel clinical study, nineteen adult, client-owned, non-food producing horses were randomly assigned to CRI group (intravenous administration of DEX at 1 µg/kg/h up to the end of the diagnostic procedure) or SC group (DEX at 2 µg /kg every 60-minutes up to the end of the diagnostic procedure). The length of diagnostic procedures influenced the time of CRI (from 65 to 130 min) and the number of SC injections (2 or 3). Dexmedetomidine extraction from equine serum and quantification were performed following an intra-laboratory validated procedure in accordance with Decision 2002/657/EC [3, 4]. Pharmacokinetic analysis was carried out with a non-compartmental approach (Phoenix® WinNonLin 8.0). The HPLC-HRMS method was validated by the required parameters: decision limit (CCα), detection capability (CCβ), recovery, trueness, linearity, specificity, repeatability, and reproducibility. In particular, CCα and CCβ were 0.014 and 0.019 ng/mL, respectively. The recovery fell in a range between 99 and 103%, repeatability and reproducibility showed a CV% range of 6-9% and 6-20%, respectively. All the parameters considered for the validation were within the limits of acceptability.

Dexmedetomidine maximum concentration (C_{max}) were 0.83±0.27 ng/mL and 1.03±0.66 ng/mL for CRI group and SC group, respectively, and obtained at a time (T_{max}) of 57.00±13.37 min and 100.56±26.97 min, respectively. Area under the curve to the last concentration (AUC_{last}) was 63.70 ± 29.34 and 66.56 ± 30.86 min*ng/mL for CRI group and SC group, respectively. Mean residence time to the last concentration (MRT_{last}) was 11.71±6.18 and 52.95±18.20 min for CRI group and SC group, respectively. The elimination half-life was 101.52±70.57 min for SC group.

In conclusion, the validated analytical method is robust and appropriate for DEX quantification also when low concentrations are attained. The SC group showed more homogenous concentration-time profiles and positive kinetic behavior with concentration levels considered clinically effective [1].

[1] Shane et al. Pharmacokinetics and pharmacodynamics of intravenous continuous rate infusion and repeated intramuscular administration of dexmedetomidine in standing horses. Journal of Veterinary Pharmacology and Therapeutics, 00:1-11, 2021.
[2] Simeonova et al. Comparative study on sevoflurane anesthesia alone and combined with partial intravenous anesthesia using dexmedetomidine in healthy horses. Pakistan Veterinary Journal, 37:155-159, 2017.
[3] Cagnardi et al. Pharmacokinetics and sedative effects of dexmedetomidine in dairy calves. New Zealand veterinary journal, 65:14-18, 2017.

Advancing green veterinary pharmacology (GVP) towards the control of gastrointestinal nematodes: the example of *Punica granatum* acqueous extract

Fabio Castagna (1), Vincenzo Musella (1), Ernesto Palma (1,2,3), Cristian Piras (1), Enrico Gugliandolo (4), Rosalia Crupi (4), Vincenzo Musolino (1,2), Antonio Bosco (5), Giuseppe Cringoli (5), Domenico Britti (1)

(1) Dipartimento di Scienze della Salute, Università "Magna Græcia" di Catanzaro, Italia (2) Centro di Ricerche sulla Sicurezza degli Alimenti e la Salute – IRC-FSH, Catanzaro, Italia (3) Nutramed S.c.a.r.l. Complesso Ninì Barbieri, Roccelletta di Borgia, Catanzaro, Italia (4) Dipartimento di Scienze Chimiche, Biologiche, Farmaceutiche ed Ambientali, Università degli Studi di Messina, Italia (5) Dipartimento di Medicina Veterinaria e Produzioni Animali, Università degli Studi Federico II, Napoli, Italia

Corresponding author: Cristian Piras (c.piras@unicz.it)

Parasites and, in particular, gastrointestinal nematodes (GIN) represent one of the main threats affecting small ruminants health, welfare, productivity and reproduction. GIN control programs are primarily based on a combination of animal management practices and on the use of anti-parasitic drugs. The intensive use of synthetic anthelmintic drugs for treating and controlling GINs infestation in sheep farms generated a high rate of single- and multi-drug resistance (MDR) worldwide. High levels of resistance to Levamisole (LEV) and Ivermectin (IVC) have been reported in the Italian territory highlighting antielmintic resistance as one of the problems to solve in the near future. Even if such a problem never represented a serious threat in southern Italy because of the favourable environmental conditions and because of the good farming management, the phenomenon is actually showing a steep increasing trend and requires alternative treatment measures and constant monitoring.

The use of phytotherapies is considered a valuable approach for nematodes control in small ruminants and could help with reducing the amount of synthetic drugs used and the forthcoming anthelmintic resistance. From this perspective, calabrian territory, offers a wide number of plants with anthelmintic efficacy that could be helpful for this purpose.

The work here presented shows the evaluation of the effectiveness of pomegranate (*Punica granatum*) aqueous extract to GINs control. The potential beneficial effect was evaluated in parallel in sheep naturally infected with GINs, using as positive control the treatment with ivermectin and albendazole (approval number 97 of 09/10/2015).

The two comparisons were performed in separated farms and evaluated as faecal eggs count reduction (FECR) percentage. The positive control treatment with ivermectin in the first farm showed a 100% FECR reduction after 14 days and a 92% reduction after 21 days. The positive control treatment with albendazole in the second farm showed a 100% reduction after 14 days of treatment and around 95% reduction after 21 days. Pomegranate aqueous extract, in both separated cases, showed a consistent reduction of FECR% of around 50% persisting from day 7 to day 21 after the beginning of the treatment. Previous studies highlighted the presence of gallic acid as main component in the methanol extract of pomegranate. Its efficacy in nematodes control has been as well previously demonstrated in other plants extracts.

With this described evidence we demonstrated the unequivocal efficacy of and endemic plant extract in easily reducing by 50% the growth of GINs. Such a result was obtained without the use of any synthetic drug and draws the way towards the use of green veterinary pharmacology (GVP) as natural and alternative method to avoid the use of chemicals and to contrast the aforementioned increasing resistance phenomena.

Snail secretion filtrate: health care from veterinary to humans

Rosalia Crupi (1), Alessio Filippo Peritore (2), Patrizia Licata (1), Cristian Piras (3), Domenico Britti (3), Salvatore Cuzzocrea (2), Enrico Gugliandolo (1)

(1) Università degli Studi di Messina, Dipartimento di Scienze Veterinarie, Viale Annunziata, Messina, Italia (2) Università degli Studi di Messina, Dipartimento di Scienze Chimiche, Biologiche, Farmaceutiche ed Ambientali, Viale Ferdinando Stagno D'Alcontres, 31 Messina, Italia (3) *Università degli Studi* "Magna Græcia" *di Catanzaro*, Dipartimento di Scienze della Salute, CISVetSUA, Catanzaro, Italia

Corresponding author: Rosalia Crupi (rcrupi@unime.it)

Snail has been used in medicine since antiquity and prepared according to several formulations. The snail secretion or mucus is a dense mucus that covers the external surface of the snail. The mucus has various functions for the life of snails with his adhesive, emollient, protective and reparative proprieties. Various snail "preparations" were recommended for external use with dermatological disorders and internally for symptoms associated with tuberculosis and nephritis. Research on the secretions of *Helix aspersa* Müller confirmed that the mucus contains an unusual combination of natural ingredients with beneficial and therapeutic qualities for skin, including allantoin and glycolic acid [1]. It has been seen that the snail mucus components were able to stimulated the formation of dermal components, in particular the formation of collagen and elastin, and to minimized the damage generated by oxidative stress and free radicals [2]; moreover, it has shown to possess other important biological properties such as antimicrobial activity and protective effect in wound repair.

To investigate the protective effect of snail secretion of *Helix aspersa* Müller on the epithelial barriers, and in particular in pro-epithelizing we performed two experimental protocols involving different substrates to analyzed the effect of mucus in different pathological conditions (approval number (650/2017-PR) of 21/8/2017). In the first step we used a murine model of ethanol intragastric administration which has been widely employed to test the drugs efficacies and to explore the underlying mechanism for gastric ulcer development; the intragastric ethanol administration causes mucosal damages and an induction of a severe inflammatory response. In the second we used a murine model of excisional wound repair. Our results show a significant protective effect of snail secretion filtrate in reducing macroscopic and histological lesions, as well as the protective effect on mucus content, oxidative stress (evaluated through the MDA, CAT and SOD in ethanol-induced acute ulcer model, whereas MMPs were investigated by western blot analysis in excisional wound repair) and inflammatory response (evaluated through ELISA assays for cytokines such as IL-6, IL-1β, TNF-α in ethanol-induced acute ulcer model and IL-1β, TNF-α and TGF-β in excisional wound repair model) demonstrating the protective effect of snail secretion filtrate in both experimental models.

These findings suggest that *Helix aspersa* extract is a natural, safe and effective alternative treatment in the management of damaged epithelial barriers.

[1] Nj N et al. Anti-ulcer potentials of phylum mollusca (tropical snail) slime, Asian Pac J Trop Med 2, 23–28, 2009.
[2] Tsoutsos D et al. The efficacy of *Helix aspersa* Müller extract in the healing of partial thickness burns: a novel treatment for open burn management protocols, J Dermatol Treat 20, 219–222, 2009.

In vitro anthelmintic efficacy of *Brassica incana* leaves extract against gastrointestinal nematodes of sheep

Monica Ragusa (1), Maria Fernanda Taviano (2), Fabio Castagna (3), Vincenzo Mollace (3), Giuseppe Cringoli (4,5), Antonio Bosco (4,5), Vincenzo Musella (3), Natalizia Miceli (2), Domenico Britti(3)

(1) Università degli Studi "Magna Graecia" di Catanzaro, Dipartimento di Medicina Sperimentale e Clinica (2) Università degli Studi di Messina, Dipartimento di Scienze Chimiche, Biologiche, Farmaceutiche e Ambientali (3) Università degli Studi "Magna Graecia" di Catanzaro, Dipartimento di Scienze della Salute (4) Università degli Studi di Napoli Federico II, Dipartimento di Medicina Veterinaria e Produzioni Animali (5) Centro Regionale per il Monitoraggio delle Parassitosi (CREMOPAR), Regione Campania.

Corresponding author: Monica Ragusa (m.ragusa@unicz.it)

Gastrointestinal nematodes (GINs) are amongst the most important ruminant production-limiting pathogens with negative impact also on animal welfare and health [1], aggravated by increasing anthelmintic resistance [2]. Therefore, the progressive anthelmintic resistance in livestock requires evaluation of alternative remedies for parasite control, as the use of medicinal plants. *Brassicaceae* are known as important sources of bioactive compounds, such as carotenoids, tocopherols, ascorbic acid, glucosinolates, and phenolic compounds [3,4,5]. The aim of this study is to evaluate the *in vitro* anthelmintic efficacy of a hydroalcoholic extract obtained from the leaves of *Brassica incana* (*Brassicaceae*) grown wild in Sicily (Italy). For this purpose, egg hatch test was used to verify anthelmintic efficacy against GINs of sheep. The study was conducted using faecal samples collected from sheep naturally infected with GINs. In order to identify the GIN genera, larval cultures were performed and showed that the genera of nematodes present were: *Trichostrongylus* spp. (43%), followed by *Haemonchus contortus* (23%), *Teladorsagia* spp. (21%), *Chabertia ovina* (7%) and *Cooperia* spp. (6%). The extraction was performed with 70% MeOH in an ultrasonic bath (50°C, 15min) four times and the filtrates were combined and evaporated to dryness by rotavapor. The obtained dry extract was diluted in deionized water and assayed at different concentrations of 1.00, 0.5, 0.25, 0.125 mg/mL; thiabendazole (0.5 and 0.25 mg/mL) and deionized water were used as positive and negative controls, respectively. The results obtained from in vitro test indicated that *B. incana* extract caused highly significant ($p<0.0001$) inhibition of egg hatching within 48 hours of exposure, showing efficacy (≥89%) at all doses tested. In particular, the extract of *B. incana* showed an egg hatch inhibition efficacy of 95.3%, 94.6%, 93.3% and 89% at concentrations of 1, 0.5%, 0.25%, and 0.125 mg/mL, respectively, while the efficacy of thiabendazole, used as a positive control, was 99.3% and 96.7% at concentrations of 0.5 and 0.25 mg/mL, respectively. A previous study showed that the extract exhibited non-toxic against brine shrimp larvae (*Artemia salina* Leach), indicating its potential safety [5].

The current study has highlighted the anthelmintic potential of the leaves hydroalcoholic extract of *B. incana* against GIN infection in sheep, justifying the need to undertake further *in vivo* studies on this medicinal plant.

[1] Charlier J et al. Initial assessment of the economic burden of major parasitic helminth infections to the ruminant livestock industry in Europe. Prev Vet Med.;182:105103, 2020.
[2] Rose Vineer H et al. Increasing importance of anthelmintic resistance in European livestock: creation and meta-analysis of an open database. Importance croissante de la résistance aux anthelminthiques chez les ruminants européens : création et méta-analyse d'une base de données ouverte. Parasite, 27:69, 2020.
[3] Jahangir M. et al. Health-a_ecting compounds in *Brassicaceae*. Compr. Rev. Food Sci. Food Saf., 8, 31–43, 2009.
[4] Cartea M.E. et al. Phenolic compounds in Brassica vegetables. Molecules, 16, 251–280, 2011.

Analytical validation of a commercial enzyme-linked immunosorbent assay for the quantification of ovotransferrin in broiler droppings

Claudia Zizzadoro (1), Valentina Meucci (2), Olimpia Lai (1), Nicola Pugliese (1), Giuseppe Crescenzo (1)

(1) Università degli Studi di Bari Aldo Moro, Dipartimento di Medicina Veterinaria (2) Università di Pisa, Dipartimento di Scienze Veterinarie

Corresponding author: Claudia Zizzadoro (claudia.zizzadoro@uniba.it)

Intestinal health is critically important to the welfare and performance of livestock and the identification of biomarkers that can allow its non-invasive monitoring under field conditions has become a research hot topic [1]. In this view, Goossens et al. [2] have recently reported that faecal ovotransferrin (OTF) may represent a valuable biomarker for some intestinal diseases in broilers. This evidence was obtained by measuring OTF concentrations in the colon content of intestinally healthy and diseased animals, as well as in litter samples, by means of a commercial enzyme-linked immunosorbent assay (ELISA) kit (Kamiya Biomedical Company). However, this kit is validated only for serum/plasma samples, and the protocol for collecting and processing the litter samples (which are more field-relevant than individual intestinal contents) was poorly described.

Since the availability of standardized and validated methods for sample preparation and analysis is prerequisite for reliable biomarker data [3], the present work was performed to evaluate the analytical performance of the commercial ELISA kit used by Goossens et al. [2] for the quantification of OTF in broiler droppings processed according to a thoroughly developed protocol. More specifically, 4 pools of fresh droppings were collected from clinically healthy broilers of 4 different farms (Ethics Approval no. 16-2020), each pool was cleaned of non faecal contaminants, homogenized in phosphate buffered saline and centrifuged, and the resulting supernatant was taken as dropping extract. Purified OTF protein standard was added at known final concentrations to either dropping extracts or homogenates in order to construct standard curves and determine dilutional linearity, spiking recovery, intra- and inter-assay variability. In addition, the assay lower limit of detection (LLOD) was established. Standard curves generated in dropping extracts assayed in a 1:50 dilution were almost identical to the reference curves generated in the assay buffer. The two-fold dilution of 4 samples generated with high OTF concentrations resulted in linear regression equations with correlation coefficients consistently >0.99. The observed to expected ratios for spiking recovery ranged from 84.9 to 112.2% (mean±SD: 94.2±8.1%) for the 4 different dropping extracts at 3 different spiking concentrations (high, medium, low). The intra- and inter-assay coefficients of variations for samples with high, medium and low OTF concentrations did not exceed the 15% limit. The LLOD, calculated as indicated by Goggs et al. [4], was 1.2 ng/ml. Overall these results indicate that the commercial ELISA kit evaluated in the present study is able to measure OTF in droppings from broilers with acceptable sensitivity, accuracy and precision. Future studies using this now validated analytical tool will determine the actual usefulness of measuring OTF in droppings as a non-invasive biomarker of intestinal health in broilers.

[1] Niewold T. Intestinal Health. Wageningen Academic Publishers, 2015.
[2] Goossens et al. Elevated faecal ovotransferrin concentrations are indicative for intestinal barrier failure in broiler chickens, Veterinary Research, 49:51, 2018.
[3] Khan et al. Recommendations for adaptation and validation of commercial kits for biomarker quantification in drug development, Bioanalysis, 7:229-42, 2015.
[4] Goggs et al. Plasma procalcitonin concentrations are increased in dogs with sepsis, Veterinary Record, 5:e000255, 2018.

ORAL COMMUNICATIONS

SIRA

Transcriptomic profile of sheep cumulus cells upon cadmium exposure during oocyte in vitro maturation

Nicola Antonio Martino (1,2), Luisa Bogliolo (3), Valeria Palumbo (2); Federica Ariu (3), Antonella Mastrorocco (2), Ernesto Picardi (2), Annamaria D'Erchia (2), Graziano Pesole (2), Maria Elena Dell'Aquila (2)

(1) Università degli Studi di Torino, Dipartimento di Scienze Veterinarie (2) Università degli Studi di Bari, Dipartimento di Bioscienze, Biotecnologie e Biofarmaceutica (3) Università degli Studi di Sassari,
Dipartimento di Medicina Veterinaria

Corresponding author: Nicola Antonio Martino (nicolaantonio.martino@unito.it)

Cadmium (Cd) is one of the most important environmental pollutants in industrialized countries. It has considerable toxicity and negatively affects female reproduction through various mechanisms: it alters oocyte and follicular development, interferes with the hypothalamus-pituitary-ovary axis, reduces steroidogenesis and can lead to pregnancy complications. We previously reported that Cd supplementation, at nanomolar concentrations, during *in vitro* maturation (IVM) affects cumulus-oocyte complex (COC) viability and oocyte fertilization in adult and prepubertal sheep [1] but its action mechanisms need to be further investigated. The aim of the present study was to determine whether exposure to 100 nM Cd during IVM affects the transcriptomic profile of cumulus cells (CCs) in adult sheep. COCs were recovered from the ovaries of slaughtered adult (2-8 years) sheep and individually *in vitro* cultured in maturation medium [1] for 24h at 38.5°C under 5% CO_2. After IVM, CCs of both groups were removed and only those isolated from matured oocytes were pooled (CCs from 20-25 COCs/sample) and stored at -80°C until analysis. Ten samples, 5 Cd-treated vs 5 controls, were processed for RNA extraction, library preparation and deep transcriptome sequencing. Raw reads were aligned onto the reference genome (Oar_v3.1 from UCSC) using STAR (PMID: 23104886). Differentially expressed genes (DEGs) were detected using the DESeq2 R package (PMID: 25516281), selecting genes with a log2 fold-change >1.5 or <-1.5. Several DEGs were upregulated upon Cd exposure. Of them, CYP19A1 (aromatase) is involved in steroidogenesis, NOS2 in inflammatory response, IGFBP4 in signal transduction, BRCA1 and HELLS in cell cycle regulation, DNA repair and chromatin modification. In conclusion, these data expand knowledge on molecular mechanism underlying the Cd-induced damage in CCs of matured oocytes and contribute to the identification of potential non-invasive biomarkers of Cd-induced damage in animals and human oocyte.

Funded by Italian Ministry of Health: Project GR-2011-02351396.

[1] Martino et al., Reproductive Toxicology, 69:132-145, 2017.

Hair cortisol and dehydroepiandrosterone concentrations in postpartum beef cows: comparison between pregnant and non-pregnant cows

Monica Probo (1), Massimo Faustini (1), Tanja Peric (2), Isabella Pividori (2) Maria Cristina Veronesi (1)

(1) Università degli Studi di Milano, Dipartimento di Medicina Veterinaria (2) Università degli Studi di Udine, Dipartimento di Scienze agroalimentari, ambientali e animali

Corresponding author: Monica Probo (monicaprobo@gmail.com)

Hair steroids measurement has received increasing attention for chronic stress assessment in cows, as it offers the advantages of being noninvasive, simple, and able to indicate steroids concentrations over long periods. Cortisol (C) and dehydroepiandrosterone (DHEA) are hormones secreted by the adrenal cortex related to resilience and allostatic load. In contrast to C, that is considered the primary stress hormone, DHEA has shown anti-inflammatory and antioxidant properties. Moreover, DHEA represents an essential pro-hormone in ovarian follicular steroidogenesis. It has been proposed that stress can impair the reproductive function of animals [1]; therefore, the object of this study was to investigate C and DHEA hair concentrations in postpartum cows, to assess possible differences in relation to reproductive outcome (pregnant vs non-pregnant). Eleven healthy late pregnant crossbred beef cows were enrolled. Hair samples were collected from the 11 cows by shaving the hair at the level of the shoulder at calving (T1) and every 20 days for six times (T2-T7) until 120 days post-partum (pp), only on the re-growth area. Hair C and DHEA were analyzed by RIA [2,3]. Starting from the 4th week postpartum, cows were checked for estrus detection and submitted to artificial insemination. Seven out of the 11 cows became pregnant (P), with a mean calving to conception interval of 82 (±18) days, while 4 cows were non-pregnant (NP). Statistical analysis (two-way ANOVA followed by independent samples T-test) revealed differenced in C hair concentrations among sampling times ($p<0.05$), with a trend of decrease from calving in agreement with previous findings [4]. No differences in C hair levels were found in relation to reproductive outcome; this latter result is in contrast with a previous study [4], in which lower C hair levels were found in cows that became pregnant by 100 days pp when compared to cows that were not pregnant by 100 days pp. Regarding DHEA hair concentrations, no differences were detected among sampling times, while significant differences were found between P and NP ($p<0.001$); specifically, P cows showed higher DEHA hair concentrations at T2 (20 days pp, $p\leq0.05$), T3 (40 days pp, $p<0.05$) and T4 (60 days pp, $p<0.05$) compared to NP cows. The present results suggest that higher DHEA hair concentrations at 20-60 days pp in crossbred beef cows may be related with higher chances of conception after artificial insemination.

[1] Dobson H, Smith RF. What is stress, and how does it affect reproduction? Animal Reproduction Science, 60–61:743-752, 2000.
[2] Peric T, Comin A, Corazzin M, Montillo M, Cappa A, Campanile G, Prandi A. Hair cortisol concentrations in Holstein-Friesian and crossbreed F1 heifers, Journal of Dairy Science, 196:3023-3027, 2013.
[3] Montillo M, Rota Nodari S, Peric T, Polloni A, Corazzin M, Bergamin C, Balestrieri A, Prandi A, Comin A. Steroids in pig hair and welfare evaluation systems: combined approaches to improve management in pig breeding? Veterinaria Italiana, 56(3):177-184, 2020.
[4] Burnett TA, Madureira AML, Silper BF, Tahmasbi A, Nadalin A, Veira DM, Cerri RLA. Relationship of concentrations of cortisol in hair with health, biomarkers in blood and reproductive status in dairy cows, Journal of Dairy Science, 98:4414-4426, 2015.

Evaluation of stiffness testes in healthy dogs by point and 2-D share wave elastography: preliminary results

Letizia Sinagra (1), Cyndi Mangano (2), Silvia Santoro (2), Nicola Maria Iannelli (1), Santo Cristarella (1), Marco Quartuccio (1), Massimo De Majo (1)

(1) Department of Veterinary Sciences, University of Messina, Italy (2) Camagna Veterinary Clinic, Reggio Calabria, Italy

Corresponding author: Letizia Sinagra (letizia.sinagra@gmail.com)

Ultrasonography-based Elastography is a simple and non-invasive technique that may be used to assess the elasticity of tissue. Two technical solutions are available for clinical use: elastography by acoustic radiation force impulse (ARFI), which uses an acoustic stimulus, and strain elastography (SE), which uses a pulsed compression stimulus caused by the operator [1]. ARFI can be divided into point shear wave elastography (p-SWE) and 2-D shear wave elastography (2-D SWE) [2]. Previous data of ARFI elastography of testes in healthy dogs were provided using SIEMENS ultrasound equipment [3].

The aim of this study is to describe testicle tissue stiffness in healthy dogs by p-SWE and 2-D SWE, using a MINDRAY DC-80 A ultrasound equipment.

Ten male dogs of different breeds (4 Italian Pointing, 4 Pointer, 1 Spinone, and 1 Drahthaar), aged between 2 and 10 years and weighing between 18 and 31 kg, of proven fertility (at least one litter in their history) were included. The animals' health was established based on general and specific physical examination (inspection and palpation of the scrotum) and blood analysis. Ultrasound studies were performed by a single operator. No sedation is needed. This study was conducted following the approval of the Ethical Committee of the Department (approval no. 051/2021). B-mode echotexture, size and contours of testes were assessed and findings were normal. Color and power doppler were performed in testes, epididymis and spermatic cord. The stiffness tissue was evaluated by SWE with a linear transducer (L12-3E). The 2-D SWE showed a qualitative evaluation, by a color code (blue for harder areas and red for softer areas). For each testis four regions of interest (ROIs) of 0.5 cm^2 at the same depth were chosen (2 above the hyperechoic line of mediastinum and 2 under that) to value them for kPa and m/s measurements. The p-SWE provided real-time measurements of shear wave diffusion in six different points in the center of 3 squared ROIs of 0.5x0.5 cm (3 above the mediastinum and 3 under that). Measurements were obtained in kPa and m/s. SWE values of ROI were: left testes 8,83 kPa (above mediastinum) and 9,36 kPa (under mediastinum), respectively 1.6 m/s and 1.7 m/s; right testes 7.9 kPa and 8,14 kPa, respectively 1.60 m/s and 1.64 m/s. SWE single point values were: left testicle 15.59 kPa, 13.31 kPa, 12.11 kPa (above mediastinum) and 19.39 kPa, 15.46 kPa, 17.08 kPa (under mediastinum); right testes 13.41 kPa, 11,10 kPa, 14,59 kPa and 18.71 kPa, 12,08 kPa, 17.58. The same values in m/s were left testes 2.23 m/s, 1.89, 1.81, 2.17 m/s, 2.17 m/s, 2.32 m/s; right testes 1.99 m/s, 1.67 m/s, 1.99 m/s and 2.43 m/s, 2.01 m/s, 2.42 m/s.

The data obtained show different results: p-SWE data emerge from a single point while 2D-SWE data derive from an average of the values of the ROIs. Data obtained in this study were different from those previously provided by Feliciano et al., maybe due to the different weights of the subjects chosen and the different equipment used, so reference data must be obtained for both.

[1] Sigrist RMS et al. Ultrasound Elastography: review of techniques and clinical applications. Theranostics, 7:1303–1329, 2017.

[2] Ferraioli G et al. Liver ultrasound elastography: an update to the world federation for ultrasound in medicine and biology guidelines and recommendations. Ultrasound in Med. & Biol., 44, 12:2419-2440, 2018.

Effect of different farrowing system on sow and piglet welfare: are we doing well?

Carla Bresciani (1), Giusy Romano (2), Righi Federico (1), Mara Bertocchi (1), Francesco Di Ianni (1), Bigliardi Enrico (1), Alberto Prandi (3), Claudio Mazzoni (2)

(1) Università di Parma, Dipartimento di Scienze Medico Veterinarie (2) Università di Udine, Dipartimento di Scienze Agroalimentari, Ambientali e Animali 3) Suivet snc – Reggio Emilia

Corresponding author: Carla Bresciani (carla.bresciani@unipr.it)

In the pig industry, labour efficiency and animal welfare have become two of the most important factors for achieving technical goals and farming competitiveness. Reducing pre-weaning piglet mortality is one of the ways to improve the total number of piglets weaned/sow/year under swine industry conditions (Mazzoni et al., 2017). The design of farrowing crates is involving in the mortality and the possible choice are to reduce the sow's mobility, saving piglets from crushing; or to allow more space for both (Buoio et al. 2020). Even if the sow's welfare is a controversial condition, cortisol serum level is a physiological parameter for the stress condition and welfare in all mammals (Scollo et al., 2019). This study aimed to compare the effects of four different designs of farrowing crates on the piglet's survival and serum cortisol level of the lactating sows. In a farrowing unit of an intensive herd located in the Po valley, from January to July 2018, corresponding to five farrowing batches: B1 to B5, 208 pregnant sows (gilts excluded) were housed in the following farrowing crates: conventional (group A=50), up&down device (group B=48), with a slide (group C=35) and liberty (group D=41). For each sow parity order, farrowing date, live born, stillbirths, mummified piglets and total mortality were reported on an individual data sheet. The subsequent piglet deaths were recorded and investigated. For each group within the same batch, a statistically representative sample of two litters were weighted at 2 - 16 - 27 days, such and ten lactating sows were sampled from the mammary vein at 3 days from parturition and the day before weaning for cortisol investigations. Statistical analysis was performed using IBM SPSS® with crate groups and batches as fixed factor; piglet weights and serum cortisol levels were analyzed adopting a mixed model for repeated measures. A total of 2766 live born piglets were considered (A=791, B=755, C=533, D=687); 546 piglets weighted (A=126, B=112, C=112, D=169) and 56 lactating sows were investigated for serum cortisol level (A=16, B=15 C=10, D=15). The mean number of live born piglets did not differ significantly. The total piglet mortality reported was 1,1 and 0,83 in group A and C respectively, while was lower in group B, especially for crushing, compared to group D (0.13 vs 1.73; $P<0.05$). The mean number of deaths for other causes (starvation, neonatal diarrhea, *Streptococcus* spp. infections, iatrogenic) for the batch B5 (0.96) was significantly different ($P=0.01$) to that for the batches B1 (0.28) and B4 (0.26). Mean of piglets weaned/sow differs significantly differed for crates and seasonality: group B reported the better results (13.11 piglets/sow), the group D was the worst (12.21 piglet/sow). The mean piglet weight at weaning was higher ($P<0.001$) in group D (6.97 kg) compared group B (6.36 kg) and group C (6.26 kg). Serum cortisol levels did not differ between the two samples and among the crate groups, while a significant difference ($P<0.01$) was reported for the batch B4 with increased mean cortisol value (21.66 ng/ml). The better piglet survival was reported in the up&down farrowing crates with confined sow, while the liberty designed reported worst value of mortality, but weaned heavier piglets. The cortisol results revealed that sow welfare is affected by seasonality and not by confinement. In conclusion, future investigations are still needed on swine farrowing unit, to improve animal welfare condition and productivity, mainly focusing on seasonality.

[1] Mazzoni C., et al. Effects of three different designed farrowing crates on neonatal piglets crushing: preliminary study, Italian Journal of Animal Science, 17:2, 505-510, 2017.

The menstrual cycle of the Baboon (*Papio hamadryas*) evaluated by vaginal cytology and hormonal variations

Maria Albrizio[1], Annarita Imperante[1], Salvatore Desantis[1], Mario Cinone[1], Piero Laricchiuta[2], Nicoletta Santamaria[1], Luca Lacitignola[1]

[1]Università degli Studi di Bari-Aldo Moro, Dipartimento dell'emergenza e dei trapianti di organi (DETO) [2] *Zoosafari-Fasano, Brindisi, Italy*

Corresponding author: Maria Albrizio (maria.albrizio@uniba.it)

Hamadryas baboons (*Papio hamadryas*) form part of a highly successful branch of the primate family (*Cercopithecidae*), commonly referred to as savannah baboons. They are broadly distributed over the African continent, and the Arabian Peninsula inhabiting the semiarid regions [1]. Baboons are menstruating primates, the average length of a normal reproductive cycles is 32 (24-38) days with close similarity to women's [2]; so that the baboon is considered a valuable model for increasing knowledge to overcome human reproductive disorders.

This study aims s to acquire knowledge on the modifications of vaginal cytology and Fallopian tubes during the menstrual cycle of females living in captivity. The stage of the menstrual cycle of 14 healthy adult females of different ages (8.5 yrs) and weights (10.5 kg) on the day of laparoscopic salpingectomy has been evaluated. The baboons breed with high fertility rates in captivity so that laparoscopic salpingectomy was employed as irreversible contraceptive surgical therapy for an extensive control birth program in the Safari Zoo (Fasano - BR). The clinical activity was authorized with written informed consent by the Zoo's property (Lion 3000 S.p.a.) and obtained the favorable opinion of the ethics committee of DETO (05/2020). The stage of the reproductive cycle was analyzed by 1) vaginal cytology (Harris-Shorr's and Diff-Quik staining), 2) endocrine changes measuring 17β-estradiol (E2) and progesterone (P4) concentrations in peripheral plasma by ELISA, 3) histological uterine tubes morphology. The stage of the cycle can be also approximated by external observation of the perineum so that the perineal turgescence, characteristic of the follicular phase, was checked during the observation of external genitalia. Laparoscopic evaluations monitored the presence of corpora lutea and the Graafian follicle and when ovulation had occurred, the increased vascularized fimbriae too. Changes in the type of vaginal cells (basal and parabasal, small and large intermediate, anuclear keratinized, erythrocytes, neutrophils) found during the follicular and luteal phases (early and late) of the ovarian cycle were analyzed. Based on hormonal evaluations and cytological observations we found 9/14 subjects in the follicular phase (mean E2 concentration 150±73 pg/ml; mean P4 concentration 0.3±0.1 ng/ml; presence of small and large intermediate, anuclear keratinized cells) and 5/14 females in the luteal phase (mean E2 concentration 50±20 pg/ml; mean P4 concentration 4±0.5 ng/ml; presence of basal and parabasal cells, neutrophils) of their reproductive cycle. Histological investigations revealed morphological and morphometric changes in the uterine tube segments related to the stage of the menstrual cycle. In addition, a different degree of hyperemia was observed in the infundibulum between follicular and luteal stages. The acquired knowledge could be the starting point to propose a hormonal contraceptive strategy for baboons living in captivity with little effect on social interactions such as grooming relationships, aggression, affiliation and sexual behavior while reducing their reproductive success.

[1] Newman TK, Jolly CJ, Rogers J. Mitochondrial phylogeny and systematics of baboons (*Papio*). American Journal of Physical Anthropology, 124: 17-27, 2004.
[2] Stevens VC, Sparks SJ, and Powell JE. Levels of estrogens, progestogens, and luteinizing hormone during the menstrual cycle of the baboon. Endocrinology, 87: 658, 1970.

Possible role of plasma procalcitonin as diagnostic biomarker of metritis in dairy cows: preliminary results

Alessio Madrigali (1), Francesca Bonelli (1,2), Valentina Meucci (1), Fabrizio Bertelloni (1), Duccio Panzani (1,2)

(1) Università di Pisa, Dipartimento di Scienze Veterinarie (2) Università di Pisa, Centro di Ricerche Agro-Ambientali E. Avanzi

Corresponding author: Alessio Madrigali (alessio.madrigali@phd.unipi.it)

Puerperal metritis is a bacterial infection of all the layers of the uterus, characterized by a red-brownish fetid vaginal discharge, abnormally enlarged uterus, with or without systemic signs of illness, occurring within the first 21 days after calving [1]. Metritis has a huge impact on dairy profitability, reducing milk yield and feed intake, impairing reproductive performance, causing severe visceral pain, and increasing culling rate [2]. Considering the economic and welfare relevance of metritis, the development of new diagnostic strategies for an accurate and early diagnosis are fundamental.

Inflammatory biomarkers could be a solution to the problem above, in fact haptoglobin have been found to be already an effective biomarker of metritis [3]. Procalcitonin (PCT) has been widely recognized as an effective biomarker of bacterial infections in human medicine [4]. In veterinary medicine, PCT has been investigated as a biomarker of bacterial infections in horses, foals, and dogs; few studies have been conducted in ruminants [5].

The aim of this preliminary study was to evaluate the plasma PCT concentration in healthy dairy cows during the first 21 days after calving. The study was conducted in clinical setting and approved by the University of Pisa ethical committee (2825/2014). All the cows enrolled were daily submitted to a complete physical examination from calving until day 21 after calving; vaginal discharge for each cow was assessed by vaginoscopy every 3 days. Cows presenting a normal physical examination and a physiological vaginal discharge during the first 21 days after calving were considered healthy and included in the study. Blood samples were collected from the coccygeal vein on day 3, 7, 14 and 21 after calving for plasma PCT determination. Procalcitonin concentrations were determined with a commercial kit for human species (Human Procalcitonin ELISA kit, Aurogene, Italy), previously validated.

A total of 15 Italian Friesian healthy cows were included in the study. The median plasma PCT concentration was 0 (0-288.5) pg/ml on day 3, 0 (0-262.5) pg/ml on day 7, 0 (0-237.5) pg/ml on day 14, and 0 (0-248.5) pg/ml on day 21. Our results about plasma PCT levels in healthy cows were even lower compared to previous findings in healthy calves [6]. Very low plasma PCT concentrations in healthy cows during the first 21 days after calving were very encouraging results for the potential use of PCT as a diagnostic biomarker of metritis. Further studies are needed to evaluate the plasma PCT concentrations in cows with clinical and puerperal metritis, comparing them with levels in healthy animals.

[1] Sheldon et al. Defining postpartum uterine disease in cattle, Theriogenology, 65:1516-1530, 2006.
[2] Azawi O.I. Postpartum uterine infection in cattle. Animal Reproduction Science, 105:187-208, 2008.
[3] Huzzey et al. Short communication: Haptoglobin as an early indicator of metritis, Journal of Dairy Science, 92:621-625, 2009.
[4] Riedel S. Procalcitonin and the role of biomarkers in the diagnosis and management of sepsis. Diagnostic Microbiology and Infectious Disease, 73:221-227, 2012.
[5] Matur et al. Biology of procalcitonin and its potential role in veterinary medicine, Journal of Istanbul Veterinary Sciences, 1:16-27, 2017.
[6] Bonelli et al. Plasma procalcitonin concentration in healthy calves and those with septic systemic inflammatory response syndrome. The Veterinary Journal, 234:61-65, 2018.

Does follicular microbiome affect oocyte competence? Preliminary observations

Slavcho Mrenoshki [1], Letizia Temerario [2], Antonella Mastrorocco [3], Marialaura Corrente [4],
Daniela Mrenoshki [4], Giovanni Michele Lacalandra [4], Maria Elena Dell'Aquila [2]

[1] Ss. Cyril and Methodius University in Skopje, Faculty of Veterinary Medicine/Visiting professor at University of Bari Aldo Moro, Department of Biosciences, Biotechnologies and Biopharmaceutics [2] University of Bari Aldo Moro, Department of Biosciences, Biotechnologies and Biopharmaceutics [3] University of Teramo, Faculty of Veterinary Medicine [4] University of Bari Aldo Moro, Department of Veterinary Medicine

Corresponding author: Slavcho Mrenoshki (mrenoski@fvm.ukim.edu.mk)

It is known that microbiome and its secondary metabolites could influence different physiological functions locally and in the distant parts of the body, either in a positive and negative way which depend of its composition [1]. Majority of the studies in this perspective are performed on the gut microbiome as most abundant, diverse and important body community, but their own, specific colonizers have other animal body areas including reproductive tract. The female reproductive tract microbiome studies are mostly focused on its lower parts (primary vagina, but also uterus) [2], while the reports in scientific databases for microbiota composition and reproductive physiology/pathology influence in distant (upper) niches, such ovaries and follicular fluid, are rare, inconsistent and mostly focused on ovarian cancer. Also, there is a lack of experimental literature data about possible effects of microbiome and its metabolites in reproductive processes simulated in IVF technologies. On the other hand, oocytes from prepubertal livestock can be used for juvenile *in vitro* embryo transfer (JIVET) programs, but their competence is lower than that of their adult counterpart. The reasons are certainly multifactorial, and we hypothesized that one of the possibilities could be the absence of interaction with metabolites present in follicles of adult females, including the microbial. In this study, we try to achieve two goals: to detect presence of microbiota in follicular fluids (FF) of adult sheep ovaries (ASO), and to evaluate any notifiable influence of its metabolites on *in vitro* prepubertal lamb oocyte (PLO) competence**.** The ASO FF were collected from healthy slaughtered animals by aspiration from a dominant follicle, or 2-3 largest follicles if the dominant one was absent. The follicular fluids were inoculated in nutrient-rich liquid medium (nrLM) to propagate enrichment and metabolite production of eventually present microbiota. Mediums with visible bacterial growth after 7 days of incubation**,** were centrifuged and supernatants (FF-S) collected**.** Two selected FF-S (FF-S1 and FF-S2), as well as nrLM supernatant (negative control to determine the growth media influence), were then used as IVM-medium supplementation in JIVET procedure with PLO [3]. Although PLO maturation rate was not affected by added FF-S (68%, 39/57 and 66%, 31/47 in FF-S1 and FF-S2 wells respectively, vs. 63%, 37/59 for nrLM control, in 2 to 3 runs with 20-25 PLOs/condition), total embryo cleavage (1 run with 20-25 PLO/condition) in FF-S2 well was more than doubled (48%, 11/23), compared to the nrLM control (16%, 4/25) and FF-S1 (17%, 4/23). Aware that obtained data are preliminary and insufficient for definitive conclusions, results lead us to initial assumptions that FF originated from healthy ASO can be inhabited with bacteria, and that their metabolites could have a potential to influence the IVF process to some extent. Further, wider and deeper experimental approaches, should provide more specific data for both, follicular microbiome existence and composition, and its influence on physiology of reproductive processes and applied artificial reproductive technologies.

[1] Agus A, Clément K, Sokol H. Gut microbiota-derived metabolites as central regulators in metabolic disorders. Gut. 2020 Dec 3:gutjnl-2020-323071.

Clinical use of Anti-Müllerian Hormone to monitor resumption of ovarian activity following removal of a 4.7 mg deslorelin implant in queens

Romagnoli S[1]. Ferro S[2]., Contiero B[1]., Andretta F[1]., Cattai A[3]., Fontaine C[4]., Badon T[1]., Gelli D[1]. Ferré-Dolcet L[1].

[1] Department of Animal Medicine, Production and Health, University of Padova, Padova, Italy [2] Department of Comparative Biomedicine and Food Science, University of Padova, Padova, Italy [3] Concordia Veterinary Clinic. Portogruaro, Venezia, Italy [4] Virbac, Carros, France

Corresponding author: Ferré-Dolcet Lluis (lluisferredolcet@gmail.com)

Anti-Müllerian hormone (AMH) is reported to be highly specific and sensitive in discriminating neutering from entire status in animals [1]. Deslorelin implants are widely used in catteries due to their efficacy although their prolonged duration of inhibitory action on the reproductive system particularly in queens (18-22 months) [2] is often too much for cat breeders who frequently request early implant removal. However, the interval between deslorelin implant removal and resumption of ovarian function in queens is unknown. Assaying AMH concentrations might be a useful tool to predict time of resumption of ovarian activity in deslorelin-treated queens following implant removal.

Twenty-two privately owned healthy queens were treated with a 4.7 mg deslorelin implant placed in the periumbilical area. In the 15 queens completing the study implants were surgically removed 3, 6 or 9 months (n=6, 3 and 6 queens, respectively) post-administration. Queens received a GnRH stimulation test as part of their pre-treatment general and reproductive health check and were implanted during postestrus-anestrus or proestrus-estrus. Starting 7-14 days following implant removal queens were checked every 1-2 weeks with an ultrasonographic exam of the reproductive tract, a vaginal smear and blood collection to assay AMH concentrations. Following implantation, all queens in postestrus-anestrus at the time of treatment came in estrus within 2-5 days. When compared to pre-treatment levels AMH concentrations decreased significantly during treatment to $\leq$2.4+0.7 ng/ml ($p \leq 0.05$) and reached a nadir at 1.7$\pm$0.9 ($p<0.05$) one-week post-removal. Following implant removal AMH concentrations started to rise reaching a value of 4.1$\pm$0.8 ng/ml on the third week and were not different from pre-treatment levels on week 6 post-removal (5.8 ng/ml +0.9, $p \leq 0.05$). AMH values did not differ depending on duration of deslorelin treatment but were lower in adult queens ($p<0.05$). The interval to resumption of ovarian function (based on observation of estrus behavior, vaginal cytology and serum progesterone assay) ranged from 3 to 7 weeks irrespective of whether the implant was left in situ for 3, 6 or 9 months and independent of age of the queen but was longer when the implant was removed at time of decreasing photoperiod ($P<0.05$). AMH assay can be a useful tool to follow resumption of feline ovarian function following prolonged period of ovarian quiescence such as after a deslorelin treatment.

[1] Axnér E, Ström Holst B. Concentrations of anti-Müllerian hormone in the domestic cat. Relation with spay or neuter status and serum estradiol. Theriogenology 2015;83:817–21.
[2] Goericke-Pesch S, Georgiev P, Atanasov A, Albouy M, Navarro C, Wehrend A. Treatment of queens in estrus and after estrus with a GnRH-agonist implant containing 4.7 mg deslorelin; hormonal response, duration of efficacy, and reversibility. Theriogenology 2013;79:640–6.

Teaching veterinary medicine students mare's uterus and ovaries palpation on live animals

Diana Fanelli, Matteo Tesi, Alessandra Rota, Duccio Panzani, Francesco Camillo

Dipartimento di Scienze Veterinarie, Università di Pisa, San Piero a Grado, Pisa, Italia

Corresponding author: Diana Fanelli (diana.equirepro@gmail.com)

In a Veterinary Medicine (VM) curriculum, hands-on practice by students', which is required in order to carry out professional activity, is mandatory [1]. The transrectal palpation of the uterus and ovaries of mares is a difficult basic practice to teach, because of the intrinsic risks of the technique and the limited number of mares available at Universities [2, 3]. This study describes the results of an intensive program intended to teach to a group of voluntary VM students about the transrectal palpation using recipient mares belonging to a veterinary teaching hospital herd. After theoretical and basic practical training, students, under the control of a supervisor, monitored the reproductive cycle of embryo recipients. To evaluate the skills of students, the question "has she ovulated?" was asked when a dominant follicle > 35 mm had been recorded during the previous day's examination. After careful palpation of the uterus and ovaries, the student had to answer YES or NO to this question and thereafter check by US whether or not the diagnosis was correct. The answer YES was classified as OV (she has ovulated), while the answer NO was classified as NOT OV (she has not ovulated). The supervisor systematically assessed the work of the student and recorded whether the diagnosis was correct or not. This study involved the prospective evaluation of 687 records on 52 Standardbred or Thoroughbred recipient mares collected from 9 right-handed students. Chi-square tests were used to evaluate the effect of breed of the mare and side of the follicle, and to evaluate overall differences between students' performance. Results were not affected by the side of the ovary, being the proportion of correct answers for OV, NOT OV and both answers combined (OV+NOT OV) 68/102 (66.67%), 268/316 (84.81%) and 336/418 (80.38%) for the left ovary and 64/113 (56.64%), 263/302 (87.09%) and 327/415 (78.80%) for the right ovary, respectively ($P>0.05$). Breed of the mare had no effect neither on both ovaries overall correct answers (Thoroughbred: 217/278, 78.06%; Standardbred 446/555, 80.36%), nor if evaluated separately by side and answer (left ovary: OV 118/148, 79.73%, NOT OV 218/285, 80.74%; right ovary: OV 99/130, 76.15%, NOT OV 228/285, 80.00% for Thoroughbred and Standardbred, respectively, $P>0.05$). As there was no difference in mare's breed, horses were pooled together to observe whether students performed differently. There was an overall (OV+NOT OV) difference between student performances: correct answers rate ranged between 60% and 92% ($P<0.05$). No injuries for students or mares were observed. It has been reported that right-handed students had more difficulties in palpating the right ovary, compared to the left [1], but this phenomenon was not shown by our study. In conclusion, after performing a similar number of transrectal palpations there were clear individual differences in the students' performances, and this training was safe for both students and mares.

[1] Nagel et al. Teaching of diagnostic skills in equine gynecology: Simulator-based training versus schooling on live horses. Theriogenology, 15 (84): 1088–1095, 2015.
[2] Claes et al. Evaluation of risk factors, management, and outcome associated with rectal tears in horses: 99 cases (1985–2006). JAVMA, 233 (10): 1605-1609, 2003.
[3] Root Kustritz et al. Availability of theriogenology training at North American and Caribbean veterinary colleges. J Vet Med Educ. 33 (1): 140–144, 2006.

Production of bovine embryos using cysteamine-treated cryopreserved bull semen: an in vitro comparative study

Nicola Antonio Martino, Antonio Avino, Gian Guido Donato, Alessandro Ricci, Alessandro Starvaggi-Cucuzza, Leila Vincenti

Università degli Studi di Torino, Dipartimento di Scienze Veterinarie

Corresponding author: Nicola Antonio Martino (nicolaantonio.martino@unito.it)

The aim of the present study was to improve the quality of cryopreserved beef bull (Piedmontese) semen by supplementation with cysteamine before in vitro fertilization procedures. Semen samples were collected from 4 proven fertile bulls, using artificial vagina, once per week for eight consecutive weeks, pooled and diluted with Bullxcell® extender before undergoing cooling, equilibration, and freezing procedures. Frozen semen was thawed at 37°C for 40 sec. and centrifuged at 300 g for 10 mins. The spermatozoa were re-suspended in Sperm Tyrode's-albumin-lactate-pyruvate (TALP) medium, supplemented with different concentrations (0 (control), 0.010, 1, 100, 1000 µM) of cysteamine for 1 h at 38.5°C and assessed for motility and kinetic parameters by using the Computer Assisted Sperm Analyser (CASA; [1]). Moreover, the developmental potential of bovine embryos produced in vitro [1] by using cysteamine-treated cryopreserved spermatozoa was investigated. The addition of 0.010 and 1 µM cysteamine statistically improved total sperm motility and sperm kinetic parameters such as VSL, ALH and STR compared with controls (ANOVA: $P<0.05$). However, no significant differences were identified for progressive motility and VAP, VCL, LIN and BCF. Interestingly, semen samples treated with 0.010 µM cysteamine improved the cleavage rate of bovine embryos compared with controls (56/105; 53.3% vs 90/234; 38.5%; respectively, Chi Square test: $P<0.05$). Moreover, the blastocyst formation rate was significantly increased in the cysteamine group compared with controls (42/105; 40% vs 33/234, 14.1%, respectively, Chi Square test: $P<0.05$). These findings suggest a potential use of cysteamine as an additive to improve the quality of cryopreserved Piedmontese bull semen. Further studies are needed to verify if cysteamine-treated semen could improve the fertility of low fertile bull semen.

[1] Elkhawagah A.R et al. Effect of relaxin on cryopreserved beef bull semen characteristics. Cryobiology 95:51–59, 2020.

Neonatal maturity: amniotic potential in the canine species

Debora Groppetti (1), Radmila Pavlovic (2), Alberto Maria Luciano (3), Joel Filipe (1), Paola Dall'Ara (1), Francesco Arioli (3), Giulia Pizzi (1), Alessandro Pecile (1)

(1) Università degli Studi di Milano, Dipartimento di Medicina Veterinaria (2) Università degli Studi di Milano, Dipartimento di Scienze Agrarie e Ambientali - Produzione, Territorio, Agroenergia (3) Università degli Studi di Milano, Dipartimento di Scienze Veterinarie per la Salute, la Produzione Animale e la Sicurezza Alimentare

Corresponding author: Giulia Pizzi (giulia.pizzi@unimi.it)

In premature human infant, one of the most frequent and critical complications is respiratory failure due to lung immaturity and surfactant deficiency [1]. Pulmonary surfactant is a complex mixture of specific lipids and phospholipids (such as lecithin and sphingomyelin), proteins including Surfactant protein A (SP-A) and carbohydrates. Surfactant acts decreasing surface tension at the air-liquid interface in the alveoli during the respiratory cycle. Lecithin and sphingomyelin (L/S) ratio in amniotic fluid is used routinely as a prognostic marker of fetal maturity in humans while amniotic SP-A protein is involved in the synthesis of surfactant and fetal innate immunity, by regulating inflammatory and defense responses against infectious agents [2,3]. To date, in the canine species, amniotic fluid composition and its potential diagnostic role in fetal maturity are far from being known. The aim of this study was to evaluate the presence and concentration of lecithin, sphingomyelin, and SP-A protein in the canine amniotic fluid collected at birth and the possible correlation with clinical aspects and neonatal maturity.

Ten pregnant bitches and theirs 63 pups undergoing elective C-section were enrolled in this study. The day of C-section was planned according to periovulatory progesterone and adjusted based on clinical and hormonal evaluations at the end of pregnancy [4]. Amniotic lecithin and sphingomyelin were measured by HPLC coupled to high resolution mass spectrometry (HRMS-q-Exactive Orbitrap). SP-A in maternal serum and amniotic fluid were titrated using a sandwich ELISA assay kit (Dog SFTPA1/SP-A Elisa Kit, LifeSpan BioSciences Inc).

Lecithin, sphingomyelin, and SP-A were detectable in canine amniotic samples. In pups affected by infectious diseases within 1 month of life, the L/S ratio was significantly lower compared to healthy ones (P=0.03). Amniotic SP-A values was related to gestational age with higher values in pups born ≥64 days from LH surge compared to the ones born earlier (P=0.0094). Three pups with severe congenital malformations showed both SP-A and L/S lower than normal pups, even without a statistical significance.

Our preliminary results require further investigation before being applied in clinical practice, however L/S and SP-A measured at delivery in amniotic fluid showed an interesting prognostic potential in assessing neonatal maturity.

[1] Chakraborty M., Kotecha S. Pulmonary surfactant in newborn infants and children, Breathe, 9:477-488, 2013.
[2] Clair et al. The probability of neonatal respiratory distress syndrome as a function of gestational age and lecithin/sphingomyelin ratio, Am J Perinatol, 25:473-480, 2008.
[3] Gupta A., Gupta R.K. In Animal lectins: form, function and clinical applications. Springer ed, 2012.
[4] De Cramer K.G., Nöthling, J.O. Towards scheduled pre-parturient caesarean sections in bitches. Reproduction in Domestic Animals, 55, 38-48, 2020.

Preliminary study on nerve growth factor in equine perinatal period

Nicola Ellero (1), Aliai Lanci (1), Giuseppe Alastra (2), Jole Mariella (1), Maura Cescatti (3), Luciana Giardino (1,2), Carolina Castagnetti (1,2)

(1) Department of Veterinary Medical Sciences, University of Bologna (2) Health Science and Technologies Interdepartmental Center for Industrial Research, University of Bologna (3) IRET Foundation, Bologna

Corresponding author: Nicola Ellero (nicola.ellero3@unibo.it)

No plasma biomarker is in current clinical use for foals with neonatal encephalopathy (NE) [1] and the role of Nerve Growth Factor (NGF) in the equine neonatal life has not been elucidated yet, although it plays a protective role in perinatal brain development [2]. The aims of the study were: (i) to examine NGF levels at parturition in amniotic fluid (AF), umbilical cord vein (UV) and foals' jugular vein (JV); (ii) to discuss NGF trend in plasma of healthy foals and foals affected by NE during the first 72h of life; (iii) to establish its role as diagnostic biomarker. Hypothetically, NGF levels should differ in foals affected by NE. Data were recorded from foals born from attended parturition or hospitalized within 24h of life. AF was collected by needle puncture of the amnion. EDTA plasma samples were obtained from UV and JV at 0, 24 and 72h from birth/admission (T0, T24, T72). Samples were analyzed by ELISA (Horse NGF ELISA, MyBiosource). The population was divided into healthy foals (group 1; N=10) and foals affected by NE (group 2; N=18). Unpaired t-test was performed to compare same parameters between groups, whereas paired t-test to compare different parameters within the same group. Pearson or Spearman test, based on data distribution, was used to evaluate biomarker frequency distribution among groups. In group 1, NGF levels were 162.9 ±40.9 ng/mL in AF, 154.8 ±29.5 ng/mL in UV, 130.7 ±59.1 ng/mL in JV at T0, 97.2 ±55.2 ng/mL in JV at T24 and 108.3 ±47.1 ng/mL in JV at T72. NGF levels in JV at T0 were negatively correlated with mares' age (P=0.035) and parity (P=0.036) and positively correlated with NGF levels in UV (P=0.002). In group 2, NGF levels were 150.7 ±35.3 ng/mL in AF, 156.8 ±45.9 ng/mL in UV, 127.1 ±48.3 ng/mL in JV at T0, 93.0 ±51.3 ng/mL in JV at T24 and 98.0 ±32.7 ng/mL in JV at T72. NGF levels in JV at T24 were positively correlated with APGAR score at birth (P=0.02). In both groups, NGF levels in JV decreased significantly from T0 to T24 (P=0.008 and 0.045, respectively). NGF levels in group 2 were lower at each time point than in group 1, but not significantly. Presumably, NGF is either transported from the mare or is produced by the placenta and seems to cross the utero-placental barrier to reach the neonatal brain through UV in a dependent manner. The source of NGF in AF and the influence of mares' age and parity on neonatal NGF levels are unknown. Increased utilization or reduced synthesis of NGF appears to characterize the first 24h of life. From a translational standpoint, due to the immature blood-brain barrier in the perinatal period, circulating low levels of NGF may reflect CNS levels, as already hypothesized in infants who have suffered from intrauterine hypoxia [3]. Although NGF appears to be an ideal biomarker, since it is stable, measurable with a high-sensitivity technique in an easy-to-access biological fluid and reaches a peak concentration early in life, it did not discriminate NE in the examined population.

[1] Toribio RE. Equine neonatal encephalopathy: facts, evidence, and opinions, Veterinary equine practice, 35:363-378, 2019.
[2] Aloe et al. Nerve growth factor: from the early discoveries to the potential clinical use, Journal of translational medicine, 10:1-15, 2012.
[3] Korhonen et al. Brain derived neurotrophic factor is increased in cerebrospinal fluid of children suffering from asphyxia, Neuroscience letters, 240:151-154, 1998.

Preliminary study on nerve growth factor in equine perinatal period

Nicola Ellero (1), Aliai Lanci (1), Giuseppe Alastra (2), Jole Mariella (1), Maura Cescatti (3), Luciana Giardino (1,2), Carolina Castagnetti (1,2)

(1) Department of Veterinary Medical Sciences, University of Bologna (2) Health Science and Technologies Interdepartmental Center for Industrial Research, University of Bologna
(3) IRET Foundation, Bologna

Corresponding author: Nicola Ellero (nicola.ellero3@unibo.it)

No plasma biomarker is in current clinical use for foals with neonatal encephalopathy (NE) [1] and the role of Nerve Growth Factor (NGF) in the equine neonatal life has not been elucidated yet, although it plays a protective role in perinatal brain development [2]. The aims of the study were: (i) to examine NGF levels at parturition in amniotic fluid (AF), umbilical cord vein (UV) and foals' jugular vein (JV); (ii) to discuss NGF trend in plasma of healthy foals and foals affected by NE during the first 72h of life; (iii) to establish its role as diagnostic biomarker. Hypothetically, NGF levels should differ in foals affected by NE. Data were recorded from foals born from attended parturition or hospitalized within 24h of life. AF was collected by needle puncture of the amnion. EDTA plasma samples were obtained from UV and JV at 0, 24 and 72h from birth/admission (T0, T24, T72). Samples were analyzed by ELISA (Horse NGF ELISA, MyBiosource). The population was divided into healthy foals (group 1; N=10) and foals affected by NE (group 2; N=18). Unpaired t-test was performed to compare same parameters between groups, whereas paired t-test to compare different parameters within the same group. Pearson or Spearman test, based on data distribution, was used to evaluate biomarker frequency distribution among groups. In group 1, NGF levels were 162.9 ±40.9 ng/mL in AF, 154.8 ±29.5 ng/mL in UV, 130.7 ±59.1 ng/mL in JV at T0, 97.2 ±55.2 ng/mL in JV at T24 and 108.3 ±47.1 ng/mL in JV at T72. NGF levels in JV at T0 were negatively correlated with mares' age (P=0.035) and parity (P=0.036) and positively correlated with NGF levels in UV (P=0.002). In group 2, NGF levels were 150.7 ±35.3 ng/mL in AF, 156.8 ±45.9 ng/mL in UV, 127.1 ±48.3 ng/mL in JV at T0, 93.0 ±51.3 ng/mL in JV at T24 and 98.0 ±32.7 ng/mL in JV at T72. NGF levels in JV at T24 were positively correlated with APGAR score at birth (P=0.02). In both groups, NGF levels in JV decreased significantly from T0 to T24 (P=0.008 and 0.045, respectively). NGF levels in group 2 were lower at each time point than in group 1, but not significantly. Presumably, NGF is either transported from the mare or is produced by the placenta and seems to cross the utero-placental barrier to reach the neonatal brain through UV in a dependent manner. The source of NGF in AF and the influence of mares' age and parity on neonatal NGF levels are unknown. Increased utilization or reduced synthesis of NGF appears to characterize the first 24h of life. From a translational standpoint, due to the immature blood-brain barrier in the perinatal period, circulating low levels of NGF may reflect CNS levels, as already hypothesized in infants who have suffered from intrauterine hypoxia [3]. Although NGF appears to be an ideal biomarker, since it is stable, measurable with a high-sensitivity technique in an easy-to-access biological fluid and reaches a peak concentration early in life, it did not discriminate NE in the examined population.

[1] Toribio RE. Equine neonatal encephalopathy: facts, evidence, and opinions, Veterinary equine practice, 35:363-378, 2019.
[2] Aloe et al. Nerve growth factor: from the early discoveries to the potential clinical use, Journal of translational medicine, 10:1-15, 2012.
[3] Korhonen et al. Brain derived neurotrophic factor is increased in cerebrospinal fluid of children suffering from asphyxia, Neuroscience letters, 240:151-154, 1998.

3D *in vitro* maturation for Juvenile *in vitro* embryo technology

Antonella Mastrorocco (1), Ludovica Cacopardo (2), Domenico Robbe (1), Augusto Carluccio (1), Maria Elena Dell'Aquila (3)

(1) Università degli Studi di Teramo, Facoltà di Medicina Veterinaria (2) Università degli Studi di Pisa, Centro di Ricerca "E. Piaggio" (3) Università degli Studi di Bari, Dipartimento di Bioscienze, Biotecnologie e Biofarmaceutica

Corresponding author: Antonella Mastrorocco (amastrorocco@unite.it)

Juvenile in vitro embryo technology (JIVET) is an assisted reproductive technology enabling the production of in vitro embryos from prepubertal-female oocytes [1]. It is a promising tool for animal breeding programs since it offers the opportunities to reduce the generation intervals and increase the genetic gain [1]. However, JIVET results are still unpredictable and further improvements are needed to use it on a large-scale level [1]. The aim of the present study was to establish a method for maintaining the three-dimensional (3D) structure of the cumulus-oocyte complex (COC) to evaluate whether it could improve in vitro maturation (IVM) and subsequent in vitro fertilization (IVF) and JIVET outcome. Two experiments were conducted to test the effects of 3D IVM on prepubertal oocytes maturation rate (experiment 1) and in vitro embryo production (experiment 2). Alginate-based microbeads were fabricated by an automated bioprinting technology already tested on adult sheep oocytes [2]. COCs recovered from the ovaries of slaughtered prepubertal sheep (<6 months) were either included in alginate microbeads (3D-IVM) or directly placed in multi-well dishes and cultured for IVM (2D IVM, CTRL) [2]. After IVM, oocytes underwent in vitro fertilization with frozen ram sperm and embryo culture up to day 7 [3]. Embryo development (number of nuclei and apoptotic index) was monitored by epifluorescence microscopy after staining nuclear chromatin with Hoechst 33258 [4]. In experiment 1, 353 oocytes of prepubertal sheep were analyzed (n=8 replicates). Significantly higher nuclear maturation rates were obtained after 3D- versus 2D-IVM (106/175, 61% vs 74/163, 45% for 3D and 2D, respectively; Chi-square test: $P<0.01$). Additional 338 COCs (n=7 replicates) were cultured for IVM with subsequent IVF and in vitro embryo culture under 3D vs 2D conditions (experiment 2). 3D IVM improved the total embryo cleavage compared with controls (118/172, 69% vs 91/157, 58% for 3D and 2D, respectively; Chi-square test: $P<0.05$). No effects were observed on blastocyst formation rates, diameter and total number of nuclei. However, blastocysts derived from oocytes matured under 3D IVM showed lower percentages of cells with apoptotic chromatin (unpaired Student's T-Test: $P<0.05$). In conclusions, in prepubertal sheep oocytes, 3D IVM allowed to reach higher nuclear maturation rates and developmental competence than conventional 2D IVM. These data could be of relevance for ovine JIVET applications.

This study was supported by the Research Grant n° VERI00086 in the framework of the Project "Demetra" (Dipartimenti di Eccellenza 2018- 2022, CUP code: C46C18000530001), funded by the Italian Ministry for Education, University and Research.

[1] Paramio M.T., Izquierdo D. Current Status of In Vitro Embryo Production in Sheep and Goats, Reprod Dom Anim, 49:37-48, 2014.
[2] Mastrorocco et al. One-step automated bioprinting-based method for cumulus-oocyte complex microencapsulation for 3D in vitro maturation, PLoS ONE 15: e0238812, 2020.
[3] Martino et al. Supplementation with nanomolar concentrations of verbascoside during in vitro maturation improves embryo development by protecting the oocyte against oxidative stress: a large animal model study, Reproductive Toxicology 65: 204–211, 2016.
[4] Somoskoi et al. Different chromatin and energy/redox responses of mouse morulae and blastocysts to slow freezing and vitrification, Reproductive Biology and Endocrinology 13:22, 2015.

Anti-Müllerian hormone in Mediterranean Buffalo heifers

Salvatore Monti (1), Gabriele Marino (1), Francesca Arfuso (1), Michelangelo La Spisa (2), Vito Biondi (1), Annamaria Passantino (1)

(1) Department of Veterinary Sciences, University of Messina (2) Veterinary practitioner, Ragusa

The anti -Müllerian hormone (AMH) has been recently indicated as a molecular marker of the ovarian follicular pool and follicular responsiveness to superovulation treatments in bovines and other species [1]. Pioneer studies on buffalo cows revealed low values of circulating AMH associated with a poor ovarian follicular reserve [2, 3]. This study aimed to evaluate AMH peripheral concentrations in buffalo heifers, investigating their correlation with the phase of the estrous cycle and the total number of follicles greater than 3 mm in diameter (follicular count, FC). Forty-two cycling 18-20 mo. old Mediterranean buffalo were enrolled in this study in March 2019 on a Sicilian farm (Italy). Uterine tone and the presence/absence of large follicle and corpus luteum were assessed via transrectal palpation and ultrasound examination to categorize animals into two groups: heifers in the luteal (n=32) and follicular (n=10) phase of the estrous cycle. On the same day, blood was collected from the caudal vein for progesterone (P4; ELISA, Virbac) and AMH (ELISA bovine AMH, ANSH labs) assays. Ultrasound was performed up to 10 consecutive days to determine FC at the emergence of the follicular wave regardless of whether it was the one in the luteal or follicular phase. Longitudinal changes in hormonal levels from follicular to luteal phase were assessed by the unpaired Wilcoxon test. Correlation between AMH and P4 and AMH and FC was assessed by the Pearson correlation coefficient. Values of $p \leq 0.05$ were considered significant. No significant difference ($p=0.59$) in AMH peripheral concentrations was found in the follicular (median=19 pg/ml) or luteal phases (median=14 pg/ml) of the buffalo oestrous cycle. No correlation ($r=0.28$, $p=0.25$) was found between AMH and P4. Instead, a strong correlation was detected between FC and AMH (Pearson's $r=0.782$, $p=0.000076$). AMH in buffalo heifers was not correlated with the phase of the estrous cycle, but with the follicular count at the emergence of the follicular wave. This finding agrees with similar studies [2, 3]. The parallelism with bovine species suggests that AMH assay may be useful to select buffalo heifers with good fertility and a long productive lifespan that may serve as candidates for reproductive biotechnology such as ovum pick up and multiple ovulation and embryo transfer.

[1] Monniaux et al. Anti- Mullerian hormone: a predictive marker of embryo production in cattle? Reprod Fertil Dev 22:1083–1091, 2010.
[2] Liang et al. Anti-Müllerian hormone (AMH) concentration in follicular fluid and mRNA expression of AMH receptor type II and LH receptor in granulosa cells as predictive markers of good buffalo (*Bubalus bubalis*) donors. Theriogenology 86:963-970, 2016.
[3] Redhead et al. The relationship between circulating concentration of AMH and LH content in the follicle stimulating hormone (FSH) preparations on follicular growth and ovulatory response to superovulation in water buffaloes. Anim Reprod Sci 188:66-73, 2018.

Conservative treatments for feline fibroadenomatous changes of the mammary gland: case series

Gabriele Marino (1), Michela Pugliese (1), Fabiana Pecchia (1,2), Giuseppe Garufi (3), Vincenzo Lupo (1,4), Stefania Di Giorgio (1), Alessandra Sfacteria (1)

(1) Università degli Studi di Messina Dipartimento di Scienze Veterinarie (2) Ambulatorio Kerastari, Roma (3) Ambulatorio Tre Fontane, Reggio Calabria (4) Almavet, Campobello di Mazara, Trapani

Corresponding author: Gabriele Marino (marinog@unime.it)

Fibroadenomatous changes (FAC) of the mammary gland in cats, are benign proliferations of the mammary ducts and mainly of the periductal connective tissue under progesterone dependence. The disease usually occurs in intact female cats at the time of puberty, during pregnancy or pseudopregnancy, or in female and male cats of any age under progestin treatment [1, 2]. Nowadays, the elective treatment of FAC is based on the progesterone antagonist and abortifacient aglepristone [2]. This study aimed to report the treatment of FAC with a combination of drugs designed to preserve mammary gland integrity, even in pregnant cats. Eight sexually intact female cats with FAC were enrolled in this trial; four of them were on day 25-32 of pregnancy at presentation. None of the cats had received exogenous progestins. The mammary glands were symmetrically enlarged, inflamed and ulcerated in six cats. Non-pregnant cats were treated with aglepristone and with a dietary supplement, containing maltodextrin and bromelain. The mammary glands were daily massaged with an *Aloe vera* emollient gel. If the gland was inflamed or ulcerated, broad-spectrum antimicrobial and anti-inflammatory treatments were given, and ulcers were treated topically with a hypericum and neem-based cream. Two of the four pregnant cats were treated with the same therapeutic schedule plus cloprostenol to facilitate uterine emptying. Two pregnant female cats underwent all the same schedule but aglepristone and cloprostenol to preserve the pregnancy. At term, they delivered respectively four and three kittens that were normally nursed and weaned after 40 days. In all the studied cases, the mammary gland reduced in size 2-3 weeks after the start of the treatment and completely remitted after 4-5 weeks. No rescue mastectomy was necessary. The low number of cases and the lack of a control group or experimental design do not allow to speculate about the efficacy of the adjuvant therapy in non-pregnant and pregnant queens. The block of the progesterone receptors caused by aglepristone is efficient to treat FAC, then the use of the drug is always recommended in non-pregnant and progestin-treated cats. This case series suggested that the adjuvant therapy may facilitate involution, reducing oedema, infection and necrotic changes. In pregnant animals, this therapy may work also without aglepristone exploiting the normal decline of progesterone at delivery. Considering the similar time of recovery in the reported cases, it is possible to hypothesize that, in non-pregnant cats, the decline of progesterone after pseudopregnancy may act in the same way and aglepristone, in some specific cases, could be not administered. Despite empirical opposite observations in literature [2], in the two pregnant cases, treated without aglepristone, the lactation was not affected after FAC resolution. These findings and considerations on FAC certainly require further studies.

[1] Zappulli et al. In Surgical pathology of tumors of domestic animals, Davis-Thompson DVM Foundation. Washington, 2019.
[2] Payan-Carreira R. In Feline mammary fibroepithelial hyperplasia: a clinical approach, IntechOpen, 2013.

Reproductive performance of Bernese Mountain Dog in Swiss: Preliminary results

Magdalena Schrank, Antonio Mollo

Università degli Studi di Padova, Dipartimento di Medicina Animale, Produzioni e Salute

Corresponding author: Magdalena Schrank (magdalena.schrank@unipd.it)

The Bernese Mountain Dog is a breed of above medium size originating in Swiss[1]. The Swiss Club for Bernese Mountain Dog contacted us to evaluate the reproductive performance (e.g. litters per year, litter size) of the breed, the incidence of c-sections and influencing factors. Data of bitches born after 01.01.2001 and their respective litters were collected using a database (DogBase, TG-Verlag Beuing GmbH) and the announcement of birth compiled by the breeders. We collected data of 403 bitches and 1127 litters. A total of 315 litters were born by c-section due to dystocia and 23 by programmed c-sections. The cause of dystocia given by the breeder was available in 286 cases. Breeders cited fetal causes (e.g. obstruction, malformation, size) in 136 cases and maternal causes (e.g. primary uterine inertia, pathologies of the bitch) in 116 cases. Motives for programmed c-sections included the presence of a single puppy and the age of the bitch. Number of litters born per year ranged between 25 and 64. Frequency of c-section increased over the years reaching a maximum of 51% in 2020. The mean litter size was 7.19±3.08 with a mean mortality of 0.85±1.15 pups per litter based on the whole population. Breeders provided information on the duration of parturition resulting in a mean length of 9.48 hours ±5.55 in eutocic births and a mean of 7.60±5.12 hours of births prior to c-section. Available data allowed investigation of inbreeding coefficient and prior c-sections in bitches within the Pedigree (e.g. mother of the bitch of interest). The inbreeding coefficient was 1.22±1.04 % (range 0-7.81%) in bitches and 1.05±0.85% (range 0-6.45%) on the litter level. Of all bitches, 176 had at least one ancestor which underwent at least one c-section. These bitches were classified as “PED positive”. Of the PED positive bitches, 77 underwent at least one c-section and 13 had exclusively c-sections in their reproductive career. Six PED positive bitches had each one programmed c-section. Litter size within our studied population is similar to previously reported ones[2,3]. A possible impact of the inbreeding coefficient on reproductive parameters has been previously investigated in the Entlebucher Mountain Dog[4] but to our best knowledge the incidence of c-sections in the ancestral line and its possible effect on reproductive parameters have not yet been evaluated. It is therefore our aim to provide a thorough statistical evaluation of the present dataset within the near future to provide a complete picture of the evolution of reproductive parameters and performance in the Bernese Mountain Dog over the last 20 years.

1. FCI Standard of the Bernese Mountain Dog. http://www.fci.be/Nomenclature/Standards/045g02-en.pdf.
2. Schrank M. et al. Bodyweight at birth and growth rate during the neonatal period in three canine breeds. Animals 10:1–11, 2020.
3. Groppetti D. et al. The role of birth weight on litter size and mortality within 24h of life in purebred dogs: What aspects are involved? Anim. Reprod. Sci. 163:112–119,2015.
4. Schrack J. et al. Factors influencing litter size and puppy losses in the Entlebucher Mountain dog. Theriogenology 95:163–170, 2017.

Effect of oral administration of *Lepidium meyenii* (Maca) on concentration and motility of mouse epydidimal sperm cells after THC exposure

Veronica Palumbo (1), Adelaide Greco (2,3), Davide De Biase (1), Sandra Albanese (3), Dario Bruzzese (4), Domenico Carotenuto (5), Francesca Ciani (1), Simona Tafuri (1), Leonardo Meomartino (2), Marcello Mancini (3), Orlando Paciello (1), Natascia Cocchia (1), Chiara Del Prete (1)

(1) Università di Napoli Federico II, Dipartimento di Medicina Veterinaria e Produzioni Animali (2) Università di Napoli Federico II, Centro Interdipartimentale di Radiologia Veterinaria (3) Institute of Biostructures and Bioimaging of the National Council of Research, Napoli (4) Università di Napoli Federico II, Dipartimento di Sanità Pubblica (5) Università di Lima (Perù), Departamento de Ciencias Biológicas

Corresponding author: Veronica Palumbo (veronica.palumbo@unina.it)

Tetrahydrocannabinol (THC) prolonged exposure has been associated with alterations of anatomy, histology, and function of male reproductive organs, reducing quality of semen. Recent studies in rats showed that the administration of antioxidants together with the intake of cannabinoids reduces the spermiotoxic effect of the latter. *Lepidium meyenii* (Maca) is used for its antioxidant power and ability to improve both male and female reproductive functions. The oral administration of Maca improved spermatogenesis, sperm motility, and count and reduced spermatogenic damage induced by different causes (i.e., lead acetate injections).

The aim of this study was to evaluate the effect of the administration of THC, of Maca, and of their association on testicular tissue and semen parameters in mice.

Thirty 8-weeks old male c57/BL mice were divided into 4 groups: 1) control mice that not received any treatment (n=6); 2) received 10 mg/kg of Δ9-THC in 0.1 ml of sesam oil subcutaneously for 30 days (n=9); 3) received 50 mg/Kg of Maca powder PO for 30 days (n=10); 4) received the same doses of both Δ9-THC and Maca (n=5). Eco Color Doppler ultrasound examination of the testicles was performed before and after treatment. Immediately after euthanasia, the epididymis, testes, liver, and kidney were collected for histological examination. For morphometry of the testis, tubular diameters and seminiferous epithelium height were measured regardless of the stage of the seminiferous epithelium cycle. Epididymis was collected and incised and placed into 500 µl of PBS solution, in order to allow spermatozoa to swim-up into the medium for at least 30 minutes at 35°C. Sperm concentration (SC) was determined with a Bürker chamber; total (TM), rapid (RPM), and slow progressive sperm motilities were assessed by placing 10 µl of pre-warmed (37°C) semen suspension onto a pre-warmed slide and examined by light microscope. Differences among the groups were assessed using the Kruskal-Wallis and Dunn's post-hoc test and were denoted by different small letters ($p<0.05$). In all groups, there were no significant changes in testicular morphology before and after treatment. Histological assessment of the testes showed no alterations in control group, no significant alterations in group 3, mild to moderate alterations in group 2, and mild alterations in group 4. Histological examination of the other organs showed no significant differences among groups. Tubular diameter showed significantly increased thickening for group 2 and 4 compared to group 1 and 3. Moreover, seminiferous epithelium height decreased for group 2 compared with that in the group 1, 3 and 4. No statistically significant reduction in the spermatogenic index was observed for group 2 compared with with groups 3 and 4. Epididymal cross-sections of groups showed no significant alterations. Mean and standard deviation of SC for group 1, 2, 3, and 4 were 50.5±8.4a, 23.1±3.6 b, 36.6±6.1c, 54±8.3ax106 spz/ml, respectively. M was higher for group 1 and 4 (76.7±2.6a and 79.4±4.4a%, respectively) than for group 2 and 3 (33±2.5b and 55.8±6.2c%, respectively). The percentage of RPM was statistically different for all groups: 1) $69.2±3.8^{a}$ 2) $15.8±5.5^{b}$ 3) $37.8±4.7^{c}$ 4) $51.6±4.7^{d}$. The percentage of SPM was lower for group 1 and 2 ($5.8±2^{a}$ and $5.9±1.8^{a}$, respectively) than for group 3 and 4 ($15.5±6^{b}$ and $13.6±2.5^{b}$).

In Vivo Maca administration reduced the deleterious effect on semen parameters caused by THC exposure.

Effects of ovine oocyte exposure to ochratoxin a during IVM on fertilization

Letizia Temerario (1), Shafaq Asif (1,2), Antonella Mastrorocco (1,2), Nicola Antonio Martino (1,3) Augusto Carluccio (2), Domenico Robbe (2), Giovanni Michele Lacalandra (4), Daniela Mrenoski (4), Fiorenza Minervini (5), Maria Elena Dell'Aquila (1)

(1) Università degli Studi di Bari, Dipartimento di Bioscienze, Biotecnologie e Biofarmaceutica (2) Università degli Studi di Teramo, Facoltà di Medicina Veterinaria (3) Università degli Studi di Torino, Dipartimento di Scienze Veterinarie (4) Università degli Studi di Bari, Dipartimento di Medicina Veterinaria (5) Consiglio Nazionale delle Ricerche (CNR) Bari, Istituto Scienze delle Produzioni Alimentari (ISPA)

Corresponding author: Letizia Temerario (letiziatem@gmail.com)

Ochratoxin A (OTA) is a mycotoxin with widespread occurrence in stored foods and feedstuffs [1] inducing reprotoxic, embryotoxic and teratogenic effects in laboratory and farm animals [2]. Undegraded OTA was detected in ovine serum [2]. OTA was found to significantly affect mouse and porcine oocyte maturation [3,4]. In other cell systems, it was shown to react in different manners at micro- or nanomolar concentrations [5]. The aim of the present study was to evaluate, in the juvenile sheep model, the effects of oocyte exposure to OTA during in vitro maturation (IVM) on fertilization outcome. Cumulus-oocyte complexes (COCs) were exposed to 1/10 serial dilutions OTA, from 10 µM to 0.1 nM during IVM [6]. Medium with 1% methanol was used as vehicle control. After IVM, COCs underwent in vitro fertilization (IVF) with frozen ram sperm and assessment of normal fertilization under epifluorescence microscopy (Chi-square test: significance at $p<0.05$) [6]. A mean n. of 130-260 COCs/condition was analyzed in 5-10 runs/condition. At any tested concentration, OTA exposure during IVM significantly affected oocyte ability to be fertilized. In detail, at 10 µM, 1 µM, 1 nM and 0.1 nM, OTA significantly reduced the fertilization rate (10%, 18%, 19% and 20% vs 31%; $p<0.05$). At 0.1 nM, it significantly increased the rate of multipronucleate zygotes (23% vs 13%; $p<0.05$). In order to assess whether these abnormal zygotes could be developed due to parthenogenesis or polyspermia, three additional sperm-free IVF runs were performed. At 0.1 nM, OTA did not induce parthenogenetic activation (14% vs 18%; $p>0.05$), thus it can be hypothesized that multiple pronuclei (PNs) could be due to polyspermia or other mechanisms which need to be further investigated. Moreover the obtained zygotes were analyzed for their 2PNs size and position. At 10 µM, 0.1 µM and 0.01 µM, OTA significantly reduced the percentage of zygotes at the PN3 stage (9%, 23% and 30% vs 60%; $p<0.05$) in which 2PNs reach maximum size and apposition. At 0.1 µM it significantly increased the rate of abnormal zygotes with juxtaposed 2PNs having minimum size (36% vs 9%; $p<0.01$). In conclusion, oocytes exposed to OTA during IVM showed reduced ability to be fertilized. Data support the hypothesis that OTA could be a risk factor for female fertility in sheep, that it affects fertilization with different mechanisms of actions according micro- or nanomolar concentrations.

This study was supported by Rep-Eat – H2020 MSCA-COFUND 2015; G.A. n. 713714.

[1] Malir et al. Ochratoxin A: 50 Years of Research, Toxins, 8:191, 2016.
[2] Gallo et al. Review on Mycotoxin Issues in Ruminants: Occurrence in Forages, Effects of Mycotoxin Ingestion on Health Status and Animal Performance and Practical Strategies to Counteract Their Negative Effects, Toxins, 7:3057-3111, 2015.
[3] Huang F.J., Chan W.H. Effects of Ochratoxin A on Mouse Oocyte Maturation and Fertilization, and Apoptosis During Fetal Development, Environmental Toxicology, 31:724-735, 2014.
[4] Lan et al. Melatonin ameliorates ochratoxin A-induced oxidative stress and apoptosis in porcine oocytes, Environmental Pollution, 256:113374, 2019.

Ultrastructure of equine amniotic fluid compartment

Aliai Lanci (1), Laura Ingrà (2), Federico Tomasello (1), Carolina Castagnetti (1,3) Francesco Dondi (1) Gabriella Teti (2), Jole Mariella (1), Nicola Ellero (1), Mirella Falconi (2).

(1) University of Bologna, Department of Veterinary Medical Sciences (2) University of Bologna, Department for Biomedical and Neuromotor Sciences (3) Health Science and Technologies Interdepartmental Center for Industrial Research, University of Bologna

Corresponding author: Aliai Lanci (aliai.lanci2@unibo.it)

Exfoliative cytology of human amniotic fluid (AF) has been extensively studied since 1940s and cell types were classified according to their maturity [1-4]. No data exist about equine AF cytology. The aim of this study was to describe for the first time the morphology of equine AF cells and amniotic membrane (AM) with optical (OM) and transmission electron microscopy (TEM).

From thirty-four healthy mares with normal pregnancy hospitalized for attended parturition, AF was collected at parturition within 5 min after the appearance of the placenta membranes through the vulva with a 50 mL syringe. AM was then cut with a scalpel. Cytospin of AF samples was prepared on glass slides, dried on air, stained with May-Grünwald Giemsa and observed by OM. Glass slides of 6-8µm AM sections were stained with Hematoxylin-Eosin for OM observation. For TEM analysis, AF and AM were fixed with 2.5% glutaraldehyde in 0.1M cacodylate buffer for 1h at 4°C and post fixed with a solution of 1% osmium tetroxide in 0.1M cacodylate buffer for 1h. Samples were embedded in epoxy resins after a graded-acetone serial dehydration steps. After 72h, samples were sectioned and stained with uranyl acetate and lead citrate solutions, and then observed by TEM. In AF nucleated and anucleated squamous cells with basophilic cytoplasm, intensely basophilic cornified cells, polymorphonuclear cells and clusters of eosinophilic amorphous substance were observed. Cells presumably derived from fetal tracheal epithelium and small round nucleated cells with eosinophilic cytoplasm presumably derived from amniotic or fetal urinary epithelium were occasionally found. Surprisingly, lamellar body-like structures (LBs) were present in some epithelial cells. In a recent study, a new and unexpected function of human AM was demonstrated due to the presence of LBs in AM cells within the native membrane and the expression of all four types of surfactant proteins, suggesting that AM could be considered the second potential source of pulmonary surfactant [5]. In AM, three layers were clearly visible with both techniques. Epithelial cells had several cytoplasmic vacuolization and microvilli were present on apical surface, as reported in cattle [6]. The connective tissue presented fibroblasts, mesenchymal and rare polymorphonuclear cells, surrounded by abundant extracellular matrix, with distribution of collagen fibers.

This is the first report on equine AF and AM observation by OM and TEM. Further studies in samples collected at different gestational ages could increase the knowledge about AF cells and their modification during pregnancy. The diagnostic role of AF cellular composition in high-risk pregnancies may also be investigated**.**

[1] Bourgeois GA. Identification of foetal squames and diagnosis of ruptured membranes by vaginal smear, American Journal of Obstetric & Gynecology, 44:80-87, 1942.
[2] Wachtel E et al. Cytology of Amniotic Fluid, The Journal of Obstetrics and Gynaecology of the British Commonwealth, 76:596–602, 1969.
[3] Gosden CM. Amniotic fluid cell types and culture, British Medical Bulletin, 39:348–354, 1983.
[4] Casadei R et al. A cytologic study of amniotic fluid, Acta Cytologica, 17:289-298, 1973.
[5] Lemke A et al. Human amniotic membrane as newly identified source of amniotic fluid pulmonary surfactant, Scientific Reports, 7:1-13, 2017.

Gender determination in *Timon nevadensis*: preliminary observations

Mara Bertocchi, Francesco Di ianni, Igor Pelizzone, Martina Fumeo, Enrico Bigliardi, Carla Bresciani, Matteo Rizzi, Alessandro Vetere

Università degli Studi di Parma, Dipartimento di Scienze Medico-Veterinarie

Corresponding author: Mara Bertocchi (mara.bertocchi@unipr.it)

In reptiles gender determination has an important role in a captive breeding context. However, this can be particularly difficult, especially in reptiles with little or no sexual dimorphism, and in general in young subjects [1]. *Timon nevadensis* (Buchholz, 1963), also known as the Sierra Nevada lizard, is a species of lizard of the family *Lacertidae* present in the south-east area of the Iberian Peninsula [2]. Although adult male and female are visually distinguished, this differentiation is not present in young specimens. The aim of the present study was to evaluate and compare the efficacy of contrast radiography and probing for early gender determination in captive-bred *Timon nevadensis*. Although ultrasound is considered a very useful method for sexing reptiles, this technique does not always offer good results in prepubescent subjects and in those of small size [1]. In small animals, sex identification through cloacal probing is often used to evaluate the presence and length of the hemipenes pouches. However, this technique could involve a high risk of injuring the subjects by breaking through the hemipenes pouches themselves [3].

Ten young lizards were enrolled with informed owner consent. For each animal a clinical examination was performed. Lizards length and weight were recorded, and a first attempt at gender determination was carried out by probing, that is by gently placing a smooth and round-ended metallic lubricated probe into the cloaca, and then directing it towards the tail measuring the length of the inserted probe (greater in males). Subsequently, a contrast medium was administered to each animal into the cloaca through a small catheter and a radiography was performed within 5 minutes. Being a minimally invasive and very fast procedure, as reported for other species of reptiles, associated with a careful monitoring of the stress of the animals by observing any increases in respiratory rate, no sedation was required. Through probing, 4 males and 3 females were recognized. In males the probe entered the cloaca for a length of about 5±1mm, while in females no more than 1±0.5mm. The test was however equivocal in three animals, in which the probe seemed to enter but opposing an intermediate resistance. By contrast radiography, the 4 males and 3 females were confirmed. In males, when filled with contrast medium, the hemipenes pouches were seen on radiographs as triangular cavities that point caudally to the vent, about 5-7mm in length. In all the females, the contrast barely outlined the cloacal rim. With regard to doubtful subjects, the hemipenal pouches in two animals were highlighted with X-ray with contrast medium, while the third was found to be female. By introducing the contrast medium into the cloaca and taking the X-ray immediately, a less invasive maneuver is performed without the risk of breaking through the blind bottom of the pockets. This has been highlighted in some species of lizards, such as *Pogona vitticeps* and *Tiliqua scincoides* [1]. Contrast radiography seems to be a sensitive diagnostic tool for identification of the hemipenes and so for gender determinations also in *T. nevadensis*. The contrast radiography requires the animal to be manipulated for a significantly shorter time, reducing the stress for the individual itself. Contrast radiography may have a greater sensitivity in gender determination compared to probing, and that could therefore represent a valid and less invasive aid in the breeding of this lizard.

[1] Di Ianni et al., Diagnostic sensitivity of ultrasound, radiography and computed tomography for gender determination in four species of lizards, Veterinary Radiology & Ultrasound, 56(1):40–45, 2015. [2] Vetere A. In Jewelled Lizards. I ed. Autopubblicato, 2021. [3] Funk RS. Lizard reproductive medicine and surgery. Veterinary Clinics of North America: Exotic Animal Practice, 5:579– 613, 2002.

Birthweight and weight gain in Dobermann pinscher puppies up to 2 weeks of age: preliminary results

Jasmine Fusi (1), Roberta Bucci (2), Massimo Faustini (1), Maria Cristina Veronesi (1)

(1) Università degli Studi di Milano, Dipartimento di Medicina Veterinaria (2) Università degli Studi di Teramo, Facoltà di Medicina Veterinaria

Corresponding author: Jasmine Fusi (jasmine.fusi@unimi.it)

The high perinatal losses in dogs are an issue for breeders and veterinarians and most of these occurred in the first 2 weeks after birth. Low birthweight (BW) and/or inadequate weight gain (WG) after birth were recognized as important prognostic factors, and some ranges for ideal BW or WG were reported in relation to the adult body weight (1). However, the range of body sizes among the different canine breeds varies from the smallest Chihuahua to the largest Saint Bernard, and most studies reported BW and WG values according to breeds grouped on the base of the body-size (small, medium, large). One big study reported 27 breed-specific BW reference values (2), and another one highlighted the need to achieve canine breed-specific information (3). To the authors experience, wide differences exists in the BW of breeds classified in the same body-size class and no specific data have been so far reported for Dobermann pinscher, a large-size breed, that, however, is morphologically very different from others grouped in the same body-size class. In the present study, aimed to define preliminary results about BW and WG in Dobermann pinscher puppies, ten 4-5 years old pluriparous bitches with normal pregnancy ware enrolled. A total of 108 puppies were born, 47 (43.5%) females and 61 (56.5%) males, with a litter-size ranging between 8 and 14 (mean 10.8). Two puppies were stillborn (1.9%), and 5 (4.6%) died along the first 2 weeks of age. Overall BW was 397±72.13 g, with significant differences ($p<0.001$) between males (419±66.6 g) and females (374±70.7g). Significantly ($p<0.01$) lower BW was detected between non-surviving (337±10.3 g) than surviving (405±64.6 g) puppies. The WG was calculated only on the surviving puppies, because those dying along the period of study did not show any WG. The average daily gain between birth and 3rd day was significantly ($p<0.05$) higher in males (34.3±9.79g; 8.2%) than in females (30.3±12.9g; 8.1%), and again between the 4th and the 7th day ($p<0.05$): 39.1±8.83g (8.6%) in males and 36.5±10.5g (7.5%) in females. The average daily WG between the 8th and the 14th day was not different between the two sexes (47.6±17.2g; 9.7% in males and 47.1±15.7g; 10.7% in females). The timing for doubling the BW was similar in both sexes (11.1±1.74 d in males and 11.4±1.81 d in females). Nor BW neither WG were affected by bitches' age, litter-size and by the litter effect. The results evidenced once more the role of BW and WG for the prognosis on puppies' survival (2) within the first 2 weeks of age. Differently to (2), a significant effect of the sex was detected, whilst no effect of the litter-size was found. The reason of these findings could lie in the enrollment of a single breed with a uniform number of puppies per litter. If on one hand this choice could preclude the acquisition of a wider amount of data, on the other it allowed to detect some breed-specific details, arising the question to group the breeds according to criteria different from the sole body-size. The association between BW and outcome, in agreement with a previous report (2), evidences, once more, the importance of BW and WG monitoring as simple tools to detect at risk puppies, under practical settings, for both breeders and veterinarians.

[1] Meyer et al. Body composition of newborn puppies and nutrient requirements of pregnant bitches. Advances in Animal Physiology and Animal Nutrition, 16:7–25, 1985.
[2] Mugnier et al. Birth weight as a risk factor for neonatal mortality: Breed-specific approach to identify at-risk puppies, Preventive Veterinary Medicine, 171:104746, 2019.
[3] Schrank et al. Bodyweight at Birth and Growth Rate during the Neonatal Period in Three Canine Breeds, Animals, 10(1):8, 2019.

Effect of uterine diseases, treatments, and β-Hydroxybutyrate on recovering time fertility in dairy cows

Sanjana Malledevarahalli Chandrappa, Nicola Antonio Martino, Samantha Drago, Alessandro Starvaggi Cucuzza, Penelope Banchi, Leila Vincenti, Alessandro Ricci

Università degli Studi di Torino, Dipartimento di Scienze Veterinarie

Corresponding author: Malledevarahalli Chandrappa Sanjana (sanjana.malledevarahallichandrappa@unito.it)

A prompt diagnosis assessing the general condition of an animal in addition to a more specific diagnosis allows applying the best treatment to restore fertility in less time. This study aims to evaluate the effect of treatment and the influence of metabolic status, on days of recovering time (dRT) and fertility restoration in cows affected by metritis (METR), purulent vaginal discharge (PVD), and combined form (COMB). In this study, 148 Holstein cows were subjected to a weekly clinical examination from days 7±3 to 45±3 postpartum. Among 148 cows, 36 were diagnosed with METR, 11 with PVD, 54 with COMB, and 7 were healthy cows. Fetid vaginal discharge and T°>39.5 were considered (first 20 days pp) to diagnose METR, vaginal discharge after day 21 pp was scored to diagnose PVD, COMB cows if showed both diseases [1]. METR was treated with systemic ceftiofur (SYS) whereas PVD with local cefapirin (IU). To evaluate the effect of the correct therapy in COMB, 12 cows underwent SYS, 9 with only IU and 33 underwent both therapies. Blood glucose and β-Hydroxybutyrate (BHB) were measured at day 7±3pp. If BHB>1.2 mmol/L, cows were considered ketotic (KET) and were treated with propylene glycol orally [2]. The incidences of METR, PVD, and COMB were 28%, 12%, 46% respectively; partum to conception (PC) was higher in all categories (141±20, 136±21, 162±15) compared with healthy cows (88±10; $P<0.01$) with an average dRT of 16±6; 22±12; 41±15 respectively ($P<0.001$). COMB cows treated only with IU, had a considerably higher average PC (342±119) and a higher number of services per conception (n=6±2) than others ($P<0.01$). However, no difference in dRT was detected. KET cows showed no difference in PC but an increased number of dRT (40 vs 28; $P<0.01$) was identified compared with the negative one. No significant difference was detected in blood glucose concentration between healthy and diseased cows ($P>0.05$). The present study highlights how early and effective diagnosis, a correct treatment administration, and the management of metabolic status are key elements to limit the impact of uterine diseases on fertility and dRT in postpartum cows. It is also important to underline the necessity to increase research on the uterine environment, to develop new preventive methods and alternatives to antimicrobials treatment for uterine diseases.

[1] Sheldon I.M. 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] Gordon J.L., LeBlanc S.J. and Duffield T.F. Ketosis treatment in lactating dairy cattle. The veterinary clinics of North America, Food animal practice, 29:433-445, 2017.

Pre and post-partum changes of uterine artery Doppler flow parameters in high-risk and normal pregnant mares

Chiara Del Prete (1), Francesca Perina (2), Aliai Lanci (2), Marina Petrucelli (2), Jole Mariella (2), Carolina Castagnetti (2,3), Francesca Freccero (2)

(1) Università di Napoli Federico II, Dipartimento di Medicina Veterinaria e Produzioni Animali (2) Università di Bologna, Dipartimento di Scienze Mediche Veterinarie (3) Health Science and Technologies Interdepartmental Center for Industrial Research, University of Bologna

Corresponding author: Chiara Del Prete (chiara.delprete@unina.it)

Doppler ultrasonography (US) is widely used in human medicine for the evaluation of high-risk pregnancies. Pulsed-wave Doppler US is a non-invasive technique introduced in equine species to assess utero-placental blood flow, indirectly expressed by a resistivity index (RI) and pulsatility index (PI) derived by means of uterine arteries flow analysis. Data regarding uterine blood flow in high-risk pregnancies in mares are spare [1].

The aim of this study was to assess uterine arteries Doppler flow changes before and after parturition in mares with high-risk (Group 1) and normal pregnancy (Group 2). Nine Standardbred pregnant mares underwent Doppler US of both uterine arteries, as previous described [1], at two time-points: within 10 days before parturition (Tpre) and within 60 hours post-partum (Tpost). The mares included in Group 1 (n=4) were on treatment with flunixin meglumine (1.1mg/kg BID) for placental edema and/or suspected placentitis, at Tpre. Diameter (D), RI and PI of gravid uterine artery (GUA) and non-gravid uterine artery (NGUA) were measured at both time points. Spearman correlation of age and parity with GUA and NGUA ultrasound parameters at Tpre and Tpost for each group were tested. For all parameters, differences between times (Tpre vs Tpost) within each group and differences between groups at each time (Tpre, Tpost) were estimated using Mann-Whitney U-test and Wilcoxon test, respectively. The median (range) of the age and parity were 11 (6-22) y and 2 (1-3) offsprings in Group 1, and 6 (5-8) y and 1 (1-2) offspring in Group 2. Age and parity did not affect GUA and NGUA parameters at any time-point in Group 1. Differently, in Group 2 there was a negative correlation of age with D of GUA at both time points (Tpre: $P<0.01$; $r=–0.975$; Tpost: $P<0.05$; $r=-0.947$). Also, in group 2, there was a correlation between parity and PI of GUA at Tpost ($P<0.05$; $r=0.876$), while, there was a tendency for correlation ($P=0.058$) between parity and both RI and PI of GUA at Tpre ($r=-0.866$). In Group 1, the US parameters did not differ between Tpre and Tpost in neither GUA nor NGUA. In Group 2, D of GUA was significantly higher ($P<0.05$) at Tpre (9.76±1.52) compared to Tpost (8.30±2.18); moreover, RI and PI were statistically lower ($P=0.043$) at Tpre (0.51±0.07; 0.69±0.14) than at Tpost (0.79±0.05; 1.32±0.15). No differences in US parameters between groups were found at any time point. This study showed that in normal pregnancies, before and after parturition, the dimension of GUA significantly decreased with age; whereas parity positively influenced PI, only after parturition. In the literature, only D is affected by the number of previous foaling, while age influenced PI of gravid and non-gravid arteries during normal pregnancy [1]. A low sample size may explain the little correlation with US parameters. Results of this study suggest an increase of uterine flow impedance and a decrease in D of uterine artery only in the gravid horn after parturition in normal pregnancies; this modification failed in high-risk pregnancies. This might be due to a hemodynamic effect of the pre-existing uterine condition during pregnancy. The effect of flunixin meglumine on uterine vascularization has to be investigated. Increasing the sample size with a more heterogeneous population could strengthen the findings that can be drawn.

[1] Klewitz J et al., 2015. Effects of age, parity, and pregnancy abnormalities on foal birth weight and uterine blood flow in the mare. Theriogenology, 83(4), pp.721-729.

Dystocia in the Standardbred mare: a retrospective study from 2004 to 2020

Aliai Lanci (1), Francesca Perina (1), Andrè Donadoni (1), Carolina Castagnetti (1,2) Jole Mariella (1)

(1) University of Bologna, Department of Veterinary Medical Sciences (2) Health Science and Technologies Interdepartmental Center for Industrial Research, University of Bologna

Corresponding author: Aliai Lanci (aliai.lanci2@unibo.it)

Some researchers defined dystocia as II stage >30 min and reported that dystocia according to this definition was associated with a higher risk of stillbirth and neonatal morbidity and mortality [1]. There are only few studies related to draft breeds regarding the effects of dystocia on mare and foal [2]. The aims of this study were: to retrospectively describe dystocia in Standardbred mares and to evaluate the effects on clinical and blood parameters of their foals. Considering the definition of dystocia as any impediment of the stage II that could result from maternal, fetal or fetal membranes causes [3], the hypothesis of the study was that dystocia could affect foal's health also when the stage II lasts less than 30 min due to obstetric corrective procedures.

All the clinical reports of 222 Standardbred mares hospitalized at the Veterinary Teaching Hospital of the University of Bologna from 2004 to 2020 were reviewed. For the mares were recorded: age, parity, type of pregnancy (normal/high-risk), duration of stage II and III, cause and categories of dystocia severity (mild/moderate/severe) and post-partum complications. For the foals were collected at birth: APGAR score, lactatemia, venous blood gas analysis, hematobiochemical and behavioural parameters, diagnosis and exitus. Differences among groups were analysed with Mann-Whitney and Kruskall-wallis test ($p<0.05$). Categorical variables were tested with chi-square test ($p<0.05$). Data were expressed as median and interquartile range.

Mares were divided in Group 1 (165, eutocic delivery) and Group 2 (57, dystocic delivery). The incidence of dystocia was 25.7%, including both normal and high-risk pregnancies and 4.9% considering only normal pregnancies. Stage II was significantly longer in Group 2 (20; 13-27 min) than Group 1 (12; 9-15 min). The occurrence of post-partum complications in mares, perinatal asphyxia syndrome and failure of passive transfer of immunity (FPT) in foals was higher in Group 2. Venous lactatemia and serum creatine kinase were significantly higher in Group 2 (3.9, 2.8-6.5 mmol/L; 262, 183-377 UI/L, respectively) than Group 1 (3.1, 2.6-4.2 mmol/L; 187, 142-243 UI/L). The APGAR score was significantly lower in Group 2 (8, 6-9) than Group 1 (10, 9-10) and significantly lower in severe dystocia (3; 0-5). The appearance of suckling reflex was significantly delayed in Group 2 (33, 15-56 min) than Group 1 (20, 12-31 min) whereas the first colostrum intake was significantly quicker in Group 2 (95, 64-130 min) than Group 1 (116, 95-136 min) due to the administration of colostrum through a nasogastric tube.

Based on the results, also when the stage II lasts less than 30 min due to obstetric corrective procedures, dystocia could pose health risks to the foal and the mare. Despite the prompt intake of colostrum through the administration via nasogastric tube, foals born from dystocia had higher incidence of FPT, likely due to gut hypoxic damage/hypoperfusion that affected IgG absorption. In case of high-risk pregnancy, it would be appropriate to recommend hospitalization of the mare at specialized structures.

[1] Norton et al. Retrospective study of dystocia in mares at a referral hospital. Equine Vet J, 39:37-41, 2007.
[2] Chiba et al. Hematological and blood biochemical characteristics of newborn heavy draft foals after dystocia. J Equine Vet Sci, 50:69-75, 2017.
[3] Frazer et al. Normal parturition and evaluation of the mare in dystocia. Equine Vet Educ, 11:41-6, 1999.

Evaluation of the correlation between some clinical parameters and malignancy of canine mammary tumors

Penelope Banchi (1), Alessia Bertero (2), Selina Iussich (1), Angela Del Carro (1), Elisa Marzano (2), Ada Rota (1)

(1) Università degli Studi di Torino, Dipartimento di Scienze Veterinarie (2) Practitioner

Corresponding author: Penelope Banchi (penelope.banchi@unito.it)

Mammary tumors are the most common neoplasms in female dogs and 30% to 60% of these tumors are considered malignant [1]. Clinical examination is a key moment for evaluating mammary neoplasm and designing the optimal treatment plan. We retrospectively investigated some clinically assessable parameters using the dataset of the Veterinary Teaching Hospital of the University of Turin, reviewing medical records of dogs with mammary tumors from 2010 to 2020. Data from 120 dogs with 298 mammary neoplasms were included. Age, intact or spayed status, number of tumors and size, malignancy based on histological diagnosis were recorded. Pearson chi-square, Student t-test, and One-way ANOVA were used for between-group statistical comparisons. The mean age of the dogs was 9.8 years (SD 2.79, range 2-16 years). Spay status was not reported in 16.6% of dogs. Only 13.3% of dogs had been spayed (N = 31). Dogs with a single neoplasm were 35%, whereas 65% had multiple masses. Nineteen percent of dogs harbored exclusively benign tumors, 53% had malignant neoplasm, whereas 25% of dogs harbored both benign and malignant tumors. The mean size of tumors was 2.1 cm (2.1± 4.8, ranging from 0.2 to 20 cm). Differences were detected in the mean age of dogs with benign and malignant tumors (9.1± 2.8 years; 10.0±2.3 years respectively; P=0.007) and in the mean tumor size based both on spayed status (intact: 1.76±2.04 cm; spayed: 2.75±2.72 cm; P=0.003) and on benign or malignant neoplasia (benign: 1.34±1.82 cm; malignant: 2.17±2.31 cm; P=0.004). No difference existed between rates of malignant and benign tumors based on spayed status (P>0.05). However, spayed dogs had higher rates of multiple tumors (P=0.03). Tumors with high degree of malignancy (histological class III) were significantly larger than less malignant tumors (0, I, and II histological classes; P=0.000002, P=0.00004, and P=0.0006 respectively). In our study, dogs harboring malignant tumors were significantly older than dogs with benign neoplasms. Malignant tumors had larger mean size when compared with benign ones. Furthermore, in agreement with other studies [2, 3], spayed status is not statistically related to the malignancy of tumors, although spayed dogs harbor larger tumors than intact ones. Other clinical parameters (e.g., mobility of the tumor) were not included due to the retrospective nature of the study, representing a limitation for a complete evaluation of the tumors. We conclude that the age of the patient and the size of the tumors should be carefully considered when performing the clinical examination of a patient with mammary tumors.

[1] Gedon et al. Canine mammary tumours: Size matters-a progression from low to highly malignant subtypes, Veterinary Comparative Oncology, 1-7, 2020.
[2] Burrai et al. A Statistical Analysis of Risk Factors and Biological Behavior in Canine Mammary Tumors: A Multicenter Study, Animals, 10(9):1687, 2020.
[3] Vascellari et al. Incidence of mammary tumors in the canine population living in the Veneto region (Northeastern Italy): Risk factors and similarities to human breast cancer, Preventive Veterinary Medicine, 126:183-189, 2016.

Evaluation of copeptin (pro-AVP) levels in mares and foals

Fausto Cremonesi, Azzurra Zuffoli, Anna Lange Consiglio

Department of Veterinary Medicine, Università degli Studi di Milano, Italy

In domestic animals as well as in humans, parturition involves an extreme degree of physical strain that triggers an endocrine response, which is involved in the maternal and fetal adaptation mechanism to this stressful condition.

Arginine vasopressin (AVP) acts as the main regulator of homeostasis of the cardiovascular and renal systems playing a crucial role in the endocrine stress response to a variety of diseases such as different states of shock [1]. Since Copeptin, the C-terminal part of the vasopressin pro-hormone, is relatively stable in blood, it can mirror vasopressin levels.

The aim of this study was to evaluate Copeptin levels in maternal, cord and neonatal plasma obtained from umbilical cord or from jugular vein immediately after birth in the horse.

Forty-one mares were clinically assisted during delivery and APGAR score was assigned to each foal according to Panzani [2]. Foals were then monitored daily during the week after. After delivery jugular blood was collected from mares and foals and cord blood was also obtained. Copeptin/AVP was detected by MyoBioSource ELISA kit.

All data were processed through ANOVA analysis. All differences were considered significant with $P \leq 0.05$. Of the 41 mares, 28 had normal delivery while 13 mares had dystocia. There was a statistically significant difference ($P<0.001$) between the AVP values in eutocic delivery and dystocia (191.65±72.60 pg/ml and 352.65±85.94 pg/ml, respectively). Of the 41 foals, 7 were classified with APGAR <7 and 34 with APGAR >7. Between foals with APGAR <7 and >7 there was a statistically significant difference ($P<0.05$) in AVP values from jugular blood (243.54±94.89 pg/ml vs 120.56±12.20 pg/ml, respectively) but not statistical difference ($P=0.336$) in AVP values from umbilical cord blood (166.89±66.81 vs 140.38±68.53).

Our data support the hypothesis that dystocia is one of the most stressful events occurring in mare reproduction and is associated with a large release of copeptin which can exceed levels of shock, sepsis and trauma [3]. The highest AVP values from jugular blood in foals with APGAR <7 demonstrate that copeptin is strongly correlated with intrapartum situations of intrauterine fetal distress. Higher level of AVP in the presence of fetal distress in jugular vein plasma compared to cord blood let hypothesize a fetal pituitary origin of copeptin. Copeptin could be considered a highly sensitive marker of fetal perinatal stress but its levels should be correlated with other maternal or neonatal stress markers to support this hypothesis.

[1] Choong and Kissoon. Vasopressin in pediatric shock and cardiac arrest. Ped. Crit. Care Med., 9:372-379, 2008.
[2] Panzani S, Veronesi C et al. Antithrombin: Could it be a Diagnostic and Prognostic Marker in Septic Neonatal Foals. J. Equine Vet. Sci., 34:1211-1217, 2014
[3] Wellmann et al. High copeptin concentrations in umbilical cord blood after vaginal delivery and birth acidosis. J Clin Endocrin Metab, 95:5091-5096, 2010.

Rapid development of anasarca in a canine fetus near term

Giulia Siena (1), Michela Corrò (2), Claudia Zanardello (2), Greta Foiani (2), Stefano Romagnoli (1), Chiara Milani (1)

(1) University of Padua, Department of Animal Medicine, Production and Health (2) Istituto Zooprofilattico Sperimentale delle Venezie

Corresponding author: Giulia Siena (giulia.siena@phd.unipd.it)

Fetal anasarca is a congenital pathology described as subcutaneous edema with or without pleural and/or peritoneal effusion, usually not involving the whole litter. It could be related to neonatal mortality and dystocia. In dogs, anasarca seems to be more common in some breeds and primiparous dams [1].

A 5-year-old healthy pluriparous Flat-coated Retriever bitch was referred to the Veterinary Teaching Hospital of the University of Padova for pregnancy monitoring. No alterations were found on ultrasound (US) performed on day 30 and 57 post-ovulation as well as on X-ray for counting fetuses (day 57). On day 60, one of the most caudal fetuses showed subcutaneous edema and anechoic fluid in the thoracic and abdominal cavities without US evidence of placental lesions. Amniotic and allantoic fluids were increased in volume. Fetal anasarca was diagnosed. Because of the risk of obstructive dystocia, a C-section was performed on day 64, and 7 live pups (2 males and 5 females) were extracted. A female pup was bigger than the expected size, with generalized subcutaneous edema and died soon after birth. At necropsy, she weighed 0.660 kg, compared to a mean of 0.472 kg for the other 6 normal fetuses (0.445 kg for females and 0.527 for males). She was 24.5 cm in length and 6.4, 7.5 and 9.0 cm width and 7.5, 7.7 and 7 cm height at the head, thorax and abdomen level, respectively. From the subcutaneous tissue, abdominal and thoracic cavities a total of 295, 40 and 27.5 ml of fluid was collected, respectively. Generalized edema and vascular congestion of all organs were found. Lungs were fully atelectatic and the right atrium and ventricle as well as the atrioventricular orifice were severely enlarged. Bacterial cultures from fetal organs and collected fluids were sterile and molecular analyses for canine herpes virus, parvovirus, adenovirus, *Leptospira interrogans*, *Chlamydia spp.*, *Neospora caninum* and *Toxoplasma gondii* tested negative. Thoracic effusion tested negative for antibodies to *Brucella canis*. A specific cause of fetal anasarca could not be determined.

Familiarity, cardiovascular and kidney abnormalities, traumas and infections have been hypothesized as causes of fetal anasarca in dogs [2, 3]. To the best of author's knowledge, this is the first reported case of a rapid onset of fetal anasarca and hydrops of fetal membranes diagnosed on day 60 of pregnancy after a normal US monitoring performed just three days before, and the first case reported in Flat-coated Retrievers.

In conclusion, ultrasonographic diagnosis of fetal anasarca is of primary importance for parturition assistance and C-section planning, due to an increased risk of dystocia. In the prepartum period, US monitoring may be useful in all breeds, because it is possible to detect US alterations occurring at term, when anasarca could still develop.

[1] Hopper B.J. et al. Spontaneous antenatal resolution or canine hydrops fetalis diagnosed by ultrasound, Journal of small animal practice, 45:2–8, 2004.
[2] Van der Linde-Sipman J.S., Mullink J.W.M.A. Hydrops fetalis in the dog; a record of ten cases. Zentralblatt für veterinärmedizin R. A., 19:8–14, 1972.
[3] Veronesi M.C. et al. In Neonatologia veterinaria. EdiSES, 2013.

Extra-cellular vesicles from bovine seminal plasma and their interaction with spermatozoa

Anna Lange Consiglio (1), Michal Andrzej Kosior (2), Valentina Longobardi (2), Riccardo Esposito (2), Federica Piscopo (2), Marco Russo (2), Giampaolo Bosi (3), Bianca Gasparrini (2)

(1) Department of Veterinary Medicine, Università degli Studi di Milano, Italy (2) Department of Veterinary Medicine and Animal Production, Federico II University, Naples, Italy (3) Department of Health, Animal Science and Food Safety, Università degli Studi di Milano, Italy

Semen is a complex fluid composed of a combination of spermatozoa and seminal plasma that contains high levels of extra-cellular vesicles (EVs). They are important regulators during the different stages of normal spermatogenesis (1) and important mediators of intercellular communication by transferring microRNAs (miRNAs). Since, in bull, a difference in miRNA content between high and low spermatozoa motility has already been observed [2], it is probably that EV presence in seminal plasma suggests a role on sperm fertility.

Our hypothesis is that the EVs isolated from seminal plasma of high quality could improve bull sperm of low quality. To test this hypothesis, at first, a preliminary work was carried out to investigate the presence and type of EVs in bovine seminal plasma and their incorporation in spermatozoa. Ejaculates of eight Holstein bulls (4–6 years age) were collected and centrifuged at 1600 g for 10 min to pellet spermatozoa and then centrifuged again at 2400 g for 30 min to eliminate cell debris. Then, the supernatants were collected and ultra-centrifugated twice at 100,000 g for 1 h. The final pellet was re-suspended in TRIS buffer and kept at -80°C until the detection of EV concentration and size by Nanosight Instruments. To trace spermatozoa incorporation of EVs by confocal microscopy, a suspension of $1x10^6$ sp/ml was co-incubated with 200 or 400 $x10^6$ EVs labelled with pKH26 for 30, 60, 90, 120, 150 and 180 minutes at 38.5°C. The endpoint of incubation was at 24h. Confocal microscopy was set to scan fluorescent images every 0.12 µm from top to bottom of the spermatozoa.

Our results showed that the size of EVs of all samples ranged from 145.1 to 187.7 nm, with an average of 166±29 nm and the number of EVs ranged from 3.62 to $6.08x10^{13}$ particles/ml, with an average of $4.37±0.61x10^{13}$. Based on size, these EVs can be categorized as shedding vesicles. By confocal microscopy, our results showed that no fluorescence signal was detectable after coincubation with $200x10^6$ EVs. At the concentration of 400 $x10^6$ EVs, up to 60 minutes no signal was visible while at 90' spermatozoa showed a fine granular fluorescent pattern within the intermediate portion. At 120' signal was within the acrosome and at 180', spermatozoa were stained for the whole length. At 24 h, the fluorescence signal decreased.

In conclusion, this is the first report demonstrating that bull spermatozoa incorporate EVs from semen. We hypothesize a transfer of molecules, like miRNAs and other non-coding RNA molecules, from EVs to spermatozoa probably involved in sperm fertility.

[1] Belleannee C. Extracellular microRNAs from the epididymis as potential mediators of cell-to-cell communication, Asian Journal of Andrology, 17:730–736, 2015.
[2] Capra et al. Small RNA sequencing of cryopreserved semen from single bull revealed altered miRNAs and piRNAs expression between High- and Low-motile sperm populations, BMC Genomics, 18:14, 2017.

Influence of the addition of Maca (*Lepidium meyenii*) acqueous extract on chilled canine semen

Chiara Del Prete (1), Barbara Merlo (2), Valentina Longobardi (1), Veronica Palumbo (1), Alfonso Calabria (1), Stefano Spada (1), Eleonora Iacono (2), Domenico Carotenuto (3), Francesca Ciani (1), Simona Tafuri (1), Natascia Cocchia (1)

(1) Università di Napoli Federico II, Dipartimento di Medicina Veterinaria e Produzioni Animali (2) Università di Bologna, Dipartimento di Scienze Veterinarie (3) Università di Lima (Perù), Departamento de Ciencias Biológicas

Corresponding author: Chiara Del Prete (chiara.delprete@unina.it)

The use of cooled semen is currently increasing in canines, requiring reliable long-term storage conditions to preserve sperm quality. Sperm survival and motility during refrigeration are impaired by increased oxidative stress. *Lepidium meyenii* (Maca) is an Andean plant that grows exclusively at high altitudes, used as nutritional supplement and to increase fertility and sexual function in traditional medicine. Previous studies reported its fertility-enhancing properties linked with antioxidant activity, especially after oral supplementation. However, there was only one study that investigated the direct effect of Maca on human and mouse sperm cells [1]. To date, there are no *in vivo* or *in vitro* studies regarding the effect of Maca on canine spermatozoa.

In this study, the effect of the addition of Maca aqueous extract on the quality of canine semen during storage at 4°C up to 7 days was evaluated. Aqueous extract of Maca was prepared from the Yellow Maca hypocotyles collected in Peru and dried according to the traditional method. Ejaculates from 9 dogs were evaluated for volume and concentration. Each ejaculate was split in 4 aliquots (CTRL, M10, M20 and M50) and diluted to reach a final concentration of $100x10^6$ spz/ml. CTRL was diluted with only egg-yolk tris-citrate glucose (EYT-G) and the other three aliquots (M10, M20 and M50) were diluted with EYT-G with increasing concentration of Maca (10, 20, 50 µl/mL). All aliquots were placed in a syringe without air and stored in the fridge at 4°C for 7 days. The evaluation of total and progressive motility, membrane integrity (HOS test) and DNA fragmentation (Tunel- Hoescht 33342 staining) was performed for each aliquot after 3hours (3h), 24 hours (24h), 4 (4d) and 7 (7d) days of storage. Friedman's ANOVA was used to measure the significance of the decrease in each semen parameter during storage times for each group. A Mann–Whitney U-test was used to compare significant differences between groups for each semen parameter at each storage time. In the present study, cooled storage of semen caused a progressive and significant reduction of sperm quality in all groups, showing a decrease in total and progressive motility ($P<0.001$) and an increase in DNA fragmentation ($P<0.05$), especially after 4d and 7d of storage. In all groups, membrane integrity decreased significantly only after 7d of storage ($P<0.05$). At 24h and at 7d of storage, total and progressive motility resulted higher ($P<0.05$) in M10 group than in CTRL, M20 and M50. A difference in DNA fragmentation among groups was found: at 3h of storage, the percentage was lower in M10 (1 [1-1.3]) group than in CTRL (3 [2.7-4]) and M50 (3[2.4-3.3]); at 7d of storage, the percentage was higher in CTRL (9[4.5-9.5]) and M50 (8[7-9,5]) groups than in M10 (6[4-7.5]) and M20 (6[5-7]) groups. In conclusion, the addition of 10 µl/mL of Maca extract to canine semen extender greatly preserved refrigerated semen quality stored up to 7 days; however, a higher dose (50 µl/mL) was detrimental. Although this study did not evaluate the production of oxidant and antioxidant, we can hypothesize that canine semen does not need high doses of antioxidants to assure a balance between oxidants and antioxidants during refrigeration. Further studies are necessary to elucidate mechanisms involved.

[1] Aoki et al. Effect of *Lepidium meyenii* on *in vitro* fertilization via improvement in acrosome reaction and motility of mouse and human sperm, 2018 Reproductive Medicine and Biology 18(1):57-64.

Prostatectomy in the dog: could it be performed by perineal access?

Daniele Zambelli, Simona Valentini, Giulia Ballotta, Marco Cunto

Università degli Studi di Bologna, Dipartimento di Scienze Mediche Veterinarie

Corresponding author: Giulia Ballotta (giulia.ballotta2@unibo.it)

Prostatectomy in dogs is not considered a routine surgery due to technical complexity and post-operative complications: few cases of total prostatectomy are reported in the literature and most of them include a celiotomic approach [1]. Perineal approach is not described and only sporadic cases of inadvertent prostatectomy are reported [2]. In case of concomitant perineal hernia repair and abdominal prostatectomy, a two-step approach is usually suggested: the perineal one to reduce the hernia followed by the celiotomy to remove the gland. There are very few reports relating prostatectomy using a perineal approach [3,4] and, to the extent of the author's knowledge, this technique has not been thoroughly reviewed in literature.

The aim of this study is to describe retrospectively the total perineal prostatectomy in the contemporary event of disease of the prostate and perineal hernia in the dog. The experience and outcome in 3 dogs with the prostate displaced within hernial contents and 3 dogs with perineal hernia and non-displaced gland are reported as well as advantages, disadvantages and limitations of the surgical procedure.

Signalment, recent medical history, clinical signs, results of pre-operative laboratory tests, imaging diagnostics and histologic examinations were evaluated. Information on the duration of the prostatectomy (from the skin incision to the apposition of the last suture of the urethro-bladder anastomosis), complications, duration of postsurgical urethral catheterization and hospitalization, discharge and survival time was also collected.

Six client-owned dogs (four intact males and two neutered males) of different breeds, age and weight were included in the study. Dogs have been referred for perineal hernia with concomitant prostatic disease (abscessations, benign hyperplasia with severe large cyst, neoplasm) for which the removal of the prostate was recommended. Dogs underwent abdominal and perineal ultrasound and radiographic examination. After perineal skin incision, the prostate was localized by palpation, the neurovascular structures were ligated and the gland was dissected free from surrounding tissues and externally tractioned. Urethra was dissected and the gland was removed. Vesico-urethral anastomosis was performed. The perineal hernia was then repaired. No major postoperative long-term complications were detected. Minor complications were recorded in only one dog, which had slipped off the urinary catheter and manifested fecal tenesmus and intermittent urinary incontinence only during the walk in the immediate post-operative period.

It is authors opinion that, in case of perineal hernia, perineal prostatectomy presents some advantages compared to the celiotomic access: less trauma for the patient, more accurate haemostasis, better visualization of the gland, less tension on the anastomosis site, decreased surgical time. The only critical issue is related to the size of the prostate: if excessive, it could prevent its passage to the hernial cavity.

[1] Bennet et al. Total prostatectomy as a treatment for prostatic carcinoma in 25 dogs. Vet Surg., 47:367-377, 2018.
[2] Haußler et al. Inadvertent iatrogenic prostatectomy and urethrectomy in 2 dogs. Tierarztl Prax Ausg K Kleintiere Heimtiere, 47(4):282-289, 2019.
[3] Cheli R. In Clinica chirurgica veterinaria. III ed UTET, 1988.
[4] White RA. Prostatic surgery in the dog. Clin Tech Small Anim Pract, 15, 46-51, 2000.

ORAL COMMUNICATIONS

SOFIVET

Influence of seasonality on boar seminal plasma steroids

Domenico Ventrella, Alberto Elmi, Nadia Govoni, Martina Bertocchi, Camilla Aniballi, Maria Laura Bacci

Università degli Studi di Bologna, Dipartimento di Scienze Mediche Veterinarie

Seminal plasma plays a pivotal role in spermatozoa survival by providing energy, inhibiting the capacitation process, protecting them from pathogens and modulating female genital tract immune response [1]. It is rich in biologically active compounds such as steroid hormones, including their precursors and metabolites, but usually in lower concentrations when compared to hematic plasma [2]. Alongside with the well characterized Testosterone (T) and cortisol (CORT), dehydroepiandrosterone (DHEA) is one of the most representative steroid in seminal plasma and seems to modulate different biological patterns [3]. When looking at the porcine species, where reproductive success equals economic income, the fluctuations in steroids levels within ejaculates may be related to seasonal hypo/infertility, well described for pigs [4]. Therefore, the aim of the present work was to evaluate the fluctuations of some steroids within boar seminal plasma throughout 1 year, and to assess the influence of seasonality on their levels.

Semen was collected every week, once a week, from 4 commercial hybrid boars housed in the same facility and immediately centrifuged at 1200 g for 10 min to separate seminal plasma from the cellular components. Samples were stored at -20°C until steroids quantification by means of RIA upon methanol extraction. Other variables included in the statistical analyses were age, season, mean daylight hours and mean temperature. Linear regression models were set up to highlight any association between seasonal factors and each hormone's level. As expected, the highest mean hormones' concentrations were detected during summer: 0.33 ng/ml DHEA, 0.92 ng/ml CORT, and 0.48 ng/ml T. Another partially expected result was the strong influence of age on all hormones that decreased as months of age increased. The multivariate analysis showed a positive relation for DHEA with both hours of daylight (p=0.02) and temperature (p=0.028), while CORT and T only with hours of daylight (p=0.004 and p=0.021respectively).

Overall, the work strengthens the hypothesis of a strong influence of daylight on steroids concentrations within seminal plasma of boars, and a milder one, yet still positive, of temperature. Quantification by RIA was easily performed and allowed for accurate analyses. Next research step would be to correlate hormones levels to semen quality, to try and uncover the mechanisms behind the seasonal hypofertility of pigs.

[1] Kelly RW. Immunosoppressive mechanisms in semen: implications for contraception. Hum Reprod. 1995 Jul;10(7):1686-93.
[2] Asch RH, Fernandez EO, Siler-Khodr TM, Pauerstein CJ. Peptide and steroid hormone concentrations in human seminal plasma. Int J Fertil. 1984;29(1):25-32.
[3] Pohanka M, Hampl R, Sterzl I, Stárka L. Steroid hormones in human semen with particular respect to dehydroepiandrosterone and its immunomodulatory metabolites. Endocr Regul. 2002 Jun;36(2):79-86.
[4] Peña ST, Stone F, Gummow B, Parker AJ, Paris DBBP. Tropical summer induces DNA fragmentation in boar spermatozoa: implications for evaluating seasonal infertility. Reprod Fertil Dev. 2019 Mar;31(3):590-601.

Uncoupling protein-1 (UCP1) in adult horse: correlation with body weight, rectal temperature and lipid profile

Francesca Arfuso (1), Claudia Giannetto (1), Maria Francesca Panzera (2), Francesco Fazio (1), Giuseppe Piccione (1)

(1) University of Messina, Department of Veterinary Sciences (2) University of Messina, Department of Biomedical, Dental, Morphological and Functional Images

Corresponding author: Francesca Arfuso (farfuso@unime.it)

In animals, free energy is derived from the oxidation of food-stuffs including fats and proteins. A regulated proton carrier, known as uncoupling protein-1 (UCP1), dissipates the mitochondrial membrane potential generated by the respiratory chain uncoupling ATP synthesis from respiration and releasing heat from oxidation of substrates [1]. It has been established that UCP1 plays important roles in metabolic and energy balance and regulation, in cold- and diet-induced thermogenesis and in control of body temperature [1]. This study aimed to evaluate the possible relationship among UCP1, body weight, rectal temperature and lipid profile in horse. Thirty clinically healthy Italian Saddle geldings, aged between 6 and 10 years, were enrolled after the informed owners consent. All horses were managed equally at the same horse training center in Sicily, Italy (latitude 38°10' 35"N; longitude 13°18'14"E) and housed in individual boxes (3.5 x 3.5 m), under natural photoperiod and environmental conditions. From each horse, body weight and rectal temperature measurement, and blood sampling were performed according to European Directive 2010/63/EU. The sera obtained after blood centrifugation were analyzed to estimate the concentration of UCP1, total lipids, phospholipids, non-esterified fatty acids (NEFAs), triglycerides, total cholesterol, high density lipoproteins (HDLs), low density lipoproteins (LDLs) and very low density lipoprotein fraction (VLDLs). Pearson's correlation coefficients were computed to evaluate the relationship among serum UCP1 concentration and the values of body weight, rectal temperature and lipid parameters. A linear regression model (y=a+bx) was applied to determine the degree of correlation between these parameters. Serum UCP1 concentration showed no correlation with body weight, rectal temperature, HDLs and LDLs values, whereas it displayed a significant negative correlation with serum total lipids, phospholipids, NEFAs, total cholesterol, triglycerides, and VLDLs values ($P<0.0001$). The findings suggest that in adult horse the role of UCP1 is linked to the lipid metabolism and, probably, not to thermoregulation [2]. The negative correlation found between this protein and the lipid parameters considered in investigated horses could be justified by the fact that the main physiological activators of UCP1 are the fatty acids resulting from hormone-stimulated lipolysis [3].

[1] Brondani et al. The role of the uncoupling protein 1 (UCP1) on the development of obesity and type 2 diabetes mellitus. Arquivos Brasileiros de Endocrinologia and Metabologia, 56:215-225, 2012.
[2] Giannetto et al. Clock gene per 2 daily rhythm: Correlation with the serum level of uncoupling protein 1 (UCP1) in goat and horse. Journal of Thermal Biology, 97:102891, 2021.
[3] Arechaga et al. The mitochondrial uncoupling protein UCP1: a gated pore. IUBMB Life, 52:165-173, 2001.

Effects of different activation media, osmolarity and temperature on European eel (*Anguilla anguilla*) sperm motility

Martina Bertocchi, Alberto Elmi, Antonio Casalini, Domenico Ventrella, Pietro Emmanuele, Aniballi Camilla, Govoni Nadia, Albamaria Parmeggiani, Oliviero Mordenti, Maria Laura Bacci

Università degli Studi di Bologna, Dipartimento di Scienze Mediche Veterinarie

Corresponding authors: Marrina Bertocchi (martina.bertocchi3@unibo.it) and Alberto Elmi (alberto.elmi2@unibo.it)

Over-exploitation of European eels (*Anguilla anguilla*) have determined a worrying decrease in its population, making it critically endangered, leading to the necessity for new techniques for captive breeding, almost impossible today. This teleost species is catadromous and spends most of its life in freshwater of lakes and rivers until spawning migration from the European coasts to the Sargasso Sea. Teleost's spermatozoa acquire motility once released into external environment due to hyperosmotic shock (marine species), thus parameters such as temperature, osmolality, ion concentration and pH can influence sperm motility. As fot the European eel, motility activation is related to changes in pH and Ca^{++} and K^{+} concentration [1], yet not a lot of data are available in literature. The aim of the work was to test the effects on sperm motility of three different activating media: Artificial Sea Water (ASW), Tank Water (TW) and IMV Actifish® diluted 1:4 (Actifish), at 4° and 20° C; these temperatures represent respectively the most used in literature for eel sperm and the physiological spawning one. Since Actifish was never tested in this species, secondary aim was to test it diluted 1:2, to mimic sea water osmolality.

The study was performed at the breeding facility of the Cesenatico Unit of the Department of Veterinary Medical Sciences. To induce semen production, eels received a weekly intramuscular injection of hCG (1 IU/g); ejaculates from 26 specimens were collected by delicate pressure on the abdomen 24 h after the last injection [2], diluted 1:10 with transport medium, and maintained at 4°C. Sperm concentration was calculated using a haemocytometer and viability by Eosin-Nigrosin staining. Each sample was activated, at 4° and 20°C, using ASW 3.7 % (pH 8.25 ±0.1; 999.67 ±12.5 mOsm), TW taken directly from the breeding tanks (pH 8.14 ±0.02; 957.50 ±3.54 mOsm), and the commercial activating medium Actifish diluted 1:4 with bidistillated water (pH 8.53 ±0.20; 592.75 ±16.74 mOsm). Additionally, to evaluate the effects of osmolarity itself, Actifish was tested at the dilution of 1:2 (pH 8.36 ±0.08; 1155.00 ±26.87 mOsm). Immediately upon activation, objective motility was assessed by Computer Assisted Sperm Analysis (CASA) [3]. All ejaculates were similar in concentration and viability, and, when looking at the same medium, temperature did not determine significant alterations. At 4°C, the different activating media did not induce differences on sperm motility, while, at 20 °C, TW statistically reduced total and progressive motility. When diluted 1:2, Actifish reduced total and progressive sperm motility, increased slow and static spermatozoa and showed negative effect on many kinematics parameters.

This study represents one of the first report considering different activating media and temperature on sperm motility of eels in a captive setting, to try and improve the production/selection of high-quality gametes in this endangered species.

[1] Vílchez M.C., et al. Role of potassium and pH on the initiation of sperm motility in the European eel, Comparative Biochemistry and Physiology, Part A, 203: 210–219, 2017.
[2] Locatello, L., et al. Sperm quality in wild-caught and farmed males of the European eel (*Anguilla anguil*la), Animal Reproduction Science, 198: 167–176, 2018.
[3] Gallego V., et al. Standardization of European eel (*Anguilla anguilla*) sperm motility evaluation by CASA software, Theriogenology, 79:1034-40, 2013.

Pre-implantation embryos from long-term stored spin-dry ram semen at room temperature

Luca Palazzese[1], Marta Czernik[2], Pasqualino Loi[2]

[1] Institute of Genetics and Animal Biotechnology of the Polish Academy of Sciences, Jastrzebiec, 05-552, Poland [2] Faculty of Veterinary Medicine, University of Teramo, 64100 Teramo, Italy

Cryo-biobank in liquid nitrogen is sturdy and undemanding methodology applied worldwide, but it is expensive, with a sizeable carbon footprint, and thus restricted to wealthy countries that have the facilities to produce liquid nitrogen and guaranty its provision. A breach was opened by an alternative way to liquid nitrogen storage in 1998, when Wakayama & Yanagimachi gave beginnings to offspring from freeze-dried mouse spermatozoa fertilized by Intracytoplasmic Sperm Injection (ICSI). The semen conservation in anhydrous state is so basics and decisive that it is potentially applicable in space long-travel, preserving the DNA integrity from ionizing irradiation. Many advances have been made since the work of Wakayama & Yanagimachi, but the stable storage of spermatozoa for long-term, at room temperature (RT) and in the anhydrous state is still difficult to apply (especially in large mammals). In this short report, we tried to apply Spin-dry (SD), a technique normally used for nucleic acids preservation, for the freeze-drying of the ram spermatozoa. The SD spermatozoa has been stored for 2 years in a drawer at RT and 4°C. Oocytes fertilized by ICSI have been able to produce pre-implantation embryos, but the most surprising aspect is that semen stored at RT has a better trend in embryonic development than semen stored at 4 degrees (8% vs 5%). In addition, the SD lyophilized semen produces blastocysts with a better trend than the canonically lyophilized ones (2% of embryo development). Moreover, a global lipidomic assay was carried out in our samples. The results confer that the SP-semen had the better cold-tress response with a percentage of omega 3 (DHA) fatty acids (47.12% vs 54.10 in canonical freeze-dried spermatozoa and SD-semen respectively). Further DNA integrity analysis via flow cytometric evaluation is underway. In conclusion, for the first time it was possible to preserve in the long term the fertilizing capacity of the lyophilized ram seed, moreover the SD allows to exploit the facilitating aspects of freeze-drying such as conservation at RT, an aspect never documented so far.

Acknowledgments. This work has received funding from the European Union's Horizon 2020 Research and Innovation Programme under the Marie Skłodowska-Curie grant agreements No. 734434. LP, and PL were supported in the framework of the Project "Demetra" (Dipartimenti di Eccellenza 2018 – 2022, CUP_C46C18000530001), funded by the Italian Ministry for Education. L.P. and M.C. acknowledge support from the National Science Centre, Poland through grant No. 2016/21/D/NZ3/02610 (Sonata) and 2019/35/B/NZ3/02856 (Opus).

Expanding laser-assisted micromanipulation in experimental embryology

Luca Palazzese[1], Marta Czernik[1, 2], Pasqualino Loi

[1]Institute of Genetics and Animal Biotechnology of the Polish Academy of Sciences, Jastrzebiec, 05-552, Poland [2]Laboratory of Embryology, Faculty of Veterinary medicine, University of Teramo

The use of 1.48nm diode laser is a portfolio's tool of human Assisted Reproduction Techniques (ART) since decades. It is prevalently used to pierce a slit in Zone Pellucida (ZP) in order to facilitate blastocyst hatching[1], or for picking up a blastomere to be processed for Pre implantation Genetic Screening (PGS)[1]. The laser beam is preferentially directed to the ZP, but sometimes it is also used to dissect trophoblast fragments for PGS[2]. Manipulating blastocyst state embryos, for any purpose, is an irksome process even for skilled embryologists. In a recent paper we have reported the exchanging of Inner Mass Cells between sheep blastocysts and the development to term of these heavily micromanipulated embryo[3]. A problem detected in that study was the difficulty experienced in injecting the ICMs into the trophoblastic vesicle. In this abstract we present our attempts undertaken to use diode laser to easy the access to the blastocele to swop ICM cells between blastocyst stage sheep embryos.

Donor ewe's estrus synchronization, superovulation and embryo recovery was conducted as routinely done in our laboratory. Twenty blastocyst stage embryos collected from super ovulated Sarda breed ewes were micromanipulated. First, the ICM was dissected out with a microblade (IMV, France), in Ca++Mg++-free PBS using a Narishighe micromanipulator (NT-88NEN, Tokyo, Japan) fitted to a Nikon Eclipse Ti2-U inverted microscope (Movie 1). The ICM cells and trophoblastic were cultured separately overnight on a bacteriological grade Petri dish to avoid sticking to the bottom. The next morning, fully expanded trophoblastic vesicles were placed in Hepes buffered SOF medium drops in a Narishighe micromanipulator fit to a Nikon Eclipse microscope, equipped with a Dynamic Laser (OCTAX, Navilaser, German; Octax EyeWare Imaging Software). The ICM was loaded (Movie 1) into a large bore injection pipette, while the trophoblast was secured with a suitable holding pipette. A laser pulse was applied to open a suitable portion of the vesicle, and the ICM was released into the blastocele (Movie 1).

A total number of 20 ICM are dissected from 20 blastocysts, that in 15 are expanded in trophoblastic vesicle. After the injection process, only 13 trophoblastic vesicles were injected and 11 of them re-expanded in 12-16 hours. Complete integration of the ICM with the trophoblastic vesicle was found in 10 embryos (50% of the number of blastocysts used).

Given the exploratory nature of the work, no statistical analysis was conducted. ICM injection is unquestionably the most aggressive manipulation carried on the embryos, as the data show. A drop in the embryo viability was evident since the first step, with 25% of trophoblastic vesicles that did not recover after the dissection. The laser allowed an easy access to the blastocoel, thus facilitating ICM injection. A collapse of the vesicle took place shortly after injection, but this did not affect the overall process, with the ICM being retained in about 77% of the manipulated embryos. To conclude, laser assisted manipulation resulted in a remarkable improvement and simplification of the injection procedures, comparing the standard approach previously used. This technological advancement might be useful for several purposes, like reconstructing "precision chimeras" between different species embryos, or to provide easy access to the ICM, to sample cells for PGS.

Acknowledgments. This work has received funding from the European Union's Horizon 2020 Research and Innovation Programme under the Marie Skłodowska-Curie grant agreements No. 734434. LP, and PL were supported in the framework of the Project "Demetra" (Dipartimenti di Eccellenza 2018 – 2022, CUP_C46C18000530001), funded by the Italian Ministry for Education. L.P. and M.C. acknowledge support from the National Science Centre, Poland through grant No. 2016/21/D/NZ3/02610 (Sonata) and 2019/35/B/NZ3/02856 (Opus).

1) Davidson et al., Laser technology in the ART laboratory: a narrative review. Reprod Biomed Online. 2019; 38(5):725-739. 2)Aoyama et al., Trophectoderm biopsy for preimplantation genetic test and technical tips: A review. Reprod Med Biol. 2020;19(3):222-231. 3) Loi et al., Development to term of sheep embryos reconstructed after inner cell mass/trophoblast exchange. J Reprod Dev. 2018;64(2):187-191.

The porcine small intestinal epithelial cell line IPEC-J2 as an *in vitro* model to study Vitamin K effect on intestinal barrier

Debora La Mantia, Chiara Bernardini, Roberta Salaroli, Augusta Zannoni, Cristina Algieri, Fabiana Trombetti, Alessandra Pagliarani, Salvatore Nesci, Monica Forni

Università degli Studi di Bologna, Dipartimento di Scienze Mediche Veterinarie

Corresponding author: Debora La Mantia (debora.lamantia2@unibo.it)

The IPEC-J2 cell line is a primary, non-transformed, established epithelial cell line obtained from the neonatal porcine small intestine. IPEC-J2 is considered an excellent functional model to investigate the possible effect of molecules on the intestinal barrier [1]. Vitamin K (VK) is a fat-soluble molecule involved in blood coagulation; vertebrates cannot synthesize it endogenously, but only acquire it from exogenous sources via intestinal absorption from the diet or gut microbiota [2]. Recently, other relevant roles of VKs have been discovered in energy metabolism in bone and blood vessels, and against colorectal cancer by inducing cellular apoptosis [3,4]. In order to analyse the VK effect on functional metabolism, IPEC-J2 cells were purchased from the DSMZ Institute. Cells were expanded in DMEM high glucose with 10% FBS until passage 30. Cell doubling time was calculated and the cell cycle was analysed by flow cytometry. To confirm the epithelial phenotype at the end of the expansion, E-Cadherin and cytokeratin 18 were quantified by flow cytometry, tight junctions' proteins (ZO-1 and OCL) immunofluorescence analysis was performed. Trans epithelial electrical resistance (TEER) was measured over time in IPEC-J2 cultured on PET membrane transwell inserts and dye efflux activity was measured to evaluate the functionality of barrier and the ABC family transporters activity respectively. Once characterised, IPEC-J2 were treated with 0, 5 and 10 μM of three different forms of VK, VK1 (phylloquinone), VK2 (menaquinones) and VK3 (menadione) and ATP production rate by glycolytic or mitochondrial path was determined in real-time by using the Seahorse XP Agilent technology. IPEC-J2 maintained the epithelial morphology in culture, cells marked positively for E-Cad and CK18 and localized positively for the junctional proteins ZO-1 and OCL confirming the epithelial phenotype even after expansion. Cell doubling time was 52±15 h and owned a regular cell cycle during expansion phase. TEER measurements indicated that cells formed a compact monolayer stable until day 10 [1]. Flow cytometric data showed the presence of two distinct subpopulations in IPEC-J2 cells regarding the efflux capability: the more abundant subpopulation showed this ability while a minority of cells did not efflux the dye.

The ATP production rate analysis highlighted that IPEC-J2, which we previously reported are characterized by oxidative metabolism under basal condition [1], showed that VKs differently modulate the OXPHOS/glycolysis ratio in relation to the vitamers used and can reflect the VK modulatory effects on the epithelial cell properties and gut functions.

These preliminary data show that IPEC-J2 are an excellent cell line model to study enterocyte metabolism and molecular mechanisms of action of the VK in different forms and could be further employed to study the VK effect on the intestinal barrier.

[1] Bernardini C. et al. Relationship between serum concentration, functional parameters and cell bioenergetics in IPEC-J2 cell line, Histochem Cell Biol, 2021.

[2] Said H.M. Intestinal Absorption of Vitamin K: Cellular and Molecular Mechanisms, Physiology of the gastrointestinal tract: Sixth Edition, Chap 53, 2018.

[3] Tsugawa N., and Shiraki M. Vitamin K Nutrition and Bone Health, Nutrients, 12:7, 2020.

Set up and standardization of a protocol for the isolation and characterization of porcine mammary epithelial cells as *in vitro* model to study the epithelial barrier – a contribution from the IMI-ConcePTION consortium

Chiara Bernardini (1), Debora La Mantia (1), Roberta Salaroli (1), Augusta Zannoni (1), Nina Nauwelaerts (2), Neel Deferm (2), Domenico Ventrella (1), Maria Laura Bacci (1), Giuseppe Sarli (1), Michele Bouisset-Leonard (3), Pieter Annaert (2), Monica Forni (1)

(1) Università degli Studi di Bologna, Dipartimento di Scienze Mediche Veterinarie (2) KU Leuven, Department of Pharmaceutical and Pharmacological Sciences (3) Novartis Pharma AG, Novartis Institutes for BioMedical Research

Corresponding author: Chiara Bernardini (chiara.bernardini5unibo.it)

The information about the risks related to the use of medication during breastfeeding is scanty and incomplete [1]. The European consortium ConcePTION [2] aims to fill this gap. Some of the various approaches include *in vivo* and *in vitro* studies with animal models. The swine was selected as the most suitable species for this research, therefore, the present research aimed to develop an efficient method for the isolation, characterization and expansion of porcine Mammary Epithelial Cells (pMECs). In full compliance of both international legislations and the 3Rs principle, mammary tissues were collected at a local slaughterhouse (n=3 sows). The abdominal breasts tissue was dissociated by an automated dissociator in combination with a mixture of enzymes. The cells were seeded in a serum free Isolation Medium [IM: Epithelial Mammary Cell Medium supplemented with 4 µL/mL Bovine Pituitary Extract (BPE), 10 ng/mL hEGF, 5 µg/mL insulin, 0.5 µg/mL hydrocortisone, 50 µg/mL gentamicin] and incubated overnight. The non-adherent spheres were cultured in IM supplemented with 20% FBS. The serum allowed the spheres to adhere while also promoting cell sprouting. The FBS level was then reduced, every 24 h, from 20% to 10% until the serum-free condition was reached. At ~70% of confluence, cells were expanded for ten passages. Doubling time was calculated and cell cycle was checked. Cytokeratins (CKs), tight junctions (ZO-1 and OCL) were evaluated by immunofluorescence; E-Cadherin and cytokeratin18 was quantified by flow cytometry (FC). In addition, pMECs were seeded on polyester permeable supports and the ability to create a barrier was evaluated by measuring Transepithelial Electrical Resistence (TEER) and sodium fluorescein transport for two weeks. Histological evaluation revealed that all the three tissue samples showed resting mammary gland with a mixture of adipose tissue and dense collagen stroma, embedding mammary interlobular and intralobular ducts. Regardless of tissue variability, primary cell cultures from all the three different animals were obtained, albeit with different quantitative percentage. Primary cells showed a typical “cobblestone” morphology that was maintained throughout the different passages. Doubling times were similar, and the three cell populations were distributed in the phases (G1, S, G2/M) as a normal proliferating cell population. Cells expressed CKs and tight junctions with a correct localization. Quantitative FC analysis showed that the three cell cultures expressed E-Cad and the specific intermediate filament protein CK18. Finally, all the three pMECs cellular lines were able to create a tight barrier, although with different strength and kinetics. In conclusion, in the present research we have reported an efficient method to obtain, from slaughtered swine, pMECs as a model highly relevant for further efforts in developing an in vitro model of the human blood-milk barrier.

The ConcePTION project has received funding from the Innovative Medicines Initiative 2 Joint Undertaking under grant agreement No 821520. This Joint Undertaking receives support from the European Union's Horizon 2020 research and innovation programme and EFPIA.

The 3Rs in the Italian veterinary context

Monica Forni

Università degli Studi di Bologna, Dipartimento di Scienze Mediche Veterinarie

Corresponding author: Monica Forni (monica.forni@unibo.it)

The 3Rs (Replacement, Reduction and Refinement) concept[1] is the ethical and scientific background on which the Directive 2010/63/EU was build.

In 2015, the European Citizens' Initiative "Stop Vivisection" was signed by 1.17 million citizens (more than half Italians), aiming to reformulate the Directive and to substantially abolish animal experiments. The European Commission (EC), clearly stated that, even if the complete replacement of the animal model is the ultimate goal of the Directive, animal experiments are still needed.

To accelerate the development of a non-animal approach, the EC identified actions for improvement including the sharing of knowledge on the 3Rs. An inventory of knowledge sources relevant to the 3Rs was created and a survey among people working in the 3Rs area was organized. Two thirds of the 351 respondents state that the knowledge is lacking. These findings resulted in a number of recommendations including the need of increasing the education and training opportunities, extending across 3 levels of learning: professional, undergraduate and school[2].

In Italy 3Rs education is not compulsory within biological or veterinary degrees. Having taught this subject since 2006 to biotechnology students with excellent feedback, starting from 2016 I offered a non-compulsory course to the veterinary students as well, but only a small number of them decided to take it (1-2/year).

To understand the reasons for the lack of interest in the topic among veterinary students, a survey was organized (year I to V). Of the 154 respondents, only 24% claim to know the 3Rs, students say they believe the field can offer employment opportunities, but do not know the existence of specific Specialization Schools and European College. To get a general perspective on student opinion of the animal use in different area, we compared the results with those obtained in a previous survey on public opinion[3]. The support given from students to the use of animal for medical research is higher respect to that of general population (61% vs 49%) but only 1% of the students believe that animal tests are the safest way to conduct medical research vs 13% of the general population.

To investigate the knowledge of the 3Rs through the application of international guidelines within the veterinary scientific community, a survey was organized during the 73° SISVET congress. Of the 127 respondents (75% veterinarians), although 88% say they are involved in research utilizing animals, only 40% and 25% are familiar with the ARRIVE and PREPARE guidelines respectively.

3Rs skills and competencies must be provided to students as early as possible to allow them to approach, without stereotypes and prejudices, the ethical and scientific aspects of research involving experimental animals, in their future life as professional researchers or ordinary citizens.

[1] Russell et al. The Principles of Humane Experimental Technique, Methuen, London. ISBN 0900767782, 1959.
[2] Holley et al. Accelerating progress in the Replacement, Reduction and Refinement of animal testing through better knowledge sharing. Publications Office of the European Union doi:10.2788/934083, 2016
[3] https://www.ipsos.com/ipsos-mori/en-uk/attitudes-animal-research-2014
[4] Kilkenny et al. Improving Bioscience Research Reporting: The ARRIVE Guidelines for Reporting Animal Research. PLoS Biol 8(6): e1000412, 2010.
[5] Smith et al. PREPARE: guidelines for planning animal research and testing. Laboratory Animals 52(2):135-141, 2018.

Plasma expression profiling of circulating microRNAs in Piedmontese cattle during different periods of skeletal muscle growth

Rupal S. Tewari, Ugo Ala, Elisabetta Macchi, Mario Baratta, Silvia Miretti

University of Turin, Department of Veterinary Sciences

Corresponding author: Silvia Miretti (silvia.miretti@unito.it)

Piedmontese cattle breed is famous for its peculiar phenotype named 'double-muscling'. For this reason, this breed has elicited attention to improve genetic selection strategies. However, the knowledge about the genetic sign attributed to its hypertrophic phenotype is still incomplete. Skeletal muscle hypertrophy is a complex process induced by the skeletal muscle satellite cells activation, proliferation, and differentiation into multinucleated myotubes [1]. MicroRNAs (miRNAs) along with other regulatory factors play a key role in the orchestration of these dynamic processes [2]. Emerging evidence show that miRNAs can be released from cells secretion into the body fluids (ci-miRNAs) and can also exert their effect on the recipient cells through cell-to-cell communication [3,4]. In this study, we hypothesized that the expression profiles of plasma miRNAs already identified as muscle tissue related can change during different growth periods. Bovine plasma was collected from the animals housed in Animal Facility of the Dept. of Veterinary Sciences, University of Turin with the authorization of Ethical Animal Welfare Committee (Prot. No. 663). Animals were divided into four groups: new-born (NB), 4-6 months old (4-6M), 10-12 months old (10-12M) and 15-17 months old (15-17M) following gain of body weight during their course of life. The plasma miRNAs for each age group were sequenced to identify a panel of ci-miRNAs during the distinct age points. Small-RNA sequencing data analysis revealed the presence of 40% of muscle-related miRNAs in the list of top 25 highly expressed miRNAs in plasma samples. Among the four age-groups, 19 miRNAs were identified differentially expressed (DE) out of which six miRNAs (miR-10b, miR-126-5p, miR-143, miR-223, miR-30a-5p, miR-99a-5p), that as per the literature are involved in skeletal muscle physiology pathways, were validated by qRT-PCR for expression analysis. When compared with NB, miR-10b, miR-126-5p, and miR-143 were found to be significantly increased in expression ($p<0.05$) along the age and reached the highest expression at 10-12M, whereas miR-223 was significantly down-regulated in the other three groups ($p<0.0005$). However, miR-30a-5p and miR-99a-5p showed no significant variation in qRT-PCR validation analysis. Our study identified several skeletal muscle related ci-miRNAs that were DE during the growth of Piedmontese cattle. Previously, some of these were demonstrated to have a role in skeletal muscle tissue through the regulation of target genes involved in IGF signalling pathway [5]. Ci-miRNAs expression patterns and their possible correlation with age and muscle growth status could be useful to improve genetic selection strategies and identify the potentiality of these molecules to become novel biomarkers in Piedmontese cattle.

[1] Keefe et al. Muscle stem cells contribute to myofibers in sedentary adult mice, Nature Commun, 6: 7087, 2015.
[2] Buckingham et al. Gene Regulatory Networks and Transcriptional Mechanisms that Control Myogenesis, Dev Cell, 28(3):225-38, 2014.
[3] Siracusa et al. Circulating myomiRs: a new class of biomarkers to monitor skeletal muscle in physiology and medicine, Journal of Cachexia, Sarcopenia & Muscle, 9:20–7, 2018.
[4] Stoorvogel et al. Functional transfer of microRNA by exosomes, Blood, 646-8, 2012.
[5] Zhang et al. miR-143 regulates proliferation and differentiation of bovine skeletal muscle satellite cells by targeting IGFBP5, In Vitro Cell Dev Biol Anim, 53:265-71, 2017.

Expression and hormone regulation of ErbB receptors and ErbB-ligands in the mammary gland of pubertal gilts

Alessia Morato (1), Eugenio Martignani (1), Paola Toschi (1), Russell C. Hovey (2), Josephine F. Trott (2) Paolo Accornero (1)

(1) Università degli Studi di Torino, Dipartimento di Scienze Veterinarie (2) Department of Animal Science, University of California Davis

Corresponding author: Paolo Accornero (paolo.accornero@unito.it)

The ErbB family of receptors is crucial in mammary development. Studies with knock-out mice proved that the stromal fraction of EGFR (ErbB1), once activated by AREG (EGFR-ligand), is essential to mediate estrogen-dependent ductal allometric growth at puberty [1]. While mice cannot depict the histoarchitecture of the breast, the mammary gland of pigs displays striking similarities to the human [2]. Still, almost nothing is known about ErbB family in the mammary gland of pigs. We hypothesize that ErbB receptors and ErbB-ligands (*Genes of Interest* GOIs) have distinct expression pattern and hormone regulation, and that a unique transcriptional profile characterizes different mammary subpopulations in pubertal gilts. We isolated fat, mammary parenchyma and stroma from 4 slaughtered Large White gilts by enzymatic digestion. The epithelium enriched fraction was digested and sorted for CD49f (integrin α6) and EpCAM (epithelial cell adhesion molecule). All fractions were analyzed with rtPCR for the GOIs. Whole transcriptome sequencing will allow us to identify gene clusters differentially expressed across subpopulations. The hormone regulation of the GOIs was investigated *in vivo* in mammary samples from 4 ovariectomized, hormone treated gilts [2] and is being investigated *in vitro* by explant culture. Of the 4 ErbB receptors, only EGFR was prevalent in the fat of mammary glands from pubertal gilts, while other ErbB were mainly epithelial and under the positive effect ($p<0.01$) of estrogen. Consistently, different ligands were highly expressed (rtPCR cycle threshold ≤26), and prevalent in the epithelium. Among these, AREG and EREG were uniquely upregulated ($p>0.01$) by estrogen *in vivo,* while TGFα was also under the positive effect of progesterone ($p>0.01$). NRG1 was also induced by prolactin ($p < 0.05$), suggesting a further role at later stages of development. In several respects, our results are in contrast with studies in mice, where ErbB3 and ErbB4 are thought to play a role later in mammary development, and AREG is the only ligand upregulated by estrogen at puberty. According to our preliminary results, 3 populations can be sorted from the mammary gland of gilts: a CD49fneg/EpCAMneg population, a CD49flow/EpCAMpos population, highly expressing Krt18, and a potentially basal CD49fpos/EpCAMneg population, highly expressing AREG, as opposed to the mouse, where AREG is a key player in the luminal epithelium [3].

All in all, we proved that ErbB receptors and ligands have a unique gene expression pattern and hormone regulation in the mammary gland of pubertal pigs, with several genes other than AREG and EGFR expressed and modulated in the epithelium. These findings, and those eventually deriving from the whole transcriptome analysis of mammary subpopulations, might help to dissect the mechanisms of development in those species characterized by a complex mammary parenchyma, providing a model to understand tumoral derangements of the human breast.

[1] Sternlicht et al. Mammary gland morphogenesis requires paracrine activation of stromal EGFR via ADAM17-dependent shedding of epithelial amphiregulin, Development 133(6):1203, 2006.
[2] Horigan at al. Hormone interactions confer specific proliferative and histomorphogenic responses in the porcine mammary gland, Domest Anim Endocrinol, 37: 124-138, 2009.
[3] Ciarloni et al. Amphiregulin is an essential mediator of estrogen receptor α function in mammary gland development, Proc Natl Acad Sci USA, 104(13): 5455-5460, 2007.

Possible interferences of bentonite on rumen metabolome highlighted by 1H-NMR spectroscopy in dairy Holstein cows

Anna Damato (1), Paola Vanzani (2), Elisa Giaretta (1), Enrico Novelli (1), Matteo Gianesella (3), Gianfranco Gabai (1), Lucio Zennaro (2

(1) Università degli Studi di Padova, Dipartimento di Biomedicina Comparata e Alimentazione (2) Università degli Studi di Padova, Dipartimento di Medicina Molecolare (3) Università degli Studi di Padova, Dipartimento di Medicina Animale, Produzioni e Salute

Corresponding author: A. Damato (anna.damato@studenti.unipd.it)

In dairy cows, diet supplementation with clay minerals, such as bentonite, is a recognised method against the negative impacts of feed aflatoxin contamination on animal health and production, and on dairy products safety. Clay minerals are capable of binding mycotoxins, reducing their intestinal absorption and distribution in animal tissues and milk. Studies conducted in vitro and in non-ruminant animals suggested that clays do not adsorb specifically mycotoxins and they may affect intestinal absorption in other ways [1]. In ruminants, bentonite has been associated with decreased energy and crude protein digestibility and mineral imbalances [2], and it is suspected of modifying ruminal microbiota [3]. Therefore, more studies are needed in cows to verify possible interferences of clays with rumen fermentations and metabolite uptake, which may affect animal metabolism and, possibly, milk characteristics. The aim of this trial was to study the effects of bentonite administration on ruminal metabolome in the absence of aflatoxins. Six multiparous lactating Holstein cows (body weight: 658.5±67.7 kg; DIM: 193±23; milk yield 26.5±4.8 kg/d) were included in a crossover 3×3 Latin square design at the end of a 24-d acclimatization period (t0). The design consisted of three 19-d periods (t1, t2, t3) and three treatments: control without bentonite (B0), prophylactic dose of 50g/day (B50) and therapeutic dose of 100g/day (B100) of bentonite (GLOBALFEED®T1). The cows were individually fed and received the same unifeed-based, aflatoxins-free ration. Bentonite was mixed with a small aliquot of unifeed and offered to the cows before feeding. A metabolomic analysis by 1H-NMR spectroscopy was performed on rumen fluid samples collected by ruminocentesis at t0 and on d 15 of each experimental period (OPBA authorization n. 0197903, 16/05/2019). Principal Component Analysis (PCA) on the ruminal metabolic profiles led to differentiate treated cows (B50 and B100) from the untreated ones (B0). However, the presence of carry-over effects can be hypothesised, in particular in animals that did not receive bentonite (B0) in t3. The concentrations of acetate, propionate, butyrate and lactate, measured on the 1H-NMR spectra, were analysed by a non- linear mixed model. A significant increase in acetate with increasing bentonite dose ($p<0.05$) and a significant decrease in propionate due to period effect ($p<0.05$) were observed. The results suggest an influence of bentonite on the metabolic profile of rumen fluid in dairy cows. On the other hand, insufficient wash out intervals between experimental periods cannot be excluded. Further analyses are in progress to identify the metabolites responsible for the PCA clustering. In conclusion, bentonite is not a completely inert additive, which can interfere with rumen metabolism with possible consequences on systemic and mammary gland metabolisms of dairy cows.

(Funded by MIPAAF, DM 27443 - 25/09/2018 project BENFELAT)

[1] Elliott et al. Mycotoxin Res, 36:115–126, 2020. [2] Rindsig and Schultz. Journal of Dairy Science, 53:888-892, 1970.[3] Ivan et al. J Anim Sci. 70:3194-3202, 1992.

Investigation of the effects of heat treatment on bovine colostrum immunoglobulins, bacterial and somatic cell counts, growth factors, and whey proteome

Giulio Curone (1), Tawny L. Chandler (2), Jessica Cha (2), Paolo Moroni (2), Ruchika Bhawal (3), Sheng Zhang (3), Sabine Mann (2)

(1) Department of Veterinary Medicine, University of Milano (2) Department of Population Medicine and Diagnostic Sciences, Cornell University (3) Proteomics and Metabolomics Facility, Cornell University

Corresponding author: Giulio Curone (giulio.curone@unimi.it)

Early intake of adequate quantities of high-quality colostrum is critical for the health and growth of the newborn cattle (Godden et al., 2019). Colostrum provides a concentrated form of nutrients and a plethora of bioactive factors that are critical to the proper development of the calf (Hammon et al., 2013). Nowadays heat treatment of colostrum is widely used to reduce the risk of transmission of infectious diseases and bacterial contaminants. The objective of this study was to investigate the effects of heat treatment on colostral low abundant proteins, immunoglobulins (Ig), insulin and insulin-like growth factor I (IGF-I), as well as bacteria and somatic cells. Colostrum samples >8 L and Brix % >22.0 were harvested from cows on a commercial dairy in NY, USA. Colostrum was split into 2 aliquots using single-use colostrum bags, and either part was cooled on ice immediately after harvest (raw; R, n=11) or heat treated in a commercial pasteurizer for 60 min at 60°C (heat; H, n=11). All samples (n=22) were analyzed for Ig concentration by radial immunodiffusion and for insulin and IGF-I concentrations by radioimmunoassay. Total bacterial counts (TBC) and somatic cell counts (SCC) were determined using standard plate culture techniques and flow cytometry, respectively. A subset of 5 paired samples (n=10) was further analyzed by nanoLC-MS/MS technique after ultracentrifugation at 100,000xg for 60 min at 4°C to enrich the low abundant protein fraction in whey. Data were analyzed using statistical analysis accounting for the paired nature of the data, or using either paired t-test (JMP v. 14.0.0, SAS Institute, Cary, NC) or free online software to analyze proteomics data (MetaboAnalyst v. 4.0, https://www.metaboanalyst.ca) for fold-change ≥1.5 between pairs, and false discovery rate (FDR)-adjusted paired t-tests with P-value <0.05. The median (range) reduction of IgA concentrations was 8.5 (0-38.0) % due to heat treatment (P=0.02), whereas IgG concentrations did not change due to treatment (P=0.36). Insulin concentrations decreased by a median (range) of 22 (7-45) % (P<0.001) and IGF-I by 10 (0-18) % (P=0.005) in H vs. R, respectively. Heat treatment was associated with a mean ± SE decline in SCC of 207,000 ± 68,000 cells (P=0.01), as well as a reduction in total bacterial count by 13,162 ± 3,472 cfu/mL (P=0.001). Proteomics analysis identified 328 unique proteins in all 10 samples. Among the 25 proteins that decreased by at least 1.5 fold in H vs. R., 9 were identified as complement proteins. In conclusion, the heat treatment of colostrum is associated with a reduction in the concentration of several of the investigated colostrum components except for IgG. Proteomics analysis of colostral whey identified a number of complement components and other proteins (fibrinogen and trypsin inhibitors) that changed in abundance due to heat treatment. Little is known about the role of colostral complement in the intestine or circulation of the newborn calf. The biological significance of the observed changes in colostral components for the health and immune function of the newborn calf will need to be assessed.

[1] Godden, S.M., Lombard, J.E. and Woolums, A.R., 2019. Colostrum management for dairy calves. Veterinary Clinics: Food Animal Practice, 35(3), pp.535-556.

Effect of ages and housing conditions on hair corticosterone, DHEA and testosterone in male laboratory mice

Alberto Elmi (1), Viola Galligioni (2), Nadia Govoni (1), Martina Bertocchi (1), Camilla Aniballi (1), Maria Laura Bacci (1), José M Sánchez-Morgado (2), Domenico Ventrella (1)

(1) Università degli Studi di Bologna, Dipartimento di Scienze Mediche Veterinarie (2) Comparative Medicine Unit, Trinity College Dublin (Ireland)

Corresponding author: Alberto Elmi (alberto.elmi2@unibo.it)

Accurate welfare assessment of laboratory rodents is both an ethical necessity and a legal requirement as reported by European and Italian laws. Steroids, key actors in stress and sexual behaviors, have been proposed as welfare biomarkers and investigated in different mice matrices such as plasma, feces, urine and saliva; nonetheless data regarding steroids hair quantification, indicative of longer timespan, are partially lacking in this species. Levels of hormones accumulated in the hair shaft are related to the hair follicle cycle, consisting of 14 days of growth followed by 14 days of rest, reflecting up to three/four months, in mice [1]. Rodents show a peculiarity in steroids physiology, with Corticosterone (CORT) being the main glucocorticoid involved in direct physiological or stressful conditions, as opposite to other mammals where it is cortisol. Dehydroepiandrosterone (DHEA), on the other hand, is generally involved in both stress and reproductive patterns, but it is not synthetized in adrenal glands when it comes to mice. Finally, testosterone (T), pivotal for the reproductive cycle, is also involved in the modulation of aggressive behavior acting on androgen and estrogen receptors in the male brain [2-3].

The aim of the present work was to analyze the hormonal hair profile of laboratory male mice and to investigate potential relationships with age and housing, as a potential tool for welfare assessment. Fifty-six adult male C57BL/6J and C57BL/6OlaHsd substrain mice were included in the study, housed in pairs or groups. After solvent extraction, T and DHEA were quantified by radioimmunoassay, CORT by ELISA. Overall mean hormone levels were: 6.42 pg/mg for T, 23.16 pg/mg for DHEA and 502.1 pg/mg for CORT. Age influenced all hormones by significantly increasing T ($p<0.001$) and DHEA ($p<0.001$) and decreasing CORT ($p<0.001$); only DHEA, significantly higher in grouped mice ($p<0.05$), was influenced by housing conditions.

The influence of age indicates the need for accurate age-related reference intervals, while the higher levels of DHEA in grouped animals suggests that such housing practice may be beneficial for social interactions. In conclusion, the study further confirms and strengthens the use of hair as a good non-invasive matrix for “long-term” endocrinologic evaluations, potentially as a good tool for welfare assessment in this species, and provides new insights on corticosterone, testosterone and DHEA levels in laboratory male mice.

[1] Carlitz, et al. Steroid hormones in hair reveal sexual maturity and competition in wild house mice (*Mus musculus domesticus*). Scientific Report, 9: 16925, 2019.
[2] Scorrano et al. Validation of the long-term assessment of hypothalamic-pituitary-adrenal activity in rats using hair corticosterone as a biomarker. FASEB Journal, 29: 859–867, 2015.
[3] Mucignat-Caretta, et al., Age and isolation influence steroids release and chemical signaling in male mice. Steroids, 83: 10–16, 2014

Alarm reactions of red deer (*Cervus elaphus*) in the Paneveggio-Pale di San Martino regional Park

Sara Moscatelli (1), Alessandro Malfatti (2), Paolo Kovatsch (3), Daniele Catorci (4), Paola Scocco (2)

(1) Etologa, libera professionista (2) Università degli Studi di Camerino, Scuola di Bioscienze e Medicina Veterinaria (3) Provincia autonoma di Trento, Agenzia provinciale delle foreste demaniali di Cavalese (4) Fotografo naturalista, Mythlands

Corresponding authors: Paola Scocco (paola.scocco@unicam.it); Alessandro Malfatti (alessandro.malfatti@unicam.it)

Increasingly, both accessible and remote areas are being used for recreational activities. The amplified anthropogenic impact is believed to be a significant factor in the disturbance of wildlife [1]. In the Red deer (*Cervus elaphus*), the heightened exposure to anthropogenic disturbance from human recreation is linked to the expression of behaviors assimilable to predation perception [2]. A population of red deer is kept in Paneveggio Pale di San Martino Natural Park (Trento-IT) for didactic purposes; the population is composed of a maximum of 20 animals and lives in a wide area, with lawns and forest. The animals are subject to intense human exposure throughout the year with elevated incidences in the summer months. Our study was aimed to develop a pasture management plan to avoid conflict between the risk of high stress levels for the animals and educational and recreative use of the area, considering the need to improve the botanical richness inside the shelter. For one week, during summer, 18 subjects were observed: 3 adult males, 10 adult females and 5 calves. The animals tended to separate in a nursery group (females and calves) and a male predominant group (all adult males and 1/2 old females). Different alarm reactions were examined in order to determine how the apparent stress level of the animals change through the course of the day and in response to different stimuli. Three types of visual stimuli were performed: a person standing, a person moving and an umbrella suddenly opened. The acoustic stimuli were two: a low tone sound produced by the observers and a grass rustling sound [3]. Three behavioural states were recorded during the observations: vigilant lying, vigilant standing and vigilant moving [2]. A growing response intensity was associated with changing the state from a vigilant lying to vigilant standing position and from vigilant standing to vigilant moving. Behavioural responses and their intensity in response to visual and acoustic stimuli were observed during different times of the day (morning and afternoon; period of 30-60min) as well as before, during, and after tourists' presence. The reactions to these disturbances were liveobserved both inside and outside the shelter by three observers; in addition, many photos and video were recorded. The stronger disturbance response was recorded in the deer during and immediately after the days of major tourist affluence, and when performing the visual stimuli inside the shelter. Deer displayed higher alert behaviours during and after weekends, with a peak of response intensity on Sunday afternoons and Monday mornings. Moreover, the visual stimuli were associated with greater responses, while acoustic stimuli caused nearly no response. Another major difference was noticeable between groups. The nursery group showed a higher number of alarm signals and a higher response intensity and tended to stay far and well hidden from the tourists. It is therefore crucial to pay severe attention during reproductive and lactation periods and plan needed pasture management measures during less disturbed days and months.

[1] Sibbald A. M. Responses of red deer (*Cervus elaphus*) to regular disturbance by hill walkers, European Journal of Wild Research, 57:817–825, 2011
[2] Jayakody S. Red deer *Cervus elaphus* vigilance behaviour differs with habitat and type of human disturbance, Wildlife Biology 14:1, 2008
[3] Hodgetts B. V. The effects of visual and auditory disturbance on the behaviour of red deer (*Cervus elaphus*) at pasture with and without shelter, Applied Animal Behavioural Science, 55:337-351, 1998.

Dogs (*Canis familiaris*) are susceptible to a contour illusion

Miina Lõoke (1), Lieta Marinelli (1), Cécile Guérineau (1), Christian Agrillo (2,3), Paolo Mongillo (1)

(1) Università degli Studi di Padova, Dipartimento di Biomedicina Comparata e Alimentazione (2) Università degli Studi di Padova, Dipartimento di Psicologia Generale (3) Università degli Studi di Padova, Padova Neuroscience Center

Corresponding author: Lieta Marinelli (lieta.marinelli@unipd.it)

Contour illusions emerge when the boundaries of an object are partially occluded, but they are nevertheless perceived by the observer. A wide range of species are known to be susceptible to contour illusions, but whether the ability extends to the dog is still unknown. Interestingly, several previous studies assessing dogs' susceptibility to visual illusions show null susceptibility [1], which suggest that dogs' visual perception is considerably different from that of humans. To shed light into this topic, we assessed dogs' susceptibility to the most well-known contour illusion, the Kanizsa's triangle illusion. The illusion consists of an arrangement of three circles with a missing sector, arranged in a way that a human observer perceives a triangle in between them. Six dogs were trained to discriminate a triangle amongst other geometrical figures using a two-alternative conditioned discrimination task on touch-screen apparatus. Once the dogs were reliably choosing the triangle, they were presented with the Kanizsa's triangle, paired with the control stimuli, where inducers were rotated around their centre, to disrupt the perception of the triangle. Each dog was presented with the illusory triangle for 25 times. Dogs as a group chose the illusory triangle significantly more often than the control stimuli (128/150, $p<0.001$, one sample t-test). At the individual level, five dogs out of six chose the illusory triangle significantly more often than expected by chance. These results are particularly interesting, since it is the first visual illusion where dogs as a group show susceptibility in the same manner as humans. Moreover, the analyses revealed that the age of the subject had a negative effect on the susceptibility ($z=-3.22$, $p=0.001$) and similar effects of age has been found in humans [2]. Conclusively, this suggests that the underling perceptual mechanisms of contour illusions are similar in dogs and humans and in sharp contrast with other categories of visual illusions that have been previously assessed in dogs.

[1] Byosiere et al. Illusion susceptibility in domestic dogs, Ethology, 126:949-965, 2020.
[2] Kurtz BA. The perception of illusory contours in young and older observers - an explorative study using psychophysics and EEG. Dissertation, Georg-August-Universität, 2011.

Raman spectroscopy for individuation of peripheral markers of fatigue during exercise in horses

Claudia Giannetto (1), Giuseppe Acri (2), Barbara Testagrossa (2), Francesca Arfuso (1), Giuseppe Piccione (1)

(1) University of Messina, Department of Veterinary Sciences (2) University of Messina, Department of Biomedical and Dental Sciences and Morphofunctional Imaging

Corresponding author: Claudia Giannetto (clgiannetto@unime.it)

The evaluation of performance levels in athletic horses is of major importance to prevent sport injuries. A current research topic in the biomedical field is Raman spectroscopy, a rapid qualitative and quantitative technique that allows the simultaneous determination of several biomolecules and chemical compounds in the biological fluids to assess the metabolic status. Raman spectroscopy is an inelastic light-scattering phenomenon that provides vibrational spectrum that contains information relative to chemical bonds and symmetry of a specific molecule [1]. The main advantage of applying the Raman spectroscopy for the analysis of protein structural characteristics is the insensitive to water and this provides an opportunity to characterize biological materials in solution, thereby preserving their biological activity, specific signature regions of amino acids existing in the Raman signal [2]. Raman spectroscopy measurement returns a spectrum over a wavenumber range constituted by several bands representing biomarkers according to investigated biological matrices.

On the basis of that, the aim of the present study was to investigate the insurgence of fatigue in five regularly trained Italian Saddle horses subjected to a standardized obstacle course (350m/min; eleven 1.25 high jumps) preceded by warm-up by the means of Raman spectroscopy. Before, immediately after, after 30 min and 60 min the end of exercise, blood samples were collected by means of jugular venipuncture into a vacutainer tubes with clot activator. Raman measurements on the collected sera were performed using a diode laser with the excitation wavelength of 785 nm. The analysis of the obtained spectra allowed the identification of peaks and bands that differed in position and intensity among the experimental conditions. The acquired spectra, obtained from horse sera collected during the experimental protocol, yielded a similar pattern, except for the large band detected in the 1250-1800 cm-1 range. In the bands (1300 - 1360) cm^{-1} and (1385 - 1520) cm^{-1} visual modifications due to physical exercise were observed. The spectral intensity decreased after training and 30 min after the end of exercise respect to the before exercise value, to come to the basal value after 60 min from the end of the exercise. At frequencies inside the 1300 – 1360 cm^{-1} and 1385 – 1520 cm^{-1} ranges serum constituents involved in the insurgence of fatigue (tryptophan, leucine, isoleucine etc.) were identified [3,4]. In particular, in the 1300-1320 cm^{-1} range two sub-bands (lipids and tryptophan) were identified; in the 1385 - 1520 cm^{-1} range seven sub-bands were identified (leucine, glycine, isoleucine, lactic acid, tripeptide, adenosine and β-carotene). The application of one-way for repeated measure analysis of variance (ANOVA) on the area of each sub-band showed a significant effect of exercise on all sub-band area considered.

In conclusion, the findings obtained in the current survey show that Raman spectroscopy is an easy, rapid, reproducible and non-invasive technique highlighting its usefulness in the biochemical field studies.

[1] Orkoula M.G., Kontoyannis C.G. Raman spectroscopy for the study of biological organisms (biogenic materials and biological tissues): A valuable analytical tool. Spectroscopy Europe, 26: 16–19, 2014.

[2] Stewart S., Fredericks P.M. Surface-enhanced Raman spectroscopy of amino acids adsorbed on an electrochemically prepared silver surface. Spectrochimica Acta - Part A: Molecular and Biomolecular Spectroscopy 55:1641–1660, 1999.

Pregnant sows clicker training: collection of blood and milk for *in vivo* lactation study

Camilla Aniballi [(1)], Alberto Elmi [(1)], Domenico Ventrella [(1)], Mie Johanne Hansen [(2)], Kirsten Rosenmay Jacobsen [(2)], Martina Bertocchi [(1)], Maria Laura Bacci [(1)]

(1) Dipartimento di Scienze Mediche Veterinarie, Università di Bologna (2) Ellegaard Göttingen Minipigs, Dalmose Denmark

The ConcePTION project (G.A.: 821520) has the goal of establishing a trusted network that can efficiently generate reliable evidence-based information regarding effects of medications used during pregnancy and breastfeeding in humans. In particular, work package 3 focusses on developing a relevant lactation animal model, translatable to humans, to perform non-clinical trials prior drug approval; the selected species, on the basis of an accurate review of the literature, is the porcine one [1]. A key factor in animal trials is behavior, that can be shaped through the consistent use of positive reinforcement training, also fulfilling the 3Rs principle for animal welfare. Clicker training , firstly described for dogs [2], is accepted worldwide as one of the most effective method to teach basic obedience to different animal species and to direct their intelligence toward productive and positive activities. It has been adapted to numerous other species, including pigs [3]. The method is based on positive reinforcement, precisely with the association of the double-click sound produced by a specific device, with a reward, such as a delicious snack or a toy. This report aims at showing how sows can be trained for milk and blood collection, looking at both “standard size” and minipig. Overall 8 pregnant sows were daily trained, 4 commercial hybrids and 4 Göttingen minipigs, provided by Ellegaard (contributing as breeding facility for the project). Pregnant sows were gradually accustomed to follow the trainer and being manipulated, with getting easy and quick access to mammary glands and ears (long term catheter for blood collection) as final goal. Training sections were short (20 min) but consistent, repeated daily (Monday-Friday), starting from the diagnosis of pregnancy. The chosen reward, to keep animals motivated, was apple slices and juice. After the first week, all animals were easily manipulated and seemed to enjoy the training session, an additional occasion to get out of the cage and explore the environment. One of the biggest challenges in pig training, especially when looking at conventional animals, is getting the animals to trust the operator and understand that no harm is involved. Once they understand it, the training session becomes a welfare enrichment itself. The clicker seems to represent a good approach, as it provides a consistent and concise message, allowing humans to clearly communicate with the sows, thus reducing confusion and frustration for both. After some repetitions, the action-click-reward association becomes a conditioned stimulus that can keep the animals motivated to accomplish different tasks having fun in the meantime. As demonstrated by previous studies, swine positive emotions could be indicated by play, barks and tail movements, while negative behaviours are suggested by freezing, defecating/urinating, escape attempts, high-pitched vocalizations and ear movements [4]. During the training sessions all the animals showed well-being associated activities, and, after the first approaches, it was themselves who manifested the desire to extend the length of the training session.

Experiment authorization number: 32/2021-PR.
Acknowledgement: this work has received support from the EU/EFPIA Innovative Medicines Initiative [5] Joint Undertaking ConcePTION grant n° 821520.

[1] Ventrella et al., Animals 2021;11:714. [2] Welsh T. Communities 1999:61. [3] Paredes-Ramos Animals 2020;10:959. [4] Reimert et al., Physiology & Behavior 2013;109:42–50. [5] Homepage. IMI Innovative Medicines Initiative n.d. http://www.imi.europa.eu/ (accessed May 18, 2021).

Assessing sound perception and localization in family dogs (*Canis familiaris*)

Cécile Guérineau, Paolo Mongillo, Miina Lõoke, Giovanni de Vincenzi, Lieta Marinelli

University of Padua, Department of Comparative Biomedicine and Food Science

Corresponding author: Lieta Marinelli (lieta.marinelli@unipd.it)

Research on dogs' auditory abilities has been very limited with only two studies based on behavioral methods [1,2] and more recent ones based on electrophysiological methods [3]. However, thresholds obtained with different methods in both humans and dogs [3] differ in terms of values, inter/intra-individual variability and biological significance. Electrophysiological methods only inform about auditory thresholds capable of generating a neurophysiological signal, while behavioral methods also highlight the appropriate use of sound information. Moreover, electrophysiological methods are more invasive, which limits their applicability. The aim of this study was to develop a behavioral, non-invasive methodology to assess different aspect of auditory abilities of family dogs, to be useful to broaden our knowledge on dog's auditory processing. Two procedures were developed to estimate respectively hearing thresholds at a specific sound frequency and dogs' ability to localize sounds in the azimuthal plane. Dogs were initially trained to discriminate a pure tone, optimized for dogs' hearing capabilities [1,4] emitted alternatively from of two speakers placed laterally of the dog. Then, for the estimation of hearing thresholds, the intensity of the pure tone (4 kHz, 750 ms) was systematically manipulated (range: 70-10 dB SPL). For the estimations of localization, speakers were places at progressively smaller angles of separation (range: 120°-4°). For each procedure, dogs underwent an ascending and a descending staircase assessment, each featuring a minimum of 10 estimations and terminating when the difference between the last two estimations was ≤ 3 dB or ≤1°, respectively for hearing threshold (as in [1]) and sound localization. Intensity threshold was assessed in 3 dogs, involved in 25±6 sessions. The minimum dB level at which the dog responded incorrectly, averaged across all dogs, was 16.4±3.6 dB; the minimum dB level at which the dog subsequently responded correctly was 21.4±3.5 dB. The intra-individual difference among the estimated thresholds obtained in the two assessments was 2.3±0.6 dB. The Minimum Detectable Angle (MDA) was estimated in one dog was between 5.0±1.0° and 6.6±1.5°, for minimum angle at which the dogs responded incorrectly and correctly respectively. Taken together, the results suggested that this methodology is feasible to assess family dogs' auditory abilities with a sensitivity of 3 dB SPL for hearing threshold, and 1° for MDA. Although repeatability within-individual is promising, more data are needed to confirm the validity of the methods and their comparison with the existing literature.

[1] Heffner H.E. Hearing in large and small dogs: absolute thresholds and size of the tympanic membrane, Behavioral Neurosciences, 97:310-318, 1983.
[2] Babushina E. Comparative analysis of spatial hearing of terrestrial, semiaquatic and aquatic mammals, In Advances in sound localization, ed. Intechopen, 477-492, 2011.
[3] Scheifele P.M. Electrodiagnostic evaluation of auditory function in the dog, Veterinary Clinics of North America Small Animal Practice 42:1241-1257, 2012.
[4] Risoud M. Sound Source localization, European Annals of Otorhinolaryngology, Head and Neck diseases, 135:259-264, 2018.

Evaluation of cortisol, dehydroepiandrosterone sulphate and testosterone concentrations in hair of wild roe deer bucks in Oltrepo Pavese area

Susanna Draghi (1), Tanja Peric (2), Davide Forni (1), Chiara Geloni (1), Isabella Pividori (2), Stella Agradi (1), Sebastiano Luridiania (4), Laura Menchetti (3), Alberto Prandi (2), Giulio Curone (1)

(1) University of Milan, Department of Veterinary Medicine (2) University of Udine, Department of Agricultural, Food, Environmental and Animal Sciences (DI4A) (3) University of Bologna, Department of Agricultural and Food Sciences (4) University of Sassari, Department of Veterinary Medicine

Corresponding author: Susanna Draghi (susanna.draghi@studenti.unimi.it)

The European roe deer (*Capreolus capreolus*) is one of the most abundant ungulate species in Europe. Roe deer is highly adaptable, living in broadleaved forests, ecotonal strips, and agricultural areas, in mountains or lowland regions. In the last few years, because of the increase of human activities, competitors (as e.g. deer) and predator species (italian wolf and golden jackal) its population is leading to several changes in habitat and behaviour with an increase of stressful situations. The best-studied component of the stress response in humans and mammals is the activation of the hypothalamic-pituitary-adrenal (HPA) axis. Glucocorticoids, in particular cortisol, are the downstream effectors of the HPA axis but also sex hormones such as testosterone are involved in the stress response. The impact of stressors can be evaluated also by dehydroepiandrosterone sulphate (DHEA-S) used in relation to cortisol (cortisol/DHEA-S ratio) that can provide additional information about allostatic load as well in relation to testosterone (DHEA-S/testosterone ratio) regarding the reproductive sphere. Measurement of free circulating steroids in blood is the most common way to obtain information on the functioning of an endocrine axis, but, although still routinely applied to domestic animals, is much less feasible for wild animals. On the other hand, hair incorporates blood-borne hormones during its active growth phase providing retrospective information and thus, can be collected also at animal's death. The aim of the work was to quantify hair concentrations of cortisol, DHEA-S and testosterone in wild roe deer bucks, using a radioimmunoassay methodology, calculate their ratios and evaluate the effect of the geographical area, altitude, and age of animals. Thirty sexually mature roe deer bucks, 24 to 72 months old, were sampled during the 2019 hunting season between 21st August and 14th September. Animals were hunted in five different zones (1-5) in the South area of Oltrepo Pavese (Italy) according to the regional hunting plan (Ordinance No. 8534 of the Lombardia region, 10th June, 2019). The association between hair hormone concentrations and age, altitude, and the geographic area was evaluated by t-test for independent samples and one-way ANOVA. Age and altitude were categorized in two levels according with their medians (3 years and 700 m a.s.l., respectively). Kolmogorov-Smirnov and Levene tests were used to verify assumptions. Mean values (±standard deviation) were 1.47±0.44 pg/mg, 172.19±86.93 pg/mg, and 7.26±2.48 pg/mg for cortisol, DHEA-S, and testosterone, respectively. Cortisol tended to be higher in animals less than 3 years old (1.57±0.43 pg/mg and 1.28±0.41 pg/mg for ≤3 years and >3 years, respectively; P=0.086) while the geographical area influenced testosterone concentrations (P=0.041). In particular, the animals in zone 4 had higher values (9.57±3.60 pg/mg) than those in zones 1 (6.84±1.83 pg/mg; P=0.027), and 3 (5.16±0.61 pg/mg; P=0.003). Altitude did not affect any variables. The fact that the cortisol tended to be higher in animals less than 3 years old is probably due to the intrinsic characteristics of the species in which young animals need to fight with old and dominant animals to obtain new areas for reproduction. In conclusion, evaluations made by hair analyses can help in studying the presence of a territorial competition between individuals in the wild roe deer buck.

Hematological parameters reference values and factors affecting them in Verzasca and Camosciata delle Alpi female goats reared with semi-extensive system in Italy

Stella Agradi (1), Giulio Curone (1), Gabriele Brecchia (1), Laura Menchetti (2), Alessia Gazzonis (1), Maria Teresa Manfredi (1), Sebastiano Luridiana (3), Davide Forni (1) Daniele Vigo (1)

(1) Department of Veterinary Medicine (DIMEVET), University of Milan, Lodi, Italy (2) Department of Agricultural and Food Sciences, University of Bologna, Bologna, Italy (3) Department of Veterinary Medicine of Sassari, University of Sassari, Italy

Corresponding author: Stella Agradi (stella.agradi@unimi.it)

The loss of biodiversity is a worldwide issue. Livestock biodiversity is pivotal to ensure agriculture and food production sustainable development and food security. In Italy, according to the national association of pastoralism (Assonapa) 46 out of 55 goat breeds are considered autochthonous. Given the greater adaptiveness to the territory and products' quality, the provision of ecosystem services and the socio-economic and cultural value, it is pivotal to improve knowledge about these breeds. To define haematological parameters physiological ranges for specific breeds is fundamental to help the management and the recovery of this important territory's heritage. The aims of this study were to establish haematological reference values for Verzasca and Camosciata delle Alpi goats, a local and a cosmopolitan breed, respectively, and to investigate the influence of breed, age, parity order, season and days in lactation on the haematological parameters.

Thirty-seven Camosciata delle Alpi and 34 Verzasca clinically healthy goats from the same farm in Varese province were enrolled. The management conditions were the same for all goats, based on traditional agro-pastoral practices: free grazing on alpine pasture from March to November. Kidding season was from January to March. Blood samples were collected monthly from January to December. The haematological parameters determined were: red blood cells (RBC), hemoglobin (HGB), packed cell volume (PCV), mean corpuscular volume (MCV), mean corpuscular hemoglobin (MCH), mean corpuscular hemoglobin concentration (MCHC), red cell distribution width (RDW), white blood cells (WBC), neutrophil, lymphocyte, monocyte, eosinophil, basophil count (NEU, LYMPH, MONO, EOS, BAS) and fraction (NEU, LYMPH, MONO, EOS, BAS fraction). Reference intervals were determined according to Friedrichs et al. [1]. A multi-way mixed model-repeated measures ANCOVA was applied to evaluate the effect of breed, age, parity, season; the effect of days in milk was evaluated with a bivariate least square linear regression model. Statistical analyses were performed with SPSS Statistics version 25 (IBM, SPSS Inc., Chicago, IL, USA). This is the first study that has investigated the hematological reference values in Verzasca and Alpine goat breeds. The reference intervals obtained for both breeds were in general narrower than the ones reported by Schalm et al. [2]. Verzasca goats had significantly higher erythrocytic parameters and lower WBC and NEU, which could be due to greater adaptation to territory and resistance/resilience to gastrointestinal parasitism than Camosciata, as testify also by age effect on EOS and EOS fraction. The age and parity effects were consistent with what was found in literature. Season influence on parameters could be explicated by gastrointestinal parasitism trend during the year, peripartum physiological changes and melatonin immunostimulanting effect. Days in milk affected nearly all parameters, partly due to the same season's influencing factors.

This study improves knowledge about these breeds and have underline some physiological mechanisms that cause fluctuation of haematological parameters in goats.

[1] Friedrichs et al., ASVCP reference interval guidelines: Determination of de novo reference intervals in veterinary species and other related topics, Vet. Clin. Pathol., 41:441–453, 2012.

[2] Schalm O. W., Jain N. C., and Carrol E. J.. Schalm's Veterinary Hematology, IV ed. Lea & Febiger, 1986.

ORAL COMMUNICATIONS

SOIPA

Comparison of two different methods to evaluate *Varroa destructor* infestation in bee farms

Roberto Bava, Fabio Castagna, Cristian Piras, Domenico Britti, Vincenzo Musella

Department of Health Sciences-University of Catanzaro Magna Græcia, CISVetSUA, Catanzaro, Italy

Corresponding author: Roberto Bava (roberto.bava@unicz.it)

V*arroa destructor* is an important ectoparasitic mite of bees. The *Varroa* control programs are mainly based on the use of chemical acaricides that are often used indiscriminately. All this has led to phenomena of drug resistance of great concern. Monitoring the level of *V. destructor* infestation is very important and allows to determine the extent of invasion of the parasite in bee colonies. This facilitates the choice of the most appropriate treatment method and timing. The powdered sugar shake is commonly used to determine mite levels in colonies.

The aim of this study was to compare the diagnostic accuracy of the classical method with a recent commercial device for field screening, the "*Varroa EasyCheck*". The investigations were carried out in two apiaries not treated with acaricides in the previous five months. Seven colonies were randomly chosen for each apiary. Two samples of adult worker bees were taken from the same open brood comb and each one was subsequently analyzed with the two different methods.

For the evaluation with the *"sugar shake method"* a sample of 300 live bees was transferred to a glass jar of 0.9 L capacity covered with a modified lid by applying a 3×3 mm mesh screen which retains the bees but allows mites to go out after shaking. Two tablespoons of powdered sugar were pushed through the shielding mesh. The jar with the powdered sugar was shaken for 1 minute. It was held still for 30 seconds and shaken for another minute. The jar was turned upside down onto a plate filled with water and shaken until no more mites came out. The parasites were then counted.

For the second diagnosis, the "*Varroa EasyCheck*" was used, a *Véto-pharma* tool based on flotation principle. For this test, 300 bees were shaken for 60 seconds in the "*Varroa EasyCheck"* filled with an alcohol solution or a winter windshield washer fluid. The mites were separated from the bees and, once they fell to the bottom of the transparent bowl, they were counted.

The measured infestation rates of *V. destructor* in the tested samples were 1.90% (±SD:1.80) and 1.95% (±SD:0.99) for the first and second group when using *"sugar shake method"* and 1.71% (±SD: 1.18) and 2.19% (±SD:1.25) for the first and second group when using "*V*arroa *EasyCheck"* device. No statistically significant differences in diagnostic accuracy were shown between the two techniques, and the results demonstrated that the two methods are both effective. *Varroa EasyCheck* can be used during the nectar import period and on humid days and has proven to be more practical and faster than the sugar shake method. The sugar shake method, on the other hand, proved to be more ethical as it does not kill the tested bees, except for a small number close to 10%.

[1] Beata Bak et al., Animal Science, 33:38-46, 2009. [2] De Jong d. et al., Apidologie, 297:306-13, 1982. [3] Lee et al., American Bee Journal, 150:1151-1155, 2010.
[4] Branco et al., Apidologie, 37: 452-461, 2006.

Epidemiology and genetic diversity of *Toxoplasma gondii* and *Neospora caninum* in cattle and sympatric rodents

Claudia Camerlo , Stefania Zanet , Elena Battisti , Ezio Ferroglio

Università degli Studi di Torino, Dipartimento di Scienze Veterinarie

Corresponding author: Ezio Ferroglio (ezio.ferroglio@unito.it)

Toxoplasma gondii and *Neospora caninum* are relevant pathogens of human and veterinary concern. The objective of the present study was to evaluate prevalence and genetic variability of *T. gondii* and *N. caninum* in cattle and sympatric rodents in 7 farms from Piedmont region. Rodents from nearby sylvatic areas were also captured and tested as reference for locally circulating genotypes. Prevalence of *T. gondii* and *N. caninum* was determined by PCR carried out on bovine diaphragmatic muscle and on central nervous system, kidney and skeletal muscle of rodents [1]. The genotype of *T. gondii* was subsequently determined by PCR-RFLP [2]. All cattle tested negative for *N. caninum* (95% CI: 0.00%-6.02%) while in captured rodents the prevalence reached 15.00% (95% CI: 5.24%-36.04%). In the bovine population, *T. gondii* was detected with a prevalence of 15.00% (95% CI: 8.10%-26.11%) while in sympatric rodent populations prevalence reached 40.00% (95% CI: 21.88%-61.34%). *T. gondii* genotype *I* was detected in 88.9% of positive cattle, followed by 11.1% of atypical genotypes. In rodents, living in sympatry with cattle, genotyping revealed a similar genotype distribution with genotype *I* detected in 87.5% of positive rodents and atypical genotypes in 12.5%. In rodents captured from sylvatic environment, genotype *I* was detected in 37.5% of the positive individuals followed by 12.5% of genotype *II*. Atypical genotypes were detected with higher prevalence, in 50% of positive rodents. In cattle, *T. gondii* prevalence was inversely related to rodent's abundance in the farm and was higher in younger animals suggesting a predominant role of vertical transmission. Genotyping allows us to hypothesize that rodents are not a main source of infestation for cattle as higher genetic variability was detected in rodent from sylvatic environment.

[1] Ferroglio et al. *Toxoplasma gondii* in sympatric wild herbivores and carnivores: epidemiology of infection in the Western Alps, Parasites and Vectors, 7:196, 2014.
[2] Battisti et al. Circulating genotypes of *Toxoplasma gondii* in Northwestern Italy, Veterinary Parasitology, 253:43-47, 2018.

Preliminary assessment of Body Condition Score as a possible marker for the targeted selective treatment of dairy sheep against gastrointestinal nematodes

Antonio Scala, Claudia Tamponi, Luisa Meloni, Francesca Porcu, Cinzia Pasini, Stephane Knoll, Maria Francesca Sini, Lia Cavallo, Giorgia Dessì, Antonio Varcasia

Parassitologia, Dipartimento di Medicina Veterinaria, Università degli Studi di Sassari, Italy

Corresponding author: Antonio Scala (scala@uniss.it)

The Body Condition Score (BCS) is generally considered the best and simplest indicator of an animal's available fat reserves for the use during periods of high energy demand, stress or sub-optimal nutrition as is for example characteristic of infections caused by Gastro-intestinal Nematodes (GIN) [1].

The purpose of this study is to assess and (possibly) validate the effectiveness of BCS as a parameter to be adopted for the implementation of Targeted Selective Treatments (TSTs) in dairy sheep farms in the context of Anthelmintic Resistance (AR) prevention.

The basis for this research were two sets of individual coproscopic examinations using the McMaster's method conducted on n=394 and n=618 (total n=1012 samples) Sarda breed X Lacaune in lactation. Individuals within this research were sampled at 3 and 5 months after lambing and their BCS was simultaneously recorded. For each sample category, 5 fecal pools were created from which coprocultures were set up for the cultivation and subsequent identification of the third-stage larvae (L3) of the GIN present.

An overall GIN prevalence rate of 85.4% (CI 95%:83.2-87.6) was found, with average EPG values of 210.1±347.3.

Prevalence rates of 92.1% (CI95%: 0.89.4-94.8) and 81.1% (CI95%: 78.0-84.2, X2=266.82; P=0.000), with average EPG levels of 280±424.9 and 165.6±278.5 (Mann-Whitney test – W=231943.5; P=000), were found for the samples collected at 3 and 5 months of lactation respectively.

Following coproculture, the genera *Teladorsagia* spp. (49.2%) and *Trichostrongylus* spp. (24.6%) were identified to be the most abundant GIN present within both categories.

A Spearman correlation of Rho=-0.163; P=0.000; R2=0.0222 was found between the EPG values of the 1,012 monitored sheep and their BCS scores. Most significant values were seen in sheep with >4.5 years of age.

Average BCS values stratified according to EPG-class showed animals with the lowest BCS, i.e. 2.25, to have the highest EPG averages (461±501).

Data obtained within this research confirmed BCS values to be negatively correlated to the EPG values for GIN found in sheep. Specifically, this study was able to point out a negative correlation between BCS and EPGs (about 200 EPG) mainly related to the presence of *Telardorsagia* spp. and *Trichostrongylus* spp. in lactating dairy sheep at 3rd - 5th month of lactation.

The evaluation of BCS was not found to be a precise enough tool for application as a criterium for TST. This finding is in accordance with Soto-Barrientos et al. [2] who consider all sheep with a BCS >2 to have a GIN burden compatible with production.

Future studies carried out on flocks with higher EPG averages could provide a clearer indications of the application of BCS as a marker for TST in dairy sheep.

[1] Coop R.L., Kyriazakis I. Nutrition–parasite interaction. Veterinary Parasitology, 84:187–204, 1999.

Distomatosis in sheep in Italy: a multicentric survey

Antonio Scala (1), Antonio Varcasia (1), Claudia Tamponi (1), Giorgia Dessì (1), Gabriella Gaglio (2), Giovanni De Benedetto (2), Manuela Diaferia (3), Giulia Morganti (3), Giulia Rigamonti (3), Sebastian Alessandro Mignacca (3), Antonio Bosco (4), Alessandra Amadesi (4), Giuseppe Cringoli (4)

(1) Università degli Studi di Sassari, Dipartimento di Medicina Veterinaria (2) Università degli Studi di Messina, Dipartimento di Medicina Veterinaria (3) Università degli Studi di Perugia, Dipartimento di Medicina Veterinaria (4) Università degli Studi di Napoli Federico II, Dipartimento di Medicina Veterinaria

Corresponding author: Antonio Scala (scala@uniss.it)

The present survey reported the results of a multicentric study on distomatosis of sheep in Italy. The study was carried out in 5 Italian regions (Basilicata, Campania, Sardinia, Sicily and Umbria) from July 2019 to June 2020 and involved 28 sheep farms from each region. A total of 278 fecal pooled samples were examined using the FLOTAC® technique with a zinc sulphate solution (ZnSO4), specific gravity (s.g.) 1.35, and eggs per gram (EPG) were determined according to Cringoli et al. [1]. All data were recorded on a spreadsheet (Microsoft Excel®, Microsoft Corp., Redmond, WA) and subsequently processed considering the sampling seasons and the animal category (adults and lambs).

An overall prevalence of 19.4% (54/278) and a mean EPG of 12±41,5 were detected for *Dicrocoelium dendriticum*, 7.9% (22/278) and 3±15.2 for *Calicophoron daubneyi* and 4.7% (13/278) e 2.3±14.1 for *Fasciola hepatica.* Significant differences between prevalence rates ($\chi2=35.04$; df=2; $P< 0.0001$), as well as between mean EPG values (Kruskal-Wallis Test = 10.08; P= 0.006) were found.

In detail, *D. dendriticum* was detected in 27.1% (38/140) of adult sheep and 11.6% (16/138) of lambs ($\chi2=10.735$; P=0.0011); *C. daubneyi* in 10% (14/140) of adults and 5.8% (8/138) of lambs ($\chi2=1.685$; P=0.1943), and *F. hepatica* in 5% (7/133) of adults and 4.3% (6/132) of lambs ($\chi2=0.663$; P=0.7967).

Prevalence rates detected for each region were: for *D. dendriticum* 32.1% in Sardinia and Umbria, 20% in Campania, 10.7% in Basilicata and 1.8% in Sicily; for *C. daubneyi* 21.8% in Campania, 10.7% in Sardinia, 7.1% in Umbria, 0% in Basilicata and in Sicily and for *F. hepatica* 18.2% in Campania, 3.6% in Basilicata, 1.8% in Sardinia, 0% in Sicily and Umbria. No significant differences were observed between the prevalence rates recorded for each parasite stratified by region and season, considering both the total of examined samples and the animal category (adults and lambs) ($P>0.05$).

According to the results of the present study, *D. dendriticum* appear to be the most widespread trematode in Italy, although, as for the other distomatosis, a lower prevalence was recorded compared to previous investigations. Conversely, the diffusion of *C. daubney* in the monitored regions seems to be increased if compared with previous epidemiological surveys.

[1] Cringoli G., Rinaldi L., Maurelli M.P., Utzinger J. FLOTAC: New multivalent techniques for qualitative and quantitative copromicroscopic diagnosis of parasites in animals and humans, 2010. Nat Protoc, 5 (3), 503-515.

POSTER

POSTER AIPVET

P51 - Investigation on Epithelial to Mesenchymal Transition (EMT) process in Equine papillomavirus-2 (EcPV-2) positive penile squamous cell carcinomas

Federico Armando (1), Samanta Mecocci (2), Valentina Orlando (3), Ilaria Porcellato (2), Katia Cappelli (2,4), Luca Mechelli (2), Chiara Brachelente (2), Marco Pepe (2,4), Rodolfo Gialletti (2,4), Alessandro Ghelardi (5), Benedetta Passeri (3), Valeria Listorti (6) and Elisabetta Razzuoli (6)

(1) Department of Pathology, University of Veterinary Medicine Hannover, Hannover 30559, Germany (2) Università degli Studi di Perugia, Dipartimento di Medicina Veterinaria (3) Università degli Studi di Parma, Dipartimento di Medicina Veterinaria (4) Università degli Studi di Perugia, Centro di Ricerca sul Cavallo Sportivo (5) Azienda Usl Toscana Nord-Ovest, UOC Ostetricia e Ginecologia, Ospedale Apuane (6) IZSLPV, Centro di Referenza di Oncologia Veterinaria e Comparata (CEROVEC)

Corresponding author: Federico Armando (federico.armando@tiho-hannover.de)

Squamous cell carcinoma (SCC) is one of the most common cancers in horses, and it can arise at any site on the skin and mucosae [1]. Recent studies associated equine papillomavirus type 2 (EcPV2) infections with this type of cancers of the oral tract and genitals [2]. Equine penile squamous cell carcinomas associated with papilloma virus (PV) infection have been recently proposed as model for human PV-induced squamous cell carcinomas. It has already been preliminarily suggested by Bonnet et al that equine penile squamous cell carcinomas might undergo epithelial to mesenchymal transition (EMT)[3]. This work mainly aims to further investigate in detail EMT process in equine penile squamous cell carcinomas. Furthermore, this work aims also to investigate on the possible role of PV oncoproteins influencing the EMT process.

To this purpose, 18 penile SCCs were retrospectively selected and tested for EcPV2 presence and oncoprotein (EcPV2-E6 and EcPV2-E7) expression. Moreover, a wide immunohistochemical EMT characterization was carried out on these samples, analyzing the main epithelial markers (E-cadherin, β-catenin, and pan-cytokeratin AE3/AE1), the main mesenchymal markers (N-cadherin and vimentin), and the main EMT-related transcription factors (TWIST-1, ZEB-1). PCR revealed a positivity to EcPV2 in 16/18 samples. Interestingly, E6 and E7 oncogenes were expressed in 54% of samples. The immunohistochemistry results suggested an epithelial to mesenchymal transition process of the neoplastic cells on the tumor invasive front. This work illustrates an example of tumor cell adaptation during the invasive process of the equine penile squamous cell carcinomas and suggests the potential influence of EcPV2 oncoproteins on the EMT process.

[1] Scott, D.W.; Miller, W.H.J. Squamous cell carcinoma. In Equine Dermatology, 1st ed.; W.B. Saunders: St. Louis, MO, USA, 2003; pp. 707–712.
[2] Sykora, S.; Brandt, S. Papillomavirus infection and squamous cell carcinoma in horses. Vet. J. 2017, 223, 48–54.
[3] Bonnet, A.S.; Willis, C.; Pittaway, R.; Smith, K.; Mair, T.; Priestnall, S.L. Molecular carcinogenesis in equine penile cancer: A potential animal model for human penile cancer. In Urologic Oncology: Seminars and Original Investigations; Elsevier: Amsterdam, The Netherlands, 2018; Volume 36, pp. 532.e9–532.e18.

P77 - Molecular detection of *Bartonella* spp. in wild rodents of two Swiss regions

Sara Divari (1), Marta Danelli (1), Paola Pregel (1), Giovanni Ghielmetti (2), Nicole Borel (3), Enrico Bollo (1)

(1) Università degli Studi di Torino, Dipartimento di Scienze Veterinarie (2) Institute for Food Safety and Hygiene, Section of Veterinary Bacteriology, Vetsuisse Faculty, University of Zurich, Zurich, Switzerland (3) Institute of Veterinary Pathology, Vetsuisse Faculty, University of Zurich, Zurich, Switzerland

Corresponding author: Sara Divari (sara.divari@unito.it)

Rodents represent a natural reservoir of several *Bartonella* species, including some zoonotic species such as *B. elizabethae*, *B. grahamii*, and *B. vinsonii* [1]. In this study, wild rodents collected from two sites in rural areas of Switzerland were screened for *Bartonella* spp. using molecular detection methods. In brief, 346 rodents were trapped between April and November 2017 in the Gantrisch Nature Park (Switzerland), in the municipalities of Plasselb (canton of Fribourg) and Riggisberg (canton of Bern). Tissues of each three animals were pooled (n=116) and extracted DNA was tested by means of an initial screening, using a qPCR amplifying the 16S-23S rRNA gene intergenic transcribed spacer region. Positive pools (84/116, 72.4%) were further analyzed by end-point PCR amplification of a citrate synthase (*gltA*) locus.

DNA was then extracted from spleen belonging to single animals (129/346) of pools that yielded bands on gels consistent with *gltA* (43/84). End-point PCR for *gltA* and RNA polymerase subunit beta (*rpoB*) was performed and amplicons consistent with *gltA* and *rpo*B loci were generated in 73/129 (56.6%) and 64/129 samples (49.6%), respectively.

Based on PCR results and further sequencing, the prevalence of infection with *Bartonella* spp. in captured rodents was 20.8%: 41/129 in *Apodemus* sp., 9/86 in *Arvicola scherman*, 22/129 in *Myodes glareolus* and 1/2 in *Microtus agrestis*. A significant association was observed between *Bartonella* spp. infection and rodent species ($p<0.01$) and between trapping region and positivity to *Bartonella* spp. infection ($p<0.001$). Likewise, prevalence of *Bartonella* DNA was significantly higher ($p<0.001$) in rodents trapped in woodland area (66/257, 25.7%) compared to those captured in open field (9/89, 10.1%).

GltA sequences showed 100% identity to *Candidatus Bartonella rudakovii* in three *Myodes glareolus* out of 129 animals (2.3%). The *gltA* sequences of *Bartonella* sp. from one *Myodes glareolus* (1/129, 0.8%) and one *Arvicola scherman* (1/86, 1.2%) showed 100% similarity to *B. grahamii* and *B. doshiae* respectively. *B. taylorii* was identified (identity 100%) by sequencing *rpo*B amplicons from 13/129 *Apodemus* sp. and 2/129 *Myodes glareolus*. One *Apodemus* sp. (1/346, 0.3%) was infected with *B. birtlesii* (*rpo*B, 100% identity). The remaining *Bartonella* positive samples (53/72) showed less than 100% identity to the closest relatives, and in particular to *B. taylorii* for 42/72 positive animals.

A BLASTn and phylogenetic analysis of *gltA* and *rpo*B loci identified in this study, revealed that most *Bartonella* sp. detected are closely related to *B. taylorii* followed by *B. grahamii* and *B. birtlesii*, which are commonly observed species in European rodents [2].

In conclusion, wild rodents infected with zoonotic *Bartonella* species were detected in two rural areas of Switzerland. This study extends our understanding of the epidemiologic significance of wild rodents and their public health importance as reservoir of *Bartonella.*

[1] Gutierrez, R. et al. *Bartonella* infection in rodents and their flea ectoparasites: an overview. *Vector Borne Zoonotic Disease.* 15, 27–39, 2015.
[2] Szewczyk et al. Molecular Detection of *Bartonella* spp. in Rodents in Chernobyl Exclusion Zone, Ukraine. *Acta Parasitologica.* 66, 222–227, 2021

P97 - mTOR and S6K1 expression in canine osteosarcoma

Lorella Maniscalco (1), Raffaella De Maria (2), Katia Varello (1), Cecilia Gola (2), Angelo Ferrari (1), Elena Bozzetta (1), Paola Modesto (1)

(1) Istituto Zooprofilattico Sperimentale del Piemonte, Liguria e Valle d'Aosta, National Reference Center for the Veterinary and Comparative Oncology (CEROVEC) (2) Università degli Studi di Torino, Dipartimento di Scienze Veterinarie

Corresponding author: Lorella Maniscalco (lorella.maniscalco@izsto.it)

Canine and feline spontaneous tumors are suitable models for human cancers due to a strong molecular, histopathological and clinical similarity. Among canine tumors, osteosarcoma (OSA) represents the most common primary malignant bone tumor, it is locally aggressive and has a high metastatic potential and a poor prognosis. Mammalian target of rapamycin (mTOR) is a conserved protein kinase playing a pivotal role in cellular signals mediated by several growth factors and tyrosine kinase receptors. One of the most important downstream effector of mTOR is the ribosomal p70 S6 kinase (S6K1) and the eukaryotic translation initiation factor 4E-binding protein (4E-BP1) [1]. The activation of S6K1 and 4E-BP1 by mTOR pathways induces specific mRNA transcription and a subsequent increase of proteins involved in cell growth, proliferation, invasion and anti-apoptotic processes. Aberrant activation and mutations of mTOR pathway are associated to several tumors in human and specific drugs able to inhibit this protein have been developed as new potential target therapy. Recently, RNAseq performed by the authors on canine OSA cell lines revealed that mTOR and S6K1 are overexpressed in all analysed cell lines suggesting an important role of these proteins in canine OSA [2]. On the basis of these molecular data, the aim of this work is to evaluate mTOR and S6K1 expression in canine OSA by immunohistochemistry in order to investigate the role of these proteins in canine OSA samples. Immunohistochemistry was performed on 22 OSA samples complete of histopathological and clinical data. Results revealed that mTOR was present in 11/22 cases (50%) while S6K1 was expressed in 17/22 cases (77,2%); additionally, we found that S6K1 was present in 6/22 (27,27%) of cases S6K1 protein was present in absence of mTOR protein. No association between mTOR and S6K1 expression and histological grade (histological grade according to Loukopoulos and colleagues – 2007) [3] was found. Our preliminary results suggest that both mTOR and S6K1 are highly expressed in canine OSA and can represent a suitable target to deeply investigate in this tumor by the use of small-molecule inhibitors, microRNAs and natural compounds. Interestingly the few cases found positive only for S6K1 (27,27%) in absence of mTOR protein can suggest a negative feedback loops that can occur in case of over-activation of upstream pathways such as PI3K, AKT and ERK. This aspect has been previously demonstrated by Rozengurt and colleagues [5]. These preliminary data are according to human OSA where the pathway mTOR/S6K1 is involved in the prognosis of this tumors and represent an attractive target for innovative therapy [5].

[1] Gordon IK, Fang Y, Kent MS. Evaluation of the mammalian target of rapamycin pathway and the effect of rapamycin on target expression and cellular proliferation in osteosarcoma cells from dogs. American Journal of Veterinary Research, 69(8):1079-84, 2008.
[2] Gola C, Giannuzzi D, Rinaldi A, Selina Iussich, Modesto P, Morello EM, Buracco P, Aresu L, De Maria R. Genomic and transcriptomic characterization of canine osteosarcoma cell lines: a valuable resource in translational medicine. Frontiers in Veterinary Medicine. In press.
[3] Loukopoulos P, Robinson WF. Clinicopathological relevance of tumour grading in canine osteosarcoma. Journal of Comparative Pathology, 136 (1): 65-73, 2007.

P102 - Changes in the fat body and hypopharyngeal glands of nurse and forager honeybees and unfavorable climatic conditions in 2019

Karen Power, Manuela Martan, Gennaro Altamura, Giuseppe Borzacchiello, Paola Maiolino

Università degli Studi di Napoli "Federico II", Dipartimento di Medicina Veterinaria e Produzioni Animali

Corresponding author: Karen Power (karen.power@unina.it)

2019, because of the unfavorable climatic conditions, will probably be remembered as the year in which the number of honeybee colonies and honey production reached its lower levels in Italy. Climate change has been recognized as a cause of loss of honeybees and of the decrease in the related productions [1]. Global warming, late spring frosts and summer storms can alter the natural life cycle of plants and be responsible for the reduction in quality and quantity of the available nectar and pollen. The decrease in the amount and variety of pollen, the only source of amino acids for honeybees, can cause extensive damage to honeybees, as it affects growth, life expectancy and immunocompetence [2]. Protein nutrition is fundamental for the formation of the fat body and for the development and correct functioning of the hypopharyngeal glands. The fat body has an important role in the neutralization of toxic substances, in nutrients storage, and it is considered the main producer of carbohydrates and proteins, including vitellogenin (VG), a lipoprotein essential for the growth and development of honeybees [3]. The hypopharyngeal glands are responsible for the production of royal jelly, the synthesis of enzymes involved in the transformation of nectar into honey and storage of glycogen, necessary to support intense flight activity [4]. In this study, 8 samples of nurse and 8 of forager honeybees were collected between June and July 2019, and 8 samples of nurse and 8 of forager honeybees were collected between September and November 2020 and used as a control group. Half of the samples was frozen at -80° and used to quantify the VG mRNA by RT-qPCR, while the other half was 10% formalin fixed and analyzed by anatomo-histopathological techniques [5]. Moreover, morphometric analysis was performed using an image analysis software to evaluate the area (μm^2) of the fat body and hypopharyngeal glands (Mean Area±Standard Deviation). The results of this study show that the mean area values of the fat body in nurse (0.559±0.1) and forager (0.142±0.1) honeybees collected in 2020 were higher than that of nurse (0.301±0.036) and forager (0.034±0,007) honeybees collected in 2019. Moreover, the mean area values of the hypopharingeal glandular acini in nurse (1.152± 0.14) and forager honeybees (0.627± 0.36) collected in 2020 were higher than that of nurse (0.756±0.19) and forager (0.210±0.067) honeybees collected in 2019. Biomolecular results showed that VG was produced in higher amounts in all honeybees from 2020, while it was present in very small amounts in samples from 2019. Concluding, the reduction of the number of honeybee colonies as well as the loss in honeybee productions which happened in 2019 could be connected to the inadequate quantity and variability of pollen available to honeybees due to unfavorable climatic conditions. However, more studies are needed to confirm this hypothesis.

[1] Le Conte Y., Navajas M. Climate Change: impact on honey bee populations and diseases. Rev Sci Tech, 27(2):499- 510, 2008.
[2] de Groot A.P. Protein and amino acid requirements of the honey bee, Physiol Comp Oecol 3:1–90, 1953
[3] Chapman R.F. The Insects: structure and function, 4th ed. Cambridge University Press, 1998.
[4] da Cruz-Landim C., Costa R.A.C. Structure and function of the hypopharyngeal glands of *Hymenoptera*: a comparative approach, J Comp Biol, 3:151–63, 1998.
[5] Power K. et al., Histopathological findings in testes from apparently healthy drones of *Apis mellifera ligustica*, Vest Sci, 2;7(3):124, 2020.

P153 - Case study of larynx entanglement in two bottlenose dolphins (*Tursiops truncatus*) found stranded along the northern Adriatic coastline

Guido Pietroluongo, Giorgia Corazzola, Cinzia Centelleghe, Davide Pedrotti, Giuseppe Sciancalepore, Emilio Cardi, Sandro Mazzariol

Università degli Studi di Padova, Dipartimento di Biomedicina Comparata e Alimentazione

Corresponding author: Guido Pietroluongo (guido.pietroluongo@gmail.com)

Within the anthropogenic threats on marine wildlife, the interaction of cetaceans with fishing gears and activities are considered of high priority for welfare and conservation of these species [1] and it can be very challenging to diagnose. Among all, this interaction can result also in the ingestion of the net with serious and fatal consequences due to the depredation feeding behaviour of some species. The characteristic anatomy of odontocetes facilitates a particular consequence represented by the larynx entanglement. In this condition, upon swallowing the net (commonly gillnets), with or without the prey, instead of reaching the forestomach becomes entrapped in the larynx [2]. Two bottlenose dolphins (*Tursiops truncatus*) were found stranded along Veneto coastline in 2020 and 2021 respectively, with a net hanging from the mouth and evidences of larynx entanglement. Within the post mortem examination, the larynxes were macroscopically analysed and sampled, and tissue were processed for histology and stained with HE for routine microscopic examination. In both cases, the gross evidences showed the presence of a gillnet at the base of the organ resulting in a strangulation of the respiratory lumen. Histologically, the laryngeal tissue showed multifocal foci of chronic-active mixed inflammation, admixed with a severe fibrous reaction forming papillary projection in the site close to the entanglement and presence of diffuse granulation tissue and edema. In accordance with other studies in the same area (North Adriatic Sea), these findings are the most frequent pathological changes affecting the larynx [2].

These two cases open a warning scenario about the long-term consequences in the conservation of the resident subpopulation of bottlenose dolphins in this area, highlighting the importance to implement mitigation measures, investigation and quantification of dolphin fishery interaction.

[1] Jusufovski et al. Competition between marine mammals and fisheries in contemporary harvested marine ecosystems, Marine Ecology Progress Series, 627, 207-232, 2019.

[2] Gomerčić et al. Bottlenose dolphin (*Tursiops truncatus*) depredation resulting in larynx strangulation with gill-net parts, Marine Mammal Science, 25: 392-401, 2009.

P161 - Clinical and histopathological characteristics of two canine gastric vascular hamartomas

Ginevra Brocca (1), Salvatore Roggio (2), Giorgio Romanelli (3), Silvia Ferro (1)

(1) Università degli Studi di Padova, Dipartimento di Biomedicina Comparata e Alimentazione
(2) AniCura, Istituto Veterinario Novara (3) CSVet, Centro Specialistico Veterinario Milano

Corresponding author: Ginevra Brocca (ginevra.brocca@phd.unipd.it)

Canine gastrointestinal hamartomas are rare presentations that have only been detected once with a gastric location, in a case of smooth muscle hamartoma [1]. Like other diseases causing an encumbrance on the gastric lumen, they can clinically manifest with gastrointestinal signs [2].

Two elderly mixed breed dogs presented with chronic vomit and lack of appetite, with ultrasound and tomographic evidence of an intraluminal gastric mass of unknown origin. In both cases, the masses were surgically resected and sent to the histopathology service of the Department of Comparative Biomedicine and Food Science.

The two masses appeared macroscopically as either an exophytic mucosal and submucosal polypoid proliferation affecting the fundic region (dog 1), or as a mural submucosal bulging mass from the pyloric region (dog 2). After fixation and processing, the histological slides were evaluated by hematoxylin-eosin (HE) staining. Sub-grossly, the polypoid mass (dog 1) was poorly demarcated, in contrast to the mass of dog 2. Microscopically, both showed an irregular proliferation of vascular structures considered veins and arteries, severely expanding the submucosa in both cases and displacing the *tunica muscularis* in one case (dog 2). The vessels were lined by factor VIII+ non-atypical endothelial cells, often supported by irregularly thickened and shaped, muscular, α-SMA+ walls. The vessels were often engorged by red blood cells and multiple intravascular thrombi were noted. The polypoid mass also showed a mild lymphocytic infiltration of the adjacent mucosa and submucosa, while the second mass had a necrotic center with dystrophic mineralization and neutrophilic inflammation.

They have been diagnosed as a gastric hamartomatous polyp of vascular type (dog 1) and as a gastric vascular hamartoma (dog 2). Both dogs recovered well and were healthy one year after the surgery. Dog 1 is still alive, while dog 2 was euthanized for unrelated causes. In both cases, complete surgical excision was conclusive for diagnosis and treatment. This approach is indeed preferred to endoscopic biopsy, allowing the evaluation of the deeper layers.

In conclusion, the two masses had a different macroscopic presentation that initially led to consider them two different entities. However, on microscopic examination, both showed a prominent proliferation of well-developed, but disorganized, non-infiltrating vascular structures characterized by strong immunolabeling for factor VIII and α-SMA protein. These findings led to a common diagnosis of gastric vascular hamartoma. Due to their good prognosis, hamartomas should be considered among the differential diagnoses and differentiated from neoplasms with which they can share the macroscopic presentation.

[1] Taulescu et al. Gastric smooth muscle hamartomas mimicking polyps in a dog: A case description and a review of the literature. Case Rep. Vet. Med., 2013: 1–5, 2013.
[2] Diana A, Penninck DG, Keating JH. Ultrasonographic appearance of canine gastric polyps. Vet. Radiol. Ultrasound, 50: 201-204, 2009.

P175 - Regucalcin as robust biomarker to detect illegal administration of sex steroid hormones

Elena Biasibetti, Alessandro Benedetto, Chiara Beltramo, Valentina Audino, Simone Peletto, Elena Bozzetta, Marzia Pezzolato

Istituto Zooprofilattico Sperimentale del Piemonte, Liguria e Valle d'Aosta, Turin, Italy

Corresponding author: Elena Biasibetti (elena.biasibetti@izsto.it)

European countries banned the sex steroids administration alone or in association as growth promoters (1). Therefore, continuous monitoring to identify resurgence of their misuse is needed. The goal of study was to evaluate whether Regucalcin (RGN) protein levels can be compare with RGN mRNA levels on paraffin fixed testis tissue, in order to setup and combine these diagnostic tests for sex steroid hormones detection in veal calves in the frame of NRCPs. This promising marker was yet study by another research (2).

Formalin fixed paraffin embedded (FFPE) testis, samples from tissue bank of National Reference Center for Biological screening of anabolic substances in producing animals, were analyzed. The experiment was authorized by the Italian Ministry of Health and the Ethics Committee of the University of Turin. All animals were treated from the sixth to the seventh month of age and slaughtered after a withdrawal period of at least 20 days. Treatments were: nandrolone (n=10; 50 mg/head/week; four intramuscular injections), 17β-estradiol (n=10; 5 mg/head/week; four intramuscular injections), a cocktail of both nandrolone and 17 β-estradiol (n= 10; 50 + 5 mg/head/week, four intramuscular injections) and 10 control animals. Moreover 10 samples, declared non suspected by histological investigation, from veal calves in field condition were evaluated only with immunohistochemistry (IHC) assay. RGN levels were determined by quantitative Real Time PCR IHC assays using anti-RGN rabbit polyclonal antibody. A quantitative analysis of immunochemistry was made with the pixel classificatory. Pixel were quantified for each slide, in 5 randomly selected fields (200×) using NIS-Elements 4.5 (3). Shapiro–Wilk test was used to test for the normality of data. The one-way Kruskal–Wallis or ANOVA test (correction by the Dunnet and Dunn post hoc test) were performed ($p<0.05$). Test performances test were compared by multiple ROC curves. For molecular analysis, copy number estimation of RGN and Peptidylprolyl Isomerase A (PPIA) transcripts was made by standard curves method, to collect PPIA/RGN copy-ratio values, needed for cut-off and ROC curve calculations: an optimal cut-off at 0.03118 was then selected. At the established cut-off limit, the analysis allowed the discrimination of positive samples from negative ones with high sensitivity and mean specificity. Immunochemistry showed a significant ($p<0.0001$) reduction in RGN expression in all treated animals compared to the control group. Similarly, results were obtained by samples collected in in field condition. Real Time Pcr analysis allowed the discrimination of positive samples from negative ones with a sensitivity of 96.67% (82.78% to 99.92%, CI 95%) with a mean specificity of 90%, (55.50% to 99.75%, 95% CI, 9.667 likelihood ratio). Both tests resulted sensitive and specific, allowing to enrich, in future field investigation, novel integrated diagnostic protocols needed to unveil sex steroid abuse. Developed RT-qPCR and IHC methods confirmed RGN as a useful and robust biomarker to detect illegal administration of sex steroid hormones in veal calves. The developed methods, successfully applied to ten years old FFPE blocks, could allow both retrospective analyses, when supplementary investigations are requested by authorities, and future implementation of current NRCPs.

[1]EU Regulation 625/2017.
[2] Starvaggi Cucuzza et al. J Agric Food Chem 65:4866-4874, 2017.
[3] Casiraghi et al. BMC Bioinformatics 19:357,2018.

P176 - Isolation of *Streptococcus iniae* associated with granulomatous inflammation in farmed Adriatic sturgeons (*Acipenser naccarii*) in Italy

Elisabetta Antuofermo (1), Marta Polinas (1), Giovanni P. Burrai (1), Paolo Pastorino (2), Vasco Menconi (2), Mattia Tomasoni (2), Claudio Pedron (3), Marino Prearo (2)

(1) Università degli Studi di Sassari, Dipartimento di Medicina Veterinaria (2) Istituto Zooprofilattico di Piemonte, Liguria e Valle d'Aosta, Laboratorio di Ittiopatologia (3) Veterinary practitioner, Settala, Italy

Corresponding author: Elisabetta Antuofermo (eantuofermo@uniss.it)

Streptococcosis caused by *Streptococcus iniae* is one of the most important bacterial diseases in the aquaculture industry worldwide. This Gram-positive bacterium has been recently involved in outbreaks of streptococcosis in farmed sturgeon in Asia and America, posing a relevant threat to aquaculture production [1, 2]. *Streptococcus iniae* is a zoonotic pathogen causing a disease characterized by a systemic necrotizing and hemorrhagic inflammatory response with lymphocytes, macrophages, and heterophilic infiltration in sturgeons both in experimental and naturally occurring infection [1, 2, 3].

This report describes the pathological and bacteriological findings in an outbreak of *S. iniae* infection in Italian farmed Adriatic sturgeons. Low mortality of sturgeons in a farm of north Italy, associated with non-specific clinical signs, was reported. Different organs from four adult moribund sturgeons were collected for bacteriological and histopathological examinations. Bacterial culture was carried out from the kidney, spleen, and brain of all animals on a primary isolation medium (blood agar). After incubation, the grown colonies were cloned and identified by a MALDI-TOF assay. Tissue samples from the spleen, liver, kidney, heart, and intestine were 10% formalin-fixed and stained with Hematoxylin and Eosin, and Gram and submitted to histopathologic examination.

The microbiological analysis detected S. iniae in one animal's brain and kidney, whereas the other specimens tested negative. Gross lesions were mainly characterized by splenomegaly, splenic and intestinal congestion. Histological evaluation of the liver, spleen and intestine revealed a mild to severe, multifocal to diffuse, mostly perivascular, chronic granulomatous inflammation. Muscular and serosal intestinal layers were involved, whereas the mucosa was unaffected. Vascular congestion and areas of hemorrhage were multifocally observed in the liver and spleen of some sturgeon. No bacteria were detected with Gram staining. This is the first description of streptococcosis in naturally infected farmed Adriatic sturgeon (*Acipenser naccarii)* in Italy. Molecular analysis and sequencing of *S. iniae* isolates are under investigation to further characterized *S. iniae* involved in the present case.

[1] Pierazan et al. Outbreaks of severe myositis in cultured white sturgeon (*Acipenser transmontanus* L.) associated with *Streptococcus iniae*, Journal of Fish Diseases, 43: 485–490, 2020.
[2] Deng et al. Outbreaks of streptococcosis associated with *Streptococcus iniae* in Siberian sturgeon (*Acipenser baerii*) in China, Aquaculture Research, 48: 1-11, 2017.
[3] Nguyen et al. Intracoelomic- and intramuscular-injection challenge model of piscine streptococcosis in white sturgeon fingerlings, Journal of Aquatic Animal Health, 32: 133-138, 2020.

P183 - Unexplained increase in calf mortality: importance of application of guidelines to forensic necropsy

Luca Bertola (1), Maria Grazia Belotti (2), Cristiano Barisani (2), Fabio Schirru (2) Carlo Rosignoli (3) e Pietro Riccaboni (1)

(1) Università degli Studi di Milano, Dipartimento di Medicina Veterinaria (2) libero professionista (3) IZSLER - Istituto Zooprofilattico Sperimentale della Lombardia e dell'Emilia Romagna

Corresponding author: Pietro Riccaboni (pietro.riccaboni@unimi.it)

Good gynecological custom in use in intensive dairy cattle rearing, requires inseminated animals being subjected to a first pregnancy diagnosis at 30 days and a confirmation at 90 days. After calving, every calf is housed in hutches suitable for containing a calf in the first 2 weeks of his life.

The early phase of calf life is characterized by the highest mortality, mostly represented by enteric diseases (e.g. rotavirosis, coronavirosis and colibacillosis).

Over a period of 120 days, in an intensive dairy cattle rearing of the Lombardy Po Valley, 40 calves died within few days after birth. The necropsy was performed on 7 animals (3 pneumonia, 1 streptococcal septicemia, 1 *Coxiella burnetii* infection) 2 of them did not reveal any plausible cause of death. The necropsy examination on other 4 animals, died in similar circumstances in the first day of life, were performed in compliance with the guidelines for forensic necropsy in veterinary medicine: one at Milan University Vet Hospital and 3 at local Section of IZSLER.

The necropsy of these 4 last cases did not show any significant pathological findings in any organ with the exception of the cranium whose case was extremely yielding. At the opening of the neurocranium, performed with a coronal cut, the presence of two diverging fracture lines starting from a roundish central focus of about 1.2 cm in diameter covered by abundant meningeal hemorrhagic spreads was evident. The underlying brain, at the center of the central fracture, was pulped and extensively hemorrhagic.

The very abundant hemorrhage surrounded the brainstem, moved ventrally to the floor of the cranial cavity, reached the foramen magnum and invaded the cervical vertebral canal up to the 4th vertebra. Presence of inflammatory findings was histologically excluded.

The lesions observed in the latter animals made it possible to conclude that the death occurred from cranial trauma, probably attributable to a blunt force trauma.

The macroscopic finding described, together with the dramatic repetitiveness of the facts in at least 4 cases, allows us to consider these events as malicious and serial.

It therefore appears clear that performing the necropsy in full compliance with the aforementioned guidelines, requires a broad view of the case under consideration, significantly increasing the chances of fully understanding the dynamics of the events.

[1] Romano JL et al. Early pregnancy diagnosis by transrectal ultrasonography in dairy cattle. Theriogenology 66:1034-41, 2006.
[2] Paciello et al. Linee guida nazionali per le autopsie a scopo forense in medicina veterinaria. Ministero Salute ISBN: 9788894453010 – 2019.

P187 - Epidemiology of cancer in cats: 15 years of cancer-registry-based surveillance in Venice and Vicenza provinces (north-eastern Italy) from 2005 to 2020

Greta Foiani (1), Claudia Zanardello (1), Antonio Carminato (1), Katia Capello (2), Franco Mutinelli (1), Marta Vascellari (1)

(1) SCS3 - Laboratorio Istopatologia, Istituto Zooprofilattico Sperimentale delle Venezie, Legnaro, PD (2) SCS4 - Epidemiologia analitica e biostatistica, Istituto Zooprofilattico Sperimentale delle Venezie, Legnaro, PD

Corresponding author: Greta Foiani (gfoiani@izsvenezie.it)

Population-based cancer registries provide data for epidemiological surveys that can be used to define and monitor cancer incidence, identify risk factors and develop prevention and control strategies. To date, there are sporadic data on feline cancer registries worldwide and published reports on Italian veterinary registries are mainly focused on the canine species [1, 2].

In April 2005, the Animal Tumour Registry (ATR) of the Venice and Vicenza provinces of Veneto region (north-eastern Italy) was established, supported by a grant from the Italian Ministry of Health [3]. The registry provides free histopathological and cytopathological evaluation of tumor samples derived from cats living in the registry's catchment area, creating a collaborative network with the veterinary pratictioners of Veneto region. The total owned feline population of the Venice and Vicenza provinces was estimated at 232,236 (IC 95%: 206,276 – 256,793) by a telephone survey at private households in 2011[4]. Assuming no significant changes for the cat-to-household ratio over the observation period, the population estimate was used as denominator for the incidence rates (IR). A total of 3,036 feline tumors were recorded during the past 15 years (2005-2020), with an average of 202 diagnosed cases per year. The estimated crude tumor IR was 87.2 new cases per 100,000 cats per year. The annual IR of tumors tended to increase through the surveillance period. The rate of malignant tumors (2,435 cases, 80.2%; IR 69.9/100,000) was 4-fold higher than that of benign tumors (601 cases, 19.8%; IR 17.3/100,000). The females had a higher IR than males (89.3 vs 82.9/100,000). Purebred cats had a higher overall tumor IR (113.4/100,000) than mixed breed cats (84.2/100,000) and a higher incidence of malignant tumors (IR 83.3 in purebreds vs 68.4/100,000 in mixed breeds). The annual IR increased accordingly to age, reaching the peak (IR 428.4/100,000) at 12-14 years of age. In the feline population, fibrosarcoma was the most commonly diagnosed tumor (514 cases, 16.9% of total tumors recorded, IR 14.8/100,000), followed by mammary tumors (384 cases, 12.6%; IR 18.4/100,000 female cats), squamous cell carcinoma (SCC - 301 cases, 9.9%; IR 8.6/100,000), and lymphomas (292 cases, 9.6%; IR 8.4/100,000). Fibrosarcomas occurred mainly in the trunk, the limbs and the head. Of the feline mammary tumors registered, 9 out of 10 cases were malignant and the great majority were represented by the simple adenocarcinoma type. SCC mainly involved the skin of the head and the oral mucosa. The most common locations of lymphoma were the alimentary tract and the mesenteric/abdominal lymph nodes.

The prevalence of tumor types and anatomical locations obtained in this study are in agreement with previous studies [5]. However, only sparse reports are actually available from registries based on the characterization and the size of the feline population at risk, making incidence data comparison challenging. Epidemiological research on feline tumors could inform on potential predisposing factors and address possible screening and therapeutical strategies.

[1] Baioni E et al. Estimating canine cancer incidence: findings from a population-based tumour registry in northwestern Italy, BMC Vet Res, 13:203, 2017.

P190 - Necrotizing encephalopathy with elevated urinary organic acids in a British shorthair cat: clinical and pathological features

Maria Teresa Capucchio (1), Elena Colombino (1), Andrea Nespro (2), Claudia Gaviglio (2), Silvia Mioletti (1), Andrea Vercelli (2), Maria Paola Puccinelli (3), Monica Giai Via (2)

(1) Università degli Studi di Torino, Dipartimento di Scienze Veterinarie (2) Libero professionista, Torino (3) Città della Salute e della Scienza, Dipartimento di Medicina di Laboratorio, Torino

Corresponding author: Maria Teresa Capucchio (mariateresa.capucchio@unito.it)

Neurometabolic disorders characterized by dysfunctions of the respiratory chain resulting in elevated urine organic acids are well known in human medicine [1]. Reports of aciduria causing neurological dysfunction are rare in dogs and cats [2,3]. The aim of this study is to describe the clinical and histopathological features of a case of necrotizing encephalopathy in a British Shorthair cat. A male neutered cat of one year and 10 months of age was presented for progressive hyporexia, disorientation and lethargy over about 7 days. Neurological examination showed a depressed mental status, ataxia, compulsive walking, bilateral menace deficits and blindness. Results of complete blood cell count, complete serum biochemistry analysis, blood ammonia, abdominal ultrasound and echocardiography were unremarkable. Urinary metabolic profile revealed an accumulation of some metabolites of the Krebs cycle, mostly citric, succinic, malic and fumaric acid, and of other organic acids such as adipic and glyoxilyc acid. MRI was performed using a 0,2T magnet (Vet Esaote). T2-weighted sequences showed bilateral symmetrical hyperintense lesions in the thalamus, mesencephalon and pons. Lesions were hypointense in T1 weighted sequences with incomplete suppression on FLAIR. Following intravenous administration of gadolinium-based contrast medium (ProHance; Bracco Diagnostics) at a dose of 0.1 mmol/kg T1W images were repeated and showed no contrast enhancement. Cerebrospinal fluid analysis, including total protein, total nucleated cell count and cytology, was unremarkable. PCR for *Toxoplasma gondii, Cryptococcus spp. and Feline Coronavirus* were negative. A supportive medical therapy including a low protein diet with carnitine and B vitamins supplements (Benexol, Bayer S.p.A.) was provided. Due to the worsening of clinical conditions the cat was humanely euthanized and submitted to a post-mortem examination. The main organs including brain, spinal cord, sciatic nerves and skeletal muscles were collected and routinely processed for histological evaluation. Luxol Fast Blue - Cresyl Violet staining and GFAP immunohistochemistry were performed on selected brain areas. No macroscopical lesions were observed. Histologically, rostral brainstem presented symmetric and severe spongiform changes in the grey and white matter due to potential cytotoxic oedema/demyelination. Also, cerebral cortex, pons and medulla were affected, showing multifocal vacuolations only. Particularly, single to confluent, clear vacuoles with well-demarcated margins, astrocytosis (GFAP positive staining) and vessels neoformations were observed. Cerebellum, hippocampus and spinal cord were not affected. Considering the alterations of the urinary metabolic profile, a hypothetical diagnosis of a mitochondrial encephalopathy was made. Further enzymatic and genetic investigations need to be performed to better characterize this neurometabolic condition in cats.

[1] Hoffmann et al. European Journal of Pediatrics, 153 (7 Suppl 1): S94-100, 1994. [2] Shea et al. Veterinary record, 179: 545, 2016. [3] Nye GJ, Major AC, Liebel FX. Journal of Feline Medicine and Surgery, 1:1,2019.

P222 - Rectal adenocarcinoma with massive liver metastasis in a Reticulate python

Alessandro Calore, Cinzia Centelleghe, Sandro Mazzariol, Giuseppe Palmisano, Letizia Moro, Laura Cavicchioli

Università degli Studi di Padova, Dipartimento di Biomedicina Comparata e Alimentazione

Corresponding author: Alessandro Calore (alessandro.calore.1@studenti.unipd.it)

An adult (5.3 meters long) female Reticulated python (*Malayopython reticulatus*), kept in a Zoological Park for the entire life, was referred for post-mortem examination. The animal was found dead by the keeper and no clinical signs were appreciated before death. Gross examination revealed a liver markedly enlarged by the presence of numerous, multifocal to coalescing, variably sized, whitish to light grey, soft nodules. On cut surfaces, the larger nodules showed multifocal areas of necrosis. The rectal mucosa presented numerous multifocal to coalescing, variably sized, light grey, exophytic nodules resembling the nodules observed in the liver. Histologically the neoplastic nodules effaced approximately 95% of the hepatic parenchyma, incarcerating islets of normal hepatocytes and they were infiltrative, unencapsulated, well demarcated and moderately cellular. The neoplastic cells were arranged in irregular tubules and cords admixed with a moderate desmoplastic reaction both within and between the nodules. Neoplastic cells were large, cuboidal to columnar, with indistinct cell borders, and a moderate to abundant amount of often apical homogeneous lightly eosinophilic cytoplasm, inconsistently presenting vacuoles either optically empty or containing feathery slightly basophilic material interpreted as mucin. Nuclei were round, eccentric often basal, frequently presenting marked fragmentation consistent with karyorrhexis. Anisocytosis was mild to moderate and mitoses were rare. Extensive multifocal areas of the masses were characterized by coagulative or liquefactive necrosis admixed with fibrin and mucin. Multiple foci of mineralization were observed. The multifocal exophytic neoplastic masses, effaced and replaced the rectal mucosa, had microscopic features similar to those described in the liver and rare neoplastic emboli could be seen inside vessels. Immuno-histochemical analysis was carried out on rectal and liver tissues and the expression of panCytokeratin (panCK) and vimentin antigens were evaluated showing diffuse intense cytoplasmic immunoreactivity for panCK of non-necrotic cells, supported an epithelial origin of the same, while they were negative for vimentin antigen. PAS staining allowed to identify a positive mucinous material multifocally produced by the neoplastic cells. Based on the location of the masses, the histopathological and IHC features, the rectal masses were considered the primary neoplasia, consistent with a rectal adenocarcinoma, associated with hepatic metastases. Prior to the 1970s, neoplasia in reptiles was thought to be a rare entity, but subsequently a broad diversity of neoplasms has been reported (1). The incidence of neoplasia in captive collections apparently has been increasing in the last decades, as report in the recent literature (12.4-23%) (1). To the best of our knowledge, this is the first case reported of a rectal adenocarcinoma with massive liver metastasis in a Reticulate python.

[1] Sykes et al. Reptile Neoplasia at the Philadelphia Zoological Garden, 1901-2002, J Zoo Wildl Med, 37:11–19, 2016.

P237 - Effects of the multistrain probiotic *Slab51*® (*SivoMixx*®) on the intestinal morphology and microbiota composition of farmed Guinea fowls (*Numida meleagris*)

Livio Galosi (1)†, Salvatore Desantis (2)†, Alessandra Roncarati (1), Patrizia Robino (3), Alessandro Bellato (3), Patrizia Nebbia (3), Ilario Ferrocino (4), Nicoletta Santamaria (2), Lucia Biagini (1), Lorenzo Filoni (1), Anna Rita Attili (1), Giacomo Rossi (1)

(1) Università di Camerino, Scuola di Bioscienze e Medicina Veterinaria (2) Università di Bari, Dipartimento dell'Emergenza e dei Trapianti di Organi (3) Università degli Studi di Torino, Dipartimento di Scienze Veterinarie (4) Università degli Studi di Torino, Dipartimento di Scienze Agrarie, Forestali e Alimentari
†Equal Contributors

Corresponding author: Livio Galosi (livio.galosi@unicam.it)

Probiotics are beneficial bacteria with positive effects on health and growth efficiency of host animals by influencing gut microbiota or modifying immune status, as well as by stimulating digestive processes [1]. To understand the effectiveness of the probiotic mixture Slab51® (SivoMixx®, Ormendes SA, Jouxtens-Mezery, CH) on intestinal morphology, mucus layer composition and caecal microbiota diversity, forty 10-days old Guinea fowls (*Numida meleagris*) were assigned to two groups: control group (C), receiving drinking water, and treated group (P), receiving water plus the multistrains probiotic ($2x10^{11}$ UFC/L). Both groups were housed in two adjacent sheds (12 m^2 each), with litter on the bottom, under controlled photoperiod and natural aeration. Through all the trial, both the groups received ad libitum the same commercial pellet feed (Cruciani, Montappone, MC, Italy), as starter followed by growing feed, that changed in proximate composition in relation to the age of the animals. At the end of the normal growth process, animals were slaughtered by electrical stunning and bleeding at 120 days of age, and intestines were collected. Samples from duodenum, ileum and caecum were processed for morphological and morphometric studies, and conventional glycohistochemistry. Caecal samples were also used to assess the microbiota by 16S metataxonomic approach. Group P showed a modification of intestinal morphology characterized by significant increase of villus height, villus width, depth of crypts, and goblet cells per villus in all investigated tracts. Caecal microbiota of birds varied considerably and, comparing the relative abundance of the main Observational Taxonomic Units (OTUs), a positive enrichment of several beneficial taxa like *Oscillospira*, *Eubacterium*, *Prevotella* and members of the *Ruminococcaceae* was observed. High levels of diversity can improve microbiota stability and resilience facing environmental stresses, enhancing its resistance against invading pathogens. *Ruminococcaceae*, which represent the most important taxon in both groups, and *Prevotella* have a key role in the gut physiology due to the production of short-chain fatty acids (SCFAs), that are a vital energy source for enterocytes, improve glucose metabolism and exert an overall anti-inflammatory effect. Probiotic administration enriches presence of *Coprococcus*, *Oscillospira* and *Eubacterium* taxa, that produce butyrate, which exerts a beneficial effect on growth performance, structure of villi and pathogen control and has anti-inflammatory properties too. This study indicates that a probiotic supplementation positively affects the morphology and microbiota diversity of Guinea fowl intestine.

[1] Patterson JA, Burkholder KM. Application of prebiotics and probiotics in poultry production. Poultry Science. 82:627–631, 2003.

MIGLIOR POSTER AIPVET - SISVET 2021

P244 - Spontaneous mucinous metaplastic changes in the stomach of laboratory mice

Andrea Cappelleri (1)(2), Camilla Recordati (1)(2), Luca Bertola (1)(2), Giulia Birolini (3)(4), Marta Valenza (3)(4), Elena Cattaneo (3)(4), Benedetta Savino (5), Raffaella Bonecchi (6), Tatiana Varanita (7),
Ildiko Szabo (7), Eugenio Scanziani (1)(2)

(1) Università degli Studi di Milano, Dipartimento di Medicina Veterinaria (2) Fondazione UniMi, Mouse and Animal Pathology Laboratory (3) Università degli studi di Milano, Dipartimento di Bioscienze (4) Istituto Nazionale di Genetica Molecolare "Romeo ed Enrica Invernizzi" (5) Università degli Studi di Milano, Dipartimento di Biotecnologie Mediche e Medicina Traslazionale (6) Humanitas University, Dipartimento di Scienze Biomediche (7) Università degli Studi di Padova, Dipartimento di Biologia

Corresponding author: Andrea Cappelleri (andrea.cappelleri@unimi.it)

Metaplasia of the gastric mucosa is considered an important precancerous lesion in humans. Depending on the phenotype of the cells involved, metaplastic changes in the stomach are classified as mucous neck cell hyperplasia, spasmolytic polypeptide (TFF2)-expressing metaplasia (SPEM), or intestinal metaplasia (IM). These lesions are widely studied in murine models of gastric preneoplasia,[3] on the contrary little is known about incidence of spontaneous metaplasia in the stomach of mice. Here we describe some cases of spontaneous mucinous metaplasia in the gastric mucosa of mice from different studies.27 mice from 3 different study groups were examined. The groups were composed as follows. G #1: 17 C57BL/6 mice (6 WT; 11 zQ175DN KI, model of Huntington's disease), G #2: 6 BALB/c mice (3 WT; 3 ACKR2KO, with 4T1-induced mammary tumor), G #3: 4 C57BL/6 mice (2 $Kc^{fl/fl}$; 2 KcKO). Formalin-fixed, paraffin-embedded stomachs were stained with H&E and Alcian-PAS and evaluated histologically for the presence of metaplastic lesions, inflammatory infiltrates and Alcian-PAS staining pattern. 14/27 mice were affected by mucinous metaplastic changes in the fundic glands, mainly located close to the limiting ridge: 8/17 mice from G #1 (7 zQ175DN KI and 1 WT), 5/6 mice from G #2 (3 ACKR2KO and 2 WT), 1/4 mice from G #3 ($Kc^{fl/fl}$). The gastric mucosa in affected mice was characterized by focal to locally extensive areas where parietal cells were partially to totally replaced by mucous cells with clear, foamy cytoplasm. The lesion was most severe in 2 WT mice from G #2 and in 1 $Kc^{fl/fl}$ mouse from G #3. Metaplasia was frequently associated with granulocytic inflammatory infiltrate, with its severity increasing with the severity of the infiltrate. In most severe cases from G #2 and G #3, numerous Alcian-positive mucous cells (acid mucins) were present, while none to rare Alcian-positive cells were present in the remaining cases, which in turn were weakly PAS-positive (neutral mucins). Based on the findings, the most severe cases of mucinous metaplasia were classified as SPEM, while the remaining cases as mucous neck cell hyperplasia. Consistently with our findings, mucinous metaplasia in mice is reported in association with neutrophil inflammatory infiltrate, and is observed in murine models of gastric preneoplasia, or associated to *Helicobacter* infection in mice and humans.[3] Mucous neck cell hyperplasia is an expansion of normal mucous neck cells of fundic glands, whereas SPEM is characterized by "antralization" of fundic glands; their features tend to overlap on histochemical stain, and immunohistochemistry is required to discriminate between the two entities. The fact that the most severe cases of mucinous metaplasia in our study were classified as SPEM, identify SPEM as a possible progressive stage of mucous neck cell hyperplasia. The significance of mucinous metaplasia in the murine models of our study is unclear, but the role of pro-inflammatory cytokines like IFN-ɣ and systemic inflammation has been suggested.[1,2]

1. Hunter et al. *Exp Mol Pathol.* 2007;82:12–24. 2. Liu et al. *Lab Investig.* 2012;92:1045–1057.
3. Petersen et al. Murine models of gastric corpus preneoplasia. *Cell Mol Gastroenterol Hepatol.* 2017;3:11–26.

P248 - Expression of NOS2/iNOS and CD163 in macrophages associated to canine malignant mammary tumor: a retrospective study

B. Passeri, F. Altomare, R. Di Lecce, A. Corradi, A.M. Cantoni

Department of Veterinary Science, University of Parma, Parma, Italy

Corresponding author: Benedetta Passeri (benedetta.passeri@unipr.it)

Macrophages represent a conspicuous component of leukocytes infiltration in canine mammary tumors (CMTs). Polarisation of macrophage subsets into classically (M1) and alternatively activated (M2) subsets has been reported in cancer and inflammation. Little is known about their role in tumor microenvironment and tumor progression. Aim of the study was to compare macrophages polarisation in canine mammary adenomas and tubule-papillary carcinomas.

Seven cases of female dogs with primary tubule-papillary carcinoma and 5 with adenoma were selected from the Archives of the Department of Veterinary Medicine of the University of Parma. FFPE (5 µm) sections were obtained after tumor surgical excision and H&E and ABC-immunohistochemistry processed (by) NOS2/iNOS (bs-2072R) for M1 and CD163 (orb13303) for M2. Positive cells for NOS2/iNOS and CD163 were counted in 10 regions of interest/sample (40x).

Tumors were classified according to Zappulli V. et al (2019). NOS2/iNOS and CD163 were more highly expressed in tubule-papillary carcinoma than in adenomas (mean cells counted: 90,5 vs. 31,5). CD163 was expressed in only one adenoma. NOS2/iNOs+ cells were localized more around necrotic and inflamed areas, while CD163+ cells, in fibrovascular stromal tissue. Overall, NOS2/iNOS were more expressed than CD163 in tubule-papillary carcinoma with a mean value of 90,5 cells counted vs. 49.

In this study, CD163+ (M2) cells are higher expressed in malignant tumor than in benign. In tubule-papillary carcinoma the conspicuous presence of NOS2/iNOS+ cells (M1) compared to M2 cells is correlated to their different localization within the neoplasia: the predominance of M2 cells in surrounding fibrovascular stromal tissue confirm an active role in the development of tumor microenvironment.

(1) Monteiro L.N., Tumour-associated macrophages: Relation with progression and invasiveness, and assessment of M1/M2 macrophages in canine mammary tumours. The Veterinary Journal 119-125 2018.
(2) Parisi F., M1 and M2 tumour-associated macrophages subsets in canine malignant mammary tumours: An immunohistochemical study. Res Vet Sci. Feb 6;136:32-38 2021.
(3) Heinrich F., Morphologic, phenotypic, and transcriptomic characterization of classically and alternatively activated canine blood-derived macrophages in vitro. PLoS One. 2017 Aug 17;12(8):e0183572 2017.
(4) Rasotto R, Prognostic Significance of Canine Mammary Tumor Histologic Subtypes: An Observational Cohort Study of 229 Cases. Vet Pathol. Jul;54(4):571-578 2017.

P252 - Bycatch-associated gas embolism in loggerhead sea turtles stranded along the Northern Adriatic coastline

Giorgia Corazzola, Cinzia Centelleghe, Guido Pietroluongo, Giuseppe Sciancalepore, Sandro Mazzariol

Università degli Studi di Padova, Dipartimento di Biomedicina Comparata e Alimentazione

Corresponding author: Giorgia Corazzola (giorgia.corazzola@gmail.com)

Fishery bycatch (i.e. accidental capture of non-targeted species) is considered one of the main anthropogenic threat for sea turtle conservation. Bycatch can cause the development of intra- and extravascular gas bubbles within supersaturated tissues after decompression, leading to several effects as compression of tissues, vascular obstruction and damage, and capillary extravasation (1, 2).

In 2020, two loggerhead sea turtles *(Caretta caretta)* were found stranded along the Veneto coastline (in Chioggia and in Caorle) and a complete post-mortem examination was performed. Grossly, a moderate to severe amount of gas bubbles were found multifocally in abdominal veins, atria, sinus venosus, and mesenteric, splenic, hepatic and renal vessels. The main histopathological findings included moderate to severe multiorgan congestion, often with the presence of extravasated red blood cells (micro-haemorrhages), and multifocal intravascular gas bubbles not associated with bacterial aggregates, causing severe dilations of the vascular lumen.

The macroscopic and histopathological lesions were consistent with the ones already described in sea turtles from the east coast of Spain, as reported in human divers and cetaceans (1, 2, 3, 4). The presence of gas bubbles in fresh carcasses in the absence of bacterial isolates, are inconsistent with decompositional bubbles from bacterial activity and allows us to hypothesize with reasonable certainty the definitive diagnosis of gas embolism as the cause of death. Diagnosis of gas embolism in sea turtles underlines the potential role of fishery as threat for the sea turtle population of the Northern Adriatic Sea and opens a new era for research in sea turtle conservation and bycatch impact mitigation.

[1] García-Párraga, D., et al. "Decompression sickness ('the bends') in sea turtles." Diseases of aquatic organisms 111.3: 191-205, 2014.
[2] Vann, Richard D., et al. "Decompression illness." The Lancet 377.9760: 153-164, 2011.
[3] Jepson, P. D., et al. "Gas-bubble lesions in stranded cetaceans." Nature 425.6958: 575-576, 2003.
[4] Fernández, Antonio, et al. ""Gas and fat embolic syndrome" involving a mass stranding of beaked whales (Family *Ziphiidae*) exposed to anthropogenic sonar signals." Veterinary pathology 42.4: 446-457, 2005.

P273 - C-reactive protein, Paraoxonase-1 activity and serum protein electrophoresis in dogs seropositive for *Borrelia burgdorferi sensu lato*

Donatella Scavone, Sergio Zanzani, Chiara Raffa, Saverio Paltrinieri

Università degli Studi di Milano, Dipartimento di Medicina Veterinaria

Corresponding author: Donatella Scavone (donatella.scavone@unimi.it)

Borreliosis is an emerging tick-born zoonotic disease, whose spread has in the last years increased due to climate and human habits changes [1]. Infection in dogs can occasionally cause non-specific symptoms such as anorexia, fever and lameness but more frequently dogs remain non-symptomatic [2]. To date, no information about changes in acute phase proteins or electrophoretic profiles of dogs seropositive for Borrelia burgdorferi is available. The aim of this study was to measure CRP concentration and PON-1 activity and to perform serum protein electrophoresis in sera collected from potentially infected dogs. This was done within a serosurveillance plan in the area of Valchiavenna (Northern Italy) in collaboration with a local veterinary facility. Among dogs admitted for medical examination, vaccinations or health checks, 151 dogs aged more than 8 months, living or frequently attending the area from April to October 2019, were included. Dogs were subjected to physical examination, blood draw and different medical procedures based on disease suspected or diagnosed. Ethical approval was granted by decision n° 2/2016 of the Ethical committee of the University of Milan as samples were collected for diagnostic purposes or health checks with informed consent of the owners. The serum level of anti-Borrelia IgM and IgG class antibodies was determined using an indirect immunofluorescence assay. Overall, 17/151 (11.3%) dogs were seropositive for IgM and/or IgG (threshold antibody titers: 1:64). Measurement of serum CRP concentration with immunoturbidimetric assay and of PON-1 activity with a paraoxon-based method already validated in dogs [3] and serum protein electrophoresis on agarose gel were performed on all seropositive sera and on 39 seronegative specimens. The Mann-Whitney U tests was used to compare the results recorded in seropositive vs seronegative dogs or in symptomatic vs non-symptomatic dogs. The Kruskall Wallis test was used to compare the results of symptomatic seropositive (n=6), non-symptomatic seropositive (n=11), symptomatic seronegative (n=17) and non-symptomatic seronegative dogs (n=22). Regardless of seropositivity, symptomatic dogs had significantly higher CRP levels ($p=0.004$), and significantly lower albumin concentration ($p=0.038$), albumin/globulin ratio ($p=0.008$) and PON-1 activity ($p<0.001$) compared with healthy dogs, supporting the presence of inflammatory processes detected with physical examination. Accordingly, seronegative sick dogs had significantly higher CRP levels ($p=0.006$) and significantly lower PON-1 activity ($p<0.001$) than seronegative healthy dogs. Interestingly, among healthy dogs, PON-1 activity was significantly lower in seropositive than in seronegative dogs ($p=0.029$). Even if indirect tests do not demonstrate an undergoing infection, our results suggest that clinically occult oxidative phenomena may be present in dogs exposed to B. burgdorferi.

[1] Kilpatrick AM, Randolph SE. Drivers, dynamics, and control of emerging vector-borne zoonotic diseases. The Lancet, 380:1946–55, 2012.
[2] Littman MP. Lyme Borreliosis, in: Clinical Small Animal Internal Medicine, pp 941-844. I ed. John Wiley & Sons Inc, 2020.
[3] Rossi G, Giordano A, Pezzia F, Kjelgaard-Hansen M, Paltrinieri S. Serum paraoxonase 1 activity in dogs: preanalytical and analytical factors and correlation with C-reactive protein and alpha-2-globulin. Vet Clin Path, 42(3), 329–341, 2013.

P291 - Lethal septic shock after dental cleaning in a dog: a case report and its forensic-medical aspects

Emanuela Vaccaro, Ilaria d'Aquino, Giuseppe Piegari, Orlando Paciello

Università degli Studi di Napoli, Dipartimento di Medicina Veterinaria e Produzione Animale

Corresponding author: Emanuela Vaccaro

Sepsis is defined as the systemic response to the invasion of all type of pathogen such as Gram– negative and Gram-positive bacteria, fungi and viruses [1]. The systemic interaction between host cells and toxins produced by bacteria results in a clinical syndrome known as "septic shock". Previous studies have observed bacteriemia in animals undergoing dental cleaning [2]. However, to the best of authors knowledge, no cases of septic shock associated to dental procedures have been described. Here we report, for the first time, a case of a lethal septic shock occured after cleaning dental procedures in a dog. An eight-year-old cavalier king Charles spaniel was submitted for dental cleaning procedures in a private veterinary clinic located in Campania region, Southern Italy. After 2 hours from the surgical procedure, the patient was found dead and, to find out the manner and cause of death, the owner submitted the cadaver to the Department of Veterinary Medicine of Naples for further forensic necropsy and ancillary examinations. The forensic necropsy was conducted using standard necropsy protocol. The macroscopic examination revealed multifocal hemorrhages on the surface of the skin. Myocardium, abdominal aorta and lung. Mucous membranes were pearly white. The gingival mucosa was hyperemic, edematous and hemorrhagic. Representative samples from multiple organs were collected for microscopic examination. Furthermore, samples of blood, lung and intestine were collected for bacteriological examination. Histologically, we observed multifocal hemorrhages in the lung, myocardium and epicardium. All organs showed to moderate vascular ectasia. Finally, bacteriological examination allowed us to isolate *Staphylococcus pseudintermedius* from blood samples and *E. coli* and *Streptococcus infantarius* spp *infantarius* from intestine samples. Based on histological, macroscopic and bacteriologic data, a definitive diagnosis of septic shock due to *Staphylococcus pseudintermedius* was made. Finally, "dental cleaning" was considered the cause of *Staphylococcus pseudintermedius* blood spreading and, consequently, the cause of the observed septic shock. Indeed, *Staphylococcus pseudintermedius* is a Gram-positive, coagulase positive, saprophytic bacteria; it is part of the normal cutaneous microflora of dogs and colonizes the skin, hair follicles/coat and mucocutaneous sites, such as the nose, mouth and anus [3]. However, it has been associated with sepsis following surgery or in immunosuppressed subjects. Overall, our findings suggest how septic shock could be a consequence not only of different types of surgical operations but also of minor non-surgical procedures; In particular, considering the normal colonization of animals' oral mucosa by saprophytic bacteria, dental procedures should always be performed using antibiotic prophylaxis to avoid massive spread of bacteria in the bloodstream and, consequently, sepsis in dogs. Furthermore, to avoid legal disputes, the possibility of sepsis should be communicated to the owner and indicated in the informed consent form.

[1] Ayres, S. M. SCCM's New Horizons Conference on sepsis and septic shock. *Crit. Care Med.* 13, (1985).
[2] Baltch, A. L. *et al.* Bacteremia following dental cleaning in patients with and without penicillin prophylaxis. *Am. Heart J.* 104, 1335–1339 (1982).
[3] Devriese, L. A. *et al. Staphylococcus pseudintermedius* sp. nov., a coagulase-positive species from animals. *Int. J. Syst. Evol. Microbiol.* 55, 1569–1573 (2005).

P292 - Validation of a chitinase assay in feline samples

Angelica Stranieri, Laura Brusasco, Gabriela Ávila Morales, Saverio Paltrinieri

Università degli Studi di Milano, Dipartimento di Medicina Veterinaria

Corresponding author: Angelica Stranieri (angelica.stranieri@unimi.it)

Chitinases are hydrolytic enzymes responsible for clearance of chitinous organisms. Chitinases are expressed mainly by macrophages and neutrophils and, in humans, they increase in diseases characterized by macrophages overactivation.[1] Feline infectious peritonitis (FIP) pathogenesis strictly involves monocytes and macrophages activation, and it is challenging to diagnose in vivo due to the lack of specific markers.[2] Therefore, the aims of this study were to validate a colorimetric assay for the measurement of serum chitinase in cats, and to assess whether cats with inflammation, and especially cats with FIP have a higher concentration of chitinase compared with clinically healthy cats or cats with diseases other than FIP. To achieve this aim, a commercially available kit (Chitinase Assay Kit, cat no. CS1030, Sigma-Aldrich, St Louis, MO, USA) was used to assess intra- and inter-assay precision on feline serum samples read in duplicate or on pooled sera. The latter were used also to evaluate the accuracy, trough linearity under dilution (LUD) of spiking recovery test (SRT). The serum samples mentioned above were collected from clinically healthy cats (n=12), cats with FIP (n=12) or cats with inflammation other than FIP (n=11) and were analyzed in two work sessions. Results of the different groups were compared to each other to assess the differences among groups and to determine the discriminating power for the diagnosis of FIP through a ROC curve analysis and to determine sensitivity, specificity and likelihood ratios at the best cut-off value. The accuracy of the method was excellent either for LUD (P=0.001; r2=0.978) or for SRT (P<0.001; r2=0.983), as well as intra-assay precision with coefficient of variation (CVs) ranging from 0.04% to 39.38% (mean: 7.78±8.16%, median: 4.82%; I-III interquartile interval: 3.00-9.56%). Conversely, the inter-assay precision was low (CVs 75.3±45.8, 74.6%, 31.3-124.2) and optical densities recorded on the two sessions on the same sera were significantly different (P<0.001) and quantitatively distant to each other (first session: 969.51 ± 1679.78, 36.93, 15.37-835.05; second session: 356.16±767.85, 38.7, IQR: 23.82-144.98). However, in both work sessions, cats with FIP had the highest OD (first session: 1881.5±2099.5, 486.5, 54,8-3999.3; second session: 129.9±170.1, 46.5, 22.1-150.9) followed by cats with inflammation other than FIP (first session: 276.1±419.8, 45.8, 22.4-604.6; second session: 70.7±71.1, 58.2, 24.7-71.8) and by clinically healthy cats (first session: 68.8±180.4, 18.4, 9.1-26.2; second session: 26.1±7.9, 27.0, 18.4-32.6), but the difference was statistically significant only in the first work session (P<0.001). These results indicate that serum chitinase increases in cats with inflammation and especially in cats with FIP and that the colorimetric method used for chitinase evaluation is appropriate for pathogenic studies based on group comparisons within a single session of tests. However, the low reproducibility limits the use of this test in routine diagnostic or on larger longitudinal studies.

[1] Pinteac, R., Montalban, X., Comabella, M. (2021). Chitinases and chitinase-like proteins as biomarkers in neurologic disorders. Neurology-Neuroimmunology Neuroinflammation, 8(1).
[2] Pedersen, N. C. (2009). A review of feline infectious peritonitis virus infection: 1963–2008. Journal of feline medicine and surgery, 11(4), 225-258.

POSTER

AIVI

MIGLIOR POSTER AIVI SISVET 2021

P2 - Estimating the impact of animal-level risk factors for *Escherichia coli* prevalence in bovine lymph nodes with Monte Carlo simulation

Beniamino Cenci Goga (1), Luca Grispoldi (1), Athanasios Chalias (2), Chrystalleni Hadjicharalambous (3)

(1) Medicina Veterinaria, Laboratorio di Ispezione degli Alimenti di Origine Animale, Università degli Studi di Perugia, Perugia, Italy (2) European Food Safety Authority, EU-FORA Programme, Parma, Italy (3) Department of Chemistry, University of Crete, Heraklion, Crete, Greece

Corresponding author: Baniamino Cenci Goga (beniamino.cencigoga@unipg.it)

A risk assessment model was built at the individual animal level using a range of possible factors that represent individual variations in risk of *E. coli* presentation in bovine lymph nodes at the slaughterhouse. Independent variables identified as potential risk factors included sex, age, breed, distance, kind of slaughter, diet and organs condemned by the vets. Specifically, data from 597 cattle carcasses were collected and the samples were distributed as follows: “sex” (357 males and 240 females), “age” (438 animals <2 years of age, 159 >2 years), “farm-slaughterhouse distance” (157 animals coming from a distance exceeding 350 km, 164 from distances 50-350 km, 276 from distances <50 km), “ordinary or casualty slaughter” (105 samples from casualty slaughter), “organs condemned by veterinary inspection” (118 carcasses had at least one organ condemned), “diet” (157 animals came from farms where >70% of feed was via polyphite grasslands) and “breed” (6 different breeds). Microbiological laboratory analysis of the samples identified prevalence of Escherichia coli in the lymph nodes and PCR analysis indicated the presence of shiga-toxin producing *E. coli* (STEC). Antimicrobial susceptibility testing was performed against 19 antimicrobials. Animal-level variables and laboratory analysis data have been categorized**.**

Initially, multiple logistic regression analysis identified “age” as a critical factor that correlates with the presence of *E. coli* in lymph nodes. Other factors, such as the “diet”, “distance from slaughterhouse” and “kind of slaughter” also related to *E. coli* positivity and the presence of genes that code for shiga toxins and were therefore categorized as critical. Variables that describe “sex”, “breed” and “organs condemned” were classified as variables that do not present any risk or “none”.

Workflow for the risk assessment is: following selection of the critical factors, a Monte Carlo simulation model was constructed in Microsoft Excel 2014 with the add-on package @Risk (version 8.0, Palisade Corporation, New York, USA) [1]. The model describes prevalence and characteristics of *E. coli* for individual animals presenting differences in the critical factors described above. The input data are fitted to distributions and by applying Monte Carlo simulation, randomly selected values from each input distribution are combined to provide output distributions that indicate the likelihood of each result. The model and the output results as «probability of *E. coli* contamination of bovine lymph nodes as a factor of age or other critical variables», will be published in a separate report.

P63 - Italian Traceability System in water buffalo for milk and processed products

Giovanna Cappelli, Gabriele Di Vuolo, Giorgio Galiero, Domenico Vecchio, Rosario Noschese, Roberta Capacchione, Francesca Bufano, Esterina De Carlo

Centro di Referenza Nazionale per l'Igiene e la tecnologia dell'allevamento delle produzioni bufaline, Istituto Zooprofilattico Sperimentale del Mezzogiorno.

Corresponding author: Gabriele Di Vuolo (gabriele.divuolo@izsmportici.it)

This document describes the online traceability system in the buffalo supply chain. On the IT platform, farmers, dairies and brokers must record the milk production from the company to the finished product. This system applied at national level is managed by the National Reference Centre on Water Buffalo Farming and Productions Hygiene and Technologies(CReNBuf) at the IZSM, and by the National Agricultural Information System (SIAN) are described in Ministerial Decree 9406 of 9 September 2014 www.tracciabilitabufala.it/ www.mipaaf.sian.it (1). Farmers are obliged to communicate the total milk production, the number of animals milked, the number of the delivery note and the name of the buyer, and within the first week of the month they must communicate the milk production of each buffalo milked. Dairies must enter the purchased milk and the processed product (mozzarella, yogurt, etc.). The intermediaries are required to enter the purchased milk, both fresh and frozen, of the semi-finished product and the sale of the same. In Italy the number of registrations is more representative in the Campania region where we have the highest concentration of herds of dairy buffaloes, followed by the Lazio region, and the Puglia region which represent the DOP area together with the province of Isernia. While for the other regions the number of buffalo farms registered in the system is less representative. In 2015 it was confirmed that the number of breeders who access and feed the system database settles and stabilizes in the years from 2016 to 2019, following the Ministerial Decree and information on the territory. By comparing the milk produced monthly over the years and the buffaloes we can know the average production of an Italian Mediterranean buffalo, which on average is about 8kg/head/day/year, going from a value of 7.70 kg in 2016 to 7.89 kg in 2019. The System highlights that the circuit of non-PDO products absorbs 35% of the total milk produced compared to 65% of the milk processed in PDO dairies, and also gives the possibility to check the production of buffalo milk on a weekly basis throughout the national territory, facilitating the control of milk that goes to freezing or that can only be used in non-dop production; this makes the supply chain more transparent. The system is in some way the computerized archive of the productions for all the Operators of the Sector (Osa) whether they are breeders, processors or intermediaries. Over the years, the system has contributed to the increase in the price of milk in the buffalo sector and could be applied to other IGP, PDO, TSG supply chains for the protection of Made in Italy.

(1) Ministerial Decree n.9406 of 9 September 2014. Available online: https/www.tracciabilitabufala.it/ www.mipaaf.sian.it.

P159 - Monitoring of the health status of slaughtered pigs by scoring of the pluck lesions

Silvio De Luca (1), Emanuela Zanardi (1), Benedetta Cordioli (1), Antonio Marco Maisano (2), Federico Scali (2), Giovanni Loris Alborali (2), Adriana Ianieri (1), Sergio Ghidini (1)

(1) Università di Parma, Dipartimento di Scienze degli Alimenti e del Farmaco (2) Istituto Zooprofilattico Sperimentale della Lombardia e dell'Emilia-Romagna

Corresponding author: Silvio De Luca (silvio.deluca@unipr.it)

The evaluation of lung lesions at the slaughterhouse allows the monitoring of swine health and welfare and provides valuable data for epidemiological purposes [1]. The aims of this study were (i) to determine the prevalence of gross lesions at abattoir on the pluck of heavy weight pigs (~160 kg, 9 months of age) destined for PDO ham production and (ii) to investigate the correlation between the severity of lung and pleura lesions and carcass weight. The study was carried out in a slaughterhouse located in Northern Italy between December 2020 and March 2021. Enzootic Pneumonia-like lesions were assessed scoring each lung lobe from 0 to 4, with a maximum score of 28. Pleuritis lesions were evaluated using the Slaughterhouse Pleurisy Evaluation System ('SPES' score) with a maximum score of 4. Liver lesions were ranked from 0 to 2, relying on the number of milk-spot lesions. Other lesions such as pericarditis, lung scars and abscesses were also recorded. Data were collected from 73 batches coming from 63 farms, for a total of 7245 pigs. Descriptive statistics of the different lesions were performed at farm-level while the association between the mean value of lung and pleura scores with the mean value of the carcass weight was assessed using a linear regression at batch-level. A p-value ≤0.05 was considered significant. Overall, 60% of the lungs presented lesions with 98% of the EP-like lesions located in the cranio-ventral lobes. The average lung and pleurisy scores were 2.48±1.43 and 1.07±0.43, respectively, with 37% of the pleura presenting severe damage (score ≥3). The average liver score was 0.38±0.34, with 29% of the livers damaged. Pericarditis and lung scars values were 5% and 1%, respectively. The results of the mean values of the examined lesions were comparable to other previously reported in the literature [2]. No statistically significant correlation was found between the lung (R^2 =0.007) and pleura (R^2 =0.001) scores and the mean carcass weight at batch level. Such findings are in contrast with other studies reporting an effect of the respiratory lesions on the productive parameters [3]. These discrepancies may depend on the different scoring methods used in the studies, duration of the finishing stage and health management of the pigs. This study supports the evaluation of pluck lesions at the slaughterhouse for the monitoring of the health and welfare status in fattening pig farms.

1. De Luca, S., Zanardi, E., Alborali, G.L., Ianieri, A., Ghidini, S. Abattoir-Based Measures to Assess Swine Welfare: Analysis of the Methods Adopted in European Slaughterhouses. Animals 11 (1):226, 2021.
2. Scollo, A.; Gottardo, F.; Contiero, B.; Mazzoni, C.; Leneveu, P.; Edwards, S.A. Benchmarking of Pluck Lesions at Slaughter as a Health Monitoring Tool for Pigs Slaughtered at 170kg (Heavy Pigs). Preventive veterinary medicine, 144: 20–28, 2017.
3. Ferraz, M.E.S.; Almeida, H.M.S.; Storino, G.Y.; Sonálio, K.; Souza, M.R.; Moura, C.A.A.; Costa, W.M.T.; Lunardi, L.; Linhares, D.C.L.; de Oliveira, L.G. Lung Consolidation Caused by Mycoplasma Hyopneumoniae Has a Negative Effect on Productive Performance and Economic Revenue in Finishing Pigs, Preventive veterinary medicine, 182:105091, 2020.

P256 - The role of organic milk among the apulian dairy farmers – future insights and consumers awareness

Giuseppe Costantino (1), Giuseppe Celano (1), Giuseppe Mangini (2), Saverio Tangorre (3), Gianluca Gigante (4), Gaetano Vitale Celano (3)

(1) Università degli Studi di Bari, Dipartimento di Scienze del Suolo, della Pianta e degli Alimenti (2) Associazione Regionale Allevatori di Puglia – ARA Puglia (3) Università degli Studi di Bari, Dipartimento di Medicina Veterinaria Scienze Veterinarie (4) Società Agricola Masserie Amiche s.r.l.

Corresponding author: Giuseppe Celano (giuseppe.celano@uniba.it)

Italy is characterized by a consolidated tradition and culture of diary product, which finds its maximum expression in about 400–450 varieties of cheese, 52 of which are PDO and with an export rate of about 35% [1]. The milk and dairy products show high potential to create synergies between tourism and economy, innovation and tradition, safety and the environment. These synergies are explained by the capability of these foods to be source of tourist attraction, leading to a better promotion and development of their territory production area [2]. Moreover, their continuing enhancement through innovative and natural biotechnological strategies ensure a more eco-sustainable impact and greater safety in terms of quality and healthiness [3]. However, to date, the organic milk and derivative products shows great variability and territorial criticalities that slow its expansion through the territory and among consumers. The objective of this study was to conduct a preliminary investigation aimed to analyze the overall issues and evaluate the potential strategies to increase the production of organic milk in Apulia region. For this purpose, in collaboration with the "Associazione Regionale Allevatori" – A.R.A. Puglia, it has been elaborated a questionnaire which was administered to ca. 200 dairy farmers whose milk is mainly produced as raw matter for its further dairy processing. The 10 questions asked to farmers according to binary schemes combined with numerical information concerned various aspects of the type and management of the farms as well as the knowledge and interest in organic milk production. From these preliminary results the 65% of dairy farms were constituted with a number of cattle above the 50 units while the remaining 35% had ca. or less than 50 units. The most common breeds resulted "Frisona", "Bruna" and "Pezzata Rossa". The 95% of the farms had outdoor spaces for the grazing and mobility of the cattle. The 92% of the total milk produced is destined for further processing. Although ca. 63% is aware on the community policies aimed to sustain organic agri-food products, almost the totality of farms subjected to this study were conventional due to the high costs of organic milk productions, due to the lack of production awards associated to organic milk and its derivative production, and due to a still lower consumers' demand. Despite this state of the art, the 65% of the farmers have shown interest in converting their farms from conventional to organic, especially whereas the 37% of the farms declared to have proper lands for organic production. From an overall evaluation of these results, there are optimal environmental factors for a major development and a more diffused production of organic milk and relative dairy products but it is essential to highlight that important economic-cultural, organizational and applied research political strategies are necessary in order to (i) enhance the nutritional and functionality values by reducing the fat and salt content through a combined approach of pro-technological bacteria and dietary fibers; (ii) increase the, hygienic-sanitary and technological qualities by a better preservation of the shelf-life through the use of selected lactic acid bacteria capable to produce bacteriocins.

[1] Gobbetti M. In: The Cheeses of Italy: Science and Technology. Springer, Cham, 2018.
[2] Cafiero et al. Traditional agri-food products as a leverage to motivate tourists: a meta-analysis of tourism-information websites. J Place Manag Dev, 195 – 214, 2019.
[3] Costantino et al. Use of Ex Exopolysaccharide-Synthesizing Lactic Acid Bacteria and Fat Replacers for Manufacturing Reduced-Fat Burrata Cheese, Microorganisms, 8, 1618, 2020.

POSTER

AMV

MIGLIOR POSTER AMV - SISVET 2021

P149 - Localization of leptin in the abomasum of the sheep: an immunohistochemical study

Francesca Mercati (1), Asia Renzi (2), Elisa Palmioli (1), Cecilia Dall'Aglio (1), Elena De Felice (3), Paola Scocco (3)

(1) Università degli Studi di Perugia, Dipartimento di Medicina Veterinaria (2) Biotecnologo, libero professionista (3) Scuola di Bioscienze e Medicina Veterinaria, Università degli studi di Camerino

Corresponding author: Francesca Mercati (francesca.mercati@unipg.it)

Adipokines are molecules involved in energy metabolism and represent important links between the nutritional status and neuroendocrine axis [1]. Leptin (Ob) is the first isolated adipokine [2] and it is one of the most important hormones involved in the control of energy homeostasis and feeding behaviour. It serves to signal nutritional status to the central nervous system and peripheral organs and, in physiological condition, it acts to reduce appetite [3]. Ob is primarily secreted by adipocytes of subcutaneous and visceral fat but it is also produced by several peripheral tissues [4]. This work aimed to investigate the presence and localization of Ob in the fundic region of the abomasum of the sheep in an attempt to shed light on those cells and structures that might locally produce this peptide. Sample collection was performed on a flock of 15 Comisana x Appenninica adult female sheep reared in a semi-natural pasture of the Italian Central Apennines. The research was approved with no. of approval 95/2018-PR by the Ministry of Health. Samples were fixed in 10% neutral-buffered formalin and processed until paraffin inclusion. Histological sections of 5 µm were microwaved in 10 mM citric acid (pH 6.0) for antigen retrieval. The endogenous peroxidase activity was blocked with a 3% peroxidase-blocking solution and non-specific binding was blocked with normal horse serum. Sections were incubated first with mouse monoclonal anti-Ob-antibody (1:150 in PBS, Fitzgerald Industries International, MA, USA) for 24 hours and successively with the horse anti-mouse biotin-conjugated antibody. The reaction was detected with a Vectastain ABC kit and visualized with diaminobenzidine. For the first time, we document that Ob is localized in the abomasum of sheep. An intense positivity to Ob was evidenced in the gastric glands of the mucous layer; leptin binding sites were mainly localized in the lower half of the fundic glands. The staining for Ob was localized in the cytoplasm of the cells. Leptin detection in the gastric glands suggests a local activity of this adipokine in the regulation of the digestive function, as already attested in other species including humans [4]. This study is a preliminary report that introduces Ob investigation in the sheep digestive system; it represents the starting point to carry out further investigations aimed to evaluate the influence of the diet on the molecule expression in sheep reared in the semi-extensive regime, as well as to search the presence of Ob receptor.

[1] Trayhurn et al. Adipose tissue and adipokines--energy regulation from the human perspective, The Journal of Nutrition, 136(7):1935S-1939S, 2006.
[2] Triantafyllou et al. Leptin and Hormones: Energy Homeostasis, Endocrinology & Metabolism Clinics of North America, 45(3):633-45, 2016.
[3] Ahima et al. Leptin regulation of neuroendocrine systems, Frontiers in Neuroendocrinology, 3: 263-307, 2000.
[4]. Sobhani et al. Leptin secretion and leptin receptor in the human stomach, Gut, 47(2):178-83, 2000.

POSTER

ANIV

P19 - Detection of *Brucella* DNA in serum of seropositive cattle and sheep from South Italy (Sicily): preliminary results

Giusi Macaluso, Ilenia Giacchino, Valeria Blanda, Marilena Alfano, Anna Candela, Antonina Tagliarini, Alessandra Torina, Giuseppina Chiarenza

Istituto Zooprofilattico Sperimentale della Sicilia "A. Mirri", Palermo – Italy

Corresponding author: Giusi Macaluso (giusi.macaluso@izssicilia.it)

Brucellosis is the most common global bacterial zoonotic disease caused by bacterial species of the genus *Brucella.* In the southern regions of Italy the annual number of reports has dropped significantly over time; it remains an important health problem in Sicily (Italy) (1). Bacterial culture is the gold standard for the diagnosis of brucellosis; it is complex, time consuming and it poses a potential risk for laboratory workers; molecular methods have been widely applied for increasing the performance of detection in term of rapidity and reducing health hazards. Polymerase chain reaction (PCR) can identify satisfactorily *Brucella* species and distinguish vaccine strains but there has been limited validation for direct diagnosis. It has been shown that PCR could be an additional tool in an outbreak situation when serological methods are still negative or it might help to identify animals which are not detected by conventional serological methods (2). This study aims to detect the presence of *Brucella* DNA in cattle and sheep sera and to investigate the usefulness of the molecular tests as an additional mean of detection of *Brucella spp.* able to control an outbreak of brucellosis. A total of 78 bovine sera and 1103 ovine sera were collected from *Brucella* positive Sicilian farms, between December 2020 and January 2021. Extensive beef cattle farms (N=3) were located in province of Palermo; extensive milk and beef ovine farms (n=3), were located in province of Agrigento. The serological response to *Brucella spp.* was assessed by Rose Bengal Test (RBT), and Complement Fixation Test (CFT), according to standard OIE procedures. Two sets of specific PCR methods for the detection of *Brucella* spp. were used to corroborate serological diagnostics. The RBT/CFT positive sera were re-tested by IS711-based real-time PCR (3) and BCSP31-based PCR (4). Real-time PCR was performed by using SsoAdvanced Universal Probes Supermix (Biorad). Positive samples were then retested by PCR by using GoTaq® DNA Polymerase (Promega).

Out of 78 bovine serum samples, 8 (10.3%) reacted positively to the RBT and CFT and 1 (1.3%) reacted positively to only CFT. Out of 1103 ovine serum samples, 44 (3.9%) reacted positively to the RBT and CFT, 1 (0.1%) reacted positively only to the RBT and 1 (0.1%) only to the CFT. Out of 9 RBT/CFT positive bovine sera 8 (88.9%) were positive by IS711 real-time-PCR and BCSP31 PCR screening. Among the RBT/CFT positive ovine sera, 14 (30.4%) were positive by IS711 real-time-PCR, 20 (43.5%) by BCSP31 PCR. Among these, 9 (19.6%) detected positive to both screenings. Application of molecular assays is a promising method for detection and identification of *Brucella spp.*, as routine clinical diagnostic procedure. A combination of RBT-CTF and PCR could be effective for future eradication programmes. Since *Brucella* DNA can readily be detected in serum of infected animals, the PCR assay could provide a good supplementary test to be used as the first step of diagnostic investigation, to control outbreak situations, for the early and accurate diagnosis of infection.

[1] Mancini et al. Trends of human brucellosis in Italy, 1998–2010, Epidemiol Infect, 142(6):1188–95, 2014. [2] Guarino et al. Detection of *Brucella* species in buffalo whole blood by gene-specific PCR, Vet Rec.147:634–6, 2000.
[3] Sola et al. Real-time PCR detection of *Brucella* spp. DNA in lesions and viscera of bovine carcasses. J Microbiol Method, 104:87-91, 2014.
[4] Imaoka et al. Simultaneous detection of the genus *Brucella* by combinatorial PCR, Jpn J Infect Dis, 60(2-3):137-9, 2007.

P20 - Serological survey of Hepatitis E Virus in Cattle in Sicily: a zoonotic perspective

Francesca Gucciardi (1), Giusi Macaluso (1), Santina Di Bella (1), Antonio Lastra (1), Calogero Castronovo (1), Pietro Niutta (2), Annalisa Guercio (1), Giuseppa Purpari (1)

(1) Istituto Zooprofilattico Sperimentale della Sicilia "A. Mirri", Palermo (2) Università degli Studi di Messina, Dipartimento di Scienze Veterinarie

Corresponding author: Giusi Macaluso (giusi.macaluso@izssicilia.it)

Hepatitis E virus (HEV) represents one of the principal causative agents of hepatitis transmitted by the fecal-oral route and an ongoing internationally challenging issue for public health (1). Among the five HEV genotypes affecting humans, 3 and 4 are zoonotic.

Domestic pigs and wild boars are the main animal reservoir of these genotypes; serological and virological evidence of HEV infection was also documented in other animal species including cattle (2) (3). The epidemiological role of these domestic species is uncertain and information about Europe is still lacking. Dairy cattle, widely distributed in the South of Italy and used for meat and milk production, could act as a potential HEV reservoir. The objective of this study was to evaluate serological evidence of HEV infection among cattle in order to identify new zoonotic sources that bear a high risk of transmission to humans. From 2018 to 2020 a total of 1173 blood samples from cattle of local breeds were collected. The cattle came from 9 sicilian provinces (Agrigento N=23, Caltanissetta N=14, Catania N= 53, Enna N=45, Messina N=241, Palermo N=200, Ragusa N=526, Siracusa N=39, Trapani N=32). We tested sera for antibodies against HEV using a commercial multi-species ELISA kit for detection of anti-HEV IgG and IgM antibodies (Wantai Biological, Beijing, China). Anti HEV total antibodies were detected in 349/1173 cattle (29.8%; 95%CI: 27.13-32.36) indicating that so far the virus seems to circulate in these animals living in the examined area. This seroprevalence rate was in line with other studies on cattle reported in eastern China (4), and in the United States (5), whilst higher than those found in a similar study carried out in Central Italy (6). Our data indicate that HEV infection could occur in cattle and that they could also play a role as a reservoir of HEV. However, recent data suggest in cattle a potential antigenic cross-reaction of anti-HEV antibody responsiveness with a related, but as yet unknown agent (5). Thus further complementary studies focusing on molecular detection of virus RNA should be carried out, to investigate about the possible role of cattle in HEV transmission. Since consumption of undercooked beef, as well as pork, might contribute to the transmission of HEV to humans, more investigations, that include detailed and continuous nationwide surveys, are required to identify unrecognized risk factors and to evaluate the contribution of cattle as reservoir of HEV infection.

[1] WHO. The Global Prevalence of Hepatitis E Viral Prevalence and Susceptibility: A Systematic Review. accessed on 19 January 2021.
[2] Zhang et al. Development and validation of a new serum standard for the measurement of anti-HEV antibodies in animals, Journal of Medical Virology, 89(3), 497–501, 2017.
[3] Ricci et al. Public health risks associated with hepatitis E virus (HEV) as a food-borne pathogen, EFSA Journal 2017;15(7):4886.
[4] Bingyu et al. Hepatitis E Virus in Yellow Cattle, Shandong, Eastern China, Emerging Infectious Diseases, Vol. 22, No. 12, December, 2016.
[5] Yugo et al. Evidence for an unknown agent antigenically related to the hepatitis E virus in dairy cows in the United States, Journal of Medical Virology, 91(4), 677–686, 2019.
[6] Mazzei et al. Detection of hepatitis E virus antibodies in domestic and wild animal species in central Italy, Clinical Microbiology, 4(06), 1–4, 2015.

P45 - Evidence of antimicrobial resistance and presence of pathogenicity genes in *Yersinia enterocolitica* isolated from wild boar

Valeria Listorti (1), Paola Modesto (1,2), Chiara De Ciucis (1), Walter Vencia (1), Maria C. Pugliano (1,2), Walter Mignone (3), Enrica Berio (3), Chiara Masotti (4), Carlo Ercolini (4), Laura Serracca (4), Tiziana Andreoli (5), Monica Dellepiane (5), Daniela Adriano (2), Simona Zoppi (2), Daniela Meloni (2), Elisabetta Razzuoli (1)

(1) Istituto Zooprofilattico Sperimentale del Piemonte, Liguria e Valle d'Aosta, Genova (2) Istituto Zooprofilattico Sperimentale del Piemonte, Liguria e Valle d'Aosta, Torino (3) Istituto Zooprofilattico Sperimentale del Piemonte, Liguria e Valle d'Aosta, Imperia (4) Istituto Zooprofilattico Sperimentale del Piemonte, Liguria e Valle d'Aosta, La Spezia (5) Istituto Zooprofilattico Sperimentale del Piemonte, Liguria e Valle d'Aosta, Savona

Corresponding author: Valeria Listorti (valeria.listorti@izsto.it)

Yersinia enterocolitica (*Ye*) is a zoonotic bacterium responsible for yersinionsis that can occur after the consumption of raw or uncooked pork meat or vegetables. Healthy pigs are the principal reservoir of Ye. However, Ye has been isolated also in wild boar in Europe with prevalence between 3.5-11% [1-2]. Data on the pathogenicity and antimicrobial resistance of the strains isolated from wild boar are still lacking. The aims of this study were to investigate the presence and biotypes of Ye in wild boar hunted in Liguria region from 2013 to 2018 and to evaluate the presence of chromosomic genes of pathogenicity (GoP) and Ye antimicrobial resistances. 4,890 liver samples were collected and tested for the presence of Ye according to ISO 10273:2003 method. All Ye strains isolated were biotyped and serotyped according to ISO10273: 2003. Ye isolated strains were checked for the presence of six chromosomic virulence genes: attachment and invasion locus (*Ail*), invasin (*inv*), Yersinia stable toxin A (*ystA*), Yersinia stable toxin B (*ystB*), mucoid Yersinia factor (*myfA*) and Yersinia modulator (*ymoA*) were investigated using protocols previously described [3,4]. The Kirby–Bauer disc diffusion test was performed following the Clinical and Laboratory Standard Institute (CLSI) guidelines. Ye was isolated in 126 samples (2.6%). 48 strains (38.1%) were serotype (ST) O:8, 13 strains (10.3%) were ST O:5, both these STs have been associated with human gastroenteritis cases [5]. 11 strains (8.7%) were ST O:9, 8 strains (6.3%) were ST O:3 and 4 strains (3.2%) were ST O:1,2. Many isolated strains (42/126; 33.3%) were not typed with the available sera. 117 (92.9%) strains were of biotype 1A, 8 (6.34%) strains of the biotype 1B and one strain (0.8%) was of the biotype 2. The *ystB* gene of pathogenicity was found in the 70%, *ail* in the 44%, *ymoA* in the 45%, *ystA* in the20%, *myfA* in the 12% and *inv* in the 8%. 78 (61.9%) of the isolated strains showed resistance at least to one drug: 85.71% were resistant to penicillins; 23.8% to sulfonamides and 7.14% to extended-spectrum cephalosporins. Low resistances towards aminoglycosides and tetracyclines (0.79%) were observed. The highest frequency of serotype O:8 suggests that, in Liguria region, the major source of Ye in wild boar is anthropogenic. The isolated strains showed many of the pathogenicity genes under study, suggesting a pathogenetic potential also in 1A biotype.

[1] von Altrock, A.; Seinige, D.; Kehrenberg, C. *Yersinia enterocolitica* Isolates from Wild Boar Hunted in Lower Saxony. Germany. Appl Env Microbiol., 81: 4835-4840, 2015.
[2] Sannö A., Rosendal T. Aspán, A.; Backhans, A.; Jacobson, M. Distribution of enteropathogenic *Yersinia* spp. and *Salmonella* spp. in the Swedish wild boar population, and assessment of risk factors that may affect their prevalence. Acta Vet Scand., 60: 40, 2018.
[3] Liang J., Bi Z., Shi G., Xiao Y., Qiu H., Kou Z., Hu B., Jing H. Wang, X. Two novel ail-positive biotype 1A strains of Yersinia enterocolitica isolated in China with unequal adhesion and invasion properties. Infect Genet Evol., 27: 83-88, 2014.
[4] PeruzyM.F., Murru N., Perugini A.G., Capuano F., Delibato E., Mercogliano R.,Korkeala H., Proroga Y.T.R. Evaluation of virulence genes in *Yersinia enterocolitica* strains using SYBR Green real-time PCR. Food Microbiol., 65: 231-235, 2017.
[5] Białas N., Kasperkiewicz K., Radziejewska-Lebrecht J. Skurnik. M. Bacterial cell surface structures in *Yersinia enterocolitica*. Arch Immunol Ther Exp (Warsz) 60: 199-209,2012

P59 - Preliminary assessment of antiviral activity of natural compounds against murine norovirus

Loredana Cozzi (1), Teresa Vicenza (1), Roberta Battistini (2), Chiara Masotti (2), Elisabetta Suffredini (1), Simona Di Pasquale (1), Carlo Ercolini (2), Laura Serracca (2)

(1) Istituto Superiore di Sanità, Department of Food Safety, Nutrition and Veterinary public health, National Reference Laboratory for Foodborne Viruses, Rome (2) Istituto Zooprofilattico Sperimentale del Piemonte, Liguria e Valle d'Aosta, La Spezia

Corresponding author: Roberta Battistini (roberta.battistini@izsto.it)

Hydrolates, or aromatic waters, are aqueous solutions obtained as co-products of essential oils production, from the steam-distillation or hydro-distillation of fresh medicinal plants. These compounds are generally safe and showed antimicrobial, antiviral and antifungal properties [1, 2]. Based on these characteristics, they are suggested to be suitable also for food safety applications [1]. The aim of this preliminary study was the evaluation of the antiviral effect of *Citrus limon, Thymus vulgaris* and *Thymus serpyllum* hydrolates on murine norovirus (MNV-1), a surrogate of human noroviruses that can be replicated *in vitro* (RAW 264.7 cell line), therefore allowing the assessment of infectivity. Different concentrations of each hydrolate (0.0125%, 0.025%, 0.05%, 0.25%, 0.5%, 1% v/v in serum-free culture medium) were treated with antibiotic/antimycotic solution and were assayed for cytotoxicity on RAW 264.7 cells. No cytotoxic effect was observed. The effect of the selected natural compounds on viral infectivity was evaluated by treating MNV-1 (2.7×10^5 $TCID_{50}$/ml final concentration) with each hydrolate (1% v/v in cell culture medium). Treated virus aliquots were stored at -80°C immediately (t=0) and after 24 h of incubation at 20±2°C (t=24). Untreated MNV-1 and 1% hydrolate solutions kept at the same conditions were used as positive and negative controls, respectively. Qualitative assay on RAW 264.7 cells showed that treated MNV-1 retained infectivity both at t=0 and t=24 and irrespective of the tested hydrolates (C*itrus limon*, T*hymus vulgaris* and T*hymus serpyllum*). Positive and negative controls provided the expected results (i.e. cytopathic effect for untreated virus and absence of cytotoxic effect for hydrolate solutions). Therefore, in order to evaluate the antiviral effect of each hydrolate, the decrease of viral infectivity was estimated by comparing the $TCID_{50}$/ml of untreated viral suspension and of the hydrolate-treated viral suspensions. The results showed a natural decrease (approx. 1 log) of the infectivity of untreated virus between t=0 and t=24 (from 2.1×10^5 to 1.0×10^4 $TCID_{50}$/ml). Compared to the untreated virus, MNV-1 treated with C*itrus limon* hydrolate1% displayed a reduction of infectivity of 1 log already at t=0 (2.1×10^4 $TCID_{50}$/ml), followed by a further reduction at 24h (1.0×10^3 $TCID_{50}$/ml). The hydrolates of *Thymus vulgaris* 1% and T*hymus serpyllum* 1%, on the other hand, showed an immediate decrease of infectious virus of 2 log at t=0 (3.2×10^3 $TCID_{50}$/ml), remaining then substantially unchanged at t=24. In conclusion, this preliminary study showed a reduction of MNV-1 infectivity by C*itrus limon*, T*hymus vulgaris* and T*hymus serpyllum* hydrolates and highlights their potential use in food sanitation.

[1] D'Amato S, Serio A, Chaves López C, Paparella A. Hydrosols: Biological activity and potential as antimicrobials for food applications, Food Control, 86: 126-137, 2018.
[2] Kaewprom K, Chen YH, Lin CF. Antiviral activity of *Thymus vulgaris* and *Nepeta cataria* hydrosols against porcine reproductive and respiratory syndrome virus. Thai J Vet Med, 47(1): 25-33, 2017.

P108 - Intramammary administration of autologous platelet concentrate in cow dry-off treatment: preliminary data

Antonio Marco Maisano (1), Giulia Quadri (1), Simone Bariselli (1), Fausto Vezzoli (1), Giorgio Varisco (1), Chiara Spelta (1), Sara Rota Nodari (1), Gianfilippo Alessio Clemente (1), Federico Scali (1), Claudia Romeo (2), Anna Lange-Consiglio (3), Rosangela Garlappi (4), Mario Vittorio Luini (1, 5)

(1) Istituto Zooprofilattico Sperimentale della Lombardia e dell'Emilia-Romagna (2) Università degli Studi di Parma, Dipartimento di Scienze degli Alimenti e del Farmaco (3) Università degli Studi di Milano, Dipartimento di Medicina Veterinaria (DIMEVET) (4) Medico Veterinario libero professionista (5) Consiglio Nazionale delle Ricerche, Istituto di Biologia e Biotecnologia Agraria

Corresponding author: Antonio Marco Maisano (antoniomarco.maisano@izsler.it)

Properly managing the dry period in dairy cows is instrumental for their next milking. Traditionally, during this period, udder health is preserved by using intramammary antimicrobials (AMs). The platelet concentrate (PC) has a demonstrated role in promoting tissue regeneration and has been successfully tested in the treatment of bovine mastitis [1,2]. The aim of our study was to evaluate the effects of administering autologous PC, as an antimicrobial use (AMU) alternative, for the dry-off treatment. A total of 53 clinically healthy cows from six farms in northern Italy were recruited for the study. Autologous PC was produced for each cow following the Lange-Consiglio et al. [2] protocol. Overall, 194 mammary quarters were treated and somatic cell count (SCC) was performed for each one immediately before treatments (T0). Sampled quarters were divided in two groups: 'PCg' (group treated with intramammary PC only) and 'AMg' (group treated with intramammary AM only). The follow-up SCCs were performed at one (W1) and two (W2) weeks after calving. Before any analysis, all SCC results underwent a $\log_2$ transformation. For each quarter, a T1 SCC was calculated as the arithmetic mean between W1 and W2 SCC. The effect of treatment (PCg or AMg), time (T0 or T1) and their first-order interaction on log-transformed SCC was analysed through a General Linear Mixed Model (GLMM), including quarter identification nested in cow identification as a random factor. Post-hoc comparisons were carried out through difference of least square means tests. The analysis showed a significant effect of treatment by time on SCC ($p=0.023$). In detail, there was no difference between the two groups at T0 ($p=0.52$), with mean values for AMg and PCg being 18.4 ± 2.8 and 18.2 ± 2.5 $\log_2$ cells, respectively. Treatment with AMs led to a significant reduction in SCC from T0 to T1 ($p<0.0001$) and so did PC treatment ($p<0.0001$). However, SCC reduction was larger in the AMg when compared with the PCg ($p=0.0121$), with mean $\log_2$ SCC at T1 being 15.5±1.9 for AMg and 16.4±2.4 for PCg. Both PC and AMs treatments led to an average SCC below the 'alert level' of 200,000 SCC cells/mL after the dry period. The larger SCC reduction in AMg confirms the known efficacy of AMs treatment. The positive effects of PC administration on SCC could provide an effective alternative to AMs reducing AMU while preserving animal health and welfare, particularly, in farms without relevant issues of contagious mastitis or within a selective dry-cow therapy program.

[1] Lange-Consiglio et al. Antimicrobial Effects of Conditioned Medium from Amniotic Progenitor Cells in vitro and in vivo: Toward Tissue Regenerative Therapies for Bovine Mastitis, Frontiers in Veterinary Science, 6:443, 2019.
[2] Lange-Consiglio et al. Intramammary administration of platelet concentrate as an unconventional therapy in bovine mastitis: first clinical application, Journal of Dairy Science, 97.10: 6223-6230, 2014.

P110 - A preliminary study on antimicrobial susceptibility of *Staphylococcus* spp and *Enterococci* spp in European wild boar (*Sus scrofa*) hunted in Campania Region – Italy

Monica Ambrosio (1), Francesca Paola Nocera (1), Emanuela Scandura (1), Filomena Fiorito (1), Luisa De Martino (1, 2)

(1) Department of Veterinary Medicine and Animal Production, University of Naples "Federico II", Naples, Italy (2) Task Force on Microbiome Studies, University of Naples "Federico II", Naples, Italy

Corresponding author: Monica Ambrosio (monica.ambrosio@unina.it)

The nasal cavity is one of the potential bio-sources of the pathogenic opportunistic bacteria resistant to standard antibiotics. The present study focuses on the isolation, identification and antimicrobial susceptibility profiles of staphylococci and enterococci detected in the nasal cavity of healthy wild boars. Among the strains of the genus *Staphylococcus*, both in human medicine and in veterinary medicine, especially methicillin-resistant *Staphylococcus aureus* (MRSA) is associated with infections that are difficult to cure due to having developed the ability to resist multiple antibiotics. The nasal swabs of the wild boars were taken at the time of capture, housed in transport medium and transported to the laboratory within 24-48 hours. The samples were seeded on selective and differential media for *Staphylococcus* spp. and *Enterococcus* spp. After 24 hours at 37°C, once microbial growth was obtained, rapid identification tests were carried out such as the catalase, oxidase and coagulase test. Then, the identification of species was carried out by proteomic analysis (MALDI TOF MS), with subsequent preparation of antimicrobial susceptibility testing by using 17 antibiotics belonging to 10 different families. The most isolated *Staphylococcus* species was *S. xylosus* (33%) followed by *S. chromogenes* (27%), *S. hyicus* (20%), *S. sciuri* (13%) and *S. simulans* (7%). While, as *Enterococcus* spp, we identified mainly *Enterococcus feacalis* (93%) followed by *Enterococcus casselliflavus* (7%). Almost all *Staphylococcus* spp. strains were found to be resistant to oxacillin (93.3%) and penicillin (66.6%). Just one strain, precisely a *S. hyicus*, did not show resistance to any antibiotic tested. Furthermore, no *S. aureus* was isolated. Regarding to the antibiotic resistance profiles of *Enterococcus* spp. resistance to penicillin was very frequently observed (92.85%), followed by resistance to ciprofloxacin (71.5%), ampicillin (71.42%) and oxacillin (64.28%). Based on the classification proposed by Margiorakos et al. (2012), we characterized 92.8% multidrug-resistant (MDR), no extensively drug-resistant (XDR) and 7.2% pandrug-resistant (PDR) *Staphylococcus* spp.; whereas based on the antimicrobial susceptibility profiles of *Enterococcus* spp. we found 85.8% MDR, 7.1% XDR and 7.1% PDR strains. Thus, both bacterial genera showed broad profiles of antibiotic resistance, which is worrying considering that these wild animals are not subjected to antibiotic selective pressure. These data suggest a possible environmental contamination to counter antimicrobial resistance and for the surveillance of wildlife.and require a continuous monitoring to counter antimicrobial resistance and for the surveillance of wildlife.

[1] Magiorakos AP et al. Multidrug-resistant, extensively drug-resistant and pandrug-resistant bacteria: an international expert proposal for interim standard definitions for acquired resistance. Clinical Microbiology and Infection, 18: 268-281, 2012.
[2] Meier RK, Ryser-Degiorgis MP. Wild boar and infectious diseases: evaluation of the current risk to human and domestic animal health in Switzerland: A review. Band 160, Heft 7/8, 443–460, 2018.
[3] Mulani MS et al. Emerging Strategies to Combat ESKAPE Pathogens in the Era of Antimicrobial Resistance: A Review. Microbiology, 10, 539, 2019.

P206 - Microbiological environmental monitoring in veterinary surgical rooms of the city of Milan and its metropolitan area: a pilot study

Piera Anna Martino (1), Lucrezia Lucchini (2), Gabriele Meroni (1), Alessandro Vetere (2)

(1) Università degli Studi di Milano, Department of Biomedical, Surgical and Dental Sciences - One Health Unit (2) Veterinary Practitioner, Milano

Corresponding author: Piera Anna Martino (piera.martino@unimi.it)

The hygienic conditions of the work environments are extremely important because the health of humans and animals also depends on the healthiness of the habitat (One Health concept). Due to the unique environment of veterinary practices, where humans come into close contact with many different species of animals/patients, also veterinary staff itself is frequently exposed to microorganisms, many of which could have zoonotic potential. Therefore, having a clean environment is of utmost importance not only for patients but also for the safety of people working in it. This study was performed in order to determine the type and the prevalence of microorganisms on some surfaces of surgical veterinarian rooms in Milan and surroundings. In literature, studies like this one were performed mainly in human hospitals, while in the veterinary field a few studies were carried out inside single entities [1, 2, 3]. The samples were collected in 30 different facilities (small veterinary practices and large veterinary hospitals) from 4 sites inside the surgical room: the table, the monitoring system's monitor, the handle of the surgical light, and air vents. In some cases, it was impossible to sample all the chosen surfaces, due to the different nature of the veterinary facilities that took part in the study. Environmental samples were obtained using contact plates of 55 mm diameter filled with Plate Count agar (Oxoid, Italy) or using sterile single wrapped swabs with Amies transport medium. At the laboratory contact plates were incubated directly at 37°C for 48/72 hours while swabs were streaked onto Plate Count Agar and then incubated at the same conditions above. The bacterial and fungal identification was performed using standard microbiological methods [4]. Of 30 veterinary facilities sampled, 18 (60%) were Small Animal Veterinary Hospitals while 12 (40%) were Small Animal Clinics. The 43 samples that were performed in the small animal clinics showed a clear prevalence of *Bacillus* spp. (53%), *Micrococcus luteus* (20%), Staphylococci (*S. aureus, S. pseudintermedius*, NCS) (13%), *Enterococcus faecalis* (4%), *Escherichia coli* (4%), *Enterobacter* (2%) and fungi (12%). From the 64 samples taken from 18 small animal hospitals, it turned out again a prevalence of *Bacillus* spp (53%), *Micrococcus luteus* (20%), *Staphylococcus* spp (9%) and fungi (6%). The viable number of bacteria and fungi in the contact plates was estimated as Colony-Forming Unit (CFU). The CFU for 46 samples ranged from 1 to 10, for 24 samples from 10 to 100 UFC; one sample had a range from 100 to 500 UFC and 11 samples over 500 UFC. This study was the first one to evaluate the environment and the hygiene of the surgical rooms in the geographical area of Milan. From the results we obtained it is evident that cleaning operations should be performed with more accuracy and the veterinary staff should be trained with the aim to perform these operations as well as possible.

[1] Boerlin et al. Transmission of opportunistic pathogens in a veterinary teaching hospital, Veterinary Microbiology, 82(4):347-59, 2001.
[2] Morley P.S. Surveillance for nosocomial infections in veterinary hospitals. Veterinary Clinics: Equine Practice, 20: 561-576, 2004.
[3] Rojas et al. High prevalence of multidrug-resistant community-acquired methicillin-resistant *Staphylococcus aureus* at the largest veterinary teaching hospital in Costa Rica. Vector Borne Zoonotic Diseases, 17(9): 645-653, 2017.
[4] Markey B. In Clinical Veterinary Microbiology. II ed. Mosby, 2013.

P214 - Emerging viruses in domestic and wild carnivores, southern Italy

Flora Alfano (1), Gianluca Miletti (1), Nicola D'Alessio (1), Gianvito Lanave (2), Roberto Scialla(1), Violetta Vasinioti (2), Antonietta Anzalone (1), Claudia Esposito (1), Martina Levante (1), Loredana Cozzolino (1), Esterina De Carlo (1), Vito Martella (2), Nicola Decaro (2), Giovanna Fusco (1)

(1) Istituto Zooprofilattico Sperimentale del Mezzogiorno, Portici, Napoli (2) Dipartimento di Medicina Veterinaria, Università degli Studi di Bari

Corresponding author: Flora Alfano (flora.alfano@cert.izsmportici.it)

The circulation of emerging viruses was evaluated in domestic and wild carnivores in southern Italy. Different samples from 237 domestic and wild animals (100 dogs, 77 cats, 14 wolves, 15 foxes, 23 badgers and 8 otters) were analyzed for the following viruses: aichivirus (AiV), sapovirus (SaV), astrovirus (AsV), hepatitis E virus (HEV), hepatitis A virus (HAV), norovirus GI (NGI), norovirus GII (NGII), rotavirus (ROTV). Sapovirus positive samples were submitted to sequencing despite the low amount of virus detected (Varela et al., 2016).

The animals testing positive for emerging viruses were: 9 for SaV (3.8% of total animals): 5 dogs (5% of dogs), 3 cats (3.9% of cats) and 1 fox (6.7% of foxes); 7 for AsV (2.9% of total animals): 4 dogs (4% of dogs) and 3 cats (3.9% of cats); 7 for ROTV (2.9% of total animals): 3 dogs (3% of dogs) and 4 cats (5.2% of cats). Single viral infection was found in only 6 animals (2.5% of total animals). The animals that showed only sapovirus infection were 3 dogs (3% of dogs) and 1 fox (6.7% of foxes). Other emerging viruses, including AiV, HEV, HAV, NGI and NGII, were not detected. To date, sapovirus sequences were obtained only from a cat.

The presence of SaV is generally associated with gastroenteritis (Usuku et al 2008; Biscaro et al. 2018 Li L. 2011; Soma et al 2015; Bodnar et al 2016). In our study, SaV was detected as single pathogen in the intestine of a dog displaying gross lesions suggestive of uremic syndrome, in the absence of gastroenteric changes. The SaV strain identified clustered with human SaV genotype GI.1, detected in children with gastroenteritis.

The constant increase in human and animal movements could trigger the rapid emergence and spread of new pathogens on a global scale, as recently observed for SARS-CoV-2. The identification of new viral agents is of fundamental importance for the assessment of the zoonotic risk, since zoonotic agents represent a major threat to human health. Our results showed that several emerging viruses circulate in Italian carnivores suggesting the need for a continuous epidemiological surveillance.

[1] Biscaro et al. Detection and molecular characterization of enteric viruses in children with acute gastroenteritis in Northern Italy, Infect Genet Evol, 60:35-41, 2018.
[2] Bodnar L. Detection and molecular characterization of sapoviruses in dogs, Infect Genet Evol, 38:8-12, 2016.
[3] Li et al. Viruses in diarrhoeic dogs include novel kobuviruses and sapoviruses, J Gen Virol, 92:2534-2541, 2011.
[4] Soma et al. Detection of Norovirus and Sapovirus from diarrheic dogs and cats in Japan, Microbiol Immunol, 59:123-8, 2015.
[5] Usuku et al. An outbreak of food-borne gastroenteritis due to sapovirus among junior high school students, Jpn J Infect Dis, 61:438-41, 2008.
[6] Varela, et al. Human Sapovirus in Mussels from Ría do Burgo, A Coruña (Spain), Food Environ Virol, 8:187–193, 2016.

P257 - Performance of MALDI-TOF in a University Laboratory of Veterinary Bacteriology

Elisabetta Mondo, Federica Giacometti, Federica Savini, Raffaele Scarpellini, Federico Tomasello, Marta Pollesel, Andrea Serraino, Silvia Piva

Alma Mater Studiorum - Università di Bologna, Dipartimento di Scienze Mediche Veterinarie

Corresponding author: Elisabetta Mondo (elisabetta.mondo2@unibo.it)

Since 2013, when FDA (Food and Drug Administration) authorized the use of MALDI-TOF (Matrix-Assisted Laser Desorption Ionisation-Time of Flight) in clinical practice, bacterial identification has been revolutionized [1]. Many studies have shown that MALDI-TOF surpassed the conventional diagnostic methods in speed, accuracy and cost [2]. In human medicine, studies report correct identification of 97.0% and 91.7%, at genus and species levels, respectively [3], while in veterinary medicine the rate of correct identification is 95.2% and 90.1%, at genus and species levels respectively [4]. Herein we report feedback on routine use of MALDI-TOF in the laboratory of veterinary bacteriology of the Department of Veterinary Medical Science of the University of Bologna. Data have been collected from 01.01.2020 to 01.04.2021 and refer to clinical samples collected from pets and horses admitted to the Veterinary University Hospital of the University of Bologna. A total of 283 strains isolated from pets' different samples (188 urine, 14 blood cultures, 1 bronchus alveolar fluid, 80 swabs, 17 body fluids, 9 biopsies, 15 skin lesions) and 32 strains isolated from horses' samples (1 stool, 20 skin lesions, 2 body fluids, 7 swabs) were collected in routine diagnostic procedures. Blood cultures and 6 urine samples were analyzed with protocols for sample direct identification and through on plate growth. Among pets' isolates, 273/283 (96.4%) were identified by MALDI-TOF after 24h of incubation, while 3 out of 283 (1.06%) were identified after 48h. Among horses' isolates, 29/32 (90.6%) were identified by MALDI-TOF after 24h of incubation, while 1 out of 32 (3.1%) was identified after 48h. No differences were observed in results obtained by direct and from colony identification of blood cultures and urines. Bacterial species most frequently isolated were *Escherichia coli* (35%) and *Staphylococcus pseudintermedius* (15%) among pets and *E. coli* (46%) and *Streptococcus equi* subsp. *zooepidemicus* (21%) among horses. Furthermore, 3 bacterial species, never described in horses (*Streptococcus uberis*, *Klebsiella variicola* and *Pantoeae agglomerans)*, have been identified by MALDI-TOF and confirmed by 16s rRNA sequencing. Data obtained show that MALDI-TOF represents an excellent method for a rapid identification of bacteria in veterinary diagnostics, with subsequent improvement in time to diagnosis and therapy. Cultural isolation represents the gold standard for clinical microbiology, but it is an extremely consuming laboratory activity, in terms of time, materials and work: the use of MA.LDI-TOF can be an efficient tool to reduce time to identification and costs, but the high initial cost of the instrument should be considered. An increase in the of the instrument in veterinary diagnostic is needed to allow an enlargement of the dataset to identify more animal pathogens.

[1] https://www.accessdata.fda.gov/cdrh_docs/pdf13/k130831.pdf, accessed 06.04.2021
[2] Sogawa K., Watanabe M., Nomura F. Rapid identification of microorganisms using MALDI-TOF mass spectrometry, Rinsho Byori, 61:44-51, 2013.
[3] Patel R. MALDI-TOF MS for the Diagnosis of Infectious Diseases, Clinical Chemistry, 61:100-111, 2015.
[4] Pavlovic M., Wudy C., Zeller-Peronnet V., Maggipinto M., Zimmermann P., Straubinger A., Iwobi A., Märtlbauer E., Busch U., Huber I. Identification of bacteria isolated from veterinary clinical specimens using MALDI-TOF MS, Berl Munch Tierarztl Wochenschr, 128:24-30, 2015.

MIGLIOR POSTER ANIV - SISVET 2021

P285 - Genotyping of *Hepatitis E virus* (*Orthohepevirus A*) spread in wild boars in the Marche region, Italy

Laura Boriani (1), Raffaele Claudio Barboni (2), Alessandro Di Cerbo (1), Maria Vittoria Alvino (1), Vincenzo Cuteri (1), Silvia Preziuso (1)

(1) Università di Camerino, Scuola di Bioscienze e Medicina Veterinaria (2) ASUR Marche.

Corresponding author: Silvia Preziuso (silvia.preziuso@unicam.it)

Hepatitis E virus (HEV), now named *Orthohepevirus A*, belongs to the *Orthohepevirus* genus in the family *Hepeviridae* and is associated in humans with outbreaks and sporadic cases of acute hepatitis. The virus is considered endemic in many tropical and subtropical countries, but its distribution is probably global. In Europe, the number of autochthonous cases in humans is increasing. In Marche region (Italy) several cases have been recently found to be caused by genotype 3 [1], which is most frequently detected in Italy in raw and dry pork liver sausages [2]. The aim of this study was to study the presence of and to genotype HEV in wild boars in Marche region, Italy.

Forty-five liver samples were collected from wild boars shot during the hunting seasons of 2018–2019 in six different municipalities in Marche region. A nested PCR protocol was used to amplify a specific part of the ORF2 [3]. Seventeen samples gave a PCR product of the expected size (348 bp) and were sequenced. The sequences were edited and aligned with representative sequences of all genotypes described so far [4]. The phylogenetic tree was inferred using the Maximum Likelihood method and the best-fitting nucleotide substitution model was Tamura-Nei with bootstrap values based on 1000 repetitions. A discrete Gamma distribution was used to model evolutionary rate differences among sites. The rate variation model allowed for some sites to be evolutionarily invariable.

In all municipalities at least one animal was infected by HEV genotype 3. Three different HEV subtypes were found. HEV-3f was found in most samples, subtype 3e was found in 5 samples, and only one wild boar was infected by 3c subtype. In one municipality all three subtypes were found in wild boars shot in the same area.

The results show that HEV genotype 3 is widely spread in wild boars. The subtypes detected in the present study have been already found in Italy both in pigs and in wild boars [4,5]. Although subtype 3f has been frequently associated to disease in humans in some European countries e.g., France and England, subtypes 3e and 3c are the most frequently reported in humans in Italy, while subtype 3c is rarely reported in Italian suids [5]. HEV-3c, HEV-3e, and HEV-3f have been reported not only in humans and suids (pigs and wild boar) but also in contaminated food items of pork and wild boar (references are reported in [5]). Considering that traditional food products are widely made with uncooked wild boar liver in the geographic area included in this study, studies on the impact of the maturing process on HEV infectivity are required. In addition, the role of wild boars in maintenance and recombination of HEV should be monitored, as well as their role in transmitting the virus to free-range pigs.

[1] Tarantino et al. Locally acquired hepatitis E virus in Marche Italy: Clinical/laboratory features and outcome, Dig Liver Dis, 52(4):434-439, 2020. [2] Di Bartolo et al. Detection of hepatitis E virus in pork liver sausages. Int J Food Microbiol, 193:29-33, 2015. [3] van der Poel et al. Hepatitis E virus sequences in swine related to sequences in humans, The Netherlands, Emerg Infect Dis, 7(6):970-6, 2001. [4] Pierini et al. Hepatitis E virus (HEV) genotype 3 diversity: Identification of a novel HEV subtype in wild boar in Central Italy, Transbound Emerg Dis, in press.

P286 - Bovine coronavirus N gene detection and characterization in cattle: a preliminary study

Maria Vittoria Alvino (1), Gilberto Zecchin (2), Corrado Maria Corradini (3), Laura Boriani (1), Rino Spighi (4), Vincenzo Cuteri (1), Silvia Preziuso (1)

(1) Università di Camerino, Scuola di Bioscienze e Medicina Veterinaria (2) Medico Veterinario libero professionista, Padova (3) Medico Veterinario libero professionista, Macerata (4) Medico Veterinario libero professionista, Forlì-Cesena

Corresponding author: Silvia Preziuso (silvia.preziuso@unicam.it)

Bovine coronavirus (BCoV) belongs to the genus *Betacoronavirus* and subgenus *Embecovirus* in the family *Coronaviridae* and causes respiratory and enteric disease in cattle. The genome is a single-stranded, positive-sense RNA of approximately 31 kb. Although most molecular phylogenetic analyses are based on the S gene, genetic variability has been found also in HE and in N genes. This preliminary work is part of a larger study aimed at analysing the genetic variability of BCoV detected in cattle recently acquired from different European countries.

Nasal swab samples were collected from 66 cattle recently acquired from Northern Italy, France, Hungary, Romania, and Poland by 15 different farms. PCR methods were used to amplify and sequence the N gene [1]. Sequences were aligned with N gene sequences available in databases and the phylogenetic tree was inferred using the Maximum Likelihood method with Kimura 2-parameter as nucleotide substitution model. The rate variation among sites was modelled with a gamma distribution. The main sites of mutation were at positions T30041C, C30059T, C30071A, T30251C and G30257T in comparison with the sequence of the Mebus isolate (GenBank ID U00735.2). In addition, one sample showed synonymous mutations at positions C30056T and A30122T. The only non-synonymous mutation A30192C, corresponding to the substitution of an isoleucine with a leucine, was found in one strain from Northern Italy. Most samples showed the highest percentage of identity with the strain BCoV/FRA/EPI/Caen/2012/07 (GenBank ID KT318089.1) isolated in France in 2012 from cattle. A few samples showed the highest percentage of identity with the strain bcovizsm (GenBank ID MW074864.1) isolated in Southern Italy in 2020 from cattle [2]. Interestingly, one sample collected from cattle reared in Northern Italy showed the highest identity with bovine-like coronavirus detected in water buffalo in Southern Italy in 2007 (GenBank ID EU019216.1) [3] and then in dromedary in Morocco in 2016 (GenBank IDs MN514972.1 and MN514975.1) [4]. N protein plays an important role during virion assembly and in enhancing the efficiency of subgenomic viral RNA transcription as well as viral replication. The sequences obtained in this study showed some synonymous mutations rarely or not detected previously. The N gene is often the target of PCR diagnostic protocols thus monitoring of sequences of this gene is important to evaluate the conservation among sequences of viruses from different geographic regions. Further investigations will be aimed at sequencing other relevant and more variable genes. Particular attention will be paid to the sample showing high identity with bovine-like coronavirus previously detected in host different than cattle [3,4].

[1] Takiuchi et al. Improved detection of bovine coronavirus N gene in faeces of calves infected naturally by a semi-nested PCR assay and an internal control, J Virol Methods, 131(2):148-54, 2006.
[2] Amoroso et al. Fatal Interstitial Pneumonia Associated with Bovine Coronavirus in Cows from Southern Italy, Viruses, 12(11):1331, 2020.
[3] Decaro et al. Biological and genetic analysis of a bovine-like coronavirus isolated from water buffalo (Bubalus bubalis) calves, Virology, 370(1): 213–222, 2008.
[4] So et al. Diversity of Dromedary Camel Coronavirus HKU23 in African Camels Revealed Multiple Recombination Events among Closely Related Betacoronaviruses of the Subgenus Embecovirus, J Virol, 93(23):e01236-19, 2019.

P287 - A cross-sectional study on the canine adenovirus type 1 (CAdV-1) infection in Southern Italy

Francesco Mira, Roberto Puleio, Giuseppa Purpari, Santina Di Bella, Giorgia Schirò, Gabriele Chiaramonte, Vincenzo Randazzo, Francesca Licitra, Francesco Antoci, Domenico Vicari, Annalisa Guercio

Istituto Zooprofilattico Sperimentale della Sicilia "A. Mirri"

Corresponding author: Santina Di Bella (santina.dibella@izssicilia.it)

Canine adenovirus type 1 (CAdV-1) is the causative agent of a systemic and potentially fatal viral disease, defined in dogs as infectious canine hepatitis (ICH) [1]. In Italy, CAdV-1 infection has been also occasionally described in dogs [2, 3, 4, 5, 6] but information on the epidemiology and the genomic features of CAdV-1 circulating strains are still limited. Aim of this study was to provide an in-depth update on the epidemiologic, pathogenetic and molecular features of ICH in domestic dogs. A cross-sectional study was conducted over 291 dogs suspected of infectious gastrointestinal disease. Rectal swabs, faeces and tissue samples from dogs were collected between 2017 and 2020. Virological and histopathological assays were carried out. The presence of CAdVs and other canine viral enteropathogens was investigated, and the sequence and phylogenetic analyses were performed. Viral pathogens were detected in 66% of the tested dogs, alone or in co-infections. CPV-2 was the most frequently detected (97.3%), alone or with other viruses, followed by the CCoV (13.6%). CAdV-1 was detected from 6 (3%) dead stray dogs, alone and in co-infection with CPV and CCoV. Gross lesions and histopathological findings referred to CAdV and CPV were observed, also involving the central nervous system tissues. Sequence analysis evidenced divergences with the circulating strains previously described from dogs or wild canids in Central and Northern Italy, and a closer relation with older CAdV-1 strains collected from other Countries. All inoculated samples were successful isolated in the continuous canine kidney cell line MDCK and were long term stored for future analyses and comparisons. These data suggest a genetic heterogeneity of CAdV-1 in Italy. The evidence of the CAdV-1 in a background of high prevalence of CPV-2 infection suggests a likely low vaccinal coverage. This condition, together with others (uncontrolled promiscuity, contact with infected animals or contaminated fomites, stressors, co-morbidities), supports the need of further control strategies and management programs for strays in urban and sub-urban areas, other than for the dog transport or in kennels/shelters. The evidence of the circulation of CAdV-1 and its genomic features suggests to not underestimate this virus as a causative agent of infection of domestic or wild animals. Moreover, it allows further comparative studies to have an in-depth knowledge on the epidemiology and evolution of the different CAdV-1 genomic variants.

[1] Decaro N, Buonavoglia C. Canine Adenoviruses and Herpesvirus, The Veterinary clinics of North America, Small Animal Practice, 38:799–814, 2008.
[2] Pratelli et al. Variation of the sequence in the gene encoding for transmembrane protein M of canine coronavirus (CCV), Molecular and Cellular Probes, 15(4):229-33, 2001.
[3] Decaro et al. Infectious canine hepatitis: an "old" disease reemerging in Italy, Research in Veterinary Science, 83(2):269-73, 2007.
[4] Balboni et al. Investigation of the presence of canine adenovirus (CAdV) in owned dogs in Northern Italy, Research in Veterinary Science, 97:631–636, 2014.
[5] Balboni et al. Novel sequence variants of viral hexon and fibre genes in two dogs with canine adenovirus type 1-associated disease, Veterinary Journal, 223:73–75, 2017.
[6] Pintore et al. Canine adenovirus type 1 and Pasteurella pneumotropica co-infection in a puppy. Veterinaria Italiana, 52(1):57-62, 2016.

POSTER
ARNA

MIGLIOR POSTER ARNA - SISVET 2021

P310 - Color of hen eggs from organic and conventional production systems

Anna Antonella Spina, Carlotta Ceniti, Nicola Costanzo, Valeria Maria Morittu

Università degli Studi Magna Græcia di Catanzaro, Dipartimento di Scienze della Salute

Corresponding author: Anna Antonella Spina (aa.spina@unicz.it)

Today consumers are more interested in products of animal origin coming from breeding systems that protect the environment, animal welfare and the nutritional quality of derived products. A characteristic that the consumer attributes as a nutritional quality index of the eggs is the color of the yolk. In turn, the visual evaluation of egg yolk can confusingly produce a wrong perception of its real nutritional value (1). Yolk color can be determined subjectively using the Yolk Color Fan® (Roche) scale (2). This method was found less accurate than color measurement performed by using spectrocolorimeters (3). The aim of this study was to investigate the effect of the production system (organic vs conventional) on some quality traits of hen eggs of the large organized distribution.

A total of 70 eggs of Class "A", weight "M" (Regulation (EC) No 589/2008) were purchased in October 2020 from the various supermarkets: 35 eggs were from 10 different conventional farms and 35 eggs were from 10 organic farms. Before analysis, the eggs were cleaned and weighed. Shell, egg white and yolk were separated and weighed individually. For color assessment, each yolk was placed on a Petri dish and then covered with a food foil to prevent direct contact between yolk and instrument. Each sample was evaluated as mean value of three different readings by a Konica Minolta CM700d spectrophotometer, with the following settings: illuminant D65, observer 10° and aperture 8 mm. All measurements were conducted at room temperature (20 ± 2°C) after the instrument was calibrated against the food foil. Results were expressed according to the CIE 1976 L*a*b* system (CIELAB) (3). Statistical analysis was performed by GraphPad Prism version 8.3.0 for Windows. As expected, we did not observe any difference in the weights of the egg and its components (eggshell, albumen, yolk.). Organic yolk eggs resulted lighter (L*= 69.18±2.96 vs 65.57±1.70, p=0.004) and yellower (b*=65.01±5.36 vs 58.89±1.82; p=0.006) than conventional ones, but no significant difference (p=0.395) was observed between the redness values (a*=15.80±3.85 vs 17.68±5.60 for organic and conventional group, respectively). In other words, color tone was similar (p=0.185) in the two groups (H°=76.19±4.06 vs 73.37±5.06, for organic and conventional group, respectively), but organic yolks can be perceived as more brilliant than conventional ones (C*=67.06±4.67 vs 61.72 ±2.11; p=0.004). Contrary to what other authors have reported (1;4;5;6), the H° of conventional yolks is no longer shifted towards red compared to that of organic yolks. It would be useful to trace the type of feeding of the hens in the two groups. It would be interesting to investigate the composition of the diets at the base of those organic farms whose eggs produced were redder, in order to identify the possible presence of natural ingredients capable of giving the product that type of pigmentation.

The pH of the albumen is significantly higher in conventional eggs (9.10±0.28 vs 8.81±0.90; p=<0.0001). However, all pH values were within the "fresh" egg category and are in agreement with those reported by Dalle Zotte (2021). As the albumen pH can varie with breed, age, and storage periods (7), further study is needed to clarify which of these factors most influenced the pH difference. This study confirms that it is possible to distinguish color based on the farming system although further study is needed to investigate the factors behind some organic eggs with H. similar to that of conventional eggs.

[1] Czarnowska-Kujawska M. Molecules 26, 2021. [2] Vuilleumier JP. Poultry Science, 1969. [3] Dvořák P. Acta Veterinaria Brno, 2010. [4] Minelli G. Italian Journal of Animal Science, 2007. [5] Lewko, L.; Gornowicz, E. Ann. Anim. Sci.2011. [6] Dalle Zotte A. Livestock Science, 2021. [7] Caglayan T.. Journal of Applied Poultry Research 23.2, 2014.

P311 - Fungal contamination detected in dairy feedstuff collected in South Italy

Carlotta Ceniti, Valeria Maria Morittu, Anna Antonella Spina, Nicola Costanzo*

Università degli Studi Magna Græcia di Catanzaro, Dipartimento di Scienze della Salute

Corresponding author: Anna Antonella Spina (aa.spina@unicz.it).

The occurrence of fungi or related mycotoxins in feed destined for animal production is one of the biggest concerns for animal and human health. Due to its ubiquity characteristic, one of the major concerns for dairy feed is fungi spoilage that represents a suitable media for the growth and proliferation of toxigenic species (1). This research provides an insight into the variety of fungi that can be isolated from hay produced in South Italy and destined to dairy cows. A total of 55 samples were collected from 20 dairy farms randomly chosen in Catanzaro area, Italy. The hay sampling methods adopted for the purposes of this research are those indicated by Regulation (EU) no. 691/2013 which amended Regulation (EC) no. 152/2009 concerning sampling and analysis methods for official feed controls. Briefly, for each batch an aggregate sample of about 1 kg was obtained by sampling a minimum of 15 bales per lot randomly chosen, using a motorized corer (length 60.0 cm, internal diameter 22 mm). Following collection, aggregate samples were transported at room temperature to the laboratory where they were carefully mixed and separated in two aliquots of 300 g for microbiological analysis. The mycological investigation focused on the taxonomical identification of filamentous micro fungi and was carried out by means of the Moist Chamber (MC) method. All hays were found to be contaminated with moulds and, in particular, a total of 33 different species of mould were identified. The most representative was *Cladosporium cladosporioides* (n=46; 84%) followed by *Alternaria alternata* (n=25; 45%) and *Rhizopus stolonifer* (n=35; 64%). *Aspergillus flavus*, a mould related to aflatoxin production, was also isolated (n=11; 20%). *Cladosporium* is one of the most frequent airborne moulds, known to secrete mycotoxins, mainly found in inside and outside environments of agricultural context and dairy farm and is responsible for damage of sheep and cow cheese surface (3). *Rhizopus stolonifer* is a typically post-harvest mould of fruit and vegetable and mould spoilage occurs mainly during storage, transport, and commercialization (4). *Alternaria alternata*, is a ubiquitous, saprophytic fungus isolated in a variety of habitat; commonly isolated in dead plant materials, it is also a plant pathogen causing disease on several crops (5). The genus *Alternaria* includes some species related to the production of toxins such as alternariol, altenuene, tenuazonic acid, altertoxin (6). *Aspergillus flavus* is the main source of AFB1, of which the ubiquitous growth can occur at any point in the pre- or post-harvest stage, making it difficult to control (7). In conclusion, the prevention of mould spoilage is mandatory to reduce the exposure of humans and animals to mycotoxins. Contamination of feedstuff by mycotoxin represents a growing global concern, thus further studies are needed to better understand the interaction relationships between mycotoxin-producing fungi and mycotoxin in feeds.

[1] Wacoo A.P. Appl. Chem.,1-15, 2014. [2] Iqbal S.Z. Jinap. Trends Food Sci. Technol., 46, 110–119, 2015. [3] Messini A. Int. Dairy J., 66, 6–12, 2017. [4] Zhang H. Effect of yeast antagonist in combination with heat treatment on postharvest blue mold decay and Rhizopus decay of peaches, 115, 53–58, 2007. [5] Leuven K.U. *Alternaria* spp.: from general saprophyte to specific parasite., 4, 225–236, 2003. [6] Panel, E., and Chain, F. Scientific Opinion on the risks for animal and public health related to the presence of *Alternaria* toxins in feed and food 1. 9, 1–97, 2011. [7] Rushing B.R. Food Chem. Toxicol., 124, 81–100, 2019.

POSTER

RNIV

P42 - The genetic variability of ORF virus in Sardinia

E. Coradduzza[1], D. Sanna[2], A.M Rocchigiani[1], D. Pintus[1], F. Scarpa[3], R. Scivoli[1], R. Bechere[1], M.A Dettori[1], M.A Montesu[4], V. Marras[4], R. Lobrano[4], C. Ligios[1], G. Puggioni[1]

(1) Istituto Zooprofilattico della Sardegna (2) Università di Sassari, Dipartimento di Scienze Biomediche (3) Università di Sassari, Dipartimento di Medicina Veterinaria (4) Università di Sassari, Dipartimento di Scienze Mediche, Chirurgiche e Sperimentali

Corresponding author: E. Coradduzza (elisabetta.coradduzza@izs-sardegna.it)

Orf virus (ORFV) represents the causative agent of contagious ecthyma, it is clinically characterized by mild papular and pustular to severe proliferative lesions, mainly occurring in sheep and goats. Similar lesions have been also reported in other animal species [1] and humans [2], thus resulting in a zoonotic disease which is worldwide spread [3]. We carried out a study aimed to assess the genetic variation of ORFV in Sardinia, with the further aim to provide hints on the evolutionary history of this virus. Tissue samples from different skin damaged areas of affected hosts were collected for histopathological and virological investigations from nine sheep, eight goats of different ages, and from the hand skin of an ORFV affected young farmer showing papular and pustular lesions. Fourteen out of 18 viral strains were isolated on VERO cell cultures and PCRs [4] were carried out for the viral genes B2L (major envelope protein), O45 (late transcription factor VTLF-1), and VIR (dsRNA-binding protein). PCR products were sequenced for both forward and reverse strands. Phylogenetic analyses were performed following the methods used in Scarpa et al. 2019 [5] and Sanna et al. 2013 [6]. We found a high worldwide mutational viral evolutionary rate which resulted higher than the rate that we detected for the strains isolated in Sardinia. In addition, a well-supported genetic structuring was found between the viral strains isolated from sheep and those from goats, but no relevant connection was evidenced between the severity of lesions produced by ORFV and specific polymorphic patterns occurring in the three genes here analyzed. Such a finding suggests that ORFV infection-related lesions are not necessarily linked to the expression of one of these genes and could be the effect of the expression of other genes or rather represent a multifactorial character.

[1] Hosamani et al. Orf: an update on current research and future perspectives, Expert Rev Anti Infect Ther, 7:879–893, 2009.
[2] Bergqvist et al. Orf virus infection, Rev Med Virol, 27:1–9, 2017.
[3] Kumar et al. Contagious pustular dermatitis (orf disease) – epidemiology, diagnosis, control and public health concerns**,** Adv Anim Vet Sci, 3:649-676, 2015.
[4] Kottaridi et al. Laboratory diagnosis of contagious ecthyma: comparison of different PCR protocols with virus isolation in cell culture, J Virol. Methods, 134:119–124, 2006.
[5]. Scarpa et al. How to achieve internal fertilization without a vagina: the study case of the genus *Archilina* Ax, 1959 (Platyhelminthes, Proseriata) from Canary Islands**,** Mar Biodiv, 49:2057–2073, 2019.
[6] Sanna D et al. Mitochondrial DNA reveals genetic structuring of *Pinna nobilis* across the Mediterranean Sea, Plos One, 8(6): e67372, 2013.

P95 - CSPG4 molecular target in canine melanoma, osteosarcoma and mammary tumors for novel therapeutic strategies

Paola Modesto (1), Floriana Fruscione (1), Isabella Martini (1), Federica Riccardo (2), Mariateresa Camerino (3), Davide Giacobino (3), Cecilia Gola (3), Luca Licenziato (3), Elisabetta Razzuoli (1), Katia Varello (1), Lorella Maniscalco (1), Elena, Bozzetta (1), Angelo Ferrari (1)

(1) Istituto Zooprofilattico Sperimentale del Piemonte, Liguria e Valle d'Aosta, Centro di Referenza Nazionale per l'Oncologia Veterinaria e Comparata (CEROVEC) (2) Università degli Studi di Torino, Dipartimento di Biotecnologie Molecolari e Scienze per la Salute, Centro di Biotecnologie Molecolari (3) Università degli Studi di Torino, Dipartimento di Scienze Veterinarie

Corresponding author: Paola Modesto (paola.modesto@izsto.it)

Canine and human melanoma, osteosarcoma (OSA) and mammary carcinomas are aggressive tumors with common characteristics making dogs a good model for comparative oncology. Novel therapeutic strategies against these tumors could be useful to both species. In humans, chondroitin sulphate proteoglycan 4 (CSPG4) is a marker involved in tumor progression and could be a candidate target for immunotherapy. The anti-CSPG4 DNA electrovaccination has shown to be an effective approach for canine malignant melanoma (CMM) [1]. An immunohistochemistry evaluation of CSPG4 expression in tumour tissue is generally performed prior to electrovaccination. To assess the possibility to perform a rapid molecular evaluation, we investigate the CSPG4 gene expression by RT qPCR, in CMM, OSA and canine mammary tumors (CMT). The total RNA was extracted from RNAlater stored tissue samples (CMM n=16; OSA n=13; CMT n=6; five paired normal tissues for CMM, five paired normal tissues for OSA and one paired normal tissue for CMT), retro-transcribed and then analysed by duplex RT-qPCR using two different TaqMan assays for the target gene CSPG4 and the internal reference gene (RG) Ribosomal Protein S19 (RPS19). RPS19 was selected from a panel of 9 candidate RGs, according to NormFinder analysis following the protocol already described [2]. Relative expression was analyzed by CFX Maestro™ Software. Student t-test and ANOVA were performed (significance set at $P<0.05$). Results showed that gene expression of CSPG4 in OSA tissues is significantly increased by 3-4 folds when compared to controls. In CMT gene expression of the target was increased from 1.5 to 19.9 folds. In melanoma, although an increasing trend was observed, no significant differences between the two groups were highlighted. Immunohistochemistry analysis of the two cancer types showed that the expression of CSPG4 within CMM is concentrated in isles of cells compared to OSA where, the distribution of positive cells is homogeneous. This evidence could explain the differences in gene expression results. CSPG4 immunohistochemistry evaluation in mammary carcinoma is progress. The evidence of CSPG4 expression in different type of canine tumors, opens the way to the possibility of extending the CSPG4 immunotherapy marker in CMM, OSA and CMT and may have an impact to translate this strategy modality to human oncology.

[1] Giacobino D, Camerino M, Riccardo F, Cavallo F, Tarone L, Martano M, Dentini A, Iussich S, Lardone E, Franci P, Valazza A, Manassero L, Del Magno S, De Maria R, Morello E, Buracco P. Difference in outcome between curative intent vs marginal excision as a first treatment in dogs with oral malignant melanoma and the impact of adjuvant CSPG4-DNA electrovaccination: A retrospective study on 155 cases. Vet Comp Oncol. 2021 Mar 9.
[2] Modesto P, Castrillo Fernandez JL, Martini Isabella, Zoccola R, Pugliano MC, De Ciucis CG, Goria M, Ferrari A and Razzuoli E. Characterization of D-17 Canine Osteosarcoma Cell Line and Evaluation of Its Ability to Response to Infective Stressor Used as Alternative Anticancer Therapy. Animals 2020, 10, 1981.

MIGLIOR POSTER RNIV - SISVET 2021

P127 - Prevalence of EcPV-2 in asyntomatic Italian horses

Katia Cappelli (1), Tiziana Nervo (2), Marco Pepe (1), Rodolfo Gialletti (1), Samanta Mecocci (1), Laura Migone (3), Luca Mechelli (1), Chiara Grazia De Ciucis (3), Gian Guido Donato (2), Paola Modesto (3), Alessandro Ghelardi (4), Elisabetta Razzuoli (3)

(1) Università degli Studi di Perugia, Dipartimento di Medicina Veterinaria (2) Università degli Studi di Torino, Dipartimento di Scienze Veterinarie (3) Centro di Referenza Oncologia Veterinaria e Comparata (CEROVEC) (4) Toscana Nord-Ovest, UOC Ostetricia e Ginecologia, Ospedale Apuane

Corresponding author: elisabetta.razzuoli@izsto.it

Squamous cell carcinoma (SCC) is the most common malignant skin neoplasia, after sarcoidis in horses. This cancer accounts for 7–37% of equine skin lesions (1) and can arise at any site on the skin and mucosa. Equine papillomavirus type 2 (EcPV2) has been consistently found in SCC of oral and genital tract, indicating a causal association of EcPV2 in the pathogenesis of these tumours (1, 2). Papillomaviruses are responsible for a subset of cancers in many species and human papillomaviruses (HPVs) are the best studied. In particular, HPV types 16 and 18, are associated with cervical, vulvar, ano-genital and head and neck cancers (3). It is known that subclinical HPV infection is more common than clinical disease, so, the identification of high-risk populations and development of screening protocols have all contributed to early detection. In contrast to HPV, our understanding of EcPV2 infecting horses is limited and little is known about the prevalence of this virus in Italy. The aim of our study was to determine the genoprevalence of EcPV2 in Italian clinically healthy horses. To this purpose 150 horses presented to the veterinary departments of the Turin and Perugia universities were sampled. Cytobrush samples from the penis/foreskin or vulva/vagina of healthy animals were collected. Horses displaying no skin or mucous membrane lesions or severe signs of other diseases were selected. To determine the genoprevalence, DNA was extracted from cytobrush samples using QIAamp DNA Mini Kit (Qiagen) according to manufacturer's instructions. EcPV2-L1 presence was tested using specific primers set and probe previously described (4). A total of 32 males and 118 mares were tested. The age of animals inserted in the study ranged between 2 month and 24 years with a mean value of 9.6±5.4. The 21.3% of tested animals resulted positive to EcPV2-L1. In particular, in 6/32 (18.8%) male and 26/118 mare (22%) was detected EcPV2. All positive samples were tested for E2, E6, E7 oncogenes. Only the 85 % was positive to EcPV2-E2. Our data showed higher genoprevalence of EcPV2 compared to Switzerland and different trend compared to the data obtained in Canada by Greenwood and co-workers (5). Indeed, our results did not show a significant difference in the genoprevalence between penis and vulva. Moreover, our findings showed that EcPV2 infection, in asymptomatic horses, is more common than previously reported and that the role of this virus in equine genital SCCs may be more complex than originally thought.

[1] Sykora, S.; Brandt, S. Vet. J. 2017, 223, 48–54. [2] Armando et al. Animals, 10: 2318, 2020. [3] Hausen. Virology. 384:260–265, 2009. [4] Porcellato et al. Cells, 9:2364, 2020. [5] Ficher et al. Vet Dermatol, 25:210-e54, 2014. [5] Greenwood et al. Vet Pathol, 57: 632-641, 2020.

P157 - Evolution of the trend of mastitogenic agents in the buffalo species in the Campania Region from 2014 to 2019

Gabriele Di Vuolo, Giovanna Cappelli, Luisa D'Angelo, Lucrezia Lucchese, Domenico Alfano, Giorgio Galiero, Esterina De Carlo, Domenico Vecchio

Centro di Referenza Nazionale per l'Igiene e la tecnologia dell'allevamento delle produzioni bufaline, Istituto Zooprofilattico Sperimentale del Mezzogiorno

Corresponding author: Giovanna Cappelli (giovanna.cappelli@izsmportici.it)

Milk is universally recognized as a complete diet due to its chemical-nutritional characteristics and can be considered one of the most important foods for humans. However, its quality can deteriorate after the onset of mastitis in both dairy cattle and buffalo. Mastitis represents one of the major problems present in dairy farms, for which prevention and control strategies take on a specific significance. The recent law on animal health establishes a connection between health and animal welfare, as better animal health promotes greater welfare and vice versa. In the buffalo, mastitis continues to be underestimated especially in company management despite the important negative effect it has on animal welfare, milk quality and herd profitability. The purpose of the work was to analyze the results of the bacteriological investigations carried out on milk samples given to the Istituto Zooprofilattico Sperimentale del Mezzogiorno (IZSM) for the search for pathogens responsible for mastitis between 2014 and 2019. Mastitis, as well as a compromising animal welfare [1;5], leads to significant economic losses [2]; direct (discarded milk, decrease in productivity) and indirect (increase in labor, diagnostic costs, drugs, early cull), in dairy breeds mastitis is the first reason for the use of antimicrobials [3], the abuse of which must be averted to avoid the onset of dangerous antimicrobial resistance (AMR) phenomena. Both of these aspects, the fight against antibiotic resistance and the protection of animal welfare, are at the heart of the new "Farm to Fork Strategy" [4]. In the period between 1 January 2014 and 31 December 2019, 2650 samples of buffalo milk from 105 livestock farms were delivered to IZSM. 88.57% of the farms that gave samples showed the isolation of mastitogenic agents. In detail of the positive samples, in 67.74% at least one contagious mastitogen was isolated, in 26.88% of the farms environmental mastitogens were isolated, and in 22.58% opportunistic microorganisms. Analyzing in detail the contagious group, as many as 93.06% of the quarters analyzed were positive for Staphylococcus aureus. In the environmental group, the most frequent pathogen was *E. coli* with 60.33% of the positive quarters, while for the opportunists the most frequent were CNS with 81%. Despite the numerous works, the problem of mastitis in the buffalo species continues to be an underestimated phenomenon. Raising the awareness of breeders and technicians is crucial to improve this perception and implement prevention through the use of structures and management procedures that are increasingly responsive to the physiology of the animals.

[1] Broom D.M., (2017). Animal Welfare in the European Union. European Parliament, Directorate General for Internal Policies. PE 583.114. [2] Pisanu, et al., Scientific Reports, 9(1), 1–14.
[3] Stevens, M., Piepers, S., & De Vliegher, S. (2016). Journal of Dairy Science, 99(4), 2896–2903..
[4] The Farm to Fork Strategy - For a fair, healthy and environmentally-friendly food system-https://ec.europa.eu/food/farm2fork_en Publication 2774/3000. [5] EFSA, (2009). Scientific Opinion of the Panel on Animal Health and Welfare on a request from the Commission on the risk assessment of the impact of housing, nutrition and feeding, management and genetic selection on udder problems in dairy cows.

P309 - Lactoferrin concentration in Italian Holstein Friesians' quarter milk at dry-off: correlation with the somatic cell count score and intramammary infections

Gianfilippo Alessio Clemente (1), Massimo Amadori (2), Valentina Lorenzi (1), Francesca Fusi (1), Giandomenico Ferrara (1), Francesca Mazza (1), Giulia Di Patrizi (1), Giorgio Franceschini (1), Luigi Bertocchi (1), Clara Tolini (1)

(1) Italian Reference Centre for Animal Welfare - Istituto Zooprofilattico Sperimentale della Lombardia e dell'Emilia Romagna "Bruno Ubertini", Brescia (2) Rete Nazionale di Immunologia Veterinaria, Brescia

Corresponding author: Gianfilippo Alessio Clemente (gianfolem@gmail.com)

Lactoferrin is an iron binding glycoprotein found in exocrine secretions, including milk. This protein has an antimicrobial and immunomodulatory role in innate immune defense.
The aim of the study was to evaluate the competence of the non-specific immune response of high-yielding cows against udder pathogens by lactoferrin concentration analysis and its relation with somatic cell count (SCC) and bacteriological results, during late lactation.
Two hundred and five quarter milk samples were collected at dry-off from multiparous high-yielding Italian Holstein Friesian cows. The animals were housed in the same farm and were subjected to feed restriction during the days preceding dry off, in order to reduce milk production. Milk samples were collected aseptically and in number of 2 for each quarter. One sample underwent bacteriological and SCC analysis, the other was used for lactoferrin determination. Based on milk bacteriological results, udder quarters were divided into 3 group: "negative" (BACTneg), "minor pathogens" (BACTmin) or "major pathogens" (BACTmaj) [1]. Milk samples having a lactoferrin concentration greater than or equal to 400 µg/mL were defined as lactoferrin positive (LFpos), otherwise they were defined as negative (LFneg). Chi-squared test was used to determine the presence of significant differences between lactoferrin status of udder quarters among BACTgroups. Moreover, Spearman's correlation coefficient (r_s) was calculated to investigate the presence of a significant correlation between lactoferrin concentration and SCC. Out of the 205 quarter milk samples examined, 67% (137/205) resulted LFpos. In particular, 100% (10/10) of the quarters in the BACTmaj group were LFpos, while 65.7% (86/131) and 64.1% (41/64) resulted LFpos in the BACTmin and in BACTneg groups respectively. Lactoferrin concentration varied significantly among BACTmaj vs BACTmin ($p<0.05$) and BACTmaj vs BACTneg ($p<0.05$) groups. No difference in the lactoferrin status was observed between BACTmin vs BACTneg groups ($p=0.12$). Quarters infected with major pathogens (BACTmaj) showed the highest mean lactoferrin concentration (1340 µg/ml), while BACTmin and BACTneg groups had similar mean values (868 µg/ml and 876 µg/ml, respectively). A significant positive correlation was found between lactoferrin concentration and SCC ($r_s=0.55$, $p<0.05$). The 75% of LFneg samples showed SCC < 200,000 cell/ml. Quarters having SCC ≥ 200,000 cell/ml were found to have a lactoferrin mean value of 1282 µg/ml, while quarters with SCC < 200,000 cell/ml had a lactoferrin mean value of 559 µg/ml. Late stage of lactation and low milk yielding have been demonstrated to be associated with an increase of lactoferrin concentration in cow milk. Nevertheless, quarters infected by major pathogens showed higher lactoferrin values than those recorded in the other two groups. The absence of differences in lactoferrin concentration between BACTmin and BACTneg groups should be further investigated and the potential influence of feed restriction during the final week of lactation should be clarified. The significant positive relation between lactoferrin concentration ad SCC values at dry-off, also reported by other authors, should be better studied in order to explore the possibility of using lactoferrin and SCC as complementary diagnostic tool for early mastitis detection.

[1] Bertocchi L. Trend in the etiology of dairy cow mastitis in Northern Italy from 2005 to 2011, Large Animal Review, 18: 51-58, 2012.

P317 - Lysozyme concentration in quarter milk of Holstein Friesian cows during intramammary infections with different pathogens

Gianfilippo Alessio Clemente (1), Massimo Amadori (2), Valentina Lorenzi (1), Francesca Fusi (1), Claudia Iacomino (1), Giulia Ventura (1), Francesca Mazza (1), Luigi Bertocchi (1), Clara Tolini (1)

(1) Italian Reference Centre for Animal Welfare - Istituto Zooprofilattico Sperimentale della Lombardia e dell'Emilia Romagna "Bruno Ubertini", Brescia (2) Rete Nazionale di Immunologia Veterinaria, Brescia

Corresponding author: G. A. Clemente (gianfoclem@gmail.com)

Lysozyme is an important humoral factor of the innate immune system, which represents the first crucial barrier against pathogens. Lysozyme is present in low concentration in milk of healthy dairy cows and it increases in mastitic milk.

The aim of the study was to evaluate the competence of the non-specific immune response of multiparous high-yielding Italian Holstein Friesian dairy cows against udder pathogens by analysing the lysozyme concentration and its relation with bacteriological results. For this purpose, 1036 quarter milk samples were collected: 556 at dry-off (T1), 352 at 10-14 days post-calving (T2) and 128 at 28-32 days post-calving (T3).

One hundred thirty-nine cows at T1, 88 at T2 and 32 at T3 were included for the study. The animals were housed in the same farm and all cows were treated with antibiotics at dry-off. Milk samples were collected aseptically and in number of 2 for each quarter. One sample underwent bacteriological analysis, the other sample was used for lysozyme determination. Twenty-one samples resulted contaminated at the bacteriological test and were excluded from the study. Based on milk bacteriological results, udder quarters were divided into 3 groups: “negative” (BACTneg), “minor pathogens” (BACTmin) or “major pathogens” (BACTmaj) [1]. Milk samples having a lysozyme concentration higher than 0.26 µg/mL were defined as lysozyme positive (LYSpos), otherwise they were defined as negative (LYSneg). Chi-squared test was used to determine the presence of a significant relationship between lysozyme status and bacteriological status of udder quarters. At T1 no differences in the lysozyme status among BACTgroups were observed ($p=0.15$), with 17% of LYSpos found among all the samples tested. Instead, at T2 and T3, lysozyme secretion resulted significantly different between BACTgroups ($p<0.05$). At T2, only 4% of all samples analysed were positive. In particular at T2, LYSpos results were more frequent in the BACTmaj group (10%) than in BACTmin (0%) and BACTneg (0.7%). At T3, lysozyme secretion followed the T2 trend. In fact, BACTmaj was the only group with a percentage of 7% of samples resulted LYSpos, while only LYSneg values were found in the other groups (0%). The percentage of total samples analysed resulting positive was 3%. The higher value of LYSpos (1.89 µg/mL) of the total study was recorded at T1, associated with a pathogen belonging to the BACTmaj. These preliminary results suggest a reduced lysozyme secretion in Holstein Friesian cows’ quarter milk, that decreases from the dry-off moment to the early lactation. At late lactation, an inefficiency of the innate immune system response was recorded, as well as an inability to discriminate potentially pathogenic bacteria. In early lactation, this discriminatory ability seems to be restored and oriented against major udder pathogens. This relation between lysozyme and major udder pathogens should be better studied due to the limited number of samples, especially at the T3.

[1] Bertocchi L. Trend in the etiology of dairy cow mastitis in Northern Italy from 2005 to 2011, Large Animal Review, 18: 51-58, 2012.

POSTER
SICLIMVET

P76 - Assessment of biological risk for dairy buffalo milkers: preliminary results

Maria Chiara Alterisio (1), Renato Gesuele (2), Raffaele D'Angelo (3), Jacopo Guccione (1), Giovanni Libralato (2), Antonio Di Loria (1), Carlo Ferravante (1), Daniele Furio (1), Ernesto Russo (3), Marco Guida (2), Paolo Ciaramella (1)

(1) Università degli Studi di Napoli Federico II, Dipartimento di Medicina veterinaria e produzioni animali (2) Università degli Studi di Napoli Federico II, Dipartimento di Biologia (3) Istituto Nazionale per l'Assicurazione contro gli infortuni sul Lavoro-Campania

Corresponding author: Maria Chiara Alterisio (mariachiara.alterisio@unina.it)

Milking parlour represents a crucial point in the potential transmission of zoonotic microorganisms between humans and animals because of the forced and repeated contact between them [1]. Although the main zoonosis present in livestock farms are under the control of the National Health System [2], information on the impact of the incorrectly defined minor diseases (e.g. pyoderma, otitis, etc.) are truly rare. The objective of this study was to explore the biological risk to which dairy buffalo milkers are exposed during their activity within the milking parlour.

During the present study a Mediterranean buffalo farm located in the province of Caserta (southern Italy) and consisting of 400 milking heads was selected. The herd was characterized by an overall bulk tank milk somatic cell count and colony-forming unit (cfu) values of 256,000 cells/mL and <100,000 cfu/mL, respectively. In order to verify the circulation of micro-organisms between animal, humans and environment a specific sampling has been perform for each of them. In detail, one composite milk sample (4 quarters pool) and individual teat skin swab were collected from 10 pluriparous buffaloes chosen by means of simple randomization during the morning milking. In addition, one swab from gloves, apron, boots and smartphones were performed from each of the two milkers; finally, other two analogous samples were performed on the return lane surface of the side by side milking parlour (3 points of sampling for each side). All the samples obtained were immediately stored at a controlled temperature (4°C), sent to the reference laboratory and submitted to a bacteriological culture for GRAM- within 4 hours from collection. Overall 30 samples were collected, of which: 8 originating from the operators, 2 from return lanes, 10 from teat skin and 10 from pooled milk. The samples showed a heterogeneous microbial population consisting of Gram- bacteria. Some of these were simultaneously present in almost all the analysed substrates and were both recognized mastitogens in the buffalo species and potentially infecting humans [3]. It should be noted that the search for *Salmonella* was fortunately negative. Although the present pilot study is based on a small sample population, it highlights how there is a circulation of potentially zoonotic bacteria inside the milking parlors, confirming the existence of a biological risk that operators in the sector can easily incur.

Further studies are necessary to define in detail the circulation of microorganisms between animal, man and the environment, in order to quantify the risk for those involved in this task and to implement all the necessary countermeasures to be able to scale it within tolerance limits.

[1] Oliver et al. Foodborne Pathogens in Milk and the Dairy Farm Environment: Food Safety and Public Health Implications, Foodborne Pathogens and Disease, 2:115-129, 2005.
[2] Ministero della Salute. D.P.R. 8 febbraio 1954. Gazzetta Ufficiale 142, 2006.
[3] Guccione et al. Clinical outcomes and molecular genotyping of *Staphylococcus aureus* isolated from milk samples of dairy primiparous Mediterranean buffaloes (*Bubalus bubalis*), Journal of Dairy Science, 97:7606-7613, 2014.

MIGLIOR POSTER SICLIMVET - SISVET 2021

P114 - Diagnostic performances of urinary neutrophilic degeneration and intracellular bacteria in detecting urine culture outcome in canine suspected urinary tract infections

Eleonora Gori, Verena Habermaass, Ilaria Lippi, Valentina Virginia Ebani, Alessio Pierini, Veronica Marchetti

Università di Pisa, Dipartimento di Scienze Veterinarie

Corresponding author: Eleonora Gori (eleonora.gori@vet.unipi.it)

In dogs, stained urinary sediment evaluation had higher diagnostic performance than wet-mount evaluation in diagnosis of urinary tract infections (UTIs) (1,2). For bronchoalveolar lavage and body cavity fluids, degenerative neutrophils, and intracellular bacteria were used to interpret the role of bacteria in inflammation (3,4). The aim of the study was to evaluate if the detection of neutrophil morphological alterations or intracellular bacteria in stained urinary sediment may predict urine culture results in dogs with clinically suspected UTI. UTIs were suspected based on clinical history and clinical signs (such as pollakiuria, dysuria, and hematuria). Each dog had urine samples taken by cystocentesis and immediately underwent urinalysis with wet-mount sediment evaluation. Urine culture, bacterial cell count and antimicrobial susceptibility test was performed within 12h from sampling. Dogs underwent antimicrobial and immunosuppressant therapy were excluded. Urine samples were prepared as cytospin, stained with Diff-Quik stain, and were microscopically evaluated for the presence/absence of neutrophils and bacteria, neutrophil degeneration, and bacterial localization (intra/extracellular). Urine cultures with <100 colony-forming units were classified as negative (5). Presence/absence of degenerated neutrophils, bacteria and bacterial localization were compared between positive/negative urine culture using Fisher's exact test. Odds ratio (OR) was calculated. Forty-five dogs with suspected UTI were prospectively included. Seventeen dogs (38%) had positive urine culture. Sixteen dogs (35%) showed bacteria at cytological evaluation, of which 14 had intracellular bacteria. Dogs showing cytologically-evident bacteria (both intra- and extracellular) had a 19-fold chance to have positive urine culture (OR 19.5 95%CI 4.2-83-5; $p<0.001$), risk that increases to a 27-fold if intracellular bacteria were highlighted (OR 27 95%CI 5-108; $p<0.001$). Twenty-two dogs (49%) showed neutrophils (both non- and degenerated), 19 of them had degenerated neutrophils. Dogs having neutrophils had a 6-fold probability to have positive urine culture (95%CI 19-22; $p=0.006$), whereas if degenerated changes were present the OR increased to 12 (95%CI 3-40; $p=0.0005$). Finally, evaluating the combination of degenerated neutrophils with intracellular bacteria, it was present in 16 dogs with OR 27 (95%CI 5-108; $p<0.0001$). Interestingly, 3 dogs (6.5%) with degenerated neutrophils and intracellular bacteria had negative urine culture. The probability to have urine culture positivity in dogs with suspected UTI increases if intracellular bacteria and degenerated neutrophils are seen. Most importantly, in some cases, the evaluation of stained samples allows to identify an infection, even with negative urine culture and it may help the clinician in the diagnostic process of dogs with suspected UTIs.

[1] Swenson et al. Evaluation of modified Wright-staining of urine sediment as a method for accurate detection of bacteriuria in dogs. JAVMA, 2004.[2] Way et al. Comparison of routine urinalysis and urine Gram stain for detection of bacteriuria in dogs. JVECC, 2013. [3] Johnson et al. Microbiologic and cytologic assessment of bronchoalveolar lavage fluid from dogs with lower respiratory tract infection: 105 cases (2001-2011). JVIM, 2013. [4] Connally. Cytology and fluid analysis of the acute abdomen. Clin Tech Small Anim Pract, 2003. [5] Bartges. Diagnosis of urinary tract infections. Vet Clin North Am Small Anim Pract. 2004.

P115 - Serum biochemical and urinary parameters of renal impairment in dogs with primary chronic enteropathy

Eleonora Gori (1), Francesca Perondi (1), Giulia Ansaldo (1), Paola Gianella (2), Ilaria Lippi (1), Alessio Pierini (1), Veronica Marchetti (1)

(1) Università di Pisa, Dipartimento di Scienze Veterinarie (2) Università degli Studi di Torino, Dipartimento di Scienze Veterinarie

Corresponding author: Eleonora Gori (eleonora.gori@vet.unipi.it)

Approximately the half of human IBD patients may show extra-intestinal manifestations, specifically 4-23% of cases develop renal and urinary impairment (1). This finding may be linked to the immune response caused by the primary chronic enteropathy itself (CE), a reduction in short-chain fatty acids, or endotoxemia (1). No specific studies have been conducted in dogs, except for those describing familiar protein-losing nephropathy and enteropathy in soft-coated wheaten terriers (2-3). The aim of the study was to describe serum biochemical and urinary parameters alterations indicating renal impairment in dogs with CE. Retrospective medical records database research of two Veterinary Teaching Hospitals including dogs with CE. CE was diagnosed after the exclusion of intestinal diseases of other etiologies and extra-intestinal diseases. Dogs with history of previous kidney or lower urinary tract diseases (previous clinicopathological finding and/or imaging alterations) and with severe proteinuria (urine protein-to-creatinine ratio >2, [UPC]) were excluded. Canine Chronic Enteropathy Activity Index Score (CCECAI), muscular condition score (MCS; 3-point scale), serum albumin, urea, creatinine, presence of glycosuria, proteinuria (UPC>0.5) and cylindruria (>1-2 casts/LPF) were recorded for each dog. Dogs with albumin <2.7 mg/dL were classified as protein-losing enteropathy (PLE). Dogs with glycosuria, proteinuria and/or cylindruria were classified as having kidney injury. No dog had immunosuppressant therapy prior the inclusion. Mann-Whitney u-test was used to compare CCECAI of dogs with and without kidney injury. Chi-square test was used to evaluate the association of PLE and kidney injury, and proteinuria. One-hundred-six dogs with CE were included. Fifty-two dogs (49%) had mild-to-severe reduction in MCS. Six out 106 dogs (6%) had azotemia (median creatinine 1.6 mg/dL; range 1.5-2.4 mg/dL), whereas 40 dogs (38%) showed kidney injury. Two dogs had glycosuria, 22 dogs had proteinuria (median 0.68; range 0.5-2.4 mg/dl), and 23 dogs had cylindruria. CCECAI was not different between dogs with and without kidney injury (both medians=4; p=0.9). Forty-four dogs were classified as PLE. The prevalence of kidney injury was not different between PLE and non-PLE dogs (p=0.3), whereas PLE dogs showed a higher frequency (61%) of proteinuria than non-PLE dogs (p=0.03 OR 2.8 95%CI 1-6.8). Serum markers of kidney injury, as creatinine, should be interpreted with caution in CE dogs, since approximately half of our dogs showed a reduction in muscular mass. On the other hand, assessment of urinary markers of kidney injury may be useful and advisable, especially due to the high risk of proteinuria in PLE dogs.

[1] Ambruzs, Larsen. Renal Manifestations of Inflammatory Bowel Disease. Rheum Dis Clin North Am. 2018.
[2] Littman et al. Familial protein-losing enteropathy and protein-losing nephropathy in Soft Coated Wheaten Terriers: 222 cases (1983-1997), JVIM, 2000.
[3] Vaden et al. Familial renal disease in soft-coated wheaten terriers. JVECC, 2013.

P134 - Is squash preparation a reliable diagnostic tool in the diagnosis of urinary bladder malignant tumors in dogs?

Alessio Pierini (1), Maria Carla Criscuolo (1), Ugo Bonfanti (2), Elena Benvenuti (3), Veronica Marchetti (1), Enrico Bottero (3)

(1) Università degli Studi di Pisa, Dipartimento di Scienze Veterinarie (2) MYLAV La Vallonea, Laboratorio Veterinario, Milano (3) Endovet Professional Association, Roma e Torino

Corresponding author: AlessioPierini (pierini.alessio2004@gmail.com)

The accuracy of cytology is controversial in predicting transitional cell carcinoma (TCC) diagnosis in dogs. Epithelial cells may show varying degrees of cytological atypia in both hyperplastic/dysplastic lesions and TCC, making a cytological diagnosis of malignancy challenging [1,2].

The aim of this study is to assess the diagnostic performance of squash-preparation cytology in the diagnosis of malignancies in dogs with low urinary tract complaints, and to investigate diagnostic performance of some cytologic features.

Sixty-one dogs underwent transurethral cystoscopy, with at least one squash-preparation sample available to be microscopically reviewed and a final histopathological diagnosis, were enrolled. Cytological samples were blinded reviewed by two cytopathologists (a third cytopathologist reviewed slides in which the diagnosis conflicted). Eleven cytologic features including macronucleated cells, abnormal nucleoli, atypical mitosis, signet ring cells, multinucleated cells, nuclear molding, anisokaryosis, cytoplasmatic microvacuolation with perinuclear distribution, cell arrangement, neutrophil and lymphocyte infiltration have been evaluated for each cytologic sample. Dogs were divided into two groups based on cytological and histopathological diagnoses: malignant tumor (cytological [cMT] and histological [hMT]) and benign lesion (cBL and hBL). The agreement between the two cytopathologists was calculated using Cohen's kappa coefficient (κ). Performance diagnostic parameters were calculated for each cytological feature. Cytological features were considered to have good diagnostic performance if sensitivity (Se), specificity (Sp), accuracy >0.7, and κ>0.6

Forty-four (72%) dogs were diagnosed with hMT, and 17 (28%) with hBL. There was a substantial agreement between operators for cytological diagnosis (κ=0.88) and histological diagnosis was significantly associated with cytological diagnosis for the two cytopathologists (p=0.002 and p=0.007). Multinucleated cells (Se 0.86, Sp 0.88, Accuracy 0.87, κ=0.75, 95%CI 0.57-0.92), and nuclear molding (Se 0.73, Sp 0.88, Accuracy 0.77, κ=0.80, 95%CI 0.65-0.95) showed the best diagnostic performance.

Cytological examination of squash-preparation samples from urinary bladder biopsies can allow a reliable diagnosis of malignant tumors in dogs. In particular, the identification of multinucleated cells, and nuclear molding showed the best diagnostic performance.

[1] Meuten, D. J., & Meuten, T. L. (2017). Tumors of the urinary system. In D. J. Meuten (Ed.), Tumors in Domestic Animals (5th ed., pp. 656–680). Wiley-Blackwell

[2] Fulkerson, C. M., & Knapp, D. W. (2020). Tumors of the urinary system. In D. M. Vail, D. H. Thamm, & J. M. Liptak (Eds.), Withrow & MacEwen's Small Animal Clinical Oncology (6th ed., pp. 645–656). Elsevier.

P260 - Iron panel in cats with chronic enteropathy: a pilot study

Fiorenza Tulone, Eleonora Gori, Alessio Pierini, Benedetta Riccardi, Ilaria Lippi, George Lubas, Veronica Marchetti

Università di Pisa, Dipartimento di Scienze Veterinarie

Corresponding author: Fiorenza Tulone (fiorenzatulone@yahoo.it)

Iron homeostasis involves complex processes and inflammation influences iron regulatory mechanisms in both animals and humans [1;2]. In human patients with inflammatory bowel disease, intestinal inflammation is associated with a dysregulated iron metabolism with consequent hypoferremia, impaired erythropoiesis and development of inflammatory anemia [3]. Inflammation may cause iron panel alterations as hypoferremia, normal or decreased total iron binding capacity (TIBC) and unsaturated iron binding capacity (UIBC), and normal or decreased iron saturation percentage (%Sat) [4;5;6]. In dogs and cats with gastrointestinal inflammation, hypoferremia seems to be a common finding as well [7]. However, veterinary literature lacks studies that evaluate iron parameters in cats with chronic enteropathy (CE) and their relationship with inflammatory markers. The Serum Amyloid A (SAA) is believed to be the most sensitive and useful positive acute phase protein in cat to detect the presence of inflammation [8]. The aim of the study was to evaluate iron panel and SAA in cats with CE. A retrospective medical record search study on data medical records of the University Veterinary Teaching Hospital was conducted for cats with primary CE. Cats with a diagnosis of primary CE were included if an iron panel and SAA were performed at the time of the diagnosis. The primary CE diagnosis was based on clinical history of chronic gastrointestinal signs (>3 weeks), blood work (complete blood count and serum biochemistry), ultrasonography exam for all cats, and endoscopic or histologic evaluation when available. The iron panel included serum total iron, TIBC, UIBC and % Sat along with SAA concentration. Twenty cats were included in the study. No one cat showed melena or hematemesis, two cats showed hematochezia. Hypoferremia was found in 2 cats (10%) with normal TIBC and UIBC and in 1 case with %Sat decreased. SAA was increased in 6/20 (30%) of cats. Patients with hypoferremia had concurrently SAA increased (100%). Cats with hypoferremia had more frequently SAA increased than cats without hypoferremia (Chi-square test, P-value=0.023). This is the first study that evaluates iron panel in cats with CE. Although occult blood loss might not be ruled out with certainty, the inflammatory state could be the cause of hypoferremia in our cats.

[1] Ganz T, Nemeth E. Iron homeostasis in host defence and inflammation, Nat Rev Immunol,15: 500–510, 2015. [2] McCown JL, Specht AJ. Iron Homeostasis and Disorders in Dogs and Cats: A Review, J Am Anim Hosp Assoc, 47: 151–160, 2011. [3] Shanmugam NKN, Trebicka E, Fu L, et al. Intestinal Inflammation Modulates Expression of the Iron-Regulating Hormone Hepcidin Depending on Erythropoietic Activity and the Commensal Microbiota, J Immunol, 193: 1398–1407, 2014. [4] Tasca S. Clinical Interpretation of Canine Sideremic Disorders, Veterinaria, Anno 22: 35–41, 2008. [5] Bohn AA. Diagnosis of Disorders of Iron Metabolism in Dogs and Cats, Vet Clin North Am - Small Anim Pract, 43: 1319–1330, 2013. [6] Naigamwalla DZ, Webb JA, Giger U. Review Article Iron deficiency anemia, Cvj, 53: 250–256, 2012. [7] Neumann S. Serum iron level as an indicator for inflammation in dogs and cats, Comp Clin Path, 12: 90–94, 2003. [8] Yuki M, Aoyama R, Nakagawa M, et al. A clinical investigation on serum amyloid a concentration in client -owned healthy and diseased cats in a primary care animal hospital, *Vet Sci,* 7: 1–9, 2020.

POSTER
SICV

P142 - Unusual association of granulosa-theca cell tumour and monodermal teratoma in the equine ovary

Valentina Palmieri (1), Giuseppe Catone (2), Cecilia Vullo (3), Stefania Di Giorgio (2), Alessandra Sfacteria (2), Antonina Zanghì (2)

(1) School of Biosciences and Veterinary Medicine, University of Camerino (2) Department of Veterinary Sciences, University of Messina (3) Department ChiBioFarm, University of Messina

Primary ovarian tumours are uncommon in mares with sex cord-stromal tumours accounting for more than 85% of them [1,2]. A 3-year heavy draft mare was evaluated for irregular heats. Transrectal palpation revealed an enlarged left ovary with no palpable ovulation fossa and a small inactive right ovary. Ultrasound examination of the left ovary showed multiple cavities giving a honeycomb appearance, concurrent with solid hyperechoic areas. A standing laparoscopic ovariectomy using the LigaSure technology was performed. Sedation and analgesia were achieved with acepromazine, detomidine and butorphanol and sedation was maintained by administration of constant infusion of detomidine. Local analgesia was achieved by infiltration of lidocaine in the paralumbar fossa. Moreover, infiltration of the ovarian pedicle was performed before cauterization with LigaSure. Verified the correct dissection and haemostasis, the neoplastic gonad was removed widening the laparoscopic portal. Grossly, the left ovary was enlarged (11x10x5 cm); the outer surface was smooth with loss of the ovulation fossa. Cut section revealed a variegated appearance due to multiloculated cystic areas and solid areas. Several solid areas were jarring on the cut, greyish in colour, hard to touch; cysts were of various sizes, from a few mm to 2 cm, many of which blood-filled. Haemorrhagic large areas were seen. Representative specimens were processed for histology and a mixed tumour was diagnosed. The predominant neoplastic tissue consisted of follicular-like cystic structures separated by a connective stroma. The cysts, varying in size, were lined by neoplastic granulosa cells, organized in layers, sometimes mixed with blood. The granulosa cells in some areas took on the typical palisade Sertoli cell-like arrangement. Around the follicular-like structures, clusters of elongated cells, likely neoplastic theca cells, could be detected. In the interstitial connective stroma, occasionally large, polyhedral cells, isolated or in groups, with abundant, eosinophilic, often vacuolized cytoplasm, similar to Leydig cells were seen. Immunohistochemistry for antimüllerian hormone revealed a strong immunoreaction confirming a granulosa /theca cell tumour. In adjacent areas, some teratomatous elements were seen as connective tissue resembling perichondrium associated with numerous bone trabeculae containing bone marrow and blood vessels. A monodermal teratoma was diagnosed. This germ cell tumour originated from a single embryonic germ layer, the mesoderm. It has not been reported in equines. Instead, the association of teratoma and granulosa cell tumour has been reported previously in two mares [3].

[1] McEntee KE. Reproductive pathology of domestic animals. Academic Press, New York, pp. 69-93, 1990.
[2] Westermann et al. Enlarged ovary in a mare: review of the literature and a case report. Tijdschr Diergeneeskd 128:692-6, 2003.
[3] Panciera et al. Ovarian teratoma and granulosa cell tumor in two mares. Cornell Vet 81:43-50, 1991.

P263 – A clinical approach to severe renal dysfunction in a critically ill Siberian tiger (*Panthera tigris altaica)*

Francesca Zanusso , Roberta Di Maggio , Luca Bellini , Giulia Maria De Benedictis

Università degli Studi di Padova, Dipartimento di Medicina Animale, Produzioni e Salute

Corresponding author: Francesca Zanusso (francesca.zanusso@unipd.it)

Chronic kidney disease (CKD) appears to be the most common metabolic disease in domestic cats. However, the prevalence of renal dysfunction in nondomestic felids is not well known and it is reported to be more frequent in animals older than 13 years [1, 2]. A 7-years intact male captive Siberian tiger (*Panthera tigris altaica*) was referred to the Veterinary Teaching Hospital of University of Padova with a 5-days history of anorexia and depression. The tiger received antibiotic, ketoprofen and betamethasone (unknown dose) in the previous week and had an episode of vomiting. It showed a progressive weight loss and episodic lethargy in the previous 5 months. At the arrival, it appeared severely depressed, cachectic and dehydrated. The animal was sedated with an intramuscular hand-injection of butorphanol 0.1 mg/kg, dexmedetomidine 6 mcg/kg, ketamine 2 mg/kg and midazolam 0.07 mg/kg. After intravenous (IV) administration of propofol, the tiger was intubated and maintained with isoflurane in oxygen. An accurate physical examination was performed and physiological parameters monitored. Electrocardiographic (ECG) alterations were observed and T waves appeared narrow and peaked. A first blood gas analysis revealed the presence of severe metabolic acidosis (pH 7.055) and hyperkalemia (serum potassium 8.5 mEq/L). To restore fluid balance and electrolyte disorders, Lactate Ringer Solution, sodium bicarbonate and gluconate calcium were administered IV. While results of blood chemistry and hematology were pending, the abdominal ultrasound revealed signs of bilateral nephropathy and presence of perirenal fluid. Serial venous blood gas analysis performed every 2 hours showed an increasing metabolic acidosis and hyperkalemia, despite intravenous replacement therapy and mechanical ventilation to reduce hypercapnia. Heart rate and blood arterial pressure were stable for almost 5 hours, then ECG alterations evolved into ventricular fibrillation. Blood chemistry revealed a severe renal dysfunction, with serum creatinine concentration tenfold higher than upper limit reported in literature [2]. Abdominal ultrasound was performed 2 and 4 hours after the first one: no increase in bladder size was observed. Caval-aortic ratio was evaluated to assess fluid responsiveness, although the reliability of this hemodynamic parameter is not reported in literature for this species. After almost 5 hours from arrival, the animal was humanely euthanized. No previous blood analysis was available for this patient, but the clinical and necroscopic findings were suggestive of an acute deterioration of CKD. Acute kidney injury is a life-threatening emergency that requires intensive care and preventive strategies are needed to reduce the risk of acute-on-CKD. Since in captive large felids not so many therapeutic possibilities are feasible as in domestic cats, early identification of renal dysfunction is fundamental. A close monitoring of clinical signs and periodical health check including blood analysis could promptly suggest the presence of renal dysfunction and possible comorbidities, not only in elderly animals, and could address the clinician to cautiously use potentially nephrotoxic drugs.

[1] Newkirk et al. Renal lesions of nondomestic felids, Veterinary Pathology 48:698-705, 2011.
[2] Liu et al. Haematological and biochemical parameters of captive Siberian Tigers (*Panthera tigris altaica*) from the Heilongjiang Province, China, Veterinary Medicine and Science 00:1-8, 2020.

MIGLIOR POSTER SICV - SISVET 2021

P283 - Do variables that affect pulse oximeter reading influence oxygen reserve index accuracy?

Luca Bellini (1), Brighton T. Dzikiti (2), Giulia Maria De Benedictis (1), Jill K. Maney (2)

(1) Università degli Studi di Padova, Dipartimento di Medicina Animale, Produzioni e Salute (2) Ross University School of Veterinary Medicine, Department of Clinical Sciences

Corresponding author: Luca Bellini (luca.bellini@unipd.it)

The oxygen reserve index (ORI) is an index calculated by a multi-wavelength pulse co-oximetry. The device estimates the arterial oxygenation by combining the measurement from both arterial and venous blood haemoglobin absorption [1]. Within its sensing range of arterial partial pressure of oxygen (PaO_2, 100-200 mmHg), ORI will read between 0.0 (no reserve) and 1.0 (full reserve). The advantage of the ORI is that it can detect impending desaturation before the pulse oximetry (SpO_2) drops below 100% [1]. The device has been evaluated in isoflurane anaesthetized donkeys breathing a fraction of oxygen above 0.95 [2]. An ORI above 0.1 and 0.3 identified arterial blood oxygen content over 150 and 200 mmHg in more than 98% and 94% of the samples, respectively. Nevertheless, that study presented 21 over 106 measurements in which ORI was 0.0 while PaO_2 was higher than 100 mmHg. Changes in pH, body temperature and arterial partial pressure of carbon dioxide ($PaCO_2$) shift the haemoglobin oxygen saturation curve, and this may influence an accurate reading of ORI. Moreover, a condition that alters peripheral perfusion at the measurement site may potentially affect the signal used to calculate ORI. The aim of this study was to evaluate, retrospectively, whether some variables that are known to interfere with pulse oximeter measurement may have influenced the correct or incorrect reading of ORI. Information from anaesthetic records and arterial blood gas analysis collected in the previous study [2] were used. A total of 106 measurements from 28 adult standard donkeys between 2 and 13 years old and weighing 109-163 kg were evaluated. Donkeys were sedated with xylazine and butorphanol. Anaesthesia was induced with ketamine and diazepam and maintained with isoflurane (1.5-2.0%) in oxygen. The ORI elastic adhesive sensor was placed over the tongue of the donkey. From the pool of data, the parameters associated with a "correct matching" (ORI=1.0 and PaO2>200 mmHg) and "incorrect matching" (ORI=0.0 and PaO2>100 mmHg or ORI=1.0 and PaO2<100 mmHg) were examined. Variables considered were: pulsatile index (PI), pH, PaO2, PaCO2, body temperature, Doppler arterial blood pressure (DoAP) and dobutamine or ephedrine administration. Mann-Whitney or Student's T-test were used to compare the variables between groups in non-normally and normally distributed variables. Correct and incorrect readings extrapolated from the pool were 28 and 29, respectively. None of the variables considered showed statistical different between groups; PI ranged between 0.5 to 3.5 (p=0.190), pH 7.22-7.45 (p=0.678), PaCO2 44-84 mmHg (p=0.195); temperature 34-38 °C (p=0.420); DoAP 73-140 mmHg (p=0.677). Inotropes or vasopressors were administered 3 and 8 times in correct and incorrect pair readings, respectively (p=0.179). In conclusion, the variables considered do not seem to influence the ability of ORI to estimate accurately the PaO2. Other factors, as the concentration of haemoglobin or the tongue width and the distance between the emitting and detecting sensor could be investigated in the future.

[1] Scheeren et al. The oxygen reserve index (ORI): a new tool to monitor oxygen therapy, Journal of Clinical Monitoring and Computing, 32:379-389, 2018.
[2] Bellini et al. Oxygen reserve index as a noninvasive indicator of arterial partial pressure of oxygen in anaesthetized donkeys: a preliminary study, Veterinary Anaesthesia and Ananlgesia, [Epub ahead of print]2021.

POSTER SIFTVET

P160 - Detection of sex steroids administration in veal calves FFPE liver samples: PLS-DA applications on molecular markers

Alessandro Benedetto (1), Marzia Pezzolato (1), Elisa Robotti (2), Elena Biasibetti (1), Emilio Marengo (2), Elena Bozzetta (1)

(1) Istituto Zooprofilattico Sperimentale del Piemonte, Liguria e Valle d'Aosta, CIBA - CRN Indagini Biologiche Anabolizzanti Animali (2) Università del Piemonte Orientale, Dipartimento di Scienze ed Innovazione Tecnologica

Corresponding author: Alessandro Benedetto (Alessandro.benedetto@izsto.it)

Abuse of growth promoters in meat-producing animals is strictly forbidden and regulated within the European Community to preserve consumers' safety. Nevertheless, illicit misuse of steroids and other veterinary drugs to increase animal production has still been found by National Residues Control Plans (NRCPs). The setup of complementary diagnostic methods, based on indirect biomarkers of exposure to anabolic substances is, therefore, needed to update current tests available in NRCPs [1].

The aim of the work was to develop a Real Time PCR array analysis applied to formalin fixed paraffin embedded (FFPE) samples for the profiling of multiple liver biomarkers of illicit treatment in cattle. This novel screening approach was proposed to identify animals suspected of illicit treatment with sex steroids, one of the most recurrent classes of growth promoters reported by EFSA [2].

The selection of differentially expressed genes (DEG) suitable as biomarkers of exposure to sex steroids in cattle was defined by analyzing the available datasets from previous studies [3-4]. Finally, 48 target genes belonging to steroids-modulated carbohydrate and protein biosynthesis patterns were selected to design a qPCR array suitable for the analysis of FFPE liver samples collected from veal calves. 92 FFPE liver samples were picked from previous animal trials performed to study the biological effects of sex steroids (namely nandrolone, estradiol and their combination) [5]. Total RNA was isolated from each FFPE sample with miRNeasy FFPE kit (Qiagen), quantified with Qubit BR-RNA kit (Thermo) and then 2.5 µg of total RNA was reverse transcribed with an RT^2 First Strand Kit (Qiagen). The qPCR runs were carried out on a StepOne Plus Real-Time PCR System (Thermo). A final melting curve stage to check expected amplicon signals and absence of primer dimers was applied. The relative quantification of each biomarker was performed by the ΔΔCq method [6]. A multivariate classification method of collected relative quantities of each target gene was performed by means of Partial Least Squares – Discriminant Analysis (PLS-DA): the models were built by coupling PLS-DA with a variable selection algorithm in backward elimination. The sensitivity (92.40-99.78%) and accuracy (92.79-99.88%) of the developed PLS-DA models were very promising. Further evaluations on a larger sample set are needed to extend and verify the performances of PLS-DA models developed on collected FFPE liver samples.

[1] EFSA. Scientific Opinion on the public health hazards to be covered by inspection of meat (bovine animals), EFSA Journal, 11(6), 2013. [2] EFSA. Report for 2018 on the results from the monitoring of veterinary medicinal product residues and other substances in live animals and animal products, EFSA Supporting Publications, 17(3), 2020. [3] Riedmaier et al. RNA-Sequencing as Useful Screening Tool in the Combat against the Misuse of Anabolic Agents, Analytical Chemistry, 84(15): 6863-6868, 2012. [4] Elgendy et al. The transcriptome of muscle and liver is responding differently to a combined trenbolone acetate and estradiol implant in cattle, Steroids, 106: 1-8, 2016.
[5] Pezzolato et al. Confirmation of the progesterone receptor as an efficient marker of treatment with 17 beta-estradiol in veal calves, Food Additives and Contaminants Part A, 33(1): 60-65, 2016.
[6] Livak et al. Analysis of relative gene expression data using real-time quantitative PCR and the 2(-Delta Delta C(T)) Method, Methods, 25(4): 402-408, 2001.

MIGLIOR POSTER SIFTVET - SISVET 2021

P180 - Antibiotic stewardship for canine and feline acute urinary tract infection: an observational study in a small animal hospital in North–West Italy

Cristina Vercelli (1), Massimiliano Della Ricca (2), Mariachiara Re (2), Graziana Gambino (1), Giovanni Re (1)

(1) Università degli Studi di Torino, Dipartimento di Scienze Veterinarie (2) Centro Veterinario Torinese di Rivoli

Corresponding author: Cristina Vercelli (cristina.vercelli@unito.it)

International institutions are pressing for finding successful strategy to limit the antimicrobial resistance phenomenon. In Veterinary medicine, it has been suggested to start with antimicrobial stewardship programs (ASPs) to reduce the prescription of antimicrobial drugs and to better target the therapy. Recent papers reported that antibiotic drugs are frequently used to treat urinary tract infections (UTIs) even if no microbiological assays confirm the clinical diagnosis. The aim of the present study was to design a specific working-flow for a decision-making process specifically addressed to create a tailored antimicrobial treatment in case UTIs in dogs and cats, referred to a small animal hospital in North-West Italy. The working-flow was specifically designed by an ASP team composed by clinician and pharmacology experts, in accordance with the entire clinical staff. From January to December 2020, urine samples were collected only by cystocentesis from 16 dogs and 12 cats, presenting acute signs of UTIs. All samples were immediately sent to a laboratory to perform the susceptibility test. It was decided to wait for the results prior to begin any treatment, but a “rescue therapy” was included in the protocol (with amoxicillin and clavulanic acid). The analysis permitted to identify only one bacteria strain per each sample and the most prevalent were *Escherichia coli*, *Proteus mirabilis*, *Streptococcus canis* in dogs and *Escherichia coli*, *Staphylococcus pseudintermedius* and *aureus* in cats. The therapy was prescribed for all patients after the receiving of antibiogram results, except one cat that received rescue therapy. The highest percentage of resistance was demonstrated for beta lactamase, fluoroquinolones and tetracyclines. No multidrug resistant (MDR) strains were detected. *Staphylococci* strains resulted all methicillin resistant. The therapy was decided according to the susceptibility test, checking the lowest MIC value. It was possible to follow up 14 dogs and 11 cats. Dogs were treated with tetracycline (1/14), fluoroquinolones (6/14), beta-lactams (6/14) and gentamicin (1/14) while cats received fluoroquinolones (3/11), nitrofurans (1/11), clindamycin (1/11) and beta-lactams (6/11). Only one dog had a recruitment. The successful rate following the working-flow was very high. Our findings could be of interest because is the first ASP in Italy and could be useful to develop similar programs in other small/medium facilities, specifically addressed to other pathologies. Clinicians are responsible to implement measures to control antibiotic resistance phenomenon without impacting animal welfare. Antimicrobials should be prescribed and used only if necessary, choosing the appropriate molecule to the causative strain, always caring about patient welfare.

[1] Guardabassi, L., Prescott, J. F. Antimicrobial Stewardship in Small Animal Veterinary Practice: From Theory to Practice. Veterinary Clinics of North America - Small Animal Practice, 45, 361–376, 2015. [2] De Briyne, N. et al. Antibiotics used most commonly to treat animals in Europe. Veterinary Record, 175(13), 325, 2014. [3] Scarborough, R. et al. Use of local antibiogram data and antimicrobial importance ratings to select optimal empirical therapies for urinary tract infections in dogs and cats. Antibiotics, 9(12), 924, 2020.

POSTER
SIRA

P8 - Effect of *Lepidium meyenii* (Maca) on buffalo frozen sperm

Valentina Longobardi, Chiara Del Prete, Maria Paz Benitez Mora, Federica Piscopo, Riccardo Esposito, Alfonso Calabria, Stefano Spada, Veronica Palumbo, Bianca Gasparrini

Università degli Studi di Napoli Federico II, Dipartimento di Medicina Veterinaria e Produzioni Animali

Corresponding author: Bianca Gasparrini (bgasparr@unina.it)

Frozen-thawed buffalo sperm are very susceptible to cellular damage associated with cold shock and oxidative stress, thus resulting in lower fertilizing potential. The *Lepidium meyenii*, known as Maca, is a root native to the Andean region, containing bioactive compounds with antioxidant and fertility-enhancing properties. In bulls, dietary supplementation with Maca improved sperm production, preserving sperm motility and DNA integrity [1]. It was also demonstrated that supplementation of the in vitro culture medium by Maca significantly improved sperm motility and fertilizing ability in mice [2]. Therefore, this study aims to evaluate whether the addition of an aqueous extract of Maca in the fertilization medium (IVF medium), could improve the quality of frozen-thawed buffalo sperm. To the best of our knowledge this is the first study evaluating the effects of Maca on buffalo sperm. Six Italian Mediterranean buffalo (Bubalus bubalis) bulls were selected for the trial. Sperm were thawed at 37°C for 40 sec, selected by Percoll gradients and incubated in IVF medium in the presence of 0 (control), 10, 20 and 50 µL/mL of aqueous Maca extract for 1 h. Both after Percoll and after incubation, visual motility was assessed by phase-contrast microscopy, viability and acrosome integrity by Trypan blue/Giemsa staining, membrane integrity by Hypo-osmotic swelling (HOS) test and DNA fragmentation by Tunel staining. Data were analyzed by ANOVA (IBM SPSS Statistics Version 22.0) Treatment of the semen with 10 µL of Maca significantly increased ($P<0.05$) motility compared to the control group, while no differences were observed with 20 and 50 µL Maca (68.3 ± 2.1; 75.0 ± 2.2, 74.2 ± 2.0 and 69.2 ± 2.0 %, respectively with 0, 10, 20 and 50 µL/mL of Maca). Similarly, viability significantly improved ($P<0.05$) with 10 µL of Maca, while a decrease ($P<0.05$) was observed at the highest concentration tested (77.2 ± 1.7, 83.0 ± 1.6, 76.0 ± 2.5 and 70.3 ± 1.9%, respectively with 0, 10, 20 and 50 µL/mL of Maca). A positive effect of Maca was also recorded on membrane integrity, as shown by the higher ($P<0.01$) percentage of HOS positive spermatozoa in the presence of 10 µL of Maca compared to all the other experimental groups (55.7±2.4, 65.8±2.20, 59.2±1.4 and 49.8±0.9%, respectively with 0, 10, 20 and 50 µL/mL of Maca). Finally, at the lowest concentration tested, a significant ($P<0.05$) reduction of the percentage of spermatozoa with DNA fragmentation was found compared to all the other groups (7.0±0.9, 4.5±0.3, 6.5±1.0 and 5.8±0.7%, respectively with 0, 10, 20 and 50 µL/mL of Maca).

In conclusion, the results of this study demonstrated that Maca, at the lowest concentration tested, showed a beneficial effect on buffalo frozen-thawed buffalo sperm quality ameliorating motility, viability and membrane integrity and reducing DNA fragmentation. These preliminary data represent an excellent starting point for further studies aimed to clarify the molecular mechanism of action of Maca and the antioxidant potential, as well as to evaluate the effect of Maca extract on in vitro fertilization and on subsequent embryonic development.

[1] Clement et al. Effect of maca supplementation on bovine sperm quantity and quality followed over two spermatogenic cycles 2010, Theriogenology , 74: 173-183.
[2] Aoki et al. Effect of *Lepidium meyenii* on in vitro fertilization via improvement in acrosome reaction and motility of mouse and human sperm, 2018 Reproductive Medicine and Biology 18(1):57-64.

P85 - Effects of treatment with ozonides and *Salix caprea* bud-extract on equine endometrium

Gian Guido Donato (1), Tiziana Nervo (1), Alessia Bertero (1), Katia Varello (2), Patrizia Ponzio (1), Elena Bozzetta (2), Simonetta Appino (3)

(1) Department of Veterinary Science, University of Torino (2) Istituto Zooprofilattico Sperimentale del Piemonte, Liguria e Valle d'Aosta (3) Department of Veterinary Medicine, University of Sassari

Corresponding author: Gian Guido Donato (gianguido.donato@unito.it)

Endometritis is a major cause of infertility in equines[1]. Recently, much attention has been paid to the search for novel therapies[2].

The purpose of the work was to evaluate the effects of an intrauterine treatment based on ozonated oil, *Salix caprea* bud-extract and their combination, investigating a possible counterirritant effect of ozone, potentially related to an improvement in fertility[3,4].

Forty-seven hypofertile Standardbred mares (non-pregnant after ≥3 AI or that had shown embryonic resorption/abortion) were subjected to gynecological examination with microbiological, cytological and histological evaluations (T0), and divided into 4 random groups: OZONE (N=16, treated with Riger Salix®, containing ozonated oil); OZSAL (N=15, treated with Riger Salix® and *Salix caprea* bud-extract); SALIX (N=8, treated with the bud-extract); CONTROL (N=8, treated with lactated Ringer's). At 24 h (T1) and at 1 (T2), 2 (T3) and 3 (T4) weeks after treatment, a cytobrush was performed. At T4 uterine swab and endometrial biopsy were repeated. Bacteriological tests were negative. Biopsy revealed more cases of endometritis in comparison with uterine cytology, demonstrating the superior specificity of this technique for diagnostic purposes[5]. Cytological examinations showed that all mares developed transient inflammation after treatment[1], which resolved more rapidly in the group treated only with the bud-extract, although in absence of statistical evidence. At T3 and T4 uterine cytology was normal in all mares. The treatments did not modify the histological category[5], with the exception of two cases in the OZONE group (from IIB to IIA), but an increased vascularization has been observed in the majority of the mares in the OZONE and OZSAL groups ($p<0.01$) and to a lesser extent in the SALIX group. In the CONTROL, vascularization did not change. This may have positively influenced embryonic implantation, given pregnancy rates >90% in the OZONE and OZSAL groups, although no statistical difference between the different groups was observed. Although this is a preliminary study, the ability of ozone to determine an increase in the endometrial vascularization, potentially related to an increase in fertility, appears evident. There is no available information on the potential angiogenetic effects of *Salix caprea* bud-extract, thus it would be interesting to investigate these properties. Ozone and bud-extract seem to have a synergistic effect: while the ozone exerts a beneficial action on vascularization, the phytotherapic determines a rapid resolution of the inflammation. Due to the limited number of animals, further evaluations are needed to confirm the positive effects of the treatment with ozonides on the equine endometrium, focusing particularly on the increase in vascularization, presumably related to embryonic implantation[3].

[1] Pasolini MP. Endometritis and Infertility in the Mare – The Challenge in Equine Breeding Industry – A Review, Genit Infect Infertil, Published online, 2016.
[2] Morris LHA. Equine endometritis: a review of challenges and new approaches, Reproduction, 160(5):R95-R110, 2020.
[3] Calderon I. The effect of ozonated sterile saline irrigation on the endometrium – A preliminary study, J Obstet Gynaecol, 36(5):635-40, 2016.
[4] Janini LCZ. Intrauterine infusion reduces inflammatory status and could be increased the embryo recovery in mares with chronic endometritis, Pubvet, 14(3):1-6, 2020.
[5] VanCamp SD. Endometrial biopsy of the mare. A review and update, Vet Clin North Am Equine Pract, 4(2):229-245, 1988.

MIGLIOR POSTER SIRA - SISVET 2021

P128 - Intra-follicular oocyte transfer in the ewe

Laura Falchi (1), Ivo Pivato* (2), Mauro Ledda (1), Valentino Melosu (1), Antonio Scanu (1), Salvatore Pau (1), Sergio Ledda (1), Maria Teresa Zedda (1)*

(1) Università degli Studi di Sassari, Dipartimento di Medicina Veterinaria (2) Universidade de Brasília, Faculdade de Agronomia e Medicina Veterinária

*L. Falchi and I. Pivato equally contributed to this work
Corresponding author: Laura Falchi (lfalchi@uniss.it)

In the ovine species, assisted reproductive technologies encounter limitations due to the anatomical characteristics of the female reproductive tract (especially the convoluted lumen of the cervix) and to the great variability of superovulatory responses in subjects submitted to multiple ovulation and embryo transfer programs. Intra-follicular oocytes transfer (IFOT) might represent a promising and innovative alternative for in vivo embryo production. In the bovine literature, IFOT resulted in birth of healthy calves [1]. The aim of the study was to assess the suitability of IFOT in the ovine species for in vivo embryo production. Procedures were conducted under European regulations on the Care and Welfare of Animals in Research, approved by the University of Sassari and the Ministry of Health (Authorization n°614/2020-PR). Two preliminary in vitro and in vivo trials were performed to test the optimal procedures and timings for IFOT. In the in vitro trial, to assess the following parameters: i) number of transferable oocytes ii) ideal volume of media for injection; iii) optimal inner diameter of the injection needle; iv) recovery rate and integrity of injected cumulus-oocyte complexes (COCs) after follicle aspiration; COCs were collected from ovaries of slaughtered adult ewes and injected into follicles >5mm. In the in vivo trial, the follicular growth was monitored in 10 adult ewes by transrectal ultrasonography to preliminary determine the ovulation and the ideal time for IFOT. For IFOT and embryo collection, 5 ewes were synchronized by CIDR insertion (CIDR Ovis® 350mg, Zoetis, Italy). Forty hours after removal of CIDR, under sedation and general anaesthesia, ovaries were exposed by laparotomy and the preovulatory follicle was injected with COCs previously collected from abattoir ovaries. The needle was then washed with PBS to check the number of COCs effectively injected in the follicle. At 4 hours from surgery, fully recovered ewes were housed in a paddock with a ram of proven fertility. Crayon on the chest was provided to allow mating control. Ovulation was assessed 24 and 48h after the transfer of oocytes by transrectal ultrasonography. On day 6 from IFOT, embryo collection was performed by uterine flushing. In the in vitro test, injection of >5mm follicles with a 28G needle loaded with 30 COCs in 5µL volume resulted in higher recovery rates and better preservation of COCs integrity. In the in vivo trial, ultrasound scanning revealed that ovulation occurred between 60 and 72h after CIDR removal in all tested animals. In one ewe submitted to IFOT, 22/24 oocytes were effectively injected in the preovulatory follicle, but no embryos were collected after flushing. In the remaining 4 animals, 85/102 oocytes were injected and 6 cleaved embryo and 13 embryos with >8cell were collected. The present preliminary investigation showed that IFOT in the ovine species results in ovulation, fimbrial capture and tubal transport of heterologous oocytes and in vivo embryo production. Further studies are needed to optimize embryo recovery rate and to provide less invasive techniques for oocyte injection and uterine flushing, such as a laparoscopic or transcervical approach.

Fundings: FAP DF (Fundacao de Apoio a Pesquisa do Distrito Federal, Brasil) fellowship; Finanziamento straordinario una tantum per la ricerca 2020

[1] Hoelker et al. Birth of healthy calves after intra-follicular transfer (IFOT) of slaughterhouse derived immature bovine oocytes, Theriogenology, 15;97:41-49, 2017.

POSTER SOFIVET

P140 - New animal-based measures to assess welfare in camels

Laura Menchetti, Barbara Padalino

Alma Mater Studiorum - Università di Bologna, Dipartimento di Scienze e Tecnologie Agro-alimentari, Bologna, Italy

Corresponding author: Laura Menchetti (laura.menchetti@unibo.it)

The assessment of animal welfare is challenging. It has been suggested that animal-based measures (ABMs) should be used as indicators of welfare consequences in animal welfare risk assessment [1]. This study aimed to propose new ABMs for camels identifying their possible associations with management factors. A set of 10 animal-based and 19 resources or management-based measures were selected from the protocol for assessing welfare in camels proposed by Padalino and Menchetti [2]. At least two ABMs were selected for each welfare principle (i.e. Good feeding, Good housing, Good health, and Appropriate behaviour). and all were "direct" indicators intended to assess physiological, behavioural, or health status of camels as well as their needs and affective states [1]. Management measures were instead collected at three levels of investigations (Animal, Herd or Caretakers [2]) and were related to husbandry practices, housing facilities, and caretaker's experience. Data were collected at the permanent camel market in Doha, Qatar, by five trained assessors as previously described [3] (Approval code 2404/2020). A total of 76 pens and 528 camels were evaluated. Associations were calculated by Generalized Linear Models including, when possible, camel's age as covariate. The ABMs selected for Good feeding (i.e. Body Condition Score and Thirst index) were negatively associated with limited space allowance, shaded, feeding and water space as well as with dirty bedding and short caretaker's experience ($P<0.05$). The ABMs selected for Good housing (i.e. Resting behaviours and Restricted movements) were associated with dirty bedding and water, rationed water distribution, positioning of the water points in the sun, presence of hobbles, and short caretaker's experience ($P<0.05$). The ABMs selected for Good health (i.e. Disease, Injury, and Pain induced by management procedures) were negatively associated with limited space allowance and shaded space, dirty bedding, several factors related to feeding and water management (e.g. frequency of distribution, resource quality, location of the troughs), presence of hobbles, and short caretaker's experience ($P<0.05$). Finally, the ABMs selected for Appropriate behaviour (i.e. Response to Approaching test, Aggressivity, and Stereotypies) were negatively associated with limited space allowance, shaded, feeding and water space as well as with rationed water distribution ($P<0.05$). Our methodological approach, even if it did not allow identifying direct cause-effect relationships, suggests that the proposed ABMs could be used as appropriate indicators of welfare consequences and it was also able to identify factors related to housing and management practices which may impair or improve camel welfare. Further studies are needed to implement the proposed ABMs particularly for some welfare principles, such as Appropriate behaviour, and to validate them in other rearing contexts.

[1] EFSA. Guidance on Risk Assessment for Animal Welfare. EFSA Journal, 10:2513, 2012.
[2] Padalino B. Menchetti L. The first tool for assessing welfare of camels. Frontiers in Veterinary Science, 7:631876, 2021.
[3] Menchetti et al. Application of a protocol to assess camel welfare: scoring system of collected measures, aggregated assessment indices, and criteria to classify a pen. Animals, 11:494, 2021.

P169 - Cytotoxic effects of *Artemisia annua* on canine osteosarcoma cell lines

Roberta Salaroli (1), Augusta Zannoni (1), Chiara Bernardini (1), Michele Protti (2), Laura Mercolini (2), Debora La Mantia (1), Giulia Andreani (1), Monica Forni (1), Gloria Isani (1)

(1) Università di Bologna, Dipartimento di Scienze Mediche Veterinarie (2) Università di Bologna, Dipartimento di Farmacia e Biotecnologie

Corresponding author: Augusta Zannoni (augusta.zannoni@unibo.it)

Since ancient times, *Artemisia annua* has been used as a medicinal plant in Traditional Chinese Medicine [1]. In addition to its recognised use as an antimalarial drug, recent studies have investigated the cytotoxic effects of *A. annua* extracts towards cancer cells, focusing on the activity of the active principle artemisinin and its derivatives [2]. Therefore, to provide new scientific evidence to support the anticancer potential of *A. annua* the purpose of the present research is to determine the concentration of artemisinin of a herbal extract and to evaluate its cytotoxic effects on different canine osteosarcoma cell lines. Osteosarcoma (OSA) shows high prevalence in dogs and is the most common primary tumor of bone in both dogs and humans.

The quantitative determination of artemisinin concentration in a commercial idroalcoholic extract of *A. annua* was carried out through the use of an instrumental analytical method based on liquid chromatography coupled with spectrophotometric detection and tandem mass spectrometry (HPLC-DAD-MS/MS). Three commercial canine osteosarcoma cell lines, D-17, OSCA-8, and OSCA-40, were exposed to different dilutions of the extract containing 0.2, 0.4, 1.1, 2.2, 4.4, and 8.8 µM artemisinin, respectively, and to the same concentrations of pure artemisinin for EC_{50} calculation.

The concentration of artemisinin determined in the hydroalcoholic extract of *A. annua* was 63.8±3.4 µg/mL. The extract showed a dose-dependent cytotoxic effect in inhibiting the proliferation of the three canine osteosarcoma lines with EC_{50} of 4.9, 3.3, and 2.6 µM for D17, OSCA-8, and OSCA-40, respectively. The extract presented significantly lower EC_{50} values than pure artemisinin, suggesting a greater cytotoxic effect, due to a possible synergistic activity of other bioactive molecules of the phytocomplex.

The research was funded by Fondazione Carisbo (Bologna) (2019.0535)

[1] Brown G.D. The biosynthesis of artemisinin (Qinghaosu) and the phytochemistry of *Artemisia annua* L. (Qinghao), Molecules, 15: 7603-98, 2010

[2] Isani G. et al. Cytotoxic effects of *Artemisia annua* and pure artemisinin on the D-17 canine osteosarcoma cell line, Oxidative Medicine and Cellular Longevity, Article ID:1615758, 2019

[3] Protti M. et al. Analysis of *Artemisia annua* extracts and commercial products by high performance liquid chromatography-tandem mass spectrometry coupled to sample treatment miniaturization, Journal of Pharmaceutical and Biomedical Analysis 174: 81–88, 2019

MIGLIOR POSTER SOFIVET - SISVET 2021

P277 - Effect of goji berry (*Lycium barbarum*) supplementation on hormonal profile and reproductive performance of rabbit does

Giulio Curone [1], Egon Andoni[2], Stella Agradi [1], Olimpia Barbato [3], Laura Menchetti [4], Daniele Vigo [1], Alessandro Necci [5], Gabriele Brecchia [1]

(1) Università di Milano, Dipartimento di Medicina Veterinaria (2) Università Agricola di Tirana, Facoltà di Medicina Veterinaria (3) Università di Perugia, Dipartimento di Medicina Veterinaria (4) Università di Bologna, Dipartimento di Scienze e tecnologie agro-alimentari (5) Università di Camerino, Dipartimento di Medicina Veterinaria

Corresponding author: Stella Agradi (stella.agradi@unimi.it)

Lycium barbarum berries, also named goji berries, have been used in Asian countries for a long time as a traditional medicinal herb given that show a wide range of pharmacological properties [1]. Lycium barbarum have become very popular also in western countries for the beneficial effects on human health, in last decades [1]. However, only a few researches evaluated the effects of the fruit in livestock animals [2]. This study aimed to investigate the effects of goji berries supplementation on the hormonal profile and reproductive performance of rabbit does (Authorization n°824/2021). Two months before artificial insemination (AI), 105 nulliparous does were randomly divided in three groups (n=35), on the basis of the dietary treatment: commercial diet (control group, C) and a diet supplemented with 1 (G1) and 3% (G3) of goji berry, respectively. On the day of AI, blood samples were collected every 60 minutes starting 120 minutes before and for 240 minutes after the GnRH administration to evaluate LH and 17-β estradiol plasma concentrations. Pregnancy was diagnosed at 12 days after AI and, then, 25 pregnant rabbit does per group were followed until the weaning of the bunnies. Milk production was evaluated from the parturition until day 18 of lactation. Reproductive and productive indices were analyzed by one-way or repeated-measures ANOVA followed by Tukey's multiple comparisons test, or Fisher's exact tests. Moreover, the LH AUC (area under the curve) was calculated for each animal by the trapezoid method and compared between groups. The results showed that, regardless of group, a peak after 60-120 minutes from GnRH inoculation was found in LH concentrations (20.3±1.1 ng/ml and 15.8±2.2 ng/ml at 60 and 120 min; $P<0.0001$). Multiple comparisons also showed a trend toward significance for differences between G1 and G3 groups in marginal means of LH concentrations (7.9±3.2 and 6.5±3.1 ng/ml for G1 and G3, respectively; $P=0.059$), and between G1 and C in LH AUC values (2510±175 ng/ml x h and 3031±149 ng/ml x h for C and G1, respectively; $P=0.078$). Estrogen concentrations showed a more fluctuating trend ($P<0.0001$) but a significant interaction effect ($P<0.001$). Regarding productive parameters, G1 group showed higher litter weight than C at birth (338±92 and 408±68 g for C and G1 groups, respectively; $P=0.008$) and weaning (3.6±0.2 and 5.6±1.2 kg for C and G1 groups, respectively; $P<0.001$) as well as higher litter size at weaning (5±1 and 6±1 rabbits for C and G1 groups, respectively; $P=0.020$). Does of G1 group also showed the highest mean milk production (122±9 g/d; $P<0.01$). Receptivity, fertility, mortality, and litter size didn't show any significant differences between groups. In conclusion, the present research suggested that the supplementation of the diet with goji berry could modulate the pattern of reproductive hormones but no differences were found in the reproductive indices, perhaps due to the small sample size. The effects on the productive performance were more evident and probably mediated by a greater secretion of milk. However, further studies will need to be performed to validate these preliminary results.

[1] Kwok et al. A Systematic Review of Poten-tial Therapeutic Use of *Lycium barbarum* Polysaccharides in Disease. Biomed Res Int, 12:4615745, 2019.
[2] Menchetti et al. Effects of Goji berries supplementation on the productive performance of rabbit. Livest. Sci. 220, 2019.

POSTER
SOIPA

P31 - Results of tick identification from wildlife in Liguria (2018-2021)

Annalisa Accorsi (1), Enrica Berio (2), Valeria Listorti (1), Monica Dellepiane (3), Tiziana Andreoli (3), Chiara Masotti (4), Carlo Ercolini (4), Elisabetta Razzuoli (1)

(1) Istituto Zooprofilattico Sperimentale del Piemonte, Liguria e Valle d'Aosta, sezione Genova
(2) Istituto Zooprofilattico Sperimentale del Piemonte, Liguria e Valle d'Aosta, sezione Imperia
(3) Istituto Zooprofilattico Sperimentale del Piemonte, Liguria e Valle d'Aosta, sezione Savona
(4) Istituto Zooprofilattico Sperimentale del Piemonte, Liguria e Valle d'Aosta, sezione La Spezia

Corresponding author: Annalisa Accorsi (annalisa.accorsi@izsto.it)

Wildlife is subjected to health surveillance since it has a role in the spread of infectious diseases [1]. In Italy, the National law 157/1992 recommends wildlife sanitary controls [2]. Besides surveillance of infectious diseases of wild animals, the Regional Plan of Monitoring and Surveillance of wildlife in Liguria Region includes the collection of ectoparasites (i.e. ticks) from animal hosts, their identification and the investigation of tick-borne pathogens. The present work aimed to identify species of ticks collected from wild mammals in Liguria and to evaluate the host-parasite association. Samples of anatomic portion of hunted game were collected during three hunting seasons (2018-2019, 2019-2020, 2020-2021) and were analysed for the presence of ticks. The involved game species were: wild boar (*Sus scrofa*), roe deer (*Capreolus capreolus*), fallow deer (*Dama dama*) and chamois (*Rupicapra rupicapra*). Ticks were also collected, in the same period, form carcasses received for necropsy of foxes (*Vulpes vulpes*), wolves (*Canis lupus lupus*), a badger (*Meles meles*), and a long-eared owl (*Asio otus*). Collected ticks were identified by species according to taxonomic keys [3-4]. A total of 942 ticks were examined belonging to 400 animals: 185 roe deers, 158 wild boars, 41 fallow deers, 5 chamoises, 6 wolves, 3 foxes, 1 badger and 1 long-eared owl. The results of tick identification are the following: 556 *Ixodes ricinus,* 271 *Dermacentor marginatus*, 108 *Rhipicephalus sanguineus*, 6 *Haemaphysalis punctata* and 1 *Ixodes hexagonus*. Considering the main results related to the single tick species, the 100% (271/271) of *D. marginatus* was collected on wild boar, the 67% (372/556) of *I. ricinus* was collected on roe deer, the 42% (45/108) of *R. sanguineus* was identified on wild boar and the 34% (37/108) on fallow deer. *H. punctata* was identified on chamois (5/6) and fallow deer (1/6). The only sample of *I. hexagonus* was collected on wild boar. Considering the canids species, that gave interesting results, *I. ricinus* was the only species identified (100%, 56/56) on wolf carcasses. *R. sanguineus* and *I. ricinus* were both identified on foxes (respectively 8/9 and 1/9). Others interesting findings are the isolation of 1 *I. ricinus* on long-eared owl and 3 *R. sanguineus* on badger. The present results suggest a strong association between host species and tick species, in particular between wild boar and *D. marginatus* [5]. *I. ricinus* was the most abundant species that was collected being the most common European species and it was mainly collected from roe deer [4]. It is worth noting that *R. sanguineus*, the so called "brown dog tick", was collected on foxes but not on wolf carcasses as expected. *I. ricinus* only was collected and the existence of a strong association between wolf and *I. ricinus* should be considered.

[1] Jones et al. Global trends in emerging infectious diseases. Nature, 451: 990-993, 2008.
[2] Legge 11 febbraio 1992, n. 157 Norme per la protezione della fauna selvatica omeoterma e per il prelievo venatorio; G.U. 25 febbraio 1992, n. 46. [3] Barker et al. Systematics and evolution of ticks with list of valid genus and species names. Parasitology. 129 Suppl. 2004. [4] Estrada-Peña et al. Ticks of Europe and North Africa. A Guide to Species Identification. Springer International Publishing, 2017. [5] Selmi et al. Rickettsia spp. in *Dermacentor marginatus* ticks: analysis of the host-vector-pathogen interactions in a northern Mediterranean area. Exp Appl Acarol. May;72(1):79-91, 2017.

P172 - Serological exposure to *Toxoplasma gondii* and *Neospora caninum* in wild boar from an anthropized area in Italy

Luca Villa (1), Alessia Libera Gazzonis (1), Carolina Allievi (1), Giordano Ventura (2), Matteo Gradassi (2), Sergio Aurelio Zanzani (1), Maria Teresa Manfredi (1)

(1) Università degli Studi di Milano, Dipartimento di Medicina Veterinaria (2) Istituto Zooprofilattico Sperimentale della Lombardia e dell'Emilia-Romagna, Sede di Cremona

Corresponding author: Luca Villa (luca.villa@unimi.it)

Wild boar populations are nowadays in expansion in terms both of number of animals and habitat range. Apart from the ecological impact and the conflicts with human activities, the increased frequency of contacts among wild boar, livestock and humans, and the increased human consumption of game meat could also influence the transmission of zoonotic and animal-specific pathogens. Among parasites of medical and veterinary interest, *Toxoplasma gondii* and *Neospora caninum* are *Apicomplexa* protozoa of relevance for human health and animal health, welfare, and productivity. Surveys on *T. gondii* infection in wild boar in Europe showed a high variability in seroprevalence values in the different countries, ranging from less than 10 to until 50%, whereas in Italy values between 14 and 43% were reported [1]. Instead, with regard to *N. caninum* infection in wild boar, serological surveys conducted in Europe reported values between 0.3 and 33.6% [2]; to date, no data are available for Italy. Since wild boar are perfectly placed in the interface of domestic and sylvatic cycle, a sero-epidemiological study was planned with the aim to investigate on the role of wild boar populations in the epidemiology of protozoa infections from an anthropized area estimating their exposure to *T. gondii* and *N. caninum*. Blood and masseter muscle samples were collected from 128 wild boar hunted within the regional population management plan and destined to human consumption in an area of the province of Cremona (Lombardy region, northern Italy). Individual epidemiological data regarding estimated age, gender and killing place were collected. For the detection of anti-*T. gondii* antibodies, serum samples were analyzed using a commercial ELISA (ID Screen® Toxoplasmosis Indirect Multi-species); a commercial IFAT (MegaFLUO® NEOSPORA caninum), using an initial screening dilution of 1:50, was performed for the detection of *N. caninum* antibodies. Pearson chi-square statistic was performed to evaluate the correlation between seropositivity and individual data. Overall, 128 wild boar, 64 female and 64 male, were included in the study; both young (<1 year of age, n=20) and adult (≥1year of age, n=108) exemplars were sampled. The animals were shot in 12 municipalities from a restricted area near the Po River. 68 and 14 wild boars were positive to *T. gondii* and *N. caninum* with a seroprevalence of 53.1 and 10.9%, respectively; besides, in six animals a co-infection with both pathogens was revealed. Seroprevalence values did not differ considering age categories, gender, and killing place. btained results confirmed a wide exposure of wild boar to *T. gondii*; besides, seropositivity to *N. caninum* was demonstrated for the first time in wild boar in Italy, suggesting a possible role of these wild populations in the epidemiology of the parasite infection. Indeed, the study area, highly anthropized, and also characterized from the presence of numerous livestock farms, is confirmed suitable for the circulation of these protozoa both in domestic and wild species, as evidenced by previous studies. Further perspectives, including molecular detection and genotyping of *T. gondii*, will allow to evaluate the risk of meat as source for human infection; besides, multilocus microsatellite genotyping of *N. caninum* would be indicative of the spatial distribution and mutual connections between the parasite isolates from different species.

[1] Gazzonis et al. Parasitol. Res., 117:2207-2215, 2018. [2] Reiterova et al. Acta Parasitol., 61:255-260, 2016.

P173 - In vitro evaluations of the efficacy of bioactive fodder and products containing condensed tannins on gastrointestinal nematodes of goats

Alessia Libera Gazzonis (1), Sara Panseri (2), Sergio Aurelio Zanzani (1), Luca Villa (1), Luca Rapetti (3), Luca Chiesa (2), Maria Teresa Manfredi (1)

(1) Università degli Studi di Milano, Dipartimento di Medicina Veterinaria (2) Università degli Studi di Milano, Dipartimento di Scienze veterinarie per la salute, la produzione animale e la sicurezza alimentare "Carlo Cantoni" (3) Università degli Studi di Milano, Dipartimento di Scienze Agrarie e Ambientali - Produzione, Territorio, Agroenergia

Corresponding author: Alessia Libera Gazzonis (alessia.gazzonis@unimi.it)

Dairy goat breeding in northern Italy represents a zootechnical reality and an economic resource in alpine and pre-alpine areas. Nevertheless, gastrointestinal parasites and particularly Strongylida still constitutes a major limiting health factor, with consequences ranging from the reduction of production performances to mortality. In recent years, the use of alternative methods, such as the integration of the diet with bioactive fodder containing condensed tannins (CT), is becoming increasingly important. The present study was aimed at evaluating the *in vitro* anthelmintic efficacy of CT contained in commercial products (Silvafeed®ByPro, SBP and Silvafeed®Quebracho, SQ) and in sainfoin hay (SH).

For each tested sample, CT were extracted in acetone/water and in ethanol, for the extraction of the solely CT and of other compounds (e.g., flavonoids), respectively. The concentration of CT was determined using the acetone-HCl-butanol method [1] and working concentrations (150, 300, 600 and 1200 µg/ml) for *in vitro* tests were then prepared. To evaluate the anthelmintic activity against eggs and third stage larvae (L3), the Eggs Hatch Essay (EHA) and the Larval Migration Inhibition Test (LMIT) were conducted, calculating the inhibition of hatching percentage (%EHI) and the inhibition of larval migration percentage (%LMI). Positive (thiabendazole and levamisole) and negative (PBS) controls were added; for LMIT, the CT inhibitor polyvinylpolypyrrolidone (PVPP) (50mg/ml) was added as a further internal control [2]. Data were analysed by one-way analysis of variance, setting the level of significance at 0.05. Concerning EHA performed on the extracts in water, SBP was effective against the eggs hatching only at the maximum tested concentration (%EHI: 54.07%), while SQ and SH were effective even at lower concentrations (SQ: 300 µg/ml, %EHI: 31.75%, SH: 150 µg/ml, %EHI: 40.87%). As for the three ethanol extracts, a statistically significant efficacy was shown starting from the first dilution tested. SBP and SQ extracted in water had an anthelmintic effect against L3 starting from the concentration of 600 µg/ml (%LMI: 69.67% and 88%, respectively), while SH only when concentrated 1200 µg/ml (%LMI: 70.42%). SBP ethanol extract showed efficacy against L3 starting from the 600 µg/ml concentration, while those of SQ and SH already starting from the lowest concentration. Finally, the data obtained with or without the addition of PVPP were analyzed. Only for SQ water extracts the difference was statistically significant: %LMI decreased from 87.475% to 51.133% with the addition of PVPP, indicating that the inhibiting effect on the migration of the L3 was entirely attributable to CT.

Data obtained suggest that the integration of CT-rich fodder into the diet may be considered for the control of gastrointestinal nematode infections in the goats. Further research perspectives include the CT characterization, and in vivo tests with the administration of supplements in the feed, necessary to evaluate the effectiveness of CT use.

[1] Grabber JH. Acetone Enhances the Direct Analysis of Procyanidin- and Prodelphinidin-Based Condensed Tannins in Lotus Species by the Butanol–HCl–Iron Assay, Journal of Agricultural and Food Chemistry, 61:2669–2678, 2013.
[2] Demeler J. Evaluation of the Egg Hatch Assay and the Larval Migration Inhibition Assay to detect anthelmintic resistance in cattle parasitic nematodes on farms, Parasitology International 61(4):614-618, 2012.

P182 - Serological survey on cat Heartworm disease in North-eastern Italy: Preliminary results

Marika Grillini, Giuditta Zanotelli, Antonio Frangipane di Regalbono, Giulia Simonato

Università degli Studi di Padova, Dipartimento di Medicina Animale, Produzioni e Salute (MAPS)

Corresponding author: Marika Grillini (marika.grillini@phd.unipd.it)

The prevalence of feline Heartworm (HW) disease in an area can be estimated as 10% of the known prevalence of the infected dogs as reported in previous studies [1]. In Italy, data on *Dirofilaria immitis* distribution in cats are limited [2] even in the endemic North-eastern (NE). In cats, diagnosis is complicated by mostly sub-clinical pictures and their natural inborne resistance [3], and combined use of serological tests could be a useful support [1]. This study aims to investigate the presence and distribution of *D. immitis* in cats from NE Italy, evaluating potential risk factors. Preliminary results are herein reported, while statistics will be performed at the end of the study. Serum samples were collected by veterinarians during routine clinical examinations from cats exposed to at least one season at risk for mosquito bites, collecting information on antiparasitic treatment and lifestyle (outdoor/indoor). *Dirofilaria immitis* antibodies (Ab) were detected by Solo Step®, HESKA-USA, and antigens (Ag) by Pet-Check® HTWM antigen, IDEXX Lab.-USA before and after heat treatment to avoid Ag-Ab complex [4]. A total of 151 cats (97 owned, 54 free-ranging) were recruited. *D. immitis* Ab and Ag were detected in 11 (7.3%) and 3 (2%) samples, respectively. No *D. immitis* Ag were detected in Ab positive samples. One/3 Ag-positive samples were detected after heat treatment. Out of 11 Ab- and 3 Ag-positive cats, 9 and 2 had outdoor lifestyle, respectively. Moreover, 10 Ab-positive cats were from Veneto (Province of BL-1 cat, PD-1, TV-1, VI-4, VR-3), and 1 was from Friuli Venezia Giulia (TS). All Ag-positive cats were from Veneto (VR, TV, PD). None of Ab-positive cats presented clinical signs, whereas 2/3 Ag-positive cats showed cardio-respiratory failure.

Most of positive cats had outdoor access exposing them to vectors' activity. Nonetheless, an indoor lifestyle can partially protect them, being some mosquito species attracted inside human dwellings, and active both night and day. In Veneto, Ab positive results are not surprising except for BL province, where data on canine HW disease are scant and, to our knowledge, no autochthonous cases have been officially reported. Thus, the Ab positive cat suggests the potential exposure to the nematode circulation, that could be also exacerbated by the introduction in the NE areas of new invasive species, competent vector of *D. immitis* [5]. In conclusion, since cats have an inborne resistance to this parasite, there is a risk of underestimating its presence and diffusion. It is therefore essential to become aware of the actual presence and spread of HW in cats from NE Italy, in order to minimize the risk of HW transmission implementing appropriate prophylaxes protocols.

[1] Venco et al. Can heartworm prevalence in dogs be used as provisional data for assessing the prevalence of the infection in cats?, Veterinary Parasitology, 176:300–303, 2011.
[2] Genchi et al. *Dirofilaria immitis* and *D. repens* in dog and cat: A questionnaire study in Italy, Veterinary Parasitology, 267:26–31, 2019.
[3] Kramer et al. Feline heartworm infection: serological survey of asymptomatic cats living in northern Italy, Veterinary Parasitology, 104:43–50, 2002.
[4] Little et al. Heat treatment prior to testing allows detection of antigen of *Dirofilaria immitis* in feline serum, Parasite & Vectors, 7:1, 2014.
[5] Montarsi et al. Development of *Dirofilaria immitis* within the mosquito *Aedes (Finlaya) koreicus*, a new invasive species for Europe. Parasites & Vectors, 8:177, 2015.

P290 - Gastrointestinal nematodes of dairy goats: which predictors for faecal egg count during the late lactation and dry period?

Sergio Zanzani (1), Alessia Gazzonis (2), Luca Villa (1), Mario Villa (2), Giorgio Zanatta (2) MariaTeresa Manfredi (1)

(1) Università degli Studi di Milano, Dipartimento di Medicina Veterinaria (2) Associazione Regionale Allevatori della Lombardia

Corresponding author: Sergio Zanzani (sergio.zanzani@unimi.it)

The use of anthelmintics is almost inevitable in small ruminants farming, and often breeders prefer to administer the treatments during the dry period to use molecules that would require a withdrawal period during lactation. To avoid/slow down the onset of anthelmintic resistance of gastrointestinal nematodes (GIN) in small ruminants, several target/selective deworming plans have been proposed. They aimed to identify the most infected hosts responsible for much of the environmental contamination with eggs excreted in faeces. In addition to the quali-quantitative copromicroscopic analyses, the identification of the most parasitised animals can be based on other individual characteristics (primiparous/pluriparous goats; high producers/low producers; cosmopolitan/autochthonous breed; body condition score (BCS); level of anaemia), singly evaluated or combined [1-3]. This survey aimed to investigate the GIN eggs' level of excretion in naturally infected dairy goats from four flocs farmed under a semi-extensive system in northern Italy during the late lactation and dry period. Individual rectal samples of faeces from 193 adult animals were monthly collected (September-December 2019) and were analysed using the FLOTAC dual technique. Furthermore, by collecting individual information (breed, number of lactations, BCS, state of the coat) of the goats, possible predictors of the faecal count of eggs per gram of faeces (EPG) were identified.

In September, October, November and December, the mean EPG values of GIN were 1170, 954, 501 and 294, respectively. Cosmopolitan breeds involved in the study, Alpine and Saanen goats, presented higher EPG values (798 and 921, respectively) than those observed in the autochthonous Nera di Verzasca goats (348). Further, goats presenting normal hair coat (shiny and sheen hair, homogeneous and well adherent to the body) showed a lower mean EPG (769) than goats with an altered hairy coat (matted, rough, or scurfy hair; 853 EPG). Statistical analysis by a mixed generalised linear model (SPSS; IBM) highlighted how breed, coat status and sampling month were significant predictors of logarithmically transformed EPG values. On the other hand, lactation and BCS were not significant. The results obtained are relevant because they provide information on how selective/target treatments could be planned in goat farms in northern Italy during dry periods. In this physiologic phase of goats, the BCS and the number of lactations seem to be not recommended as selection criteria for untreated animals that could act as refugia for GIN. Treatments could be mainly given to goats of non-autochthonous breeds and presenting a poor coat status. Furthermore, the decrease in UPGs observed in November and December suggested that treatments in the dry period at these latitudes should be carried out by the beginning of November to avoid the administration of anthelmintics during the fourth stage larvae hypobiosis.

[1] Hoste et al. Control of gastrointestinal parasitism with nematodes in dairy goats by treating the host category at risk, Veterinary Research, 33:531-545, 2002.
[2] Burke et al. Accuracy of the FAMACHA system for on-farm use by sheep and goat producers in the southeastern United States, Veterinary Parasitology, 147:89-95, 2007.
[3] Zanzani et al. Gastrointestinal nematodes of goats: Host–parasite relationship differences in breeds at summer mountain pasture in northern Italy, Journal of Veterinary Research (Poland), 63:519-526, 2020.

P307 - Molecular identification of *Encephalitozoon pogonae* in central bearded dragon (*Pogona vitticeps*) from Italy

Alessandro Marigliano, Lisa Guardone, Francesca Mancianti, Michela Maestrini, Stefania Perrucci

Università degli Studi di Pisa, Dipartimento di Scienze Veterinarie

Corresponding author: Lisa Guardone (lisaguardone@gmail.com)

Microsporidia are intracellular eukaryotes, recently reclassified from protozoa to fungi, that can infect and cause disease in invertebrates, fish, amphibians, reptiles, and mammals [1]. The aim of the present study is to report the molecular identification of *Encephalitozoon pogonae* in captive bearded dragons (*Pogona vitticeps*) in Tuscany, central Italy.In the framework of a larger study on faecal samples from 14 different species of reptiles (including saurians, tortoises and snakes) kept as pets by private owners or in pet shops, 18 faecal samples of bearded dragon (*Pogona vitticeps*), 20 of different tortoise species and 6 of different species of snakes where submitted to total DNA extraction, followed by PCR amplification of a 250-280 bp fragment of the 18S rRNA gene, using a primer pair (V1 and PMP2) commonly used from different genera of microsporidia, including *Encephalitozoon* sp. and *Enterocytozoon* sp. [2]. Amplified PCR products were sent for Sanger sequencing to an external company. Sequences were compared with those deposited in GenBank by using the National Center for Biotechnology Information (NCBI) Basic Local Alignment Search Tool (BLAST).

Four faecal samples of *P. vitticeps* were positive for microsporidia DNA, while all snakes and tortoises were negative. The BLAST analysis retrieved 100% identity with *E. pogonae,* a recently described species [3].

The possibility of transmission of *E. pogonae* from the insect preys to bearded dragons was also evaluated, but all the examined insects were found negative at PCR analysis.

In *P. vitticeps,* encefalitozoonosis was first reported in the USA [4], and a few subsequent reports followed [5-8]. Bearded dragons tested positive in this study showed similar clinical signs as those described in previous studies, such as weight loss, anorexia, and lethargy [1,3,4,7].

Results obtained highlight the need to perform specific parasitological examinations for microsporidia in bearded dragons kept in captivity.

[1] Vergeneau-Grosset C, Larrat S. Microsporidiosis in vertebrate companion exotic animals. Journal of Fungi, 2: E3, 2015.
[2] Fedorko et al. Identification of microsporidia in stool specimens by using PCR and restriction endonucleases. Journal of Clinical Microbiology, 33:1739–41, 1995.
[3] Sokolova et al. Establishing a new species *Encephalitozoon pogonae* for the microsporidian parasite of inland bearded dragon *Pogona vitticeps* Ahl 1927 (*Reptilia, Squamata, Agamidae*). Journal of Eukaryotic Microbiology, 63:524–535, 2016.
[4] Jacobson et al. Systemic microsporidiosis in inland bearded dragons (*Pogona vitticeps*). Journal of Zoo and Wildlife Medicine, 29(3):315-23, 1998.
[5] Richter et al. Encephalitozoonosis in two inland bearded dragons (*Pogona vitticeps*). Journal of comparative pathology, 148:278–282, 2013.
[6] Schilliger et al. Triple infection with agamid adenovirus 1, *Encephaliton cuniculi*-like microsporidium and enteric coccidia in a bearded dragon (*Pogona vitticeps*). Tierarztl Prax Ausg K Kleintiere Heimtiere, 44:355–358, 2016.
[7] Shibasaki et al. First report of fatal disseminated microsporidiosis in two inland bearded dragons *Pogona vitticeps* in Japan. JMM Case Reports, 4:e005089, 2017.

P318 - *Lernea* sp. in a pet axolotl (*Ambystoma mexicanum*): a case report

Lisa Guardone (1), Daniele Petrini (2), Francesca Mancianti (1)

(1) Università degli Studi di Pisa, Dipartimento di Scienze Veterinarie (2) Ospedale veterinario San Concordio, Lucca

Corresponding author: Lisa Guardone (lisaguardone@gmail.com)

Axolotls (*Ambystoma mexicanum*) are large neotenic salamanders (Amphibia) sometimes kept as aquatic pets (1), that can be affected by several endo and ecto-parasites [1,2].

The aim of the present study is to describe a clinical case due to *Lernea* sp. in a pet axolotl from Tuscany (Italy), including therapy and follow-up.

An exotic veterinarian was called by the axolotl's owner after noticing small, whitish, elongated structures (~5 mm) attached to the animal body, particularly affecting the neck region and the abdominal flanks. The axolotl was kept in an aquarium. It had been bought from an aquatic pet shop, where it was kept with freshwater fish. During the visit a poor general clinical status was observed. The structures where identified by the vet as potential ectoparasites, and they were manually removed and stored in 5% formalin before being transferred to the Department of Veterinary Sciences for identification. An oral administration of ivermectin was given, as reported in [3]. The animal was also transferred in another aquarium, changing water daily.

The morphological analysis by optical microscopy confirmed that the structures were ectoparasites, identified as belonging to the genus *Lernea*, commonly called "anchor worm", on the basis of these characteristics features: a cephalic region with the typical anchor shaped attach organ showing two pairs of branches, and the elongated abdominal region bearing paired egg sacks. Species-specific identification was not attempted as high intraspecific morphological variability and a lack of morphological distinction between species is known [4]. Parasites of the genus *Lernea* are crustacean copepods commonly parasitizing freshwater fish species and, less frequently, amphibians [5]. Previous reports in axolotl are limited. *Lernea* sp. was described for the first time affecting this host in Uruguay, where it was responsible of a farm epizooty [5]. Subsequently it was described in Mexico [1] and in a pet axolotl (presumably) in Italy [3]. In this last case the axolotl was kept in an aquarium with fish at the pet shop, as the case here reported.

In conclusion, the described clinical case points out the possible presence of *Lernea* copepods clinically affecting axolotls, especially after contact with fish. The follow-up showed that the animal fully recovered, confirming the efficacy of ivermectin associated to sanitary measures to interrupt the cycle.

[1] Recuero et al. Urban aquatic habitats and conservation of highly endangered species: The case of *Ambystoma mexicanum* (*Caudata, Ambystomatidae*). Annales Zoologici Fennici 47(4):223-238, 2010.

[2] Bulletin of The Chicago Herpetological Society. Crustacean Ectoparasites of Amphibians. 54(4),85-91, 2019.

[3] Melidone et al. *Lernaea cyprinacea* infestation in an axolotl. Exotic DVM, 6(5):24-25, 2004.

[4] Hua et al. Morphology is not a reliable taxonomic tool for the genus *Lernaea*: molecular data and experimental infection reveal that *L. cyprinacea* and *L. cruciata* are conspecific. Parasites & vectors, 12(1), 1-13, 2019.

[5] Carnevia D and Speranza G First report of *Lernaea cyprinacea* L., 1758 in Uruguay, introduced by goldfish *Carassius auratus* (L., 1758) and affecting axolotl *Ambystoma mexicanum* (Shaw, 1798). Bulletin of the European Association of Fish Pathologists 23(5):255-256, 2003.

P325 - Survey on dermatophytoses in ruminant farms of Basilicata region

Roberta Galuppi (1), Antonio Bosco (2), Filippo Maria Dini (1), Giuseppe Mangieri (2), Paola Pepe (2), Laura Rinaldi (2), Giuseppe Cringoli (2)

(1) Alma Mater Studiorum - Università di Bologna, Dipartimento di Scienze Mediche Veterinarie
(2) Università degli Studi di Napoli Federico II, Dipartimento di Medicina Veterinaria e Produzioni Animali

Corresponding author: Roberta Galuppi (roberta.galuppi@unibo.it)

Dermatophytoses are significant pathogens in animal health due to their zoonotic potential, also impairing the productivity [1]. In the frame of a more extensive investigation on ectoparasitosis, this survey aimed to evaluate the spread of dermatophytes in ruminants reared in the Basilicata region. During the monthly clinical visits to farms registered at the Basilicata Farmers Association, the veterinarians performed a clinical examination in cattle from 115 farms (ranged from 30 to 280 animals) and sheep from 143 farms (ranged from 50 to 450 animals). Samples (skin scraping) from 99 cattle (from 31 farms) and 11 sheep (from 6 farms) showing skin lesions, were collected in petri dishes, which were sealed and sent to the laboratory of Mycology for the research of dermatophytes. Each sample was in parts observed for microscopic examination after clarification in potassium hydroxide and in part cultured in duplicate onto Mycobiotic agar (BD) with inositol and thiamine, incubated at both 25°C and 37°C. All sheep samples were negative for dermatophytes both at microscopic and cultural examination, while 35 out of 91 (38.5%) bovine skin skraping were positive for dermatophytes at microscopy and 34 out of 99 (34.3%) were positive for *Trichophyton verrucosum* at cultural examination (at 25°C or 37°C or both). By the combination of microscopic examinations and fungal cultures, the presence of dermatophytes, and specifically *T. verrucosum*, was therefore ascertained in 37 cattle belonging to 12 farms out of the 115 (10.4%) visited by the veterinarians. Based on these results, the frequency of dermatophytosis in the bovine farms of Basilicata seems lower than described in other survey, reporting from 30% to 100% of farms positive in Northern [2] and Central Italy [3,4] respectively. Microscopic examination is the elective method for diagnosis in cattle, but the association of culture at different temperatures is useful, particularly in the presence of scarce material and with few spores, and allows the identification of the dermatophytes. In this survey only *T. verrucosum,* the most frequent dermatophyte in cattle, was found. However, also *Trichophyton mentagrophytes* var. *mentagrophytes* was described in bovine in Central Italy [4] and outbreaks of *Trichophyton erinacei* were recently reported in Northern Italy [5]. In conclusion, in Basilicata Region the presence of dermatophytosis seems low, unlike other Italian regions. Although the lesions in cattle are usually pathognomonic, laboratory confirmation may be helpful to clarify doubtful cases and to identify the species involved. This could be of interest because bovine vaccination programs may be useful in controlling the infection but there is no evidence of vaccine efficacy for dermatophytes other than *T. verrucosum*.

[1] Bond R. Superficial veterinary mycoses, Clin Dermatol, 28(2):226-36, 2010.
[2] Rossi et al. Indagine sulla dermatofitosi in allevamenti bovini del Nord Italia, Large Animal Review, 18: 235-240, 2012.
[3] Papini et al. High Infection Rate of *Trichophyton verrucosum* in Calves from Central Italy, Zoonoses and Public Health, 56: 59–64, 2009.
[4] Agnetti et al., *Trichophyton verrucosum* infection in cattle farms of Umbria (Central Italy) and transmission to humans, Mycoses, 57: 400–405, 2014.
[5] Agnetti et al. Ringworm by *Trichophyton erinacei* in calves: description of two Italian outbreaks, Large Animal Review, 26: 141-143, 2020.

P328 - Succesful treatment of pleural and peritoneal larval mesocestodiasis with fenbendazole in two dogs

Alice Vismarra (1), Luigi Venco (2), Laura Kramer (1), Marco Genchi (1)

(1) Università degli Studi di Parma, Dipartimento di Scienze Veterinarie (2) Clinica Veterinaria Lago Maggiore, Arona, Italy

Corresponding author: Alice Vismarra (alice.vismarra@unipr.it)

Mesocestoides spp. are common intestinal tapeworms of dogs and cats. The presence of numerous larval stages in the pleural or peritoneal cavities causes larval mesocestoidosis, that is a potentially life-threatening disease in massive infection (1).

In vivo diagnosis of larval mesocestoidosis is challenging and confirmed diagnosis is often made post-mortem. For this reason, therapeutic recommendations are empirical and there is currently no data on the best treatment options in dogs and cats. Usually fenbendazole and praziquantel have been used to treat canine larval mesocestoidosis. Treatment with fenbendazole at 100 mg/kg orally twice a day, for 1–3 months has been reported as being effective. However, it is known that administration of high, off-label doses of fenbendazole can lead to adverse reactions.

Here, we reported two clinical cases of dogs with peritoneal and a rare pleural mesocestodiasis, treated with fenbendazole at a lower dosage than reported in the literature. Thoracic ultrasound examination clearly showed cystic structures covering the pleural surface in dog 1 and the peritoneal surface in dog 2. Ultrasound-guided fine needle biopsy yielded a clear fluid with numerous suspended, whitish structures of few millimeters diameter which are diagnostic for cestode stromal tissue (2). Moreover, fluid samples were submitted to a PCR for molecular identification (60F and 375R primers used for the amplification of a mitochondrial 12S rDNA fragment) (3). Copromicroscopic examination was negative for parasites, including cestode proglottids and eggs. Based on a presumptive diagnosis of pleural/peritoneal mesocestoidosis, treatment with oral fenbendazole 50 mg/kg twice a day was started in both dogs.

Respiratory signs of dog 1 started to improve rapidly after 2 weeks of treatment, and 78 days later, the dog was clinically normal, without dyspnea. Treatment was discontinued, and both thoracic radiographs and ultrasound were completely normal 90 days later. At 940 days from diagnosis, dog 1 was in perfect physical condition.

Dog 2 clinical condition started to improve quickly and 70 days later abdominal ultrasound showed the resolution of effusion and peritoneal reactivity. The number of cysts was dramatically reduced. Therapy was continued but unfortunately, after 57 days from the beginning of the therapy, a malignant lymphoma was diagnosed. Euthanasia was performed and a necropsy limited to the abdominal cavity was done. No cysts or signs of abdominal reactivity were observed. Morphology of aspirated material collected at diagnosis from the two dogs was typical of mesocestoideal tetrathyridium and PCR was positive for *Mesocestoides* spp.

In the present cases, treatment with 50 mg/kg of fenbendazole twice a day led to complete clinical and parasitological cure, without the need for surgical curetage. The advantages of this lower dose regimen include fewer potential adverse effects, lower cost and greater owner compliance.

[1] Eleni et al. Proliferative peritoneal and pleural cestodiasis in a cat caused by metacestodes of *Mesocestoides* sp. anatomohistopathological findings and genetic identification. Parasite. 14:71–76, 2007.

[2] Wirtherle et al. First case of canine peritoneal larval cestodosis caused by *Mesocestoides lineatus* in Germany. Parasitology International 56(4):317-20, 2007.

[3] Nickisch-rosenegk. et al. Contributions to the phylogeny of the *Cyclophyllidea* (*Cestoda*) in-ferred from mitochondrial 12S rDNA. Journal of Molecular Evolution 48:586–596, 1999.

P329 - Negligible risk of zoonotic parasites in Italian aquaculture

Andrea Gustinelli, Monica Caffara, Perla Tedesco, Maria Letizia Fioravanti

Department of Veterinary Medical Sciences, Alma Mater Studiorum Università di Bologna, Italy

Corresponding author: Maria Letizia Fioravanti (marialeti.fioravanti@unibo.it)

The diffusion of new eating habits and the increase of fish products demand have been raising concerns in the European countries, including Italy, about the transmission risk of zoonotic fish parasites to consumers, leading to the current EU "Hygiene Package" Regulations. In relation to the mandatory freezing treatment for *"products intended to be consumed raw, or marinated, salted and any other treated fishery products, if the treatment is insufficient to kill the viable parasite"*, an exception is included in the Reg. (EU) No 1276/2011 for farmed Atlantic salmon (*Salmo salar*), for which the risk was considered negligible according to EFSA Opinion (2010), but not for other fish species farmed in Europe. With the aim to collect the necessary epidemiological information on the presence/absence of zoonotic parasites in EU aquaculture, in the framework of the EU H2020 project ParaFishControl (www.parafishcontrol.eu), an extensive parasitological survey has been carried out on the main farmed fish species in Italy and other countries such as Spain, Greece, Denmark, Norway and Hungary. The present work reports the results from Italy. From 2016 to 2018 a total of 4728 farmed fish have been examined from 6 freshwater and 5 marine farms located in Italy: 1594 rainbow trout (*Oncorhynchus mykiss*), 1571 European sea bass (*Dicentrachus labrax*) and 1563 gilthead sea bream (*Sparus aurata*) have been collected based on a random polietapic and stratified sampling plan with a confidence level of 99% and a margin of error (MoE) of 4-8%. Besides harvest quality fish, runts were also examined in order to consider even the most predisposed hosts to acquire parasites through the natural food web. Parasitological analyses to search for anisakid nematodes in marine fish and diphyllobothriid cestodes and Opisthorchioidea digeneans in freshwater fish were performed utilizing methodologies such as visual inspection and candling as provided by the EU regulation, integrated by UV-press method, muscle compression/artificial digestion followed by microscopic examination when required (e.g. for *Opisthorchioidea*). No zoonotic parasites were found in any of the examined fish, including runts. The data obtained from this extensive survey show the absence of zoonotic parasites in the fish examined and are comparable to those reported in previous studies, leading to consider as negligible the risk of infections due to zoonotic helminths in the most important fish species of Italian aquaculture. Similar results have been obtained for the examined farmed fish in other countries involved in ParaFishControl Project. The results allow to assess the risk of the presence of zoonotic parasites in farmed rainbow trout, European sea bass and gilthead sea bream as negligible, indicating that also these farmed fish species should be considered suitable to benefit from the exemption from freezing treatment provided by EU Regulation No 1276/2011 for Atlantic salmon. Furthermore, the monitoring approach employed could be adopted for planning surveillance activities in EU fish farming systems, as it appears implementable also by industry, to better guarantee the safety of aquaculture products.

Funding: ParaFishControl H2020 project (634429).

[1] European Food Safety Authority (EFSA). Scientific Opinion on risk assessment of parasites in fishery products. EFSA panel on Biological Hazards (BIOHAZ). EFSA J. 2010;8(4):1543.

P330 - Preliminary investigation on the correlation between *Toxoplasma gondii* infection and epilepsy in dogs

Giulia Morganti, Giulia Rigamonti, Maria Chiara Marchesi, Giovanni Angeli, Iolanda Moretta, Fabrizia Veronesi

Università degli Studi di Perugia, Dipartimento di Medicina Veterinaria

Corresponding author: Giulia Morganti (giulia.morganti@collaboratori.unipg.it)

Toxoplasma gondii is a protozoan Apicomplexa capable of replicating in neuronal cells and causing necrotizing foci, both in primary infection and after reactivation. On that basis a correlation between acquired toxoplasmosis and epilepsy has been suggested, in dogs as well as in humans [1,2]. *Toxoplasma gondii* can be vertically transmitted by infected bitches to their litters, without any apparent pathological effect [3]. However, as observed in humans, the pathogenicity expressed during the intrauterine life could affect brain electrophysiology over the long term, concurring in the determination of Idiopatic Epilepsy [1,3]. To date, no evidence on the impact of *T. gondii* in the occurrence of canine epilepsy is available. The present retrospective study aimed to investigate the correlation between seropositivity to *T. gondii* and epilepsy in a group of 92 dogs, admitted to the Veterinary University Hospital of Perugia, in the period between 2017 and 2020. The 92 dogs were divided into two homogeneous groups: the first group (Group E) included 46 dogs with epilepsy, the second group (Group C) included 46 dogs with pathologies of other kind (es. blindness, ataxia, pulmonary diseases, urinary incontinence, etc.). All 92 dogs were tested serologically by IFAT for *T. gondii* and *N. caninum*, protozoa commonly considered in tandem by a clinician in diagnostic panel of central e peripheral neurological disorders. The positivity rates found in both groups were evaluated by inferential analysis (Chi-Square Test), with a significance threshold at $P<0.05$. In Group E, 13 out of 46 (28.26%) dogs tested positive for *T. gondii* and 12 (26.09%) for *N. caninum*. In Group C 14 out of 46 (30.43%) dogs tested positive for *T. gondii* and 6 (13.04%) for *N. caninum*. By comparing the results for *T. gondii* in the two groups, we can see a higher percentage of positivity in Group C rather than Group E, while the results for *N. caninum*, show higher percentage of positivity in Group E rather than Group C, however, these differences were not statistically significant ($P>0.05$). There was no statistically significant difference ($P>0.05$) between the percentages of positivity for *T. gondii* and *N. caninum* in Group E (28.26% vs 26.09%); thought *T. gondii* positive dogs with active and progressing infection were 4 (8.70%) vs 6 (13%) with *N. caninum* infection. The results obtained don't seem to support the role of *T. gondii* as a cause of epilepsy in dogs and in addition we could speculate that this parasite could be associated with other clinical forms rather than those of epileptic nature. Nevertheless, considering the limited number of subjects in our sample population and the lack of data related to intrathecal antibodies production, it is suggested to further investigate the role of *T. gondii* in the pathogenesis of this disorder, through the evaluation of brain lesions and genetic profile of the parasite recovered from the brain of dogs died due to the infection.

[1] Sadeghi et al. An updated meta-analysis of the association between *Toxoplasma gondii* infection and risk of epilepsy, Trans R Soc Trop Med Hyg, 113:453-462, 2019.
[2] Plugge et al. Occurrence of antibodies against *Neospora caninum* and/or *Toxoplasma gondii* in dogs with neurological signs, Rev Bras Parasitol Vet, 20:202-206, 2011.
[3] Al-Qassab et al. Isolation of *Toxoplasma gondii* from the brain of a dog in Australia and its biological and molecular characterization, Vet Parasitol, 164:335-339, 2009.

MIGLIOR POSTER SOIPA - SISVET 2021

P332 - Serological diagnostic approach in experimentally infected dogs with *Dirofilaria repens*

Lavinia Ciuca (1), Alice Vismarra (2), Wilfried Lebon (3), Frederic Beugnet (3), Rodrigo Morchon (4), Laura Rinaldi (1), Giuseppe Cringoli (1), Laura Kramer (2), Marco Genchi (2)

(1) Università degli Studi di Napoli Federico II, Dipartimento di Medicina Veterinaria e Produzioni Animali (2) Università di Parma, Dipartimento di Scienze Medico-Veterinarie (3) Boehringer Ingelheim Animal (BI-AH) Health Research Centre, in France (4) University of Salamanca, Group of animal and human dirofilariosis, Parasitology area, Faculty of Pharmacy

Corresponding author: Laura Rinaldi (lrinaldi@unina.it)

Diagnosis of *Dirofilaria repens* in dogs is a challenge: many infected dogs are asymptomatic and there is a limited number of reliable diagnostic tools available. Indeed, diagnosis is most often based on detection of circulating microfilariae (mff) during patent infection, followed by morphometric or molecular species identification [1]. The lack of a commercially available test for serological diagnosis is likely one of the most important limitations for *D. repens* diagnosis [2,3]. Therefore, the aim of the present study was to provide new insights from experimental infections of dogs with *D. repens*, focusing on the evaluation of: 1) the pre-patent period and 2) the antibody response against *D. repens* somatic antigens and against the *Wolbachia* endosymbiont. Briefly, on Day 0, 20 purpose-bred Beagle dogs were experimentally infected with 50 infective larvae (L3) of *D. repens*. Starting from Day 58 until the last day of the study (Day 281), blood samples were collected on a monthly basis for detection of antibodies against *D. repens* (Dr) and recombinant *Wolbachia* surface protein (rWSP) by non-commercial IgG-ELISAs. Additional samples were collected on Days 220, 245 and 281 for the detection of mff using the modified Knott's test and biomolecular analysis, following two PCR protocols: Gioia et al. (2010; protocol A) [4] and Rishniw et al. (2006- protocol B) [5]. The results were analysed by univariate statistical analyses using 2x2 contingency tables and K Cohen was calculated to assess the agreement among all the diagnostic techniques. Overall, the outcome of the study revealed that out of the 20 dogs experimentally infected with *D. repens*, 16 (80%) were microfilaraemic, 17 (85%) were positive at DNA detection in the blood, 18 (90%) had *D. repens* antibodies and 16 (80%) had *Wolbachia* antibodies on the last day of the study. The overall k agreement between Knott's and PCR protocol B was 0.442 (P=0.0001) and increased throughout the study, reaching 0.828 (P=0.0001) on Day 281. To the authors knowledge, this is only the second study reporting antibody response to *D. repens* somatic antigen in experimentally infected dogs. Results from both ELISAs, anti-*D. repens* and anti-WSPr, confirm that the development of serological tests for *D. repens* infection could be a starting point for application in epidemiological studies and as an aid in the diagnosis of infection in dogs, in particular for early stage infections and in absence of mff.

[1] ESDA, Guidelines. Guidelines for clinical management of subcutaneous dirofilariosis in dogs and cats. 2017, https://www.esda.vet/guide-lines-tutorials (accessed on January 2020). [2] Simón et al. Human and animal dirofilariasis: the emergence of a zoonotic mosaic, Clin. Microbiol. Rev. 25:507–544, 2012. [3] Capelli et al. Recent advances on *Dirofilaria repens* in dogs and humans in Europe, Review. Parasit. Vectors 11:663, 2018. [4] Gioia et al. Highly sensitive multiplex PCR for simultaneous detection and discrimination of *Dirofilaria immitis* and *Dirofilaria repens* in canine peripheral blood, Vet. Parasitol. 172: 160–163, 2010. [5] Rishniw et al. Discrimination between six species of canine microfilariae by a single polymerase chain reaction, Vet. Parasitol. 135:303–314, 2006.

Segreteria Organizzativa

SAFOOD srl

L.go Braccini 2 – 10095 Grugliasco (TO)

Tel.+39.011.670.9263