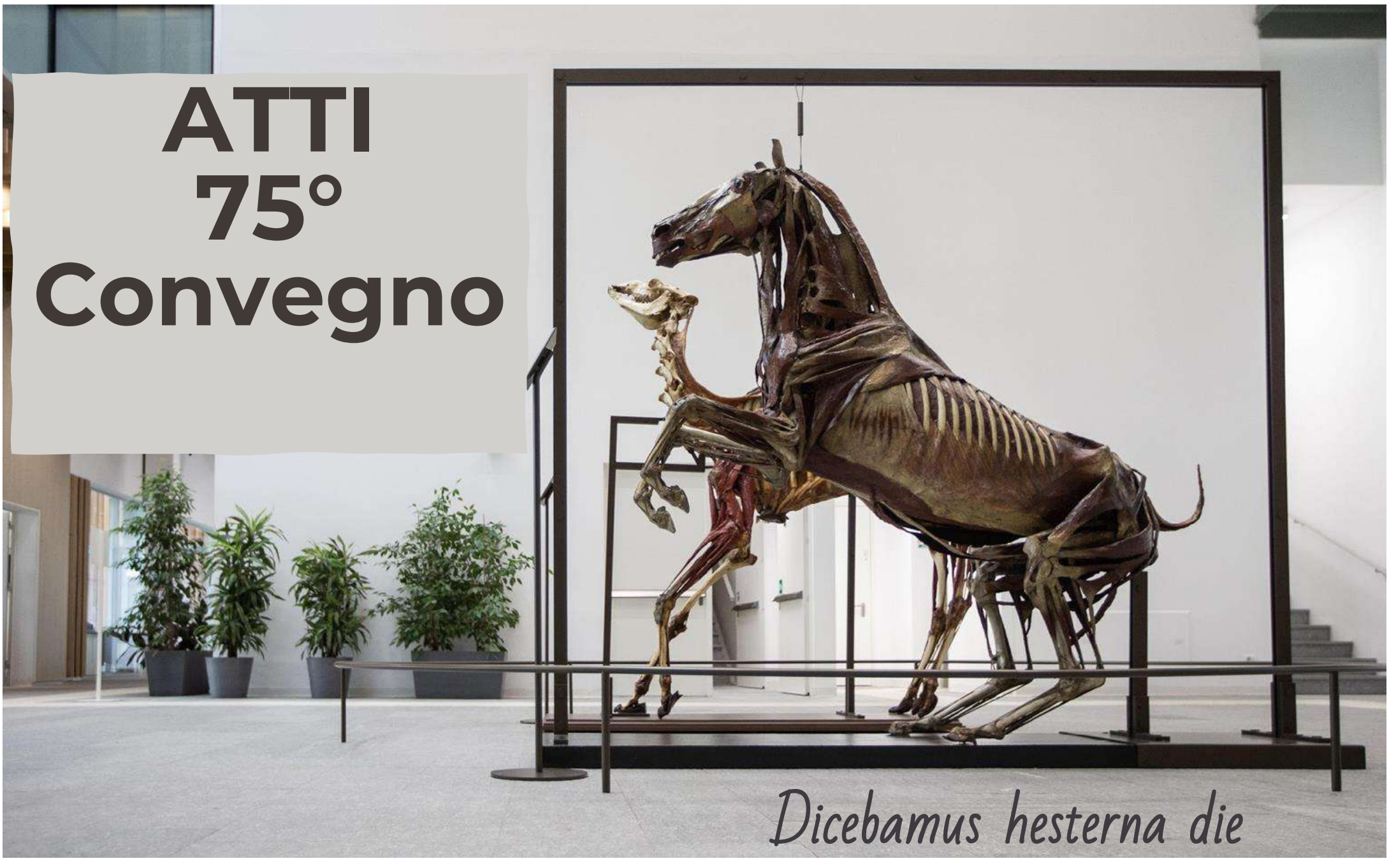
UNIVERSITÀ DEGLI STUDI DI MILANO

S.I.S.Vet

divas
DIPARTIMENTO DI MEDICINA VETERINARIA E SCIENZE ANIMALI

ATTI 75° Convegno



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15 - 18 Giugno 2022

Dipartimento di Medicina Veterinaria e Scienze Animali
Università degli Studi di Milano
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SALUTO DEL PRESIDENTE

Carissimi Colleghi,
in qualità di neo Presidente della Federazione SISVET, permettetemi di esprimere il mio ringraziamento a tutti voi per la fiducia accordatami e il grande attestato di stima che fin da subito intendo onorare.

I Presidenti che mi hanno preceduta nella gestione della Federazione hanno definito iniziative importanti di rinnovamento che, nei prossimi anni, richiedono interventi di consolidamento, di supporto e di adeguamento organizzativo. Tra questi il Convegno Nazionale, che tornerà finalmente in presenza a Lodi dal 15 al 18 giugno p.v. presso la sede del Dipartimento di Medicina Veterinaria dell'Università degli Studi di Milano, e che rimane uno dei primi impegni a cui dobbiamo dare lustro e continuità. Il Comitato Scientifico in stretta collaborazione con le Società Federate ha programmato alcuni Workshop di forte attualità e di interesse trasversale per la nostra comunità. Tra questi un Workshop sarà incentrato sull'aggiornamento in merito alla *Peste Suina Africana* e uno affronterà il tema della *One-Health*. Il 16 Giugno a seguire l'Inaugurazione del 75°Convegno SISVET si dialogherà in merito al *Ruolo del Medico Veterinario durante la Pandemia*. Il programma è inoltre arricchito da alcune *Main lectures* e da un *Simposio sugli animali selvatici* e *sui Pet non convenzionali*, nello spirito della multidisciplinarietà cara alla Federazione.

Ritengo che la vita della Federazione vada alimentata da un nuovo impulso in particolare per quanto riguarda il ricambio generazionale, la creazione di un percorso condiviso e un nuovo approccio attivo, coeso e partecipato, grazie all'entusiasmo e all'indiscussa competenza di tutti noi e attraverso iniziative divulgative e attività di collaborazione e di formazione. In questo contesto si innesca la *Young SISVET*, una nuova iniziativa promossa dal Comitato Scientifico e supportata dal Comitato Esecutivo della Federazione, rivolta ai giovani neolaureati che volessero approfondire alcune specifiche competenze.

Sono da sempre certa che la Federazione SISVET rappresenti il naturale punto di incontro fra le diverse anime delle Scienze Veterinarie e credo fermamente che le qualità e le capacità di tutti noi siano indispensabili per garantire al mondo della Medicina Veterinaria quel ruolo politico, istituzionale e sociale che nella società civile, e fuori dall'Accademia, le è proprio.

Noi abbiamo le professionalità, le capacità e la visione per farlo insieme e, sotto la casa comune della Federazione, oggi più che mai, possiamo affrontare le nuove importanti sfide che il futuro ci sta per riservare.

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ABSTRACT WS e MAIN LECTURE

Di seguito vengono riportati i programmi dettagliati e gli abstract dei contributi pervenuti

WORKSHOP

Sostenibilità e One Health

Moderatori:

Prof. Aniello Anastasio
[Dipartimento di Medicina veterinaria e Produzioni animali - UNINA]

Prof. Giuseppe Bertoni
[Istituto di Zootecnica della Facoltà di Agraria - UNICATT]

10.40	**Saluti e apertura del WS**
11.00	**Linee di indirizzo europee** *Felice Adinolfi* [Dip. di Scienze Mediche Veterinarie – UNIBO]
11.30	**Attualità e prospettive italiane per le filiere zootecniche** *Bruno Ronchi* [DAFNE – Dip. di Scienze Agrarie e Forestali - UNITUS]
12.00	**Ruolo della stewardship del farmaco, dell'antibioticoresistenza e del benessere: l'esperienza classyfarm** *Loris Alborali* [IZS Lombardia e Emilia Romagna] *Sergio Ghidini* [Dip. di Scienze degli Alimenti e del Farmaco - UNIPR]
12.30	**Sanità di prevenzione: il ruolo del Medico Veterinario** *Antonio Limone* [FNOVI]
13.00	**Discussione**

WORKSHOP

15 Giugno 2022

Peste Suina Africana
[AULA L04]

Moderatori:
Dr. Angelo Ferrari [Istituto Zooprofilattico di Torino]
Prof. Sergio Rosati [Dipartimento di Scienze Veterinarie di Torino]

14.15	**Saluti e apertura del WS**
14.30	**Il ruolo dei servizi veterinari nel controllo della PSA** *Pierdavide Lecchini* [Direzione Generale della Sanità Animale e dei Farmaci Veterinari]
14.50	**Peste suina africana: le strategie di eradicazione e controllo dell'Unione Europea** *Vittorio Guberti* [ISPRA]
15.20	**Peste suina africana: aspetti generali e aggiornamenti epidemiologici** *Francesco Feliziani* [IZSUM – Centro di referenza]
15.50	**Clinical and pathological features of ASF** *Francisco J. Salguero* [UK Health Security Agency]
16.20	**Vaccines: The missing tools for effective prevention of ASFV** *Chris Netherton* [The Pirbright Institute]
16.50	**Discussione**

Alimenti e salute umana

GIORNATA ARNA
[AULA L113]

Moderatore:

Prof. Giuseppe Bertoni [Presidente ARNA]

14.30	**Ruolo degli alimenti di origine animale in una dieta sana ed equilibrata** ***Franca Marangoni*** [Nutrition Foundation of Italy] ***Andrea Poli*** [Nutrition Foundation of Italy]
15.15	***Consumo di alimenti ultra-processati e salute: cosa dicono gli studi epidemiologici?*** ***Giovanni de Gaetano*** [Dip. di Epidemiologia e Prevenzione, IRCCS NEUROMED] ***Marialaura Bonaccio*** [Dip. di Epidemiologia e Prevenzione, IRCSS NEUROMED]
15.50	**Contaminanti naturali, di processo e antropici negli alimenti vegetali** ***Terenzio Bertuzzi*** [Dip. Scienze animali, della nutrizione e degli alimenti - UNICATT]
16.25 **16.25**	**L'infiammazione low-grade: da dove origina, cosa fa, come si combatte** ***Francesco Visioli*** [Dip. Medicina Molecolare – UNIPD] **Discussione**

WORKSHOP

16 giugno 2022
[Aula L04]

Il ruolo del medico veterinario ai tempi della pandemia

Moderatori:
Prof. Nicola Decaro [Dipartimento Di Medicina Veterinaria di Bari]
Prof. Domenico Bergero [Dipartimento di Scienze Veterinarie di Torino]

11.00	**Saluti e apertura del WS**
11.15 **11.30**	**Breve cronistoria delle epizoozie tra il XIX ed il XX secolo** *Ivo Zoccarato* [A.I.S.Me.Ve.M] **COVID-19 ed animali** *Umberto Agrimi* [Istituto Superiore Sanità]
11.45	***Alea iacta est*. Se non ora, quando?** *Alessio Lorusso* [IZS Teramo]
12.15	**Impatto della pandemia sulla professione veterinaria** *Carla Bernasconi* [FNOVI - Medico Veterinario]
12.45	**Sopravvivere alla professione in tempo di pandemia: medici veterinari e società, in equilibrio tra curare e "prendersi cura".** *Alessandro Schianchi* [Medico Veterinario e Psicologo]
13.15	**Discussione**

SIMPOSIO FEDERALE

17 giugno 2022

Ore 10.30

La medicina veterinaria e la fauna selvatica

Apertura:

Prof. Alessia Di Giancamillo [Dip. Scienze Biomediche per la Salute - UNIMI]
Prof. Giuseppe Cringoli [Dip. Medicina veterinaria e Produzioni animali - UNINA]

Moderatori:

Prof. Pierluigi Di Ciccio [Dip. Scienze veterinarie - UNITO]
Prof. Fiammetta Berlinguer [Dip. Medicina veterinaria – UNISS]

10.30	**Saluti e apertura del Simposio**
11.00	**Passaggio fenotipico dal selvatico al domestico nel genere *Ovis*** ***Salvatore Naitana*** [SOFIVET – Dip. Medicina veterinaria - UNISS]
11.20	***La patologia da emoparassiti nell'avifauna*** ***Frine Eleonora Scaglione*** [AIPVET – Dip. Scienze veterinarie - UNITO]
11.40	**Approccio innovativo della figura del Medico Veterinario nell'ambito della selvaggina da caccia** ***Vincenzo Veneziano*** [AIVI – Dip. Medicina veterinaria e Produzioni animali - UNINA] ***Raffaele Marrone*** [AIVI – Dip. Medicina veterinaria e Produzioni animali - UNINA]
12.00	**Gestione degli asini bianchi** ***Oliviero Olivieri*** [ARNA - Dip. Medicina veterinaria - UNIPG]
12.20	**Influenza aviara** ***Calogero Terregino*** [ANIV – IZS delle Venezie]
12.40	**Ungulati selvatici: anestesie "normali" con accorgimenti speciali** ***Giuliano Ravasio*** [SICV – Dip. Medicina Veterinaria e Scienze Animali - UNIMI]
13.00	**Ecotossicologia dei grandi vertebrati marini: principali effetti avversi** ***Annalisa Zaccaroni*** [SIFTVET – Dip. Scienze Mediche Veterinarie – UNIBO]

AIPVET

17 Giugno

Ore 14.30 -15.30 **[AULA L03]**

Moderatore:
Prof. E. Lepri

Main Lecture

Pathology of animals models of COVID-19

Prof. Francisco Javier Salguero

SICV

17 Giugno

Ore 14.30- 15.15 **[Aula L02]**

Moderatori

Prof. Giuliano Ravasio
Prof. Gerardo Fatone

Main Lecture

Per una storia della medicina veterinaria: una prospettiva clinica nei libri a stampa

Prof. Fonda

SIMPOSIO FEDERALE

18 giugno 2022

Ore 08.30

"La medicina veterinaria e i PET non convenzionali"

[AULA L05]

Moderatori:

Prof. Laura Rinaldi [Dip. Medicina Veterinaria e Produzioni Animali- UNINA]
Dott. Michele Capasso [Medico Veterinario]
Prof. Annamaria Grandis [Dip. Scienze Mediche Veterinarie - UNIBO]

8.30	**Saluti e apertura del Simposio**
9.00	**Anatomia comparata dell'apparato respiratorio nelle tre principali classi di animali esotici (mammiferi, uccelli e rettili)** ***Claudio Tagliavia*** [AMV – Dip. Scienze Mediche Veterinarie- UNIBO]
9.20	**Impatto sanitario delle parassitosi nei nuovi animali da compagnia** ***Fabrizia Veronesi*** [SOIPA – Dip. Medicina veterinaria - UNIPG] ***Manuela Diaferia*** [SOIPA – Dip. Medicina veterinaria - UNIPG]
9.40	**Aspetti innovativi sulla riproduzione dei rettili** ***Francesco Di Ianni*** [SIRA – Dip. Scienze Medico-Veterinarie - UNIPR]
10.00	**Ecocardiografia bidimensionale e analisi dei flussi con metodo doppler nel pitone reale (*Python regius*)** ***Mara Bagardi*** [SICLIMVET – Dip. Medicina veterinaria - UNIMI]
10.20	**Lo stato dell'arte della medicina veterinaria negli animali esotici** ***Stefano Cusaro*** [SIVAE]
10.40	**L'immunologia dei rettili** ***Elisabetta Razzuoli*** [RNIV – IZS del Piemonte, Liguria e Valle d'Aosta]
11.00	**Discussione**

SICV

15 Giugno

Ore 14.30 -15.30 **[Aula L02]**

Main Lecture

Digital dermatitis in cattle still debate 50 years later

Prof. Carlo Maria Mortellaro

SIRA

15 Giugno

Ore 14.30 – 16.30 **[Aula L123]**

Moderatore

Prof. Mario Cinone

Main Lecture

Valutazione del neonato canino

Dr.ssa Jasmine Fusi

SICV

16 Giugno

Ore 08.30-09.30 **[Aula L02]**

Main Lecture

Malattie dell'accrescimento del cane e del gatto: le 7 sorelle + 1 (ma non c'è il tempo per tutte)

Prof. Carlo Maria Mortellaro

AIPVET

16 Giugno

Ore 17.00 – 18.00 **[AULA L03]**

Moderatore: Prof.ssa Raffaella De Maria

Main Lecture

Vescicole extra-cellulari nel mondo della patologia

Prof.ssa Benedetta Bussolati

AMV

16 Giugno

Ore 17.00 -18.00 **[AULA L112]**

Moderatore

Prof. Giuseppe Radaelli

Main Lecture

Cellule staminali e precursori endogeni: dall'embriologia alla neuroriparazione

Prof.ssa Laura Calzà

SOIPA

16 Giugno

Ore 17.00 – 18.00 **[AULA L119]**

Moderatori

Prof.ssa Fabrizia Veronesi
Prof. Giuseppe Cringoli

Main Lecture

Does bovine besnoitiosis spread from Europe to Italy follow the beef cattle trade?

Prof.ssa Maria Teresa Manfredi

PREVENTIVE HEALTHCARE: THE VETERINARIAN ROLE

Antonio Limone[1]

Corresponding Author: A. Limone (antolim@izsmportici.it)

The veterinary medicine has always been applied in a thousand areas. Most known as the profession that guarantees the health and welfare of pets, actually express its action in several fields:

- ✓ Veterinarian is responsible for infectious diseases outbreaks management and prevention
- ✓ Veterinarian guarantees the quality and safety of food and feed of animal origin
- ✓ authorization and certification of products of animal origin intended for export are up to the Veterinarian
- ✓ Veterinarians, in close collaboration with human doctors, improve the public health, follow the collaborative and multidisciplinary approach of "One Health, which recognizes that human health is strictly linked to animal health and the environment"

The change in professions arises precisely from the environment.

Although the veterinary world has always safeguarded the territory, protect biodiversity and animal species, it is necessary, today, to pay more attention to it. In fact, only by a deep knowledge of the risks, it is possible to implement the correct management policy "from farm to fork ", committing to reformulate the supply chains management to simultaneously guarantee quality, healthiness and sustainability.

PERSPECTIVES FOR ITALIAN LIVESTOCK SUPPLY CHAIN

Bruno Ronchi

Università degli Studi della Tuscia – Dipartimento di Scienze agrarie e Forestali
Corresponding author: B. Ronchi (ronchi@unitus.it)

The primary meaning of "One Health" (OH) approach is a multidimensional interface of components related to human, animal, and environment (1). A good state of animal health and welfare positively affects productive efficiency and profitability, environmental sustainability, and food safety. The outbreak of animal diseases can severely affect the food supply chain, influencing the market demand and prices, as demonstrated by price transmission analysis during BSE crises (2). It has been estimated that around 60% of emerging infectious diseases of human derives from animals, and for the most part (70%) from wildlife (3). Over recent time, OH meaning has changed from a medical and veterinary science approach, to include many other disciplines, such as food science, health economics, environmental, and social sciences (4).

The health of the environment, including plants, is of basic importance for agricultural and livestock production systems, due to strong interactions with provision of ecosystem services, animal health and food safety. But to date, a limited integration of environmental sector in OH can be highlighted in practice, such as the evaluation of effects of stressors to ecological systems due to climate variability and climate change, and/or degradation of agricultural land and natural habitats (1). On addition, little attention has been devoted to political and social factors involved in OH implementation in practice, since OH represents also complex social systems, with multiple components and expertises (5).

The "Farm to Fork" strategy, elaborated by EU Commission in 2020, includes the concept of OH, and highlights the need of a holistic transdisciplinary approach to improve overall sustainability of food system. If from one side there is a strong consensus regarding the conceptual framework, on the other side there is a necessity of a cumulative impact assessment on targets set by EU, to verify problems and potential solutions. The multidimension and complexity of OH components, to be successfully applied in livestock farming systems and relative food chains, require implementing strategies, effective coalitions of actors involved, mobilization of scientific community.

Further problems relate to COVID-19 pandemic disease and Ukraine war, responsible for a global economic recession, supply chain disruption, price volatility, and cascading risks for farmers.

The perspectives of OH successful application for Italian livestock supply chains appear conditioned by the possibility to design a system-based approach of decision making to guarantee, as a priority, an economic and social sustainability of farmers and of other partners of food chain. Clear goals must be established, tailored around the specific characteristics of supply chain, and especially on the farmer's needs, together with standard operating procedures, good record keeping, and proper training (6).

Supply chains should complete the transition towards more climate-friend models, applying concepts of circular economics for energy production and saving, by-products recycling, reduction of environmental impact. Livestock sector can play a fundamental role in circular economy, for example absorbing residual biomass from other food and non-food activities to produce foods of high nutritional values. In such perspective, a competitive access of the feed industry to residual biomass for animal feeding should be privileged.

The development of livestock systems according to OH principles can be founded around the paradigms of "sustainable intensification" and "precision livestock farming" (7), and can be facilitated by the technological and digital transformation, by the application of modern biotechnologies and genomic

testing, by the availability of detection tools and robotics, by the availability of modern additives and advanced vaccines.

The cost for the process of transition of Italian livestock farming systems, such as dairy farming, pig and poultry farming, are not negligible, since many farms need investments to renovate the buildings and the equipment for animal husbandry and waste management. The EU Commission has recently published a list of potential eco-schemes, which will support, starting from 2023, the future of Common Agricultural Policy to address the Green Deal targets. The inclusion of precision livestock technologies into the eco-schemes can facilitate the adoption of more sustainable practices, improving farmers' working conditions and animal welfare.

Some Italian extensive or semi-extensive livestock farming systems must deal with the risk of diseases transmission of public health significance, posed by wildlife-livestock interactions, and associated economic and social impact (8). New professional responsibilities of veterinarians in the perspective of OH can be highlighted, including also the necessity to interact with other competences (e.g., medical profession, environmentalists, and wildlife specialists) (9).

Following emerging examples in other countries, the commercial business of typical Italian animal food can benefit from a "one health" certification for labelling of food, produced according to best responsible practices to guarantee: safety food (e.g., restricted use of antibiotics), good animal health (e.g., advanced disease prevention programs), and low environmental impact (e.g., low carbon footprint).

Pillars of a national road map to achieve OH targets could be:

- promote a science-based governance, specific for each animal food chain, with the challenge of translate expertise into public policy;
- stimulate finalized research for solution of critical points, and facilitate research transfer;
- define and develop OH education programs, thinking and acting across disciplines, to improve the professional competences, working on academic degree programs, and on continuous technical education programs;
- develop a "supra-country" (or trans-border) approach, with the involvement of international organizations;
- design an integrated risk assessment project, focusing on national intersectoral response plans across main components;
- define a programmatic action at national level, based on priorities, with major impact at minimal costs;
- promote a multisector collaboration, with multi-stakeholders' partnership, facilitating information flow, demonstrating economic benefits of OH approach, and defining clear stakeholders' rules.

The OH approach, if well-structured and operating in practice, can give a substantial contribution to promote healthy ecosystems, with the aims of a more effective disease prevention and biosecurity, and to improve the competitive position of the Italian agri-food sector, together with social well-being and sustainability.

[

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AFRICAN SWINE FEVER IN ITALY: EPIDEMIOLOGICAL DATA AND RISK MANAGEMENT

Carmen Iscaro, Francesco Feliziani

(Centro di Referenza Nazionale per lo studio delle malattie da Pestivirus ed Asfivirus Istituto Zooprofilattico Sperimentale dell'Umbria e delle Marche "Togo Rosati"
Corresponding author: Feliziani (f.feliziani@izsum.it)

African Swine Fever (ASF) is a contagious viral disease that is currently the greatest threat to pig farming worldwide. The causative agent is a DNA virus (ASF Virus, ASFV) that is the only representative of its family called Asfarviridae (1). The original host of this virus has been identified as some arthropods commonly referred to as soft ticks, belonging to the genus Ornithodoros (2); the shift first to wild African suids (Warthog and Potamoceros) and even more to domestic and wild pigs (Sus scrofa) was essential to catch the attention of scientist and researchers. Although research activities has been directed understanding the complex immunological mechanisms that regulate the interaction between virus and host, to date there is no safe and effective vaccine available to prevent ASF Virus infection (3).
In Africa, 24 genotypes of ASFV are known (4), but only two of them escaped from the continent: the first epidemic wave initially involved the Iberian Peninsula and then it spread to several countries in Western Europe, as well involved other countries in Central and South America (5). This first emergency has taught us that infection often starts as an epidemic, but could become endemic in the affected territories; indeed, some territories gained the free status by applying impressive measures of direct prophylaxis in a reasonable time, but in other contexts, the eradication process was complex and long. The second wave is still in full progress and it is already considered a full-blown pandemic. ASF cases have been notified in all five continents: the disease continues to be present in Africa (where it is probably underestimated), a large endemic territory characterises Europe and Asia, and ASF is reported on some islands of Oceania and Central America (6). The index case was found in Georgia 2007 (7) and to date it has not been possible to stop the spread of the virus. In fact, with a few exceptions (Czech Republic and Belgium), ASF remained endemic in any territory it has reached, either through contamination by contiguous domestic and wild pig populations or using indirect means of transportation mediated by the so-called human factor (jumping diseases).
It is now well known that a number of factors heavily influence both the likelihood of the virus spreading and its capability to persist in endemic territories. Undoubtedly, farms with biosecurity deficits are highly exposed to the introduction of ASFV and, at the same time, may play an important role in the spread of infection (8). It is worth to remember that wild boar, which until a few years ago were confined to the spill-over function in the infection, are now playing a very active epidemiological role, especially in Europe (9).
The presence and permanence of infection in feral pigs obliged the experts to turn around the strategic approach to be applied in infected territories; in the absence of a safe and effective vaccine, direct measures remain the unique choice to contain the infection, both in terms of prevention and eradication (10). In order to have any chance of success, however, the eradication plans must rely on early diagnosis and rapid intervention. As far as diagnosis is concerned, it should be remembered that biomolecular tests to search for the viral genome are extremely sensitive and specific, so therefore absolutely reliable; surveillance and sampling systems, on the other hand, are more complicated and are influenced by the characteristics of the territory. However, all studies and experts agree that passive surveillance is more effective than active surveillance (12). It is therefore necessary to organise a system for tracing dead animals (both domestic on farms and wild animals in their typical habitat); this target increases the likelihood of detecting any new introduction of the virus at an early stage. In the absence of suspicion based on clinical or anatomical-pathological evidence, it is neither convenient nor useful to analyse samples collected from farms and slaughterhouses or from hunted animals and road-accident victims.
The tendency of ASF to remain as endemic in infected areas is a familiar feature in Italy: In Sardinia, the disease arrived as far back as 1978 and unfortunately, the free status has not still regained. In this region, it was ascertained that the persistence of the infection was linked to the traditional practice of rearing free ranging pigs; these animals, often illegally kept, had frequent contacts with either farms or wildlife (12). In recent years, the epidemiological situation has definitely improved, thanks to the measures applied by the regional government and supported by the Central Authority (13). The number and the role of illegal free ranging pigs has been definitively reduced and currently the surveillance system has not detected any virologically positive animals for at least two

years. A small number of sero-positive animals are continuing to emerge each year, but experts are confident that Sardinia is next to become ASF-free, at least in domestic pigs. Considering the experience gained over the years in fighting ASF in Sardinia and the risk of the infection being introduced into free regions, the Ministry of Health adopted a national surveillance plan with the aim of detecting any new introductions of the virus at an early stage (14). By the means of sampling activities carried out by the Veterinary Services in application of this plan, a positive RT-PCR sample was found on a wild boar found dead in the mountains on the border between the Piedmont and Liguria regions (north-west Italy).

The first cases were detected in the first days of January 2022 and the measures provided for in the contingency plan were immediately applied to prevent the spread of the infection. At the same time, passive surveillance was reinforced and consequently the number of cases rose steadily in Liguria and Piedmont. The national alert system had an adequate reaction, and the emergency response system demonstrated the suitability of the procedures developed by the Ministry of Health with the collaboration of CEREP and ISPRA. The restrictive measures, of course, were applied not only to wild boars population, but to even to the pig farms; it should be remembered that in the area affected by the infection, there are only a few holdings and just a small number of which are classified as commercial.

The current challenge is to adapt the approach that has enabled to eradicate the ASF clusters of Belgium and the Czech Republic. The orographic characteristics of the territory, the density of wild boar population and, above all, the size of the affected area complicate the strategy to be applied in order to achieve eradication, but the competent authorities are determined to make every effort to achieve the objective in the shortest possible time. It will be essential to apply barriers that can effectively contain the infected wild boar population and, at the same time, organise depopulation activities that can combine maximum effectiveness with minimum disturbance of the resident wild boar population. The aim is to prevent contact between infected and healthy animals, but above all to avoid the spread of the virus. If the eradication plan can achieve these objectives before the summer season, confidence in eradicating the disease within a reasonable timeframe will be high.

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AFRICAN SWINE FEVER IN WILD BOAR: STRATEGIES FOR THE CONTROL AND THE ERADICATION OF THE VIRUS APPLIED IN THE EU

Vittorio Guberti (1)

(1) Istituto Superiore per la Protezione e la Ricerca Ambientale.

Corresponding author: V. Guberti (vittorio.guberti@isprambiente.it)

The epidemiology of African swine fever in wild boar in Europe is everywhere characterised by the same evolution (1). The virus is introduced either by chance through human-caused risk activities (e.g. Piedmont/Liguria) or by continuity of the infected wild boar's range (Germany on the border with Poland). Once the virus has been introduced, if it finds a sufficient number of wild boars, it begins a process of invasion of the host population (successful invasion), which gradually culminates in an epidemic phase consisting of a wave that spreads geographically according to a gradient defined by the continuity of the distribution area and abundance of the wild boar.
Behind the epidemic wave there is - invariably - a very long endemic phase, the duration of which is influenced both by the extension of the viral circulation area and by the number of wild boars present.

Since 2007 (when the virus was first introduced into Georgia) the disease has been combated using three different strategies implemented with three different tactics.
A) Drastic depopulation: this strategy has been applied mainly in countries outside the EU. This involves depopulating at least 80% of the wild boar population in the area of virus circulation by applying the simple equation: no wild boar no virus. This strategy has failed everywhere; on the contrary, it has resulted in a sudden spatial spread of the virus due to the escape mechanisms put in place by the infected population under very high hunting pressure.
B) Soft hunting: this strategy was initially applied in Poland, Lithuania, Latvia and Estonia. The idea was to eradicate the virus by progressively reducing its incidence (number of new cases) by changing the abundance and sex and age structure of the infected population. Hunting was to be directed mainly towards adult and sub-adult females (the so-called demographic drivers of the population); hunting activity was to be conducted following strict biosecurity measures, thus limiting the probability of further spread of the virus caused by the handling of tools and materials contaminated with the virus during hunting. Finally, the strategy called for the removal of at least 60% of infected carcasses in the area of virus circulation. The end result of the application of this strategy was a slow but inexorable spatial spread of the virus and its continued endemicity in all areas of virus circulation regardless of their extent.
C) Fencing of the viral circulation area and near extinction of the infected population within the fenced area. The essential idea is to block the epidemic wave and subsequently drive the wild boar population to extinction through specific culling and with it the virus. This strategy was first applied in the Czech Republic and then in Belgium where it led to the successful eradication of the virus; the same strategy is struggling to achieve success in the infected area of Germany on the Polish border. The strategy may be easier to apply in the case of single point/local introductions than in situations where the virus may be introduced multiple times.
However, the European experience has defined 5 essential pillars to build an eradication strategy for African Swine Fever in wild boar.
1) Early detection: this is a prerequisite for achieving virus eradication. Effective early detection allows for a small infected area, which is easier to manage, as well as an abundant wild boar population residing there.

2) Blocking the epidemic wave: the epidemic wave is responsible for the extension of the area of viral circulation that will then become the area of endemic virus persistence. The sooner the epidemic wave is blocked, the greater the likelihood of eradicating the infection due - again - to the small size of the viral circulation area and the abundance of the host population residing there; the epidemic wave is usually blocked by natural or artificial barriers.
3) Continuous –enhanced- passive surveillance: passive surveillance allows the geographical spread of the virus to be constantly monitored and the epidemiological phase of the disease to be assessed. Precise knowledge of the epidemiological phase makes it possible to modulate the actions planned for any specific phase.
4) Stopping hunting activity in the area of virus circulation: it is a fact that recreational hunting activity does not have the technical capacity (probability of hunter-prey encounters at low prey densities; sustainability of the hunting effort in the long term) to bring wild boar abundance to the limits necessary for virus eradication. Hunting induces a range of escape behaviours, hunter avoidance mechanisms and triggers demographic responses that negate the momentary reduction in wild boar numbers. On the contrary, hunting in the area of viral circulation increases the probability of viral contamination of hunting tools and consequently the passive - involuntary - transport of the virus outside the infected area.
5) A strong increase in hunting activity outside the viral circulation area: even in the presence of fences it is very likely that the virus can find an escape route to the outside. In such a situation it is crucial that the virus does not find the necessary number of wild boars to initiate a succesful invasion phase. Outside the viral circulation zone, hunters are advised to double their usual number of hunted animals.

The eradication of African Swine Fever in wild boar is a particularly difficult challenge that can only be met if it is carried out with extreme care, in a timely and coordinated manner between the different entities involved in animal health and wildlife management. The experiences gained in Europe provide a clearer picture of the key points that underlie the likelihood of success. It is up to the infected countries to gather and modulate these experiences in order to adapt them successfully to local epidemiological conditions.

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CLINICAL AND PATHOLOGICAL FEATURES OF AFRICAN SWINE FEVER

F. Javier Salguero

United Kingdom Health Security Agency (UKHSA). Porton Down, Salisbury, U.K.

Corresponding author: F.J.Salguero (javier.salguero@phe.gov.uk)

African Swine Fever was first described in in East Africa at the beginning of the twentieth century as an acute disease characterised by high morbidity and mortality and fatal haemorrhages in imported domestic pigs from Europe.
ASF has caused outbreaks in numerous countries and it continues to be devastating nowadays for the porcine sector in those countries affected, and a massive threat for those free of the disease. ASF was confined to the African continent until 1957 when it reached Portugal via contaminated waste containing infected pork products that were used to feed local pigs. Even though, this outbreak was quickly controlled, ASF re-entered Portugal in 1960 and spread rapidly to Spain and produced sporadic outbreaks in several European countries, including Belgium, the Netherlands, Italy, Malta and France. The disease jumped to the Americas with sporadic outbreaks in Brazil, the Dominican Republic, Haiti and Cuba. ASF was eradicated from all these countries out of Africa, except the Italian island of Sardinia, where the disease has persisted since 1978. However, the disease continued to persist and spread within Africa and entered the Republic of Georgia in 2007 most likely via contaminated food used to feed domestic pigs sprerading rapidly within the Caucasian region and neighbouring countries. ASF continued to spread to the West, including European Union countries, mostly associated to wild boars, and to the East, with the disease causing abundant outbreaks and affecting dramatically the pork industry in China, and South East Asia.

The clinical presentation and the gross pathological lesions of ASF in domestic pigs (*Sus scrofa*) may vary depending on the virulence of the virus isolate, the route and dose of infection and host characteristics, including the immune status of the animal. ASFV isolates can be classified as highly virulent, moderately virulent and low virulent . The clinical courses observed in ASF in domestic pigs can be described as peracute (or hyperacute), acute, subacute or chronic.

The key features of the pathogenesis of the disease in domestic swine are a) a severe lymphoid depletion including lymphopenia and a state of immunodeficiency, and b) haemorrhages. However, African wild swine like bushpigs (*Potamochoerus larvatus*), red river hogs (*Potamochoerus porcus*) and warthogs (*Phacochoerus africanus*) can be infected by ASFV showing no clinical signs of disease and acting as natural reservoir hosts.

The hyperacute form of ASF is typical of highly virulent isolates and characterised by a very rapid clinical course, with high fever (up to 42°C), anorexia, lethargy and sometimes sudden death without signs of disease. This is often observed when the virus enters a naïve farm causing death of some animals before the explosion of clinical cases. Some animals can show respiratory distress due to the high fever, but no gross lesions are usually found at the post mortem examination.

The acute clinical form is caused by highly or moderately virulent isolates, and it is the typical course observed in naïve farms very quickly after the first fatal cases are reported. The clinical course is characterised by high fever, with temperatures of 40-42°C, lethargy, anorexia, and inactivity. Many affected animals show a centripetal cyanosis. Respiratory distress is usually observed associated with pulmonary oedema. Skin lesions are frequent, with presence of petechial haemorrhages or ecchymosis. Other clinical signs may include nasal discharges, sometimes stained with blood (epistaxis), vomiting and diarrhoea, that can be also blood-stained (melaena). Abortions may occur in pregnant sows and the mortality rates may reach up to 100% in affected farms within 7 days of the onset of the disease. At the post mortem examination, the most characteristic lesion

of acute ASF is the haemorrhagic splenomegaly. The second most important lesion described in acute ASF is a multifocal haemorrhagic lymphadenitis. The most affected lymph nodes are the gastrohepatic, renal and other abdominal lymph nodes as ileocaecal or mesenteric. Petechial haemorrhages are often observed in the kidney surface and at sectioning. Other lesions can also be observed, mostly haemorrhages in the mucosa or the serosa of other organs, as the large and small intestinethe epicardium or the urinary bladder.

The subacute clinical form is usually observed in animals infected by moderately virulent isolates, with similar clinical signs as those observed in acute ASF, although normally less marked. Affected pigs show moderate to high fever and the mortality rate ranges from 30 to 70%, with pigs dying at 7-20 after infection. The vascular changes, mostly haemorrhages and oedema, in the subacute form of the disease can be more intense than the acute form.

The chronic clinical form is caused by the infection of low virulence isolates and has been observed, quite infrequently, in the Iberian Peninsula and the Dominican Republic. It has been hypothesized that this low virulence isolates, and the associated chronic form, has evolved from ASFV isolates employed in early vaccine trials carried out in the Iberian Peninsula in the 1960s. The evolution of highly and moderately virulent isolates in other areas where the virus has been present for long periods of time has not produced this chronic form of the disease.
This clinical form is characterised by multifocal necrosis in the skin and arthritis, growth retardation emaciation, respiratory distress and abortion. No remarkable vascular changes are observed in the chronic form of ASF, and many observed lesions are associated with bacterial secondary infections, inducing fibrinous polyserositis, necrotic or chronic pneumonia, necrosis of the skin, tongue and tonsils.

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BRIEF HISTORY OF EPIZOOTICS BETWEEN 19TH AND 20TH CENTURIES

Ivo Zoccarato

Associazione Italiana di Storia della Medicina Veterinaria e della Mascalcia
Corresponding author: I. Zoccarato (ivzccrt@gmail.com) (segreteria.aismevem@unito.it)

The aim of this presentation is to recall, from an historical perspective, the most important epizootics that affected livestock and companion animals during the 19th and the first half of the 20th century. Fortunately, today, many of these diseases are unknown to young veterinarians. In fact, improved knowledge of the microbiology of pathogens, the availability of vaccines, strict rules of prevention and enforcement of stamping out, and the progressive availability of various drugs have made it possible to reduce the presence of the "great" diseases that ravaged livestock and pets in the past. Nevertheless, hitherto, the only two viral diseases eradicated worldwide have been human smallpox (1980) and rinderpest (2011). Communicable diseases, in particularly viral diseases, are not eradicated, and veterinarians must maintain a high level of surveillance. For instance, in order of time, the recent outbreak of African swine fever, in Italy, is the latest example of the great danger that an epizootic disease can provoke.

The need to combat infectious livestock diseases was one of the reasons that led to the foundation of veterinary schools. We should remember that already in the 18th century, when the first veterinary schools were founded including the one in Turin, in Europe about 200 million cattle were lost due to rinderpest (Bosticco and Pagano Toscano, 1984-1985). Still throughout the 19th century rinderpest will be the disease that will determine the highest mortality among cattle worldwide.

In Europe, rinderpest, known since ancient times and coming from the East, had spread inexorably accompanying the herds following the armies at war. It soon became endemic and, in June 1865, it appeared in England due to the commercial introduction of livestock in the London market, coming from the Baltic port of Tallinn. The disease, practically unknown in the British island, soon caused the loss of more than half a million heads of cattle (Leclainche,1955). In Europe, one of the last outbreak of rinderpest, immediately stopped, took place in 1920 in Antwerp, due to the transportation of some zebu coming from India and destined to Brazil. After this episode, it was decided to create the Office International des Epizooties. In Italy, during the 20th century there were two outbreaks, which were promptly solved: the first, in 1918, in the province of Vicenza due to the introduction of frozen beef from India and the second, in 1949, in the zoo of Rome where some antelopes from Somalia had been introduced. Cattle plague was the most important epizootic during the 19th and 20th centuries but not the only one. Many other epizootics, less lethal than rinderpest but no less serious from an economic point of view, ravaged livestock. We can remember foot and mouth disease (FMD); the contagious bovine pleuro-pneumonia (CBPP); the sheep pox, that spread from the Mediterranean to the North and Baltic Sea; the anthrax and the symptomatic anthrax; the glanders, endemic across military cavalry and in the agricultural areas; the problems in the pork production with the outbreaks of erysipelas and the “red diseases”, the rabies which represented a continuous alarm for the people both in town and in the countryside; the distemper, which, until the development of a vaccine around 1930s, caused the death of thousands of puppies and many others diseases.

FMD, until the introduction of the vaccine in the first half of the 20th century, was characterized by periodic waves of variable intensity and unpredictable duration and severity. Although present since ancient times, because of its relative benignity, its outbreaks began to be recorded only since the mid-nineteenth century, thanks to the awareness of the veterinary class. In Europe, the great FMD epidemics were observed between 1845-46, 1855-57, 1862, 1869, 1871-74, 1875-77, 1883-84, 1893-94 and the

damage was incalculable. In Italy, for a long time FMD was considered a contagious disease of little importance due to its mild course and almost no mortality. However, from 1898 the situation changed abruptly when FMD penetrated from France. In 1901, more than 180,000 cattle were infected and, despite the low mortality rate of about 5%, the economic damage was enormous: it was calculated that losses ranged from 50 to 150 Italian liras (about 500 euros in today's value) per infected animal, depending on the severity of the infection. There was a widespread belief among veterinarians and livestock producers that epizootics recurred every seven years (Sali, 2021). Unlike FMD, CPPB, which was present for much of the 19th century mainly in central Europe and northern Italy, spread slowly through the breeding trade. Until the end of the 18th century it was only present in the area coinciding with Bavaria, Wurttemberg, Switzerland, Tyrol, Franche-Comté and northern Italy. By the increasing of livestock trade and the practice of genetic improvement by the crossbreeding, the contagion seems to have spread from the canton of Vaud in Switzerland at the end of 18th century and, by 1852, all of Europe had been invaded. The damage caused by the CPPB was considerable; for example in England it is estimated that, until 1860, losses amounted to over 187,000 heads and between 1870 and 1890 another 70,000 heads were culled. In France, in the North department, between 1827 and 1846, losses exceeded 210,000 animals.

Among the epizootics that interested equines, it is worth mentioning glanders, a disease known since ancient times. In the mid-eighteenth century the "first veterinarians" were divided into two opposing factions: on the one hand those who believed that the glanders was spread by contagion and on the other those who denied it. The School of Lyon was contagionist, while that of Alfort sided with the deniers. It was necessary to arrive until the mid-1800s to finally recognize the infectiousness of the disease that, in addition to being contagious for equids, was also contagious for humans. In Italy, approximatively 10% of military horses were infected with glanders. The French army, during the First World War had about 55,000 heads, including horses and mules, affected by the disease (Milhaud, 2017).

It is not possible in this context to discuss all the various epizootic diseases that occurred during the 19th and 20th centuries, but we must briefly mention African swine fever (ASF). The adjective "African" comes from the fact that the first report, just a century ago in 1921, was made in Kenya by Eustace Montgomery (Calisher, 2021) who reported data from a series of observations conducted between 1909 and 1915: 98.9% of affected pigs died. The first outbreak in Europe took place in 1957 near Lisbon and from there, in the next years, it first penetrated Spain (1960), then France (1964) and then Italy (1967) before appearing in Belgium (1985) and the Netherlands (1986) in the mid-1980s. Since 1995 in the Iberian Peninsula there have been no more reports and the disease, with enormous efforts, is eradicated. In Italy the situation is particular, the disease is endemic in Sardinia, where it appeared in 1978.

According to Dunlop and Williams (1996), the second half of 19th century can be defined as the “golden age of microbiology”. In this period, that we can define as heroic, veterinarians have been able to develop a particular attention and sensitivity not only to the knowledge of communicable diseases of animals, but also to the ability to manage from the organizational point of view in the surveillance and in the fight against the spread of the diseases themselves. Now we can consider this skill as a legacy that allows veterinarians to play a fundamental role in the context of "one health".

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PANDEMIC IMPACT ON VM PROFESSION

Carla Bernasconi Medico Veterinario (1),

(1) Consigliere FNOVI e Presidente Ordine Medici Veterinari di Milano

Corresponding author: C. Bernasconi (carla.bernasconi@fnovi.it)

In the last 15 years Veterinary Medicine of pets has considerably evolved, due also to increasing social sensibility towards animals.

This development has obviously had to be accompanied by scientific and cultural progress, but also by all non-medical aspects of management, digitalization, communication, and organization. The so-called soft skills.

Pandemic gave an important acceleration to this process, we were faced with new and unexpected scenarios, to be faced in a few days; then the passing of months allowed us to better evaluate and think about a new organization and management. What happened has to be seen as a growth opportunity, most of our certainties and established habits were reset in a few days, and we had to reinvent a way of living and working.

We understood the need to prepare for change by quickly changing approach and response capabilities and solutions.

Today everyone has understood the need / opportunity to change healthcare delivery system, making greater use of work planning, also through aggregate offers (health plans, prophylaxis, etc.), improving quality processes within facilities.

Telemedicine use also, in its possible forms, has great development potential; for this reason, FNOVI drawn up guidelines subjected to the scrutiny of the Ministry of Health. Tele-reporting is a reality today. REV also has certainly given a great help, but we must not forget that prescription is a Medical Act and as such it must be managed.

Given that skills and continuous updating are now more necessary than ever, it becomes essential to learn communication techniques, organizational and managerial notions.

VMs must understand that there is no going back, and that Veterinary Facilities must be managed as companies that carry out activities of high social value, as they are not only concerned with animal health but also with public health and environmental health; through care and disease prevention and with responsible use of drugs, primarily antibiotics.

On post-pandemic many studies, surveys and evaluations were run, in our sector also, and various keys of reading or analysis of particular aspects were given.

A survey conducted by SWG for Federchimica Aisa – National Association of Animal Health Companies – presented public opinion data on relationship between pandemic and animals. For 85% of respondents there is a clear link between human health, animal welfare and environment. For 62% animal health counts "a lot": on a 1 to 10 scale, it counts between 8 and 10. Combination of humans and animals, and poor farms' health control, for 76% of respondents contributed to the pandemic. Alerts on future animal-derived pandemics are high for 49% of respondents.

Reducing pollution and improving farms' health are the two factors on which more investment is required, although One Health concept turns out as unknown to over 80% of respondents.

When Italians are asked to try to hypothesize which could be the most urgent interventions to be implemented at the institutional level, 92% agree on the need to invest in reducing pollution, as well as in implementing significant practices to improve farmed animals' life quality (92%). Percentages of those who consider essential to intervene to protect biodiversity are high also (91%); finally, the need to improve animals' care capacity, livestock and domestic, developing new veterinary drugs (88%).

Veterinary Doctors of the future will need to be in possession of a "toolbox", that is to say a wealth of knowledge and operational skills to allow them to go and fill the gaps left by others, and at the same time succeed in establishing themselves in present new spaces and in those that will be created in coming years.

Exposure to animal suffering, adult attachment styles, and professional quality of life in a sample of Italian veterinarians

Alessandro Musetti (1), Alessandro Schianchi (2), Luca Caricati (1), Tommaso Manari (1), Adriano Schimmenti (3)*

1 Department of Humanities, Social Sciences and Cultural Industries, University of Parma, Parma, Italy, 2 Private Practice, Fornovo di Taro, Parma, Italy, 3 Faculty of Human and Social Sciences, UKE - Kore University of Enna, Cittadella Universitaria, Enna, Italy)

Corresponding author: A. Musetti (alessandro.musetti@unipr.it)

Contextual and individual risk factors of veterinarians' professional quality of life are being debated. Research suggests that attachment styles are relevant predictors of professional quality of life; however, their role in work-related well-being of veterinarians is yet to be ascertained. In the present study, self-report measures on exposure to animal suffering, adult attachment styles, and professional quality of life were administered to 1,445 Italian veterinarians (70% females) aged 24 to 74 years old; sociodemographic information and information on workload were also collected. Female gender, higher levels of ordinary work- load, on-call hours per week, exposure to animal suffering, together with fearful and preoc- cupied attachment styles were significantly associated with lower levels of veterinarians' quality of life. This suggests that work-related factors may combine with individual psychological features in promoting or disadvantaging the professional quality of life of veterinarians. Implications of these findings for promoting veterinarians' quality of life and directions for future research are discussed.

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Challenging suicide, burnout, and depression among veterinary practitioners and students: text mining and topics modelling analysis of the scientific literature

Marta Brscic1, Barbara Contiero1, Alessandro Schianchi2 and Cristina Marogna3*

[1]Department of Animal Medicine, Production and Health (MAPS), University of Padova, Agripolis - Viale dell'Università 16, 35020 Legnaro, PD, Italy. [2]Fornovo di Taro, Italy. [3]Department of Philosophy, Sociology, Education and Applied Psychology (FISPPA), University of Padova, Piazza Capitaniato 3, 35139 Padova, PD, Italy.

Correspondence: marta.brscic@unipd.it

Background: Worldwide, veterinary practitioners and students are reported to be at higher risk of suicide, burnout, and depression compared to other occupational groups. The aim of the current study was to apply text mining and topic modelling analysis on scientific literature regarding suicide, burnout, and depression among veterinary practitioners and students to extract meaningful and synthetic information. These statistical approaches can be used to comprehend more in deep the phenomena involving veterinarians and veterinary students and to suggest the potential changes needed in admission to veterinary school, veterinary curricula, and post-graduation initiatives as preventive actions.

Results: A systematic search protocol was set up to identify scientific literature that published on the topic from 1985 to 2019. Two-hundred-eleven records were selected with abstracts/texts submitted to text mining and topic modelling analysis. Student, stress, work, anim*, and euthanasia resulted the most frequent terms. Topics modelling allowed to differentiate groups of words and papers in 3 areas of interest: 1) students' difficulties encountered during their studies that increase stress and anxiety impairing their psychological health; 2) exposure to death and euthanasia as risk factor for mental health; and 3) need of support among those providing medical and health care, and of supportive group work to cope with such profession.

Conclusion: Based on the most frequent words included in the clouds and on the contents of the papers clusterised in them, some suggestions are interfered. It is emphasized that the veterinary curricula should include courses that prepare them early to deal with animal death and post-death grief of pet owners, to handle ethical dilemmas and moral stressors, to communicate with clients and staff members, to work in team, to balance work- family life and to promote individual and team resources. Specific courses for veterinary practitioners could keep them updated on their new roles and ways to handle them among functioning as potential feedbacks to monitor their psychological wellbeing.

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TWO-DIMENSIONAL AND DOPPLER ECHOCARDIOGRAPHIC EVALUATION IN TWENTY-ONE HEALTHY PYTHON REGIUS

Mara Bagardi, Martina Manfredi, Stefano Romussi, Paola Giuseppina Brambilla

Department of Veterinary Medicine, University of Milan, Milano, Italy.
Corresponding author: M. Bagardi (mara.bagardi@unimi.it)

In the last few decades, the popularity of snakes (ball pythons in particular) as pets has increased worldwide. From being the prerogative of a few reptile enthusiasts, snakes have come to be considered by many as members of the family, such as with dogs and cats. This has led to a higher demand for high-quality veterinary care for snakes. It is the duty of the reptile specialist to provide it and to bridge this gap in small animal practice. Specifically, echocardiography is an integral part of the cardiac evaluation of humans and domestic animals, but its use is still rarely reported in reptiles, and little emphasis has been placed on ultrasonographic examination of the reptilian heart (Snyder et al., 1999). Echocardiographic evaluation is a diagnostic tool for the in vivo diagnosis of heart diseases. Specific and unique anatomical characteristics of the ophidian heart, such as the single ventricular cavity, a tubular sinus venosus opening into the right atrium, the presence of three arterial trunks and extreme mobility in the coelomic cavity during the cardiac cycle, directly affect echocardiographic examination. Twenty-one awake, healthy ball pythons (*Python regius*), average age 6.5 years, mean weight 1.39±0.46 kg and mean length (nose-to-cloaca) 110.78±14.36 cm were analysed based on guidelines for performing echocardiographic examinations. The population was 36.8% female and 63.2% male and included subjects of any morph. To ensure the standardization of the physiological parameters, the snakes were individually housed in 120×80×60 cm glass cages with water ad libitum and shelter for 7 days at a standard temperature (26–30°C) and controlled humidity (50%–70%). Their diet consisted of thawed or freshly killed prey (mice and rats) provided once a month. The snakes had been fasting for at least 1 month prior to measurement to assure that they were in a post-absorptive condition.

The docile nature of these animals allowed to carry out all echocardiographic examinations without difficulty. Even in the smallest subjects, it was possible to evaluate all the investigated cardiac structures. Imaging in the sagittal plane demonstrated the caudal vena cava, sinus venosus valve (SVV) and right atrium and the various portions of the ventricle, horizontal septum, left aortic arch and pulmonary artery. Transverse imaging depicted the spatial relationship of the left and right aortic arches, the pulmonary artery and the horizontal septum. Basic knowledge of cardiac blood flow in reptiles is necessary to understand the echocardiographic anatomy. The flow of the arterial trunks and SVV was analysed using pulsed-wave Doppler based on the approach used for humans and companion mammals. The walls and diameters of the cavum arteriosum, cavum venosum and cavum pulmonale were also evaluated. Understanding the normal anatomy and flow is mandatory to improve the ultrasonographer's ability to identify cardiac abnormalities such as congenital heart defects, endocarditis, cardiac neoplasia, thrombi and cardiomyopathy. The repeatability obtained in the measurements allows to propose these data as reference values for *P. regius*. In conclusion, echocardiography is a useful, non-invasive diagnostic tool to evaluate the anatomy of the ophidian heart and can contribute to the development of cardiology in reptiles in general.

[1] Schilliger et al. Proposed standardization of the two-dimensional echocardiographic examination in snakes. Journal of Herpetological Medicine and Surgery, 16, 76–87. 2006.

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INNOVATIVE ASPECTS ON REPTILES REPRODUCTION

Francesco Di Ianni

Università degli Studi di Parma, Dipartimento di Scienze medico Veterinarie.

The reproductive strategies of the reptiles are numerous and well diversified. Professional reptile breeding is becoming increasingly important, and it is therefore particularly useful to develop reliable techniques to monitor reproduction and gestation, maximizing the potential of the breeding itself. Some reptiles are oviparous, some viviparous and cases of parthenogenesis have been demonstrated. To describe these reproductive mechanisms behavioural data were integrated with ultrasonography, a non-invasive diagnostic technique, minimizing animal stress. In reptile ultrasound, the presence of air trapped under the scales could cause artefacts. Most species of Boa, such as Boa constrictor sp., are viviparous, with females giving birth to live young. In the female's body, individual membranes protect each embryo by regulating temperatures. Once born, the offspring must break these membranes and get out of them. Viviparity has evolved many times within squamate reptiles, mostly in cool climates, but the selective advantages of the uterine retention of eggs remain obscure. In ovoviviparous females, at the beginning of the breeding season, multiple follicles develop into Graafian follicles along with vitellogenesis to store yolk in ova. After ovulation, the ova pass along through the infundibulum into the oviduct, and all ova are fertilized. In viviparous snakes, the embryo will develop in its individual foetal membrane within the oviduct. Follicle-stimulating hormone (FSH) stimulates follicle growth and leads to production of vitellogenesis hormones, while luteinizing hormone (LH) stimulates ovulation and changes follicles to the corpus luteum to produce progesterone. The independent origins of placentation have resulted in a variety of placental morphologies indifferent taxa, ranging from simple apposition of foetal and maternal tissues to endotheliochorial implantation that is homoplasious with mammalian placentation. In viviparous squamates, the functions of extraembryonic membranes found in oviparous species are retained or even enhanced. In species with simple placentae, the chorioallantois is remains a highly vascularized membrane that surrounds the embryonic hemisphere of the egg. Chorioallantoic capillaries are closely aligned with maternal capillaries, so gas exchange is still thought to be its primary function in viviparous squamates, although the transport of inorganic ions and histotrophic transfer are also functions. Ultrasonography is a very important diagnostic tool in any veterinary field, with many potential uses in reptile medicine [1]. Some studies have been performed to monitor the reproductive activity of oviparous snakes. These studies confirm the different phases of a female ball pythons reproductive cycle identified by ultrasonography. This technique has also proved to be important to early recognize the presence of slugs. Furthermore, our finding highlights the utility of Colour-Doppler in monitoring the embryo viability, evaluating vascularization and early heart activity. This study suggests that the evaluation of sex steroid faecal metabolites levels, particularly progesterone, may provide further information on the reproductive cycle of females of this species. We believe that the female reproductive cycle of royal pythons kept in captivity can be schematized. In conclusion, the association between ultrasonography and the analysis of sex hormone faecal metabolites allows us to monitor the reproductive activity of female royal pythons bred in captivity. Detection of sex steroid levels in the serum is the most direct method of data collection; however, blood sampling can be difficult due to venous access and animal stress. Therefore, non-invasive detection methods were used, including the analysis of faecal and urinary hormone metabolites. However, biological and technical factors related to the sampling and analysis (such as sex, age, and reproductive status of the animal, sample storage and transportation, the representativeness of the sub-sample selected for extraction and analysis if is not possible to collect the entire faecal mass) could complicate the interpretation of these results and must be considered and possibly standardized [2]. Parthenogenesis, or the production of embryos from unfertilized eggs, is a form of reproduction that occurs predominantly in different taxa of invertebrates where various types of such reproduction are recognized. Some animal species consist exclusively, or nearly so, of females that reproduce by parthenogenesis generation after generation; others tend to alternate between parthenogenic and sexual generations or always have the ability to reproduce in both ways. Among vertebrates, parthenogenesis is a rare phenomenon and true parthenogenetic

lineages can only be found in reptiles. Except for a single species which consists entirely of parthenogenetic females, snakes show the greatest amount of cases where parthenogenesis occurs occasionally in species that normally reproduce sexually (facultative or occasional parthenogenesis). Although some cases of snakes suspected of parthenogenetic reproduction have been reported in the past, the first documentation of facultative parthenogenesis (FP) occurred in 1997, following the application of molecular methods for parentage analyses. Since then, it has been described in different species of both viviparous and oviparous snakes. These cases have received a lot of scientific attention because, on the one hand, they can allow a better understanding of the general mechanisms that lead to parthenogenesis, on the other hand they can have important consequences for the breeding programs of reptiles. However, the evolutionary and adaptive role of FP in snakes, as well as its real diffusion in nature, requires further investigation in future studies [3].

IMMUNE RESPONSE IN UNCONVENTIONAL PETS

Elisabetta Razzuoli

Istituto Zooprofilattico Sperimentale del Piemonte, Liguria e Valle d'Aosta. Centro di referenza per l'Oncologia Veterinaria e Comparata (CEROVEC)- Genova.

Corresponding author: E Razzuoli (elisabetta.razzuoli@izsto.it)

In recent decades, news species of pets have emerged in Italy, including rodents, lagomorphs, birds and reptiles. The interest of numerous enthusiasts has in particular shed light on reptiles. Their diffusion in our home as pets has determined the need to study more accurately the pathologies of these animals, which are often due to poor management. In fact, the importance of the immune response also in the development of pathologies related to mismanagement is well documented. In this work we will therefore try to summarize the peculiarities of the immune system in these animals (1). The immune system is constituted by organs, cells, and molecules that are involved in the defense activity versus pathogens and transformed cells (tumors). The evolution has finely shaped this system producing different levels of organization, spanning from the simple and primitive systems of the elementary invertebrate organisms, to the complex and specialized systems of higher vertebrates. Reptiles represent a key link between fishes and amphibians, and birds and mammals. For this reason, a greater understanding of their immunity will provide important insights into the evolutionary history of vertebrate immunity and in eco-immunology. Reptile immunity is complex and involves innate, cell-mediated and humoral compartments (2). The innate system includes the complement system, non-specific leukocytes and antimicrobial peptides. Usually, the responses given by this first line are stronger than those of mammals. However, the lack of appropriate reagents, resulted in a big gap on the knowledge about the cell-mediated and humoral responses in this animals (1). It is very important to remember that cell-mediated response (like T cell proliferation and allograft rejection) is significantly affected by season, so any study on the reptile immune system needs to take season into account (3). As regards humoral response, after immunization in reptiles, antibody titers increase little, if at all, and do not increase in binding affinity. Moreover, the recent identification of two additional isotypes of immunoglobulins in leopard gecko highlights the lack of information about the humoral responses, of reptiles (2). To date many opportunities exist to enhance our understanding of reptilian immunity, thereby increasing our knowledge of vertebrate immunity and the evolution of this complex and diverse system, as well as provide significant information to the growing field of eco-immunology. In particular, reagent development will be critical in allowing more detailed characterization of non-mammalian immunity, such as cell markers to differentiate T cell types and cytokines. Another area of great interest is the investigation of immune function in natural populations, including a suite of biotic and abiotic characters that have the potential to affect immunity (e.g. pathogens, pollutants, temperature). Finally, as we explore the evolution of the vertebrate immune system, it is important to consider how life history may influence resource allocation patterns. Animals that are constrained to maintain their body temperature physiologically (i.e. endotherms) will probably face very different resource demands than those that are not so constrained, and this may affect how these groups allocate resources to innate versus adaptive immune compartments (3).

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TWO-DIMENSIONAL AND DOPPLER ECHOCARDIOGRAPHIC EVALUATION IN TWENTY-ONE HEALTHY PYTHON REGIUS

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Department of Veterinary Medicine,University of Milan, Milano, Italy.
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ECOTOXICOLOGY OF LARGE MARINE VERTEBRATES: MAIN ADVERSE EFFECTS

Annalisa Zaccaroni

Università di Bologna, Dipartimento di Scienze Mediche Veterinarie
Corresponding author: A. Zaccaroni (annalisa.zaccaroni@unibo.it)

The large marine Vertebrates are considered excellent environmental indicators because they live in an environment that is the final sink of all contaminants regardless of their place of origin. Despite the large volume of water that characterizes these water masses, continuous discharge of pollutants has caused an increase in concentrations such that even in the oceans, we begin to see the adverse effects.

The large marine Vertebrates are chosen as monitors of their living environment because they are generally large species with reasonably long life, generally live close to the coast, and thus are exposed to environmental stressors and experience bioaccumulation and biomagnification. Therefore, they are excellent indicators of the degree of contamination of large bodies of water such as the Mediterranean Sea.

Age, diet, and position along the food chain affect the ability to accumulate; in particular, the more the species is at the top of the trophic chain, the greater its ability to accumulate and its ability to biomagnify.

As in many other terrestrial species, important factors that affect the accumulation and levels found in marine organisms are the age and sex: there is a tendency to increase concentrations with the age of the animal (due to direct accumulation of contaminants with the diet and from the environment) and are often observed (even if it is not the rule) differences between sexes, with females usually having levels of contaminants higher than males, even of similar age.

This is because reproduction determines, particularly at the first reproductive event, a release of high quantities of contaminants that are passed to the offspring, whether they are oviparous or viviparous species, that determines a strong removal of pollutants from the mothers, thus reducing the risks for the female. On the other hand, a greatest risk is run by the unborn exposed to very high concentrations of contaminants that may also be responsible, according to some authors, for the high percentage of abortions observed, for example, in marine mammals primiparous females.

Aquatic species are exposed to a very high number of contaminants. Among these, the most studied ones are probably trace elements and POPs (Persistent Organic Compounds) and “new” compounds like phthalates or microplastics and the compounds that they carry by adsorption.

All these molecules have effects at various levels of the body. They can induce endocrine disruption, liver injury, alteration of calcium metabolism, alteration and damage to the reproductive system, immunosuppression, and induction of tumors. Undoubtedly among these effects, those most studied are endocrine disruption and immunotoxicity because they have had very strong impacts on populations of large marine Vertebrates, particularly marine mammals.

The phenomena of endocrine disruption involve oviparous species and marine mammals. It determines important alterations in the endocrine balance, mainly the reproductive and thyroid system. An alteration of the reproductive ax is easily detectable as it implies impairment of the reproductive capacity, causing an alteration of population structure and reducing population numerosity. An impairment of the thyroid axis can delay the progression of embryonic development or alter phenomena such as molting, which is essential for species such as pinnipeds, or metamorphosis.

Numerous contaminants among those listed can alter both the reproductive and thyroid axes, causing severe damage to populations affected by pollution. Among the most studied species from this point of view, we have the polar bear in which the effects on the productive axis and on the thyroid have been studied, finding in the first case even phenomena of pseudohermaphroditism in female polar bear cubs that had secondary male sexual characters and in the second case a strong alteration of thyroid hormone levels also responsible of altered reproduction and a reduced adaptation to climate change.

Significant is also the effect on the immune system as alterations of this system can cause mass mortalities in entire populations of animals, as seen in the 90s in harbor seal populations. These populations experienced an outbreak of morbillivirus that led to death a very high number of animals. The studies carried out in the period were aimed at understanding why there was this episode and allowed to identify in dead animals for morbillivirus also high concentrations of PCBs. Studies carried out in vitro and in vivo showed how these levels of PCBs were able to inhibit the activity of the immune system of harbor seals, therefore exposing a very high number of animals to a greater risk of infection by morbillivirus and subsequent death, for the inability to counteract the virus itself. Similar observations were then carried out in other marine mammals, such as *Stenella ceruleoalba* subject to an outbreak of Morbillivirus, finding the same situation: high levels of contaminants and reduced activity of the immune system point.

In the presentation a practical example will be used, focusing on the effects of oil in marine mammals, as the latter summarizes and includes all the adverse effects that contaminants can exert in large marine vertebrates.

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INNOVATIVE APPROACH ON THE ROLE OF THE VETERINARIAN IN THE HEALTH MANAGEMENT OF GAME ANIMALS

Vincenzo Veneziano, Raffaele Marrone

Università degli Studi di Napoli Federico II, Dipartimento di Medicina Veterinaria e Produzioni Animali.
Corresponding author: V. Veneziano (vincenzo.veneziano@unina.it)

Game animals are any wildlife hunted for animal products (mainly meat), for recreation ("sporting"), or for trophies.

The species of animals hunted as game varies in different parts of the world and by different local jurisdictions, though most are terrestrial mammals and birds. In Italy the most common big game mammals are wild boar (*Sus scrofa*) and roe deer (*Capreolus capreolus*). The wild boar in Europe have been growing exponentially in both size and distribution range, in Italy this ungulate is widely distributed throughout the national territory. The roe deer is the most widespread wild ungulate in Europe, in Italy it is permanently present in the northern and central regions and is emerging in the southern territory [1, 2].

The abundance of wild ruminants has favored the increase of pathogens and their circulation between domestic and wild animals. Some pathogens can increase their virulence and extend the range of competent hosts through spill-over. In this way, numerous pathogens can infect more hosts and therefore can cause an outbreak of infectious diseases in wildlife, livestock and humans [3]. Domestic pigs and wild boar share most diseases. Notably the wild boar may represent a reservoir or play a crucial role in the transmission of many diseases in the livestock-wildlife and human interface such toxoplasmosis, trichinellosis, brucellosis, tuberculosis, salmonellosis, African and classical swine fever, Aujeszky's disease and foot and mouth disease [4].

A higher frequency of emerging diseases, which present pandemic risks, accelerated biodiversity loss and understanding that disease emergence is driven by the nature of interactions between humans, animals, and the environment highlights an urgent need to reinforce One Health strategies. Wildlife health requires investment and attention. The veterinarians play a main role in the wildlife value chain, managing the risk of disease emergence and protecting wildlife health. The National Veterinary Service can promote early detection of diseases in wildlife through surveillance systems.

Another very important aspect is related the consumption of wild meat, in particular of boar. The use of game meat as a food source is currently a growing trend in Italy. These products have strong and historic ties with cultural and culinary tradition, but are also appreciated for their sensory and nutritional characteristics. For example, the total consumption of wild ungulate meat in some Italian regions rises to significant levels, especially for hunters' families (1.0–4.0 kg per capita/year) [5]. Wild boar carcass contamination is very variable because it depends on natural microbial population on the skin and in the digestive tract, hunting hygiene and slaughtering process [6]. Due to the request of wild meat, its optimal nutritional characteristics [5] and the feasibility of a microbiologically safe production [7], is undeniable that consumers would accept to pay for game meat. However, a major contributor to the supply of theese products is hunting. The European Regulations (EC) No. 178/2002, No. 853/2004 and No. 854/2004 establish that hunters are responsible for wild game meat traceability and safety for human consumption [8]. The hunting activity cannot be compared with conventional food production in terms of standardization of processes; in this sense it is objectively difficult for hunters to comply with hygienic requirements for the food trade unless strong actions are introduced for their training by the veterinarians. According to EU Regulation 853/2004, meat can be placed on the market only if a "trained person" has evaluated the absence of macroscopic lesions on site and the bodies and viscera of hunted animals are transported to an Approved Game-Handling Establishment as soon as possible after killing. Hunters training by the veterinarian certainly plays a very important role in this sector also because until hunters can prove the safety of the meat they produce, it is to be expected that food manufacturers will not participate in the supply chain because of the reputational risks they would have to assume when buying unconventional meat.

It is crucial the interaction with stakeholders (physician, hunters, hikers), as well as a multisectoral collaboration between the Universities and the net of Istituti Zooprofilattici to optimize and harmonize the different skills.

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FROM WILD TO DOMESTIC PHENOTYPE IN THE OVIS GENRE

Salvatore Naitana (1), Paolo Mereu (2), Monica Pirastru (2), Mario Barbato (3) Laura Manca (2), Giovanni Leoni (1)

(1) Department of Veterinary Medicine, University of Sassari. (2) Department of Biomedical Science, University of Sassari; (3) Zootecny Institut, University Cattolica del Sacro Cuore.

Corresponding author: S. Naitana (snaitana@uniss.it)

Currently, domestic sheep breeds are characterized by 5 different haplogroups (APGs), from A to E, each deriving from a wild ancestor (1). It seems likely that the first phase the domestication process started with the spread of the idea that keeping local animals in enclosures guaranteed safe food and eliminated the occasional nature of hunting. Among domestic sheep, APG B is the most widespread, evolved from an ancestor of the European mouflon, currently living in Corsica and Sardinia, where the oldest known mouflon genotype has been identified. Before the domestication process, the primary product searched by humans was meat, requiring the elimination of the animal. After that, observations of the animal characteristics lead to a more accurate choice of their use to search for secondary products. This has favored an action of selection of favorable characters in the wild species in relation to the phenotypic aspects such as the color of the coat, moult and the length of the tail. These characters, resulting in part of the mutation / selection processes, have been widely spread from the original areas to new settlements through the use of males, carrying the positive phenotypic characters. The coat color was regulated from the expression of the ASIP gene (Agouti signaling protein). The presence of a double allele in domestic sheep determines the synthesis of pheomelanin which, by blocking the hormonal interaction of α-MSH with the MC1R receptor, is responsible for the clear coat; on the contrary, a single copy expression of the ASIP allele produces eumelanin which characterize a dark phenotype, typical of the mouflon coat. The coat of mouflon consists of a layer of long, coarse, medullated fibers of variable diameter (Primary), which cover an undercoat of shorter, finer, non-medullated fibers (Secondary). Both fibers undergo annual growth and moulting cycles starting from the belly and neck regions. The mutation/selection led to the prevalence of secondary fibers over the primary ones, giving rise to the continuous growth fleece. The fleece was shorn by recovering the wool, which becomes the royal symbol instead of leather and its processing became prerogative of women's such as the processing of cotton wool. Ovine can express 4 different types of tail: short and thin, typical of the mouflon; short and fat, long and fat, long and short expressed by domestic sheep. The fat tail, preferred in some regions, would be determined by the expression of the BMP2 and PGDFD genes. The length of the tail is under the control of the TBXT gene: in the domestic sheep it determines the presence of 22-24 coccygeal vertebrae while in the mouflon it determines a shorter tail, with only 11 vertebrae. In the domestic ovine-mouflon hybrids of the first generation, an intermediate phenotype of the three previously listed is observed which, in subsequent generations, leads back to those of the backcross species. In relation to this, in the actions of reintroduction and recolonization, it is essential to carry out preventive genetic analyses to avoid restocking with animals in which there are signs of introgression of the domestic component (2). (Supported by Fondo di Ateneo per la ricerca 2020-Naitana).

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PATHOLOGY DUE TO HAEMOPARASITES IN AVIFAUNA

Frine Eleonora Scaglione

Università degli Studi di Torino, Dipartimento di Scienze Veterinarie.
Corresponding author: F.E. Scaglione (frineeleonora.scaglione@unito.it)

Hemosporidia are a heterogeneous and cosmopolitan group of obligate parasites, having amphibians, reptiles, birds and mammals as hosts, and using some bloodsucking insects as vectors. Malaria is one of the best known haemosporidioses and it is considered a particularly important parasitic disease in tropical and subtropical countries, where it affects several million people every year (Snow and Omumbo, 2006).

Malaria is an ancient disease, and the first evidence of this pathology is found in Chinese documents from 2700 BC, in clay tablets from 2000 BC in Mesopotamia and in the Egyptian papyri of 1570 BC.

The first description of the clinical picture dates back to the ancient Greeks: Homer, Empedocles of Agrigento and Hippocrates described the typical intermittent fever (Cox, 2010) and associated this symptomatology to the proximity to the marshes (Manson-Bahr, 1961).

The etymology of "malaria" derives from the Italian term "mal 'aria", that is unhealthy air, also called "paludismo", a term already used in the eighteenth century to describe a fever, often fatal, which appeared only in summer (Cox, 2010) and due to noxious vapors from marshy soils.

For over 2,500 years, malaria has been thought to be transmitted by miasmas. The discovery of the true causes and dynamics of transmission was very complicated and it was the result of numerous researches conducted on human and animal models, in particular, on birds (Cox, 2010).

Avian malaria is a common disease transmitted by bloodsucking insects of birds, caused by parasitic protozoa of the genus *Plasmodium*. However, the same term also identifies haemoparasitosis due to the genera *Haemoproteus* and *Leucocytozoon*. These three genera, although they use different vectors, are closely related to each other and have similar morphological and developmental characteristics.

Plasmodium avian species have played a fundamental role as a model for human malaria studies, since they were the first to be recognized as intra-red cell parasites (Danilewsky, 1889). In fact, the main studies in the field of human malariology have been carried out on the basis of discoveries obtained on bird parasites (Hewitt, 1940; Garnham, 1966; Valkiūnas, 2005). Also important were Danilewsky's (1889) first descriptions of the anatomo-pathological lesions characteristic of malaria in birds, the discovery of *P. relictum* transmission via the mosquito by Sir Ronald Ross (1898) (Bynum, 1998), the discovery of the exoerythrocytic merogony of *P. elongatum* in the cells of the reticulo-endothelial system of birds (Raffaele, 1934), and the development of the theory of premunition or resistance to reinfection conferred by chronic malarial infection in avian hosts (Sergent and Sergent, 1956).

Italy as a whole constitutes a paradigm of the utmost importance for a wide range of species and vast contingents of migrants, who are confronted with overcoming the ecological barrier represented by the Mediterranean basin. The Alpine chain represents an ecological barrier which notoriously shapes the migration directions followed by species widely distributed in Europe (Berthold, 1996). Many birds avoid crossing it directly, channeling themselves along northern Italy to follow an autumn route with a strong east-west direction. For birds engaged in overcoming extended sea stretches (for example those encountered in the Tyrrhenian Sea), the system of the Italian islands constitutes a network of important resting opportunities, leading to high concentrations of birds in sometimes very restricted territorial areas. For migratory species that are primarily based on gliding, areas of particular importance for crossing the Mediterranean are represented not only by the coast lines, but also by the Strait of Messina, the Strait of Sicily and a series of Alpine and Apennine passes.

In the last 10 years our research group, in cooperation with many other authors, tried to improve the knowledge about the presence of avian malaria in Italy and to analyze the effects of this parasitosis on the affected populations.

In a study conducted on piedmontese pigeons, without clinical signs or macroscopical lesion, out of 51 animals, 15 were positive for *Haemoproteus/Plasmodium* spp. and 8 for *Leucocytozoon* spp., and DNA sequencing of

Leucocytozoon spp. showed six different lineages, and one of *Haemoproteus* and *Plasmodium*, respectively (Scaglione et al., 2015).

A further research conducted in hooded crows showed that neither macroscopical nor histological lesions were present, and out of 47 crows tested by nested PCR, 31 (59.6%) were found positive for *Haemoproteus/Plasmodium* spp., and 46 (97.9%) for *Leucocytozoon* spp. (Scaglione et al., 2016).

Nine raptorial birds investigated at the Pombia Safaripark (Northwest Italy) showed loss of stamina, developing listlessness, anorexia and regurgitation. Within one month three animals died due to *Plasmodium* infection and all other raptorial birds were treated: clinical improvement was observed in all the birds, and blood smears made after one month resulted negative for parasites (Scaglione et al., 2016b).

A necropsy was conducted on a female grey-headed parrot (*Poicephalus robustus suahelicus*) that died following signs of depression, ruffled feathers and inappetence. Microscopic examination revealed the presence of hemoprotozoa in the liver and nested PCR identified *Leucocytozoon* species (Galosi et al., 2019).

These studies confirm the widespread infections in birds which, however, show different manifestations of the infection, probably attributable to host-parasite co-evolution.

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HIGH PATHOGENIC AVIAN INFLUENZA IN WILD BIRDS: WHAT'S GOING ON?

Calogero Terregino

EU/OIE/National Reference Laboratory for Avian Influenza and Newcastle Disease; FAO Reference Centre for Animal Influenza and Newcastle Disease - Istituto Zooprofilattico Sperimentale delle Venezie.
Corresponding author: C. Terregino (cterregino@izsvenezie.it)

Avian influenza (AI) is a highly contagious and diffusive disease of birds, characterized by symptoms and lesions of varying severity in relation to the ability of the viral strain to replicate in specific organs and systems.

The disease has to be reported to national and international authorities and can have enormous economic consequences, not only for the high mortality rate that some strains are able to cause, but also for the measures to be taken in the field and for the trade restrictions imposed on the affected countries.

Considering the ability of some strains to infect humans, AI poses a major challenge also to public health and in some cases may lead to severe diseases.

In recent years, the host spectrum of High Pathogenic Avian Influenza (HPAI) viruses has progressively expanded to wild birds as a consequence of the HPAI H5N1 virus outbreaks in Asia of the early 2000s. Human activities and the massive involvement of domestic species reared outdoors (ducks in particular) had favoured the circulation of these viruses in the wild bird populations that had been in contact with infected poultry. Subsequently, there was a further evolution of AI eco-epidemiology and what was once considered an exception has now become a consolidated reality. Today we can say that wild birds are able to allow HPAI viruses to persist in nature for a certain length of time, giving them the opportunity to generate novel strains with new and unpredictable genotypic and phenotypic characteristics and to spread with dynamics comparable to those known for Low Pathogenicity Avian Influenza viruses. This new phenomenon was at the origin of the epidemics of HPAI from viruses of the H5 subtype (H5N8, H5N6, H5N5, H5N4, H5N3, H5N2, H5N1) introduced by wild birds in many countries of Asia, Europe and the USA in the years 2014-2022.

The persistence and continuous circulation of HPAI viruses in migratory and resident wild birds represent an extremely high risk for the poultry industry in Europe and requires an in-depth study of the epidemiology of these viruses in the wild in order to identify adequate surveillance, preventive and control strategies to safeguard such an important zootechnical sector.

EXTRACELLULAR VESICLES IN THE PATHOLOGY WORLD

B. Bussolati

(1) Department of Molecular Biotechnology and Health Sciences, University of Torino, Torino, Italy.
Corresponding author: B. Bussolati (benedetta.bussolati@unito.it)

Extracellular vesicles (EVs) are membranous vesicles containing active proteins, lipids, and different types of genetic material, including small non coding RNA species, related to the characteristics of the originating cell. Although the heterogeneous and apparently diverse nature of EVs, their central role in numerous physiological and pathological conditions is clearly defined and is sustained by a solid literature. EVs are highly present in biological fluids, from which they can be easily isolated. The complex cargo of EVs represents an import source of information, as it can reflect the type of originating cell, as well as its pathophysiologic status. In addition, EVs can be internalized by target cells in an archaic form of long-distance cell to cell communication, retained along the evolution in all the 4 kingdoms of life. EVs have brought great momentum to the non-invasive liquid biopsy procedure for the detection, characterization, and monitoring of different diseases. EVs appear to play a relevant role in kidney physiology, starting from the initial phase of nephrogenesis. Moreover, EVs present in the urine or in the circulation are considered to participate to the modulation of kidney functions and to the communication between different nephron districts. Urinary extracellular vesicles released mainly from resident kidney cells might provide an alternative tool for detection of kidney injury. In particular, EV surface markers or associated miRNAs are promising biomarkers for disease processes, like diabetic kidney disease, kidney transplant, and lupus nephritis. EVs are an important player in tumor progression and diffusion, and their characterization within the extracellular matrix will represent a tumor molecular fingerprint. The presentation will focus on strategies for EV detection in biological fluids as well as in tumor matrix, and on their potential diagnostic application for oncological and non-oncological pathologies.

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NEWBORN DOG EVALUATION: CLINICAL ASSESSMENT AT BIRTH

Jasmine Fusi

Università degli Studi di Milano, Dipartimento di Medicina Veterinaria e Scienze Animali.
Corresponding author: J. Fusi (jasmine.fusi@unimi.it)

In mammals, the event of birth represents one of the most challenging process for the fetus that must quickly cope with the transition to the new-born individual. To cope with this delicate process, a number of factors must be finely orchestrated, to allow the neonatal adaptation to the extra-uterine life and therefore, his or her survival. Among these factors, a pivotal role is played by the fetus itself. It should be, indeed, well-grown and developed, physically mature, without malformation or defects, with a correct weight and thus "prepared" for birth. At birth, the newborn should start, as a first step, the autonomous respiration, that will, in turn, activate a cascade of physiological changes allowing the proper functioning of many organs and systems of utmost importance for short- and long-term puppy survival. Given the importance of the event of birth, a prompt newborn clinical evaluation and a correct management with possible assistance is pivotal to assure the maximum survival rates, thus limiting the perinatal mortality. Differently to humans and other domestic species, in the dog, neonatology did not receive enough consideration until recent times, despite perinatal mortality faces impressive rates, with peaks of >20% [1]. The causes for such a high perinatal mortality are addressable to many factors, such as genetics, breeder's selection, maternal factors, reproductive-related issues, feto-neonatal issues, management and environment inadequacy, infective and non-infective diseases, and the process of birth. The dog is a polytocous species, with bitches giving usually birth to multiple puppies for each litter, so that the expulsion stage of parturition can lasts several hours, impairing the survival of those puppies suffering from hypoxia. Moreover, the strong morphological and physiologic differences among canine breeds leads also to an increased risk for dystocia in some breeds [2]. For all these reasons, the proper evaluation at birth, and consequent tailored assistance to the newborn puppies requiring medical support, represents the first-line for a more specialistic neonatological approach. However, the still limited knowledge about newborn dog's physiology and pathophysiology, the very small size of neonatal puppies and the almost absence of dedicated equipment for the newborn dog, restrict the possibilities of neonatal evaluation and assistance in comparison to humans or other domestic species. For this reason, beside the need for a continuous improving of research strictly focused on canine neonatology, the veterinarian assistance to newborn dogs must be recognized as part of the peripartal management, and must include the evaluation of those parameters proved to be related to newborn dog survival. Of course, the newborn clinical evaluation should be based, as much as possible, on quick and simple systems, performable on all the puppies of each litter, under different clinical settings, without the use of sophisticated instruments. However, beside the "basic" clinical approach, also some laboratory analyses can be helpful for the better evaluation of newborn puppies, especially for those requiring special assistance. The "basic" assessment should include the evaluation of viability, the assessment of possible malformations, and the measurement of temperature and birthweight, while the first-line laboratory analysis should include the assessment of asphyxia/mixed acidosis and hypoglycemia through blood gas analysis. Viability in newborns can be assessed by the Apgar scoring system, well known since more than 70 years in humans, and adapted to several domestic species. In 2009, [3] proposed an Apgar scoring system also for the newborn dogs, and, since then, it proved to be useful for viability evaluation and classification of puppies, allowing a tailored assistance/resuscitation. Moreover, the Apgar scoring demonstrated also to be useful for the evaluation of short-term survival prognosis [1, 3, 4, 5]. Normal newborns should have effective typical reflexes, necessary for survival, such as the suckling, swallowing, rightening and rooting reflexes. Therefore, the evaluation of their presence is also useful for newborn viability assessment at birth [1, 6] and could be associated to the Apgar scoring in puppies. During the clinical evaluation of a newborn at birth, the prompt detection of gross physical malformations or physical defects incompatible with life is strictly necessary, to avoid wasting time and resources for a newborn puppy eventually destined to humane euthanasia. At birth, newborns experience a physiologic transitory hypothermia, protective for possible brain damages caused by hypoxia, and reducing newborn metabolism. However, when prolonged or exacerbated, hypothermia can impair

neonatal adaptation or the newborn response to assistance and resuscitation, causing neonatal mortality [7]. Therefore, the monitoring of body temperature should be part of the clinical evaluation at birth, allowing its prompt adjustment through warmth support. The clinical assessment of a newborn should include the measurement of the birthweight. This parameter, in fact, mirrors maternal environment, placental efficiency and fetal development and is an essential prerequisite for newborn survival. It is then not surprising that low birthweight was associated with poorer prognosis for survival or health of the newborn [8], becoming a recognized negative prognostic factor [9]. Although some authors reported a correlation between the Apgar score and some blood gas parameters [5], the Apgar score alone does not provide information about the possible asphyxia and related acidosis of the newborn, conditions that can impair the neonatal adaptation and survival. These conditions should therefore be diagnosed through blood gas analysis, performable also on newborn puppies thanks to the availability of portable instruments working on very small volume of blood, usually collected from the umbilical or jugular vein. These instruments have also the advantage to measure a list of parameters, including glycemia and lactatemia. Notably some blood gas parameters were found to be good indicators of clinical prognosis and outcome of newborn puppies [5]. Because severe hypoxia could be associated to myocardial ischemic injury, [6] showed the usefulness of cardiac troponin 1 measurement in peripheral blood as specific marker of cardiac damage in newborn dogs. According to glycaemia, because of the typical hepatic immaturity, limited energetic depots, and immaturity of glucose metabolism, the newborn dog has been considered at high risk for hypoglycemia, so that this condition has been largely recognized as one of the main causes of neonatal mortality in these species [1, 7]. However, it is interesting to note that, recently, some authors found hyperglycemia in canine newborns with poorer survival prognosis [5]. Lactatemia, as indirect marker of acidosis, was also reported to be useful not only as diagnostic, but especially as prognostic factor in newborn dogs [10]. In conclusion, although the continuous developing of canine neonatology will allow a more adequate clinical evaluation of the puppy at birth, even with the present knowledge a systematic evaluation of canine newborns is needed for a tailored assistance to those puppies needing special cares and, in turn, reducing the effect of birth on perinatal mortality in this species.

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PATHOLOGY OF ANIMAL MODELS OF COVID19

Francisco J. Salguero

United Kingdom Health Security Agency (UKHSA). Porton Down, Salisbury, U.K.
Corresponding author: javier.salguero@phe.gov.uk

A novel coronavirus, named SARS-CoV-2, was identified as the causative agent of the current COVID-19 pandemic originated in Wuhan, China in 2019. Animal models have been essential to understand the pathogenesis of this and other emerging diseases and to the development of safety and efficacious vaccines and therapeutics to control them.

From the beginning of the COVID19 pandemic, the scientific community started establishing different animal models of the disease while novel vaccines where being developed. Among the animal species, non-human primates (NHPs) were key to replicate some of the clinical features observed in human patients suffering from COVID19. The main NHP species used were the rhesus macaques (Macaca mulatta) and the cynomolgus macaque (*Macaca fascicularis*). SARS-CoV-2 replicates in the upper and lower respiratory tract and causes pulmonary lesions in both rhesus and cynomolgus macaques, resembling the mild clinical cases of COVID-19 in humans. Immune responses against SARS-CoV-2 are also similar in both species and equivalent to those reported in milder infections and convalescent human patients. Gross pathological changes consist of multifocal mild to moderate consolidation distributed in cranial and caudal lobes, that are resolved after 2-3 weeks post infection. Histological changes in the lungs show multifocal to coalescing areas of pneumonia, surrounded by unaffected parenchyma. Overall, diffuse alveolar damage is a prominent feature in the affected areas, characterised by individual, shrunken, eosinophilic cells in alveolar walls, with pyknotic or karyorrhectic nuclei. In these areas, alveolar spaces were often obliterated by collapse of the thickened and damaged alveolar walls which contain mixed inflammatory infiltrates; or had obvious, alveolar type 2 pneumocyte hyperplasia (alveolar epithelialisation), as well as expanded alveolar spaces that can show different degrees of oedema and mixed inflammatory infiltrates. In distal bronchioles and bronchiolo-alveolar junctions, degeneration and sloughing of epithelial cells is frequently observed present, with areas of attenuation and foci of plump, type 2 pneumocytes representing regeneration. Multinucleated cells resembling syncytial cells are unfrequently observed. Virus RNA is found in the areas of pneumonia as well as in the BALT.

The ferret is an excellent animal model for some respiratory diseases, including Influenza. Ferrets have been also used as animal models for COVID19, showing a very good virus replication in the upper respiratory tract, but only minimal/mild changes in the lower respiratory tract.

One of the most valuable animal models to study COVID19 to date has been the Syrian golden hamster (*Mesocricetus auratus*), also used for other respiratory infections. After intranasal infection, hamsters show progressive body weight loss starting very early after infection, being a key clinical feature used in vaccine preclinical evaluation. Apart from the weight loss, respiratory signs are also observed, and animals recover from the infection after 2-3weeks post infection. Viral RNA is detected in the upper and lower respiratory tract and histologically, inflammatory infiltrates with abundant viral antigen expression is observed from the early infection time point, being most severe at 4 dpi and resolving almost completely at day 14 post-infection.

Mouse models have also been used, including transgenic mice expressing the human ACE2 receptor. Conventional mouse lines do not get infected by the original SARS-CoV-2 variants, but the model is permissive to some of the most recent variants, making the mouse model an useful tool to study some variants of concern

Other animal species have been used as potential models of disease, including cats, minks, pigs or rabbits with variable success although some animals show good opportunities to study immunity after experimental vaccination.

One of the big gaps for the scientific community is the existence of an animal model of severe COVID19 disease. Most of the NHPs species can develop the milder forms of clinical disease observed in humans, but not the most severe or fatal clinical disease observed in elderly or immunocompromised patients. The scarcity of aged NHPs makes this search even more difficult. The Syrian golden hamster has proven to be a very good model of moderate

to severe clinical disease, with the recovery of infected animals after a severe weight loss and resolving bronchointerstitial pneumonia.

The use of animal models of COVID19 has been very useful in preclinical studies of the rapidly developed vaccines used to control the pandemic. Moreover, they have been crucial to study the pathogenesis and immunology of the disease, the possible presence of Vaccine Enhanced disease and the transmission of the different variants of concern that have been appearing during the course of the pandemic.

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DIGITAL DERMATITIS IN CATTLE: STILL DEBATED AND CHALLENGING 50 YEARS FROM ITS DISCOVERY

Carlo Maria Mortellaro

Università degli Studi di Milano
Corresponding author: C.M. Mortellaro (carlomaria.mortellaro@unimi.it)

Digital Dermatitis (DD) firstly discovered in 1972 in a dairy farm of Po Valley, and officially reported in 1974 under the misnomer of Interdigital Dermatitis by Cheli and Mortellaro 1974 [1], at that time the only condition sharing some "ambiguous" similarities with DD, is a multifaceted disease characterized by ulcerative or reactive or proliferative lesions of the skin bordering the plantar aspect of the interdigital space. Alternative locations are less commonly found and involve the dew claws, palmar aspect of the interdigital cleft and coronary band. DD is a major problem in dairy cows, and leading cause of lameness, causing significant economic losses worldwide. The prevalence in herds is quite variable and range from 20% to more than 50%. DD is currently considered endemic in almost all countries were the cows are housed. Nevertheless, mainly over the last decade, this disorder is increasingly recognized in beef cattle of the United States, not sparing some European countries, sheep (Contagious Ovine Digital Dermatitis – CODD) [2] and, more recently, elk and goats. With specific regard to beef cattle, Veterinarians working in this field anecdotally report that the problem is occasionally present in some farms in northern Italy since 2019. Moreover, recent clinical field observations allowed us to verify a more aggressive and rapid progression of the disease causing severe lameness, mainly because its control is quite problematic and challenging in these animals. Similarly, the CODD results in acute severe lameness and the progression of the disease, characterized by ulcerative lesions of the coronary band, sometimes mimicking a footrot, with the disruption of the abaxial wall until the exungulation (2). The most common clinical sign is lameness, although a significant number of animals are asymptomatic, also depending upon the anatomic location of the lesions, DD being representing an "incidental finding" during foot trimming sessions. However, the typical lesion, the ulcerative stage, is very painful upon palpation and prone to bleeding. Further manifestations include decreased milk yeld, poor reproductive performance, loss of body weight and decreased average daily gain [3]. In addition, DD is a reason for further concern for farmers due to the cost of treatment and sometimes premature culling both responsible, often difficult to quantifie, for economic losses. The several clinical presentations of DD reflect very often the stage of the disease although some geographical regions or farms are more predisposed to one or the other of the different forms, ulcerative vs reactive vs proliferative. To better describe the stage of the disease, avoiding misleading terms like strawberry foot rot, Italian foot rot, raspberry heel, hairy heel warts, footwart, verrucose/papillomatous DD, Mortellaro's disease, numerous classification systems have been proposed, especially for the purpose of objectifying a research methodology requested for scientific comparison of clinical and experimental studies [4]. The M-stage scoring system developed by Dopfer et al. in 1997 [5] and subsequently modified and more extensively described by Berry et al. in 2012 [6], is still the most widely adopted to date. Five categories are identified being M0 indicating healty digital skin with no signs of dermatitis, whereas the stages from M1 onwards identify the progression of the disease. M1, early/subclinical stage: small, circumscribed, red to grey epithelial defect less than 2 cm. in diameter; surprisingly M1 lesions, sometimes more than one, can be found in the interdigital space which has raised many doubts for interpretation especially against Interdigital dermatitis, the existence of which, as a separate lesion, is questioned by many clinicians [7] and who writes among these. M2, painful/acute ulcer: red active ulcer or red to grey granulomatous lesion both 2 cm. or greater in diameter. M3, healing stage: scab-like lesion; usually occurs two or more days after topical treatment and no longer painful. M4, chronic stage: hyperkeratosis or filamentous proliferations, exceptionally mimicking a tumor mass. M4.1 chronically recurring lesion: hyperkeratosis or filamentous proliferation and concurrent M1 lesion within its boundaries. This scoring system has been further amended by Dopfer [8] during the 20th International Symposium and 12th International Conference on Lameness in Ruminants held in Tokyo in March 2019, with the addition of a more specific and detailed subclasses aimed for providing some kind of therapeutic algorithm: Decision Tree Chart . Several risk factors are involved in the

pathogenesis of DD including housing (abrasive walking surfaces) bad hygiene, high stocking density, lactation stage, high milk production, intensive feeding and too frequent or aggressive foot baths, risk the latter often overlooked. In DD free farms, purchasing animals and herd replacement are considered additional risk factors. According to a survey of NAHMS (National American Health Monitoring System) issued in 1996, the odds of infection were nearly eight time greater for herds that introduced replacements from off-dairy sites than those that did not [9] [10]. But more recently this finding was not confirmed by Holzhauer [11]. Even more recently a further predisposing factor has been ascribed to the lack of disinfection of trimming instrumentation. A study presented at the aforementioned congress showed that Treponemes can be grown from hoof knife blades for up two hours confirming that contamination of knives during foot trimming could be a potential risk for transmission of DD, both between cows in the same herds and between herds [12]. The ultimate cause of DD is still debatable but the very prompt response to topical antibiotics indicates a likely underlying bacterial infection. DD lesions are consistently associated with an abundant and diverse population of species of Treponemes (*T. phagedenis*-like, T. *medium, T. vincentii-like, T. putidum-like, T. denticola, T. pedis, T. refringens, T. brennaborense,* etc.) that represents only a portion of a much more varied and complex bacterial community forming the total microbiota of the disease (4). In fact, in addition to members of *Treponema* genus, *Mycoplasma, Porphyromonas, Fusobacterium, Bacteroidites, Prevotella* and other anaerobic bacteria have been consistenly identified in the majority of DD lesions as emerged from a very recent meta-analysis on Digital dermatitis microbiota [13]. Furthermore, if we accept that Spirochetes are the primary aetiologic agents associated with DD, it must be admitted that is to be acknowledged as "polytreponemal" disease condition. The question arises as to why DD requires the presence of more treponemal species instead of one [4]. Moreover, the previous meta-analysis also revealed that members of Mycoplasma, Porphyromonas and possibly other bacteria may play a significant role in DD pathogenesis. For this reason, it would perhaps more appropriate to define this disease as polymicrobial and not simply polytreponemal, as well as obviously polyfactorial. Further studies are required to uniquely elucidate the disease pathogenetic mechanisms in order to achieve a more effective treatment [13]. Contagiousness of DD undoubtedly represents an important issue. Transmission of the disease by inoculation with pure cultures of Treponemes has never been obtained according to the rules of Henle and Koch. However, a successful transmission was achieved by Read and Walker in 1996, by determining a prolonged maceration of epidermal barrier and anaerobiosis of the foot prior to inoculation (14). Some of the feet, previously wrapped to mimic, as just mentioned, conditions of prolonged moisture, showed lesions compatible with DD around the dew claws. In 2012 another experimental model to induce DD was developed by Gomez et al. [15] but in this study the lesions were induced only near the dew claws similarly from what has been obtained by Read and Walker. Very recently Krull et al. [16] evaluating 21 different protocols in 126 immature Holstein calves (504 feet) of 3 months of age, on their effectiveness to reproduce DD lesions, were able to develop a final protocol, among five different experiments, capable of inducing the lesions in 42 of 44 feet (95%) over a 28-day period. Furthermore, the lesions thus induced were similar to naturally occurring DD as far as the location is concerned, that is plantar aspect of the interdigital space. However, on day 0 of this protocol all four feet were mechanically abraded in order to remove the entire epidermis and partially the dermis, then wrapped with waterproof tape left in place for 28 days. On day 3 of the trial an inoculum was prepared from tissue lesion biopsies obtained from adult cows with natural DD. The bioptic material, placed in Induction Broth containing a mixture of growth media, was then macerated in anaerobic chamber, placed in a syringe in the amount of 1,5 ml. of the supernatant and injected under the wrap. The Induction Broth was administered also on day 11,18 and 25 to maintain and preserve the moisture. But, borrowing what is written by Orsel et al. (4), "all these studies are somewhat disappointing and problematic to interpret regard to aetiology, especially the last one in wich it emerged that even the negative control animals, wrapped and inoculated with sterile media alone, housed in the pens with animals that were induced with maceration, actually had lesions not dissimilar to those induced with macerated inoculum (organisms plus media)". Further and more robust studies will therefore be necessary to resolve the many discrepancies that still concern the aetiopathogenesis of DD. Individual therapy is based on topical antibiotic, Oxytetracycline being the most effective, sprayed on the affected area after thorough cleaning and even more accurate trimming, often omitted by farmers. This therapeutic protocol gives excellent and rapid results in M1 and M2 lesions while it is ineffective in M4 and M4.1 , characterized

by hyperkeratosis and/or filamentous proliferations. M4 lesions actually represent the true reservoir of the disease because Treponemes are often encysted deep in the epidermis, even the dermis, not reached by the antibiotic and non-antibiotic substances. Therapeutic rational for this stage of DD is still pending. Surgery is discouraged by some (8) (Dopfer, personal communication) but other efficacious and cost effective modalities are scarcerly available. Several non-antibiotic preparations are also effective especially those based on copper and zinc, chelated better than if sulphated. To be successful the preparations must be applied also in the interdigital space, "hidden" site of M1 lesions. A bandage is not always necessary but, if considered useful, must be removed alfter 2-3 days at the latest. Systemic antibiotics are contraindicated unless there are ongoing complications, while the use of anti-inflammatory drugs can sometimes be useful. Large outbreak of DD conversely require collective treatments by means of walk-through foot-baths which contain antibiotics (not allowed in our country) and non-antibiotic substances (copper, zinc, formalin). However antibiotic foot-baths lose their efficacy after a relatively small number of cow passages and can promote a potential antimicrobial resistance, a growing concern of this century! The 3-5 percent formalin still remain the first and ultimate choice for many farmers but an unregulated use is a matter of great concern for human health as it can cause cancer. Fewer concerns raise copper-based foot-baths, used at concentrations of 2-8%, but their elimination and dispersion into environment is however not a negligible problem. Furthermore, an overuse of foot-baths, too frequent or too aggressive, because of too high concentrations of product used (especially formaldehyde) must be avoided possibly causing an increased number of chronic lesions that act as a reservoir of DD. In conclusion, the individual therapy of DD aimed to eradicate the disease, and ever more management of large outbreaks, remain challenging and partly elusive. Periodic retreatment is therefore necessary to achieve a manageable control of the disease paired with appropriate preventive measures including, among these, the meticulous feet exam of every purchased heifer and, possibly quarantine adoption. Many unanswered questions still remain relative to DD in cattle, both in dairy ones and even more so in beef cattle. We can assume that Treponemes are the primary causative agents but the role of other anaerobic microorganisms cannot be completely ruled out. The high rate of recurrence has no definitive explanation and other reservoirs, in addiction to infected cows, might be involved. Topical antibiotic, especially Oxytetracycline or Lincomycin and some non-antibiotic preparations, based on copper chelates or sulphates, zinc, alginates are effective in controlling the disease in individual cows, always paired with a careful hoof trimming. However, regardless of the medication chosen for treatment of this condition, recurrence rates are unfortunately high requiring continuing therapy to achieve an optimal and so called "manageable control". Severe outbreaks of DD conversely require an integrated and multifaceted, yet non-standardized, approach. Equally, if not more complicated, is the therapy in beef cattle in wich individual treatment is rarely feasible and often nonprofitable. Herds free of DD must avoid the purchase of animals coming from infected farms despite the definitive proof of its contagiousness is still pending. Moreover, appropriate and convincing biosecurity measure for trimmers or veterinarians and other off-farm personnel, including visitors, must be pursued [7]. The ultimate goal is to reach and maintain a" manageable control" of the disease assuming that its eradication is far from being achieved until more epidemiological information will be available.

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DOES BOVINE BESNOITIOSIS SPREAD FROM EUROPE TO ITALY FOLLOW THE BEEF CATTLE TRADE?

Maria Teresa Manfredi)

Università degli Studi di Milano, Dipartimento di Medicina Veterinaria e Scienze Animali.
Corresponding author: M.T. Manfredi (mariateresa.manfredi@unimi.it)

Bovine besnotiosis is considered an emerging disease in Europe having consequences on productions which could arise a reduction in milk production, in the value of the hides and poor body conditions of affected bovines. The infection also affects reproduction; in fact, although the animals may occasionally have an abortion, the consequences on the fertility of the bulls are not negligible. The disease can have an acute, subacute and chronic form; in the majority of cases, animals are found to be infected with the chronic debilitating form characterized by systemic symptoms and typical skin lesions. Dermal lesions consist of thickening, hardening and folding or wrinkling of the skin, especially around the neck, shoulders and rump. Hyperkeratosis, hyperpigmentation and alopecia are also present (1). Due to these lesions the bovine besnoitiosis is also known as the elephant skin disease of cattle. The disease is caused by the cyst-forming apicomplexan protozoan named *Besnoitia besnoiti* in 1916 (Franco and Borges) but the first report related to bovine besnoitiosis occurred in France and was published in 1884. The bovine besnoitiosis currently has a worldwide distribution; in Europe, from 2010, it is considered an emerging disease due an increased number of cases and geographic expansion (2). Cattle affected herds were now reported both in the mainland and southern european countries and even in the British isles (3).

In Italy, the first clinical case concerning imported beef cattle was reported in 1994 (4), further autochthonous outbreaks involving local breeds and/or native individuals of any breed occurred in the central mainland part of the country (5-8). According to diagnostic tests, a few serosurveys revealed higher prevalence values in southern Italy (44.1% and 83% at individual and farm level respectively) and central Italy (29.4-52% and 94.6-100% at individual and farm level, respectively) compared to northwestern and insular Italy (0.3% and 3.9% at individual and farm level respectively) (9-11). The latest study (11) only confirmed positives two Holstein Friesian cows and eight beef cattle. Clinical cases and higher serological positivities were found in bovines from a purebred beef herd (36,5%); the infection was associated with age and sex, with males presenting a greater risk (prevalence 56.7%). In these herds, breeding bulls represent one of the relevant epidemiological factors that favour the spread of the disease; indeed, the results showed that mating with a seropositive bull enhances infection risk for a seronegative cow (OR=1.678) (12). Recently an autochthonous outbreak of bovine besnoitiosis with serological positivities and clinical cases in Limousine bovines was reported in two nearby farms located in north-western Sicily (13). Although these data seem to confirm the greater impact of besnoitiosis in beef cattle linked to management factors typical of this breeding, the risk for dairy cattle should not be underestimated. In fact, recent studies, in addition to demonstrating that besnoitiosis in Italy is becoming a reality, confirmed its significant spread also in dairy herds (14). Indeed, in a dairy herd with 43.5% of seropositives cows, 75% of the serologically positive and clinically examined animals had the typical cysts localized in the skin, sclera, and/or vulva. Furthermore, in seropositive and clinically affected cows hematological alterations have been demonstrated; particularly in primiparous seropositive cows, the leukocyte differential with a higher percentage of granulocytes and a lower percentage value of lymphocytes were evidenced (15). Recently, in a further outbreak of bovine besnoitiosis in an intensive dairy herds with acute clinical forms a greater number of distress events in infected animals compared to seronegative (40% vs. 21.7%), with a decrease in rumination in 36.6% vs. 13% of cases was reported (16). Bovine besnotiosis cannot be longer considered a negligible disease; its real spread could be greater than what is normally believed as shown by the numerous reported outbreaks and its impact on the health and sustainability of cattle breeding cannot be underestimated. Although its distribution is probably not as widespread as other parasitic diseases, such as neosporosis, among cattle herds, knowledge through dissemination plans among breeders and veterinarians is needed to implement specific control programs. Unfortantely, the exact origin of the infection cannot be established although it is possible to hypothesize that the disease was introduced by the entry of infected bovines into these

herds and, in at least one case (16), it is possible that the infection spread among animals even through mal practices. Considering that there are many aspects of the *Besnoita besnoiti* life cycle that are not yet known well, such as which hosts are receptive, and that, currently, there are no effective drugs and vaccines, it is urgently necessary to adopt a health scheme that also includes the besnotiosis in the cattle herds. The latter could protect the herds both from the entrance of the protozoan and from the spread within the herd itself so by preventing the disease from reaching high prevalence and incidences. A correct and early diagnosis is also crucial to implement control plans (17). Finally, another aspect that needs to be strengthened is besnoitosis as a systemic disease, which was demonstrated by the tissutal cysts detected in many organs and even in the muscle, and is a further reason that should convince breeders to implement control plans. (14).

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ENDOGENOUS STEM AND PRECURSOR CELLS: FROM EMBRYOLOGY TO NERVOUS SYSTEM REGENERATION

Laura Calzà (1), Vito Antonio Baldassarro (2), Luca Lorenzini (2), Luciana Giardino (2)

(1) Università di Bologna, Dipartimento di Farmacia e Biotecnologie. (2) Università di Bologna, Dipartimento di Scienze Mediche Veterinarie.

Corresponding author: L. Calza (laura.calza@unibo.it)

Past-century anatomy has been marked by the emergency of neuroanatomy, through the "neuron theory" by Ramon Cajal made possible by the Camillo Golgi silver staining, and by the extensive use of histochemical and immunohistochemical techniques that leaded to the "chemical neuroanatomy". Since the 80s of the last century, the modern neuroanatomy was marked by the coupling of molecular biology and new microscopy techniques, leading to the "molecular neuroanatomy". Finally, cell biology techniques and live imaging microscopy provided an unexpected plastic vision of the central nervous system (CNS) structure and cellular composition, also through the identification of new cell types.

In this context, one of the most revolutionary discovery during the last decades has been the identification of stem/progenitors and precursor cells in the CNS of adult vertebrates, including mammals until humans. Neural stem/progenitor cells are resident in the subventricular zone of the telencephalon and in the sub granular zone, from which migrate to guarantee the renewal of selected neural populations in the olfactory bulb and the hippocampus. Moreover, a new cell population in the CNS was identify and defined as "NG2-positive" cell or "polydendrocytes", and finally named Oligodendrocyte Precursor/Progenitor Cells (OPCs). OPCs are generated during development as precursors of oligodendrocytes (OL), and a pool of undifferentiated precursors remains all over the mature CNS. These quiescent unipotent stem cells can be activated by different stimuli able to trigger the proliferation, migration, and differentiation process once more, allowing the myelin repair and axonal re-myelination, thus indirectly contributing to neuroprotection. Remyelination is currently the only known self-repair ability of the CNS, potentially providing full anatomical and functional neuroregeneration.

Over the past years, our laboratory has been actively involved in the study of OPCs biology during development, adulthood and in pathological conditions, taking the opportunity to study an immature cell able to resume embryonic molecular signature in the context of a mature and stabilized microenvironment. A combination of in vitro, ex-vivo and in vivo experiments, supported by morphological, molecular, genetic tools and advanced microscopy, was used to investigate the molecular signature of OPC until OL differentiation, focusing on the role of nuclear receptors (thyroid hormone and retinoic acid receptors). These knowledges were then applied to study OPCs in pathological contexts during the adulthood, in view of the myelination enhancing therapies. A general question has been the *fil rouge* of this work: can cellular and molecular mechanisms regulating stem and precursor cells during brain development be reactivated in case of injury in the adulthood, and eventually used as target for therapies?

In this talk, both methodological aspects and experimental results will be reviewed, moving from cellular and molecular mechanisms of stem/precursor cells lineage and differentiation during embryogenesis toward neuroregeneration in the adulthood. OPCs will be the cell type and white matter the anatomical compartment considered. Finally, the significant on embryology in the context of the regenerative medicine will be discussed.

How to study OPCs lineage and maturation. OPCs can be identify in tissue sections by cell markers as the surface markers NG2 proteoglycan and the platelet-derived growth factor alpha receptor (PDGF-Ra), and the transcription factors Olig1-2. Moreover, OPCs can be isolated as primary cells from different CNS areas or generated by neural stem cells (NSCs) derived either from embryonic of mature CNS. These last cells fully recapitulate developmental maturation in vitro. The metabolic profile of these cells during differentiation stages [1], and the role of nuclear receptors (thyroid hormone nuclear receptors and retinoic acid receptors) [2] in this process will be reviewed, focusing on OPCs cycle exit and terminal differentiation induction.

Thyroid hormone and developmental myelination. The importance of an appropriate thyroid hormone (TH) signaling during brain development is dramatically illustrated in humans by the severe mental retardation and growth impairment observed in conditions leading to a substantial decrease of fetal TH availability, such as congenital hypothyroidism. The developmental role of TH on myelin formation will be reviewed, focusing on OPCs biology, the expression regulation of gene encoding for myelin proteins, and impact of 3D microenvironment [3].

OPCs and myelin repair in the adulthood. OPCs are present in the mature CNS, where they represent a major proliferating cell population, constituting around 5% of the total cell population. OPC is responsible for myelin repair during adulthood, being remyelination is the only true regenerative capability of the CNS. In response to myelin loss or increased demand, "adult" OPCs have the capacity to differentiate into mature myelinating OL, to guarantee an appropriate white matter turnover. This significant capability, demonstrated in physiological conditions, led to the so-called "recapitulation hypothesis" for myelin repair which suggested that remyelination after myelin damage follows several steps and molecular mechanisms occurring during developmental myelination. The impact of TH on myelin development, homeostasis and repair will be reviewed, focusing on the regulation of TH tissue signaling under physiological and pathological conditions affecting myelination and/or myelin repair during early postnatal age and during adulthood.

TH-based myelination enhancing therapies. Tetraiodothyronine, also called L-thyroxine, or T4, and L-triiodothyronine, or T3, are blood circulating THs in which more than 99% of both T4 and T3 is bound to serum proteins. The thyroid gland mainly secretes T4, which is considered the inactive form of TH or a pro-hormone, while T3 is mainly produced by the target tissues, and has a much greater biological activity (about 10x) than T4. In mammals, the thyroid secretes most T4, and a low amount of T3 per day. T3 is produced by peripheral tissues from T4, mainly in the liver and kidneys. In addition to T3, an equal amount of "reverse T3" which has no biological activity may also be formed. Thus, the tissue concentration of TH is mainly due to the local T3 production. Several pathological conditions, characterized by inflammation, alters the T3 tissue production, leading to tissue hypothyroidism that impair the biological function of TH-dependent cells as OPCs. Starting from the TH role during development, our laboratory proposed a TH supplementation as re-myelination enhancing therapy. Data supporting this indication will be reviewed, focusing on effect of inflammation on TH metabolism in the CNS; role on inflammation on OPCs differentiation; effects of exogenous TH administration on myelin repair in experimental models of demyelinating diseases in rodents and non-human primate. Moreover, TH therapeutic use will be also discussed in the contest of multidrug therapy and considering biomaterial-based strategies for local TH delivery by drug-loaded implantable electrospun devices and nanoparticle assisted drug delivery.

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ULTRA-PROCESSED FOOD CONSUMPTION AND HEALTH: EVIDENCE FROM EPIDEMIOLOGICAL STUDIES

Marialaura Bonaccio (1), Licia Iacoviello (1,2), Maria Benedetta Donati (1), Giovanni de Gaetano (1)

(1) Department of Epidemiology and Prevention, IRCCS Neuromed, Pozzilli (IS), Italy. (2) Department of Medicine and Surgery, Research Centre in Epidemiology and Preventive Medicine (EPIMED), University of Insubria, Varese-Como, Italy

Corresponding author: M. Bonaccio (marialaura.bonaccio@moli-sani.org)

For decades, the diet–health relationship has been explained almost exclusively by food composition (i.e. calorie, macro- and micronutrient contents), therefore leading to recommendations of reducing sugar, salt and fat in the diet (1), with no or little attention paid to degree of food processing.

However, such a nutrient-centred approach, also referred to as the 'nutrient gate' (2), has some important limitations, since other aspects in the diet-health-disease relationship are increasingly acknowledged as important as nutrients in shaping health risk at population level.

The NOVA classification, developed by Brazilian researchers (3), was proposed as a novel way to look at foods based on the degree of processing of foods rather than on their nutritional composition, postulating that processing may be as relevant to health as food composition, possibly through mechanisms that are triggered by non-nutritional components of these foods, such as degradation and artificialization of the food matrix, cosmetic additives, food contact materials, or neo-formed compounds (1,2,4). The term ultra-processed food (UPF) indicates industrially manufactured ready-to-eat or ready-to-heat formulations made mostly or entirely from substances extracted from foods or derived from food constituents often containing added flavours, colours, emulsifiers and other cosmetic additives (3,4). Examples of typical UPF are carbonated drinks, fruit yogurt, sweet or savoury packaged snacks, ice-cream, chocolate, candies (confectionery), mass-produced packaged breads and buns, and many others (3).

In the last decade, the number of studies examining the relationship of UPF and health has dramatically increased, going from 23 papers published in 2009-2015 to nearly 500 issued only in the last year and a half.

This growing attention to the impact of UPF to human health relates to the fact that their consumption is on the rise worldwide. Actually, UPF constitute a large part of the world's food consumption and is remarkably high in non-Mediterranean countries, representing almost 60% of total calories in the USA (5) and in the UK (6), 42% in Australia (7) and 46% in Canada (8), with substantial differences between adults and children/adolescents. In Mediterranean countries such as Spain and Italy, the proportion of food that is ultra-processed among adults is about 24% and 17%, respectively (9,10), possibly because home cooking is still part of a traditional Mediterranean diet.

Robust and well-conducted cohort studies worldwide found that a large dietary share of UPF is associated with shorter survival (11) and an increased risk of non-communicable diseases, including cardiovascular disease (CVD) (12), type 2 diabetes (13) and some cancers (14).

More recently, data from the Moli-sani Study cohort, in Italy, reported a 65% increased risk of CVD mortality associated with an elevated UPF intake among individuals with pre-existing CVD, as compared to those consuming less UPF (15). Of interest, most of these studies, although not all, considered the overall diet quality in their analyses. This means that the detrimental health impact of UPF should be understood as net of its nutritional quality. If on the one hand it is undeniable that these foods are nutritionally inadequate (i.e. having a low content of fibre, vitamins and other nutrients, while being rich in calories, unhealthy fats and salt), on the other it is necessary to search for other possible

mechanisms, other than the nutrient pathway, which may explain the link between heavy UPF intake and non-communicable chronic diseases. As first, any type of processing fundamentally alters food matrix, typically in a detrimental manner (16). Moreover, the additives found in highly processed products also have uncertain effects on health, as well as the packaging of UPF which is a major source of chemicals (e.g. bisphenols and phthalates) that have the potential to act as xenohormones (16) and are also associated with altered concentrations of inflammatory biomarkers (17).

Finally, both modification of the food matrix and the inclusion of certain food additives during processing may adversely influence gut microbiota composition, function, and bacteria–host interactions (18).

In conclusion, epidemiological evidence available to date supports the notion that a large dietary share of UPF represent a major threat to human health. Given the rising popularity of UPF globally, and also in Mediterranean countries, the issue of food processing should be prioritized in relevant dietary recommendations with emphasis on consumption of minimally/unprocessed foods.

This is paramount also in light of the efforts put at EU-level for enabling uniform front-of-pack (FOP) labels intended to help consumers make healthier food choices. Among candidates, the Nutri-Score, which is an interpretative FOP labelling system grading the nutritional quality of foods (19), is gaining increasing attention. However, the Nutri-Score, as well as the majority of FOPs, rates foods only on the basis of their nutritional content and therefore disregarding the food processing.

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THE ROLE OF ANIMAL FOOD IN A HEALTHY AND BALANCED DIET

Andrea Poli (1) and Franca Marangoni (1)

(1) NFI - Nutrition Foundation of Italy

Corresponding author: A. Poli (poli@nutrition-foundation.it)

The role of foods of animal origin in the human diet undergoes, nowadays, conflicting evaluations; on the one hand it is well known that the quality of animal proteins and the bioavailability of some vitamins and minerals, such as iron and vitamin B12, are better in animal foods than in foods of vegetable origin; the long-term health effects of the consumption of animal foods are on the other hand controversial, and a number of epidemiological data suggest the existence of a correlation between their consumption, especially at high levels of intake, and a greater risk of developing non-communicable diseases such as cancer, cardiovascular diseases, etc. In fact, many guidelines, including the most recent ones, suggest a more or less marked limitation of the intake of foods of animal origin (especially red meat, processed meats, dairy products with a high fat content); also, some well-known graphic displays of the optimal dietary pattern, such as the so-called Mediterranean pyramid, confine most of these foods in the "tip" of the pyramid itself, among those whose consumption should be limited to one or a few times a week.

It must be considered that the most recent epidemiological data support a significant modification of this situation; the evaluation of the correlation between diet and mortality (both of cardiovascular origin and from tumors) obtained by the research group called GBD (Global Burden of Disease) seems for example to exclude a significant contribution of foods of animal origin to the overall risk of mortality. The GBD, in particular, does not attribute a significant role in this regard to saturated fats, typically present in dairy products and in the meat of ruminants; also, the role attributed to red meats is less significant than expected, and a small although significant risk is associated only with the consumption of processed meats (1).

The downsizing of the unfavorable role of saturated fats on cardiovascular risk, generally attributed to the effect of these fats on LDL cholesterol level, emerges also from all the most recent meta-analyses; the largest, which combines the results of more than 50 cohorts, does not detect any increase in the risk of cardiovascular events in the groups of subjects with high saturated fat intake as compared to subjects with low dietary consumption of these fats (2).

It is important to underline, in this regard, that we eat foods, and not isolated nutrients. In fact, rich information suggests the importance of the food matrix in which nutrients (such as saturated fats) are incorporated in conditioning their biological effect; the effects of these nutrients must in any case be evaluated in the context of the entire composition of the food: milk and its derivatives (such as cheese or yogurt), as an example, exert significant protective effects on the risk of cerebrovascular diseases such as stroke, probably mediated by the control of blood pressure values, due to their content in calcium, anti-hypertensive peptides, probiotics. Avoiding or banning the consumption of these foods in light of their high content of saturated fats, therefore, seems of uncertain significance, and could be potentially counterproductive.

Even the association between red meat and health has become more complex to interpret in the light of the most recent epidemiological evidence. While, on the one hand, the existence of a positive (and therefore not favorable) correlation between the high consumption of red meat and the risk of certain cancers and cardiovascular diseases seems to be confirmed (even if in not all the papers published on the topic), it is also evident that such correlation is strongly dose-dependent, and that the direct health effects of red meat, at the average intake levels of the Italian population, is probably low or negligible. A model developed by the NutriRECS group (3) has in particular allowed to estimate the contribution of red meat to the risk of occurrence of cardiovascular events over time; the impact in absolute terms of its consumptions was very small, and the theoretical advantage deriving from the restriction

of red meat consumption emerged from this evaluation and communicated to a population sample, was considered by the majority of the interviewed persons completely insufficient to justify a significant reduction of the consumption of red meat.

The data on so-called processed meats confirm that their consumption is associated with an increased risk of some non-communicable diseases. Processed meats are however a very heterogeneous category of products, which includes foods with a very low processing (such as some typical hams of the Italian tradition, produced starting from pork meat and salt) up to others much more complex (such as some sausages and the frankfurters); a complete understanding of their health effect is still largely unavailable.

The health effects of egg consumption have also been reconsidered in light of the most recent studies. It is in fact established that the cholesterol contained in the egg yolk can increase, albeit slightly, the plasma LDL cholesterol levels, however, this effect is very variable in the population. In fact, it is more marked among subjects with a pattern, in which the content of cholesterol of food origin, effectively absorbed by the intestine, plays an important role in determining the levels of LDL cholesterol; however, it plays a minor role among subjects with a "synthetic" pattern, where it is essentially the speed of hepatic synthesis that determines the plasma levels of LDL cholesterol. The synthesis, starting from the choline contained in the egg yolk, of a potentially atherogenic compound (TMAO) is also highly variable from person to person, probably due to the crucial intervention of the intestinal microbiota

In fact, it is more marked among subjects with an "absorptive" pattern, in which dietary cholesterol is efficiently absorbed from the gut and plays a large role in determining the levels of LDL cholesterol, while it plays a smaller role among subjects with a "synthetic" pattern, in which LDL cholesterol are essentially determined by the liver synthesis rate. Also, the synthesis, starting from the choline contained in the egg yolk, of a potentially atherogenic compound (TMAO) is highly variable from person to person, probably due to the crucial intervention of the intestinal microbiota. In fact, the scientific literature continues to report discordant overall evaluations of the possible effects of egg consumption on health, and specifically on cardiovascular risk. However, most studies agree that moderate consumption (within two to three eggs per week, but probably up to one per day) has no significant negative effects in this regard (4).

In conclusion, the data from the most recent literature confirm on the one hand the importance of the vegetable components of the diet, which should be significantly increased compared to the current consumption volumes, especially for whole grains, fruit and vegetables, which are rich in fiber and in polyunsaturated fatty acids, etc, but also suggest that the adverse effects associated with some foods of animal origin are limited to very high consumption levels or are even non-existent. In moderate amounts, these foods can therefore be part of a balanced diet, also providing components with favorable health effects.

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NATURAL, PROCESS AND ANTHROPIC CONTAMINANTS IN VEGETABLE FOODS

Terenzio Bertuzzi

Università Cattolica del Sacro Cuore, Facoltà di Scienze Agrarie, Alimentari e Ambientali, Dipartimento di Scienze Animali, della Nutrizione e degli Alimenti (DIANA), Piacenza

Vegetables foods may be contaminated with several toxic compounds during different steps of their production. The quality of raw materials plays a significant role, in particular the control of mycotoxin contamination levels. Recently, some studies evaluated the concentration ratio in several products of the cereal supply chain during the milling steps and in the bakery products. Moreover, emerging and not regulated mycotoxins have been considered, as alternariols, moniliformin, citrinin and sterigmatocystin.

Besides the natural contaminants, other toxic compounds can occur after transformation processes at high temperatures. In particular, acrylamide, furans and chloro-propanediols show high toxicity and have been considered in recent EFSA and European Commission reports.

Finally, anthropic contaminants can also occur in vegetables foods; recent EFSA opinions concerned with risk assessment of polycyclic aromatic hydrocarbons (IPA) and toxic elements (Pb, As, Cd).

L'INFIAMMAZIONE LOW-GRADE: DA DOVE ORIGINA, COSA FA, COME SI COMBATTE

Francesco Visioli (1)

(1) Dipartimento di Medicina Molecolare, Universita' di Padova)

Corresponding author: F. Visioli (francesco.visioli@unipd.it)

The global population is steadily aging and this has become a public health concern with an important socio-economic dimension. Ageing is multifactorial and poorly understood, but is characterized by an increase in the concentration of inflammatory markers detectable in the bloodstream, a phenomenon that has been termed "inflammageing". Acute inflammatory response is beneficial if it's a transient reaction to harmful conditions. Acute inflammation stimulates the defense, repair, turnover and adaptation of many tissues. Alas, there is also chronic and low grade inflammation, which detrimental for many tissues and for normal organ's function. This lecture will give an overview of low grade inflammation (LGI) and underscore the potential drivers and the effects of the "inflamed" phenotype observed in the elderly. Among many factors, the lecture will address the role of gut microbiota, the immune system crosstalk, and the gut-brain axis. Finally, we will discuss the major health complications associated with LGI in the elderly, including mental health and wellbeing, metabolic abnormalities and infections. Because there is the possibility of manipulating LGI by nutritional interventions, the lecture will provide an overview of the evidence that exists in the elderly for micronutrients such as omega-3 fatty acids, probiotics, prebiotics, vitamins, and (poly)phenol interventions as potential food components that slow LGI. Indeed, there is evidence to support specific dietary or pharma-nutritional interventions as a strategy to control LGI. Future research will further clarify this issue and provide validated preventive strategies.

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ABSTRACT ORAL PRESENTATION

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

AIPVET

REPRODUCIBILITY AND FEASIBILITY OF CLASSIFICATION AND NATIONAL GUIDELINES FOR HISTOLOGICAL DIAGNOSIS OF CANINE MAMMARY GLAND LESIONS: A MULTI-INSTITUTIONAL RING STUDY

V. Baldassarre(1), B. Brunetti(2), G.P. Burrai(3), C. Cocumelli(4), M.I. Crescio(5), V. Grieco(6), S. Iussich(7), L. Maniscalco(7), F. Mariotti(8), F. Millanta(9), O. Paciello(1), S. Papparella(1), R. Rasotto(10), M. Romanucci(11), A. Sfacteria(12), V. Zappulli(13)

Authors, listed in alphabetical order, are from the AIPVET working group on Standardization of diagnostic criteria for canine an feline mamamry tumors, Italy. 1) University of Naples Federico II, Department of Veterinary Medicine and Animal Production; 2) University of Bologna, Department of Veterinary Medical Sciences; 3) University of Sassari, Department of Veterinary Medicine; 4) Istituto Zooprofilattico Sperimentale del Lazio e della Toscana M. Aleandri, Roma; 5)Istituto Zooprofilattico Sperimentale del Piemonte, Liguria e Valle d'Aosta, National Reference Center for the Veterinary and Comparative Oncology (CEROVEC), Torino; 6) Università degli Studi di Milano, Dipartimento di Medicina Veterinaria e Scienze Animali;7) Università degli Studi di Torino, Dipartimento di Scienze Veterinarie; 8) University of Camerino, School of Bioscience and Veterinary Medicine; 9) University of Pisa, Department of Veterinary Sciences, 10)Veterinary Histopathology Consultant, Verona; 11) Università degli Studi di Teramo, Facoltà di Medicina Veterinaria; 12) Università degli Studi di Messina, Dipartimento di Scienze Veterinarie; 13) University of Padua, Department of Comparative Biomedicine and Food Science.

Corresponding author: V. Zappulli (valentina.zappulli@unipd.it)

A standardised histological diagnosis provides the basis for clinical treatment and management decisions but also contributes to monitoring cancer incidence, using cancer epidemiology to enlighten the causes of the disease, and framing the activity of cancer research. However, the accuracy of histological diagnosis is inadequately understood in veterinary oncology and often lacks standardisation and includes misleading terminology, causing variable/personal interpretation of histological criteria and resulting in non-uniform diagnosis and impossibility to compare studies and interchange data.

The aim of the present work was to quantify interobserver agreement of canine mammary tumours (CMTs) among pathologists with different diagnostic experiences and belonging to different institutions when the same classification (including application of ICD-O codes), grading systems, and guidelines were applied.

A group of 15 pathologists reviewed the recently published Davis-Thompson Foundation classification for mammary lesions, identified critical points and agreed on guidelines to overcome these points. The guidelines were applied in a blinded ring test on 36 CMTs.

The interobserver agreement, feasibility, and reproducibility of guidelines, were statistically assessed by Kappa analysis.

The overall concordance rate of diagnostic interpretations of participating pathologists expressed in terms of identification of Hyperplasia-Dysplasia vs Benign vs Malignant lesions showed a substantial agreement (average k ranging from 0.66 to 0.82, with a k-combined of 0.76). Instead, outcomes expressed in terms of ICD-O morphological code/diagnosis of histotype had only a moderate agreement (average k ranging from 0.44 and 0.64, with a k-combined of 0.54).

Results were encouraging, demonstrating that internationally established classifications and consensus panel guidelines can produce moderate to substantial agreement, however additional efforts are needed to further reduce interobserver variability in CMTs diagnosis, grading and classification.

MILK FAT GLOBULE MIRNOME RESPONSE TO AN LPS CHALLENGE IN HOLSTEIN COWS

Matteo Cuccato (1), Karol Pawłowski (2), Yannick Faulconnier (3), José Pires (3), Sebastien Bes (3), Paola Sacchi (1), Francesca Tiziana Cannizzo (1), Christine Leroux (3)

((1) Università degli Studi di Torino, Dipartimento di Scienze Veterinarie, Grugliasco, Italia. (2) Warsaw University of Life Sciences, Faculty of Veterinary Medicine, Department of Pathology and Veterinary Diagnostics, Poland. (3) INRAE, Université Clermont Auvergne, VetAgro Sup, UMR Herbivores, Saint Genes-Champanelle, France.
Corresponding author: M. Cuccato (matteo.cuccato@unito.it)

Bovine mastitis is an inflammatory disease which can affect animal health and welfare, and decrease profitability due to reduced milk production, increased treatment costs and culling [1]. microRNAs (miRNAs) are small non-coding RNAs, with 20 to 22 nucleotides, which regulate gene expression through mRNA silencing at post-transcriptional level [2]. In the last decades, miRNAs have been largely studied and have attracted an ever-increasing interest as innovative biomarkers [2]. Milk fat globules (MFG) are an easily accessible source of miRNAs and this study aimed to investigate the miRNAs cargoes in MFG as possible biomarkers of mastitis using an LPS challenged dairy cow model. As previously described [3], multiparous Holstein cows (n=5) at 27±3 days in milk were injected with 50 µg of LPS from *E. coli* in one healthy rear mammary quarter. All procedures were approved by the Auvergne -Rhône-Alpes regional ethics committee on animal experimentation (APAFiS #2018062913565518). Residual milk was collected just before and 6.5 h after the LPS challenge. Total RNA was extracted from 1 g of MFG obtained after centrifugation of collected milk at 2000xg at 4°C as previously described [4]. Transcriptomic analysis was achieved using a customized 8x60K ruminant miRNA microarray kit. One-color (Cy3) method with 100 ng of total RNA (100 ng) was used. FDR values obtained using Storey correction were considered significant with a p-value ≤0.05. In silico functional analyses were performed using OmicsNet, Mienturnet and Cytoscape software. A total of 37 differentially expressed miRNAs were identified before and after the LPS challenge, 27 and 9 downregulated and upregulated, respectively. PANTHER BP analysis was conducted in OmicsNet to identify biological processes regulated by the LPS challenge. In particular, the analysis revealed that 11 out of 37 miRNAs were mainly involved in cell life and gene expression mechanisms, among the different biological processes. The list of validated miRNA-gene interactions, obtained using Mienturnet, was filtered according to the genes involved in cell life and gene expression processes, previously identified with OmicsNet, allowing the identification of the main miRNAs involved in the regulation of the abovementioned biological processes. Reactomes, visualized with Cytoscape, showed that 8 miRNAs out of 11 were mainly involved in the regulation of biological processes after the LPS challenge. Furthermore, among these 8 miRNAs, *miR-362-3p* and *miR-190a-3p* were involved in many biological processes, including cell life and gene expression. In addition, these two miRNAs represented an important node of the regulation of biological processes by targeting the majority of genes. *miR-362-3p* and *miR-190a-3p* are known to regulate inflammation and apoptosis [5, 6] and their downregulation may better explain the pathogenesis of LPS-induced mastitis. In conclusion, this study confirmed MFG as an easy and rapid source of miRNAs. LPS challenge modified the expression of miRNAs, showing their role in the modulation of the acute inflammatory response to LPS. Further studies will be conducted for a better understanding of miRNAs role during mastitis, especially during spontaneous diseases.

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RAPID EVAPORATIVE IONIZATION MASS SPECTROMETRY-BASED LIPIDOMIC FOR IDENTIFICATION OF CANINE MAMMARY PATHOLOGY

Jessica Maria Abbate (1), Domenica Mangraviti (2), Carmelo Iaria (2), Francesca Rigano (2), Luigi Mondello (2), Fabio Marino (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: J.M. Abbate (jabbate@unime.it)

Rapid Evaporative Ionization Mass Spectrometry (REIMS) is an emerging ambient ionization technique which allows the identification of tissues in real-time through the analysis of the informative aerosols generated during their intraoperative electrosurgical dissection. The coupling of the REIMS method with a classic surgical electrocautery is known as the intelligent knife (iKnife) [1, 2]. The MS spectra obtained are used to build a predictive statistical model (PCA/LDA, Principal Components Analysis/Linear Discriminant Analysis), which allows the immediate identification and differentiation of tissues based on their differences in the lipidomic profiles [1, 2]. Currently, REIMS technology has been validated for metabolic phenotyping tumors intraoperatively in human surgical oncology, exhibiting high diagnostic accuracy, improving margin assessment and surgical outcomes [3, 4]. The ability to rapidly identify tissues based on their metabolic phenotypes using a REIMS approach may represent an important advantage over routine methods in monitoring tissue resection in the field of veterinary medicine. Therefore, this study aimed to investigate REIMS-TOF-MS technology in identifying pathological canine mammary tissues, differentiating them from normal mammary gland based on different metabolic phenotypes, using histologically validated ex-vivo samples. Thirty-nine mammary gland samples were specularly sectioned and routinely processed for histology (HE) and stored at -80°C for REIMS-TOF-MS analysis. Histological examination classified our samples in 4 classes: normal (n=12), hyperplastic (n=6), inflammatory (n=7), and neoplastic (n=14). The neoplastic samples included 6 benign and 8 malignant canine mammary tumors of different grade of malignancy [5]. Healthy mammary tissues were obtained from necropsied dogs. Mass spectrometric data generated from histologically validated samples (n=20) representative for each histological type were used to build the multivariate statistical model, further validated for robustness and predictive capability by performing two different in silico validation tests (1-file out and 5-fold validation) using LiveID software version 1.2 (Waters Corporation, UK). The confusion matrix of both approaches revealed a very low rate of failures and outliers, with classification accuracy of 90% and 95%, respectively. The validated model showed a distinct clusterization of the samples based on histological classification, in particular between healthy and pathological/neoplastic tissues along the main linear discriminations. A second MS analysis of mammary samples, with known histological diagnosis and not previously included in the statistical model, was performed to test the recognition capability in real-time. All samples were correctly identified with very high correctness score (98-100%). The REIMS method has proved to be a valid, fast and accurate technology in the differentiation of pathological canine mammary tissues based on differences in lipid metabolism. Real-time intraoperative tissue identification would offer a significant advantage especially in the complete surgical excision of tumors. Further validation studies on a larger number of samples and different histotypes will be required before applying REIMS technology to intraoperative veterinary diagnostics.

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POSTMORTEM ELECTRICAL CONDUCTIVITY CHANGES OF DICENTRARCHUS LABRAX SKELETAL MUSCLE: ROOT MEAN SQUARE (RMS) PARAMETER IN ESTIMATING TIME SINCE DEATH

Jessica Maria Abbate (1), Gabriele Grifò (2), Fabiano Capparucci (3), Luca Cicero (3), Giancarlo Consolo (2), Giovanni Lanteri (3)

(1) Università degli Studi di Messina, Dipartimento di Scienze Veterinarie. (2) Università degli Studi di Messina, Dipartimento di Scienze Matematiche e Informatiche, Scienze Fisiche e Scienze della Terra (3) Università degli Studi di Messina, Dipartimento di Scienze Chimiche, Biologiche, Farmaceutiche ed Ambientali.
Corresponding author: G. Lanteri (glanteri@unime.it)

The estimation of postmortem interval (PMI) is a critically important investigative tool in some animal death investigations, and nowadays it represents a major challenge for veterinary pathologists globally [1]. Investigations on the electrical properties of tissues have been of great scientific interest for decades and several practical applications have been proposed [2]. Since the electrical properties of tissues are strongly dependent on morphological and functional cells' integrity and intra- and extracellular conductivities, electric impedance spectroscopy techniques have been explored to predict carcass decomposition and estimate PMI [3, 4]. However, the most relevant, quantitative parameter in approximating PMI has not been recognized so far. The present study investigated the electrical conductivity changes in skeletal muscles of sea bass specimens (*Dicentrarchus labrax*; n=18) (approval no. 037/2019) kept at different room temperatures (15°C; 20°C; 25°C) during a 24-hour postmortem period, using a signal generator/oscilloscope system as innovative technology. After 10-days acclimatization, fish were euthanized (1 fish/day) and blood was sampled to assess haematological profile and serum concentration of electrolytes. Signal was applied to each specimen by placing 2 electrodes in the epaxialis/hypaxialis skeletal muscles, behind the operculum. The oscilloscope was used to record the input and output signals, measured by 2 electrodes at 10 cm of distance. The Root Mean Square (RMS) voltage value, detected by the oscilloscope, was recorded every 15 minutes for 24 hours. The resulting time series were statistically analysed using MATLAB. The time evolution of the RMS signal in the 24h postmortem underwent the same qualitative behaviour for all specimens. In particular, after a short period during which the RMS signal decreased, the RMS values increased until they reached a maximum and subsequently decreased progressively over time, returning to the initial value. A strong linear correlation ($r>0.978$) was obtained among all RMS time series, confirming that the above time behavior was applied to all animals. Regression analysis was applied to deduce the mathematical peak function which best describes the aforementionated trend of the RMS values over the 24 hours ($r^2>0.86$). Interestingly, the time at which the maximum of the RMS value was reached strongly depended on room temperature, ranging from 6 hours in fish kept at 25.0°C to 12-14 hours at 15.0°C. The use of a signal generator/oscilloscope system has proved to be a promising technology in studying the dielectrical properties of muscle during early PMI, with the advantage of being a fast, non-destructive and inexpensive method. Of course, further electrical and theoretical investigations will be required to standardize and validate this technology before moving from the laboratories to the field applications.

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VET-ICD-O-CANINE-1: THE GIVCS INITIATIVE FOR CANCER CODING IN COMPANION ANIMALS

Valeria Baldassarre (1), Katia Pinello (2), Orlando Paciello (1), Katja Steiger (3), Isabel Pires (4), Renée Laufer-Amorim (5), Anna Oevermann (6), João Niza-Ribeiro (2), Luca Aresu (7), Brian Rous (8), Ariana Znaor (9), Ian Cree (9), Franco Guscetti (10), Chiara Palmieri (11), Maria Lucia Zaidan Dagli (12)

(1) Department of Veterinary Medicine and Animal Production, University of Naples Federico II, Italy. (2) Departamento de Estudo de Populações, Vet-OncoNet, ICBAS, Instituto de Ciências Biomédicas Abel Salazar, Universidade do Porto, Portugal. (3) Institute of Pathology, School of Medicine, Technical University of Munich, Germany. (4) Animal and Veterinary Research Centre (CECAV), Associate Laboratory for Animal and Veterinary Science-AL4AnimalS, University of Trás-os-Montes and Alto Douro (UTAD), Portugal. (5) School of Veterinary Medicine and Animal Science, São Paulo State University (UNESP), Brazil. (6) Division of Neurological Sciences, DCR-VPH, Vetsuisse Faculty, University of Bern, Switzerland. (7) Department of Veterinary Sciences, University of Turin, Italy. (8) National Disease Registration Service, NHS Digital, Cambridge, UK. (9) International Agency for Research on Cancer, IARC, Lyon, France. (10) Institute of Veterinary Pathology, Vetsuisse Faculty, University of Zurich, Switzerland. (11) The University of Queensland, School of Veterinary Science, Gatton Campus, Australia. (12) School of Veterinary Medicine and Animal Science of the University of São Paulo, Brazil.

Corresponding author: V. Baldassarre (valeria.baldassarre@unina.it)

In recent years, the interest in the one health perspective has also involved the field of oncology resulting in an increased attention in comparative studies. Domestic animals develop cancers with similar features to, and share the same environment as humans, thus representing promising translational models and potential sentinels of environmental risk factors. The absence of a standardized method for collecting validated and comprehensive cancer data in veterinary medicine, as provided by human cancer registries, is one of the main obstacles to this approach. Since a pivotal aspect for a widely accepted and comparable veterinary cancer registration system is the availability of standards for cancer coding, the Global Initiative for Veterinary Cancer Surveillance (GIVCS) developed and proposed a comparative coding system for canine neoplasms. An international collaboration between veterinary pathologists and epidemiologists analysed the currently accepted classification of canine tumours and defined topography and morphology codes for each organ system, in alignment with the International Classification of Disease for Oncology ICD-O-3.2. The codes were reviewed by members of the International Agency for Research on Cancer (IARC). The proposal, developed specifically for canine tumors, has been named Veterinary International Classification of Diseases for Oncology – Canine, 1st edition (Vet-ICD-O-canine-1). It consists of 335 topography and 534 morphology codes. Most canine tumors exhibit a high level of similarity with corresponding human entities leading to the application of the same code of the human ICD-O-3.2. *De novo* codes were created for specific tumor entities and topographic sites specific to the canine species. This coding system represents a comprehensive resource for veterinary pathologists, veterinary epidemiologists and cancer researchers interested in establishing and maintaining a canine cancer registry, as well as collecting and sharing comparative cancer data.

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HISTOPATHOLOGICAL FEATURES OF CANINE MAMMARY GLAND LESIONS IN RELATION WITH SHEAR WAVE ELASTOGRAPHY FINDINGS

Marcella Massimini, Mariarita Romanucci, Alberto Contri, Alessia Gloria, Leonardo Della Salda.

Università degli Studi di Teramo, Facoltà di Medicina Veterinaria
Corresponding author: (mmassimini@unite.it)

Due to the heterogeneity of canine mammary gland lesions, the usefulness of ultrasound techniques, including Shear Wave Elastography (SWE), to discriminate between benign and malignant lesions is still unclear [1, 2]. In particular, SWE is the most advanced technique that allows imaging tissue elasticity enabling objective measurement of stiffness expressed in kPa or m/s, and it can differentiate benign and malignant human breast lesions with acceptable to excellent performance, reducing the frequency of unnecessary biopsies, particularly when coupled with B-mode ultrasonography [3]. The aim of this study was to evaluate possible correlations between several histopathological features of canine mammary gland nodular lesions such as percentages of fibrosis, non-epithelial (myoepithelial/stromal) cells, chondroid and/or bone tissues, necrosis and intratubular secretion, and SWE values, as well as to investigate SWE diagnostic performance to distinguish benign *vs* malignant lesions, or its possible relationship with tumor histotype and/or grade. Mammary nodules were initially displayed in B-mode using a Logiq S8 (GE Healthcare, Milwaukee, WI, USA) and then examined with the Shear Wave Elastography tool, using a 9L-D multi-frequency linear probe (GE Healthcare) at a frequency of 8 MHz. Tissue stiffness was measured on a region of interest (ROI) manually defined by the same expert operator within the margin of the lesion and the value was recorded (ELStiff, kPa). Mammary nodules (n=27 from 16 dogs) were fixed in 10% neutral-buffered formalin and routinely processed for histology. Histological classification (hyperplasia n=3, benign tumors n=1, malignant tumors n=23) and grading (I n=15; II n=7; III n=1) were assessed according to Zappulli et al. (2019) and Penã et al. (2013), respectively [4, 5]. Fibrosis and non-epithelial cell components were evaluated through Masson Trichrome and Movat Pentachrome stainings, and immunohistochemistry for Vimentin, respectively, and quantified by means of specific tools of the ImageJ programme. The percentage of the other histopathological features was assessed by area measurement tools of Leica Application Suite X. Correlation coefficients were calculated through Pearson's coefficient, whereas Mann-Whitney test was used to evaluate significant differences between groups. Statistical analysis revealed that % of fibrosis was the main feature showing a significant positive correlation with SWE values (Pearson's $r=0.768$; $p<0.001$) together with % of chondroid tissue (Pearson's $r=0.772$; $p<0.039$). A low but significant negative correlation was also present between % of fibrosis and % of Vimentin+ cells (Pearson's $r=-0.385$; $p<0.027$). Although significant differences between benign vs malignant lesions, tumor histotypes or grades were non observed in our study, further investigations with a higher number of cases are still necessary to better evaluate the diagnostic performance of SWE for canine mammary gland tumors, and to establish optimal cutoff SWE values to differentiate benign vs malignant lesions.

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PERIOSTIN EXPRESSION IN CANCER ASSOCIATED FIBROBLAST IN CANINE UROTHELIAL CARCINOMA OF THE URINARY BLADDER: A PILOT STUDY

Eleonora Brambilla (1), Rafal Ciaputa (2), Stanislaw Dzimira (2), Marcin Nowak (2) Stefano Romussi (1), Damiano Stefanello (1), Valeria Grieco (1)

(1) Università degli Studi di Milano, Dipartimento di Medicina Veterinaria e Scienze Animali. (2) Wroclaw University of Environmental and Life Sciences, Faculty of Veterinary Medicine, Department of Pathology, Division of Pathomorphology and Veterinary Forensics, Poland.

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

Periostin (POSTN) is a non-structural protein of the extracellular matrix (ECM) mainly secreted by stromal cells such as cancer-associated fibroblasts (CAFs), and also by tumor cells, especially cancer stem cells. POSTN has been shown to regulate multiple biological behaviors of tumor cells, including proliferation, survival, invasion, angiogenesis, metastasis and chemoresistance [1].

Unlike in most tumors, POSTN expression appears to be downregulated in human urinary urothelial carcinoma of the bladder (UCB) compared with the normal tissue [2]. However, the expression of this marker has not yet been investigated in veterinary medicine.

UCB represents 1.5–2% of all naturally-occurring cancers in dogs, a rate similar to that reported in humans [3]. Aiming to investigate their POSTN expression, for the present retrospective study 20 cases of canine UCBs (10 cystoscopic biopsies and 10 surgical biopsies) were considered. Related paraffin blocks were retrieved from the archive of the Pathology Unit of the Veterinary Medicine Department of the University of Milan and from University of Environmental and Life Sciences of Wroclaw. Serial sections were obtained and stained with hematoxylin-eosin for histological evaluation and immunohistochemically tested (ABC method) using antibody direct against the POSTN antigen. POSTN intensity was scored and compared with normal bladder expression, moreover the correlation between the tumor expression, the tumor behavior and mitotic count were evaluated. A decrease in POSTN expression in CAFs was observed in UCBs compared with normal urinary bladder and POSTN expression seems to negatively correlate with the tumor mitotic count.

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DISSECTING THE IMMUNE LANDSCAPE OF CANINE DIFFUSE LARGE B-CELL LYMPHOMA BY NANOSTRING TECHNOLOGY

Luca Licenziato (1), Lucia Minoli (1), Laura Marconato (2), Diana Giannuzzi (3), Raffaella De Maria (1), Selina Iussich (1), Giulia Orlando (4), Antonella Fanelli (1), Ugo Ala (1), Luca Aresu (1)

(1) Università degli Studi di Torino, Dipartimento di Scienze Veterinarie. (2) Università di Bologna, Dipartimento di Scienze Mediche Veterinarie. (3) Università degli Studi di Padova, Dipartimento di Agronomia, Animali, Alimenti, Risorse naturali e Ambiente. (4) Università degli Studi di Torino, Dipartimento di Oncologia.
Corresponding author: L. Licenziato (luca.licenziato@unito.it)

Canine diffuse large B-cell lymphoma (cDLBCL), one of the most investigated tumors in veterinary oncology, is characterized by high mortality and clinical heterogeneity. The recent addition of immunotherapy (APAVAC) to standard chemotherapy (CHOP) dramatically improved the outcome; however, treatment response remains unpredictable [1]. To gain insight into the complex mechanisms regulating the immune system-cancer interaction and to identify genes aberrantly regulated and potentially impacting prognosis, we explored the tumor immune landscape in dogs with DLBCL that were uniformly treated. To this aim, a Nanostring gene expression panel containing 800 genes involved in immune response was employed. Forty-eight dogs with histologically and immunohistochemically confirmed DLBCL were included. All dogs underwent complete staging work-up and received the same chemo-immunotherapeutic protocol (CHOP plus APAVAC) [1]. In addition, *TP53* mutational status was evaluated. The immune tumor landscape was quantified with the Nanostring nCounter Canine IO Panel using RNA extracted from paraffin blocks.

Among the clinico-pathological features included in the multivariate Cox proportional-hazard analysis, *TP53* mutation and serum LDH level were significantly associated with a shorter overall survival (OS) ($p<0.001$ and $p=0.03$, respectively) and time to progression (TTP) ($p<0.001$ and $p=0.03$, respectively). A total of 615 genes were identified over the detection threshold and considered in the following analyses. K-means clustering showed two clusters of 27 ("good responders") and 21 ("bad responders") dogs, characterized by a significantly different OS (544 and 196 days, $p=0.003$) and TTP (227 and 134 days, $p=0.005$). Genes and pathways characterizing the two clusters and putatively driving treatment response were investigated. Seventy-eight differentially expressed genes between the two groups were found, including 77 up-regulated and 1 down-regulated genes in "good responders" compared to "bad responders". By GSA, "good responders" were characterized by an enrichment in gene sets regulating antigen processing (e.g. *CD8A, B2M, DLA88, DLA-12*), T-cell functions (e.g. *CXCL10, IDO1, CD8A*), and cytotoxicity (e.g. *NKG7, IFIT2, IFIH1*).

Together, our results demonstrate that the immune landscape of cDLBCL is heterogeneous and clinically meaningful. Dogs with an efficient tumor antigen recognition and enhanced cytotoxic activity showed a more effective treatment response and longer survival. Previously, the relevance of the immune response was hypothesized in dogs with DLBCL by RNA-seq, and molecules such as PD-1, PD-L1 and CTLA-4 were associated with survival [2]. Here, by Nanostring technology we identified numerous genes that play roles in multiple biological pathways involved in both immune activation and suppression, highlighting the complexity of this tumor. Our observations also suggest that Nanostring may be a promising molecular tool for predicting survival in cDLBCL when only formalin-fixed paraffin-embedded samples are available.

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CANINE MAST CELL TUMOR-IMMUNE SYSTEM INTERACTION: IMMUNOHISTOCHEMICAL INVESTIGATION OF TUMOR ASSOCIATED MACROPHAGES AND TUMOR INFILTRATING LYMPHOCYTES

Luca Bertola (1,2), Benedetta Pellizzoni (1,2), Roberta Ferrari (1), Lavinia Elena Chiti (1), Damiano Stefanello (1), Martina Manfredi (1), Donatella De Zani (1), Chiara Giudice (1), Valeria Grieco (1), Cristina Lecchi (1), Camilla Recordati(1, 2)

(1) Università degli Studi di Milano, Dipartimento di Medicina Veterinaria e Scienze Animali (DIVAS). (2) Mouse and Animal Pathology Laboratory (MAPLab), Fondazione Unimi.

Corresponding author: Luca Bertola (luca.bertola@unimi.it)

Mast cell tumor (MCT) is one of the most common skin neoplasms in dogs. Although various methods have been proposed for prognostic assessment, the prognosis of grade II/low grade MCTs still remains controversial (1-3). In the last years, the interaction between tumors and immune system has been extensively studied allowing important progresses in prognostic assessment. In particular, the role of tumor-associated macrophages (TAMs) and tumor-infiltrating lymphocytes (TILs) has been proven useful as a prognostic marker in various human neoplasms (2). The aim of this study was to characterize the immune cell infiltration in canine MCT, and evaluate its relationship with location (cutaneous, subcutaneous) and nodal metastatic spread.

Thirty-eight MCTs [26 grade II/low-grade cutaneous MCTs, 12 subcutaneous MCTs with different growth patterns] from 33 dogs with known sentinel lymph node (SLN) metastatic status (according to Weishaar *et al*, 2014) were selected. Samples were routinely processed for histopathology and immunohistochemistry for Iba1 (macrophages), CD20 (B cells), CD3 (T cells), and FoxP3 (Tregs) was performed. A semiquantitative grading of interstitial and perivascular CD3+, CD20+ and FoxP3+ cells and a morphological evaluation of Iba1+ cells were performed. For each marker, immunopositive % area in 6 hot spot fields at 40x was evaluated by digital imaging analysis (ImageJ software).

All MCT (38/38) were markedly and diffusely infiltrated by round, spindled or stellate IBA1+ cells, and variably infiltrated by lower numbers of CD20+ and CD3+ cells. FoxP3+ cells were rare and found only in 19/38 cases (50%). Perivascular aggregates of CD3+ cells and CD3+ % area were significantly increased in subcutaneous MCTs when compared to cutaneous MCTs, and interstitial CD3+ cells were significantly increased in cutaneous MCTs with HNO SNLs when compared to cutaneous MCTs with HN1, HN2, HN3 SNLs. Concerning CD20+, FoxP3+ and Iba1+ cells no significant differences between cutaneous and subcutaneous MCTs and among sentinel lymph node groups were identified.

The results suggest that MCT are markedly infiltrated by TAMs, and less and variably infiltrated by TILs. Only the presence of CD3+ T cells was affected by tumor location and SLN metastatic status, suggesting that cutaneous and subcutaneous MCT have a different tumor immune microenvironment and that increased intratumoral T cell infiltration might contribute to the control of nodal metastatic spread of cutaneous MCT.

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MUTATIONAL PROFILE AND PROGNOSTIC SIGNIFICANCE OF TP53 IN CANINE DIFFUSE LARGE B-CELL LYMPHOMA

Antonella Fanelli (1), Diana Giannuzzi (2), Laura Marconato (3), Luca Licenziato (1), Raffaella De Maria (1), Andrea Rinaldi (4), Luca Rotta (5), Nicole Rouquet (6), Giovanni Birolo (7), Piero Fariselli (7), Afua A. Mensah (4), Francesco Bertoni (4, 8), Luca Aresu (1)

(1) Università degli Studi di Torino, Dipartimento di Scienze Veterinarie. (2) Università degli Studi di Padova, Dipartimento di Agronomia, Animali, Alimenti, Risorse naturali e Ambiente. (3) Università di Bologna, Dipartimento di Scienze Mediche Veterinarie. (4) Università della Svizzera Italiana, Istituto Oncologico di Ricerca (IOR). (5) Istituto Europeo di Oncologia (IEO), Dipartimento di Oncologia Sperimentale. (6) URODELIA. (7) Università degli Studi di Torino, Dipartimento di Scienze Mediche. (8) Ente Ospedaliero Cantonale, Istituto Oncologico della Svizzera Italiana.
Corresponding author: A. Fanelli (antonella.fanelli@unito.it)

In dogs, diffuse large B-cell lymphoma (DLBCL) is the most common lymphoid neoplasm and is characterized by a remarkable degree of clinical heterogeneity. While the majority of dogs relapses after treatment, survival varies among patients and is difficult to anticipate [1]. Several prognostic variables were recently proposed, but molecular data were never considered. Transcriptomic profiling has partially explained the pathogenesis of canine DLBCL (cDLBCL) revealing similarities with the human counterpart, but also differences [2]. Recent studies in canine B-cell lymphoma have identified some recurrently mutated genes, but data were neither associated with clinical features nor histotype, reducing the significance of the results [3].

To overcome these limitations, we performed an integrated analysis of exome (n=77) and RNA sequencing (n=43) in a cohort of dogs with confirmed cDLBCL to investigate the genetic fingerprint and to retrieve possible associations with clinico-pathological features, including outcome.

Recurrently mutated genes (*TRAF3, SETD2, POT1* and *TP53*) and copy number aberrations (gains of CFA13 and CFA31) were catalogued. Mutations associated with clinico-pathological features and affecting prognosis were identified. While it was found that a wide range of signaling pathways and cellular processes were shared with human DLBCL, including NF-κB, chromatin remodeling, histone modifications and cell cycle, the frequencies of the most recurrently mutated genes differed between the two species.

Using a machine learning approach, a prognostic model integrating clinical, exonic variants and transcriptomic features to predict outcome (https://compbiomed.hpc4ai.unito.it/canine-dlbcl/) was developed. Risk scores for *TP53* mutation were calculated, revealing an association with a shorter overall survival and time to relapse in dogs treated with CHOP or CHOP plus APAVAC. The model was further validated in an independent cohort of cDLBCLs (n=56).

In conclusion, the results presented here describe the genetic landscape of cDLBCL, define mutations with clinical significance and identify possible new therapeutic opportunities.

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DOES TLS EXIST IN CANINE MAMMARY GLAND TUMORS? PRELIMINARY RESULTS IN SIMPLE CARCINOMAS

Giada Giambrone (1), Stefania Di Giorgio (1), Giuseppe Mazzullo (1), Cecilia Vullo (2), Alessandra Sfacteria (1)

(1) Università degli Studi di Messina, Dipartimento di Scienze Veterinarie. (2) Università degli Studi di Messina, Dipartimento di ChiBioFarm.

Corresponding author: G. Giambrone (ggiambrone97@gmail.com)

The neoplastic development is influenced by the expression of tumour antigens that activate an anti-tumour immune response committed to eliminating cancer cells. Human medical studies on the tumour microenvironment show that this body's defence is not only carried out in secondary lymphoid structures (e.g. in lymph nodes) but also directly in the tumour through organized cellular aggregates that are called tertiary lymphoid structures (TLSs) (1). They have been found in the stroma of different tumours, and are generally composed of T-lymphocyte-rich areas, dendritic cells juxtaposed to B-lymphocyte follicles with characteristic germinal centres and surrounded by plasma cells. Some high endothelial venules can be seen nearby and serve to allow the passage of lymphocytes (2).

While TLSs were first interpreted as an attempt by the body to mount a local anti-tumour response, their role has since then been revised to conclude that their occurrence has different meanings in different tumour types. In breast cancer, the presence of TLSs is associated with the most aggressive subtypes, representing a negative prognostic factor (3).

Although canine mammary tumours have long been proposed as a model for studying breast cancer, there is still little research on the tumour stromal microenvironment in veterinary medicine and TLSs evidence could allow advances in understanding the process of tumour immunoediting.

This study aimed to study TLSs in mammary simple carcinomas. Fifty cases were selected and a careful morphological assessment of the inflammatory infiltrate was performed on H&E sections. Immunohistochemistry was then carried out to typify inflammatory cells and molecules of interest in the tumour microenvironment.

Results showed that the stromal infiltrate was mainly perivascular and variable according to the histotype with conspicuous lymphocytic and macrophagic infiltrates close to malignant epithelial proliferation. T lymphocytes were higher than IgG positive cells. Sometimes, inflammatory infiltrates were organized in follicles close to high-grade carcinomas. Indeed, in these last areas numerous lymphoid cells assumed concentric patterns around vessels with plumped endothelium, or vessels containing neoplastic emboli, and IgG-positive cells were in the majority. The concentric pattern simulated a lymphoid organization similar to that described in breast cancer (2). The composition of these formations was mainly plasma cells, macrophages, T lymphocytes and mast cells in order of the highest representation. Although the organization of tertiary structures did not fully reflect secondary lymphoid structures, it is now recognised that TLSs are dynamic entities with a varying organization from simple clusters of lymphocytes to more complex structures (4). Therefore, we can assume that even in canine mammary tumours TLSs exist and that are entities to take into account due to their presence in the most aggressive histotypes or tumours with a high degree of malignancy.

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MACROSCOPICAL EVALUATION OF THE CARCASSES TO ESTIMATE PMI IN THE DOMESTIC CAT

Frine Eleonora Scaglione (1), Lorenzo Ressel (2), Arturo Nicoletti (1), Erica Ottonello (1), Chiara Parodi (1), Michela Clerici (1), Paola Pregel (1), Emanuele Ricci (2)

(1) Università degli Studi di Torino, Dipartimento di Scienze Veterinarie. (2) Department of Veterinary Anatomy, Physiology and Pathology, University of Liverpool, Neston, UK

Corresponding author: F.E. Scaglione (frineeleonora.scaglione@unito.it)

In recent times, pets' role in society has increased and a high number of cruelty episodes have been reported and prosecuted. Therefore, we aimed at investigating a method to estimate the post-mortem interval (PMI) in the domestic cat on the basis of macroscopical evaluation of carcasses. Few studies are currently available on PMI determination in the veterinary field and the most investigated species are pigs, usually considered as proxies to the human species [1]. The available methods are various and different (total body score, entomology, molecular biology, histology), but to the best of our knowledge no study about macroscopical evaluation of carcasses are available in cats.

Twenty-four carcasses of domestic cats have been left in the field from February to June for 1 day, 3 days, 14 days or 28 days. Three evaluation scales have been created, on the basis of the observation of the carcasses, respectively for head and neck (score range 1-15), trunk (score range 1-8) and limbs (score range 1-6). A total body score has been then calculated for each animal, as a sum of the previous ones. The relations among the scores and the accumulated degree-days (ADD) have been investigated.

In agreement with some authors [2], also in the present study the head and neck region was the first to show signs of decomposition, accelerated by larval colonization. Differently from other studies [3], skin discoloration, cadaveric swelling and leakage of body fluids from the natural orifices have not been observed. The identified one-phase association models revealed to be a promising tool to estimate the PMI on the basis of the visual evaluation of corpse decomposition.

The proposed evaluation represents a simple and fast method to estimate PMI, which can also be applied in the place where the carcass was found, differently from other described methods requiring laboratory settings. Nonetheless, it should be avoided the use of this protocol as the only method in determining the time of death. In fact, since multiple factors can affect the presentation of decomposed animal carcasses, histopathology, forensic entomology and circumstantial information should accompany the gross assessment for a more accurate esteem of the PMI.

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A STEP FORWARD IN VETERINARY FORENSIC MEDICINE: A HISTOLOGICAL SCORING SYSTEM TO ESTIMATE THE POST-MORTEM INTERVAL IN CATS

Emanuele Ricci (1), Paola Pregel (2), Arturo Nicoletti (2), Chiara Parodi (2), Erica Ottonello (2),Michela Clerici (2), Lorenzo Ressel (1), Frine Eleonora Scaglione (2)

(1) Department of Veterinary Anatomy, Physiology and Pathology, University of Liverpool, Neston, UK. (2) Università degli Studi di Torino, Dipartimento di Scienze Veterinarie.

Corresponding author: P. Pregel (paola.pregel@unito.it)

The estimation of the time elapsed since death is a fundamental challenge in the practice of Forensic Pathology, but it is also a complicated topic, as the decomposition rate is influenced by different internal and external factors and each organ undergoes a peculiar series of alterations. In the veterinary field, only few studies have been conducted, and most of them focus on the macroscopical evaluation of carcasses [1, 2], while those who take into consideration the microscopical changes generally focus on one or few tissues only [3].

The aim of the present work was to create a scoring system based on the histological analysis of different organs, allowing to estimate the post-mortem interval (PMI) in cats. In particular, the objectives were: to identify those parameters whose changes are correlated with the time elapsed since death; to assign a score to the different phases that follow each other during the decomposition process, for each of the selected parameters; to investigate the relations between the evaluated parameters and the accumulated degree-days (ADD), in order to obtain a precise estimation of the PMI; to recognize the reliability of the analysis of each organ for the estimation of the PMI, revealing the convenience to use them for a specific range of accumulated degree-days.

Twenty-four cats, variable in age, breed and cause of death, have been enrolled. The corpses were donated to the Department of Veterinary Sciences, University of Turin, by the owners. The cats were placed in a dedicated area where the cadavers maintained their contact with the soil, which allowed a total, undisturbed exposure to the action of insects and environment, but covered by a metal cage to protect from scavenging. ADD were used as they are better correlated with the post-mortem alteration than time [4] and the number of days elapsed since the death can be subsequently deducted. One-phase association models have been identified, relating the histological score determined for each analyzed organ to the ADD.

The estimation of the PMI based on a thorough and wide histological scoring system is an innovative tool for the veterinary forensic practice. Some organs reveled to be more useful to determine shorter PMI, while others could be useful for higher ADD. Therefore, in addition to the detailed routine gross and entomological examinations, the results of this study provide useful information on the stage of tissue autolysis and decomposition in cats by the mean of histopathology, in order to further improve the accuracy of PMI esteem.

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AUTOMATED HEMATOLOGICAL ANALYSIS USING SYSMEX XN-V IN Testudo hermanni: AGREEMENT WITH MANUAL COUNT

Sara Meazzi, Valeria Martini, Amanda Moretti, Emanuele Lubian, Saverio Paltrinieri, Alessia Giordano

Università degli Studi di Milano, Dipartimento di Medicina Veterinaria e Scienze Animali.
Corresponding author: S. Meazzi (sara.meazzi@unimi.it)

The haematology of non-mammalians, including tortoises, does not rely on automated methods due to the presence of nucleated red blood cells (NRBC) and thrombocytes (Thr) that interfere with the automated count. Thus, the use of specific hemocytometer counting chambers is required [1]. This method, being operator-dependent, may have an inherent error up to 10% and it is time consuming [1]. The use of an automated analyser, instead of the manual count, for the evaluation of tortoise whole blood would allow a decrease in turnaround time for reporting results and an increase of accuracy and precision, due to the higher number of cells counted by the analyser compared to those evaluated by the operator. The new haematology analyser Sysmex XN-V offers the possibility to count NRBC into a new white and nucleated red blood cells channel (WNR), where whole blood reacts with an acidic haemolysing reagent and nucleic acid stains. In humans, NRBC are differentiated from leukocytes (WBC) thanks to a weaker fluorescence signal [2]. The aims of this study were to evaluate the possibility to perform a complete blood cell count (CBC) based on WNR data from Sysmex XN-V and its agreement with the manual counts in *Testudo hermanni,* one of the most diffused tortoises in Italy [3]. Fifty heparinized blood samples, collected for diagnostic purposes, were counted using the Neubauer improved chamber on a sample diluted 1:200 with Natt and Herrick's solution, as already described in literature [3]. The same samples were then counted twice with Sysmex XN-V and fourteen out of fifty samples were counted again after 24 hrs. All the WNR scattergrams were then re-analysed using a gate panel created *ad hoc* to separate two main populations: NRBCs (weak fluorescence signal) and WBC + Thr (high fluorescence signal). The intra- and inter-assay precisions of the automated method were assessed. Agreement between methods was assessed using Passing & Bablok regression analysis and the Bland - Altman difference plot test. Intra - assay precision was optimal for NRBCs (CV 0.98%±1.96) and moderate for WBC + Thr (9.24%±16.61). Nevertheless, no significant differences between the two automated counts were found, either for NRBCs or WBC + Thr. Similar results were obtained for inter - assay repeatability (NRBCs CV 1.31%±2.98; WBC + Thr CV 12.69%±10.35). No proportional and constant errors were observed between the methods for NRBC (intercept: 51.91; 95% CI: -20–120; slope: 0.85; 95% CI: 0.68–1). However, the automated measurement always slightly underestimated RBC count compared to the manual count (bias 31.1; 95% CI: 4.98–57.22; P=0.02). Concerning WBC + Thr no constant and proportional errors were observed (intercept: 0.29; 95% CI: -0.89–1.53; slope: 0.85; 95% CI: 0.66–1.10). However, a slight overestimation of the automated count was highlighted (bias -1.04; 95% CI: -1.79–-0.29; P=0.007). Results obtained in this study suggest that Sysmex XN-V may be used to perform haematological analyses in *Testudo hermanni*, even in the absence of perfect overlap with the manual count. Indeed, manual count, due to its subjective nature, may be inaccurate [1]. It is mandatory to assess new reference intervals for the examined species using the automated method. Moreover, it would be interesting to assess the performances of the Sysmex XN-V in other type of tortoises and turtles to investigate possible differences among species.

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HEREDITARY CATARACT, DEGENERATIVE MYELOPATHY, AND MULTIFOCAL RETINOPATHY IN ITALIAN PYRENEAN MOUNTAIN DOG: A GENETIC SURVEY

Riccardo Moretti (1), Stefania Chessa (1), Stefano Sartore (1), Margherita Profiti (1), Guido Massimello (2), Paola Sacchi (1)

(1) Università degli Studi di Torino, Dipartimento di Scienze Veterinarie. (2) Freelance veterinarian.
Corresponding author: R. Moretti (riccardo.moretti@unito.it)

Pyrenean Mountain Dog (PMD) is an autochthonous dog breed from the Pyrenean Mountains, which divide France from Spain. It is an ancient breed, originally used to guard both castles and flocks of sheep from wolves and bears [1]. Data on genetic characterization of this breed is still scarcely available, and the few studies found in scientific literature are mostly case reports [2]. Recently, the occurrence in PMD of diseases for which their genetic causative mutation has been identified in other canine breeds was reported. Aim of this genetic survey was to investigate the genetic variability of three known causative mutations in Italian PMD: 5:g.82198113del (primary hereditary cataract [3]), 31:g.26540342G>A (degenerative myelopathy [4]), and 18:g54478586G>A (multifocal retinopathy 1 [5]). A total of 39 PMD were sampled (blood or buccal swab) by veterinarians during routinary visits, DNA was extracted, amplified by PCR for each of the three loci, and sequenced to obtain the genotype at each locus of interest. None of the investigated animals carried the deletion responsible for primary hereditary cataract. Regarding the degenerative myelopathy, 7 dogs (17.95%) carried the nucleotide substitution, all of them in a heterozygous asset. The allelic frequency for the mutated A allele in our population was therefore 9%. Lastly, as for the multifocal retinopathy 1, only 2 dogs (5.13%) carried the nucleotide substitution, both in an heterozygous asset. The allelic frequency for the mutated A allele in our population was therefore 3%. Since both are autosomal recessive disorders, dogs carrying the nucleotide substitution for myelopathy and retinopathy did not show clinical signs of the relative disease, but, as healthy carriers, can pass this mutation to their offspring. Therefore, according to these results, genetic testing of the sires and dams should be performed, in order to avoid mating between healthy carriers of these pathologies.

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INVOLVEMENT OF MICRORNAS IN THE PATHOGENESIS OF OSTEOSARCOMA: THE CANINE MODEL

Simona Perga (1), Chiara Beltramo (1), Raffaella De Maria (2), Katia Varello (1), Maria Ines Crescio (1), Valentina Campia (1), Simone Peletto (1), Elisabetta Razzuoli (1), Lorella Maniscalco (1), Elena Bozzetta (1), Paola Modesto (1)

(1) Istituto Zooprofilattico Sperimentale del Piemonte, Liguria e Valle d'Aosta, Torino as National Reference Center for the Veterinary and Comparative Oncology (CEROVEC), Genova, Italy. (2) Department of Veterinary Science, University of Turin, Turin, Italy.

Corresponding author: P. Modesto (paola.modesto@izsto.it)

Osteosarcoma (OSA) is the most common primary malignant bone tumor in dogs and represents 80-85% of primitive bone tumors. Canine OSA is highly comparable to the human disease due to similar genetic and clinical features. These characteristics make dogs a good model in comparative oncology [1]. MicroRNAs (miRNAs) controls post-transcriptional genes expression, their altered regulation has been described in different malignant tumors, among which OSA [2, 3]. The aim of this study was to validate the canine OSA as an experimental model of the human OSA, therefore we studied the expression profile of a panel of miRNAs already described to have an important role in tumor development and progression in human OSA, and the existence of prognostic markers and molecular targets for novel therapeutic approaches was investigated. Nineteen samples of OSA and paired healthy bone were collected and stored in RNAlater at -80° C, five blood samples of the same animals were also collected. Twenty-three blood samples were collected from healthy dog and from 15 dog with canine malignant melanoma and other pathologies. Total RNA enriched in miRNA was extracted using silica membrane columns (miRNeasy Mini and miRNeasy Serum/Plasma Advanced Kit, Qiagen); a custom panel of 57 miRNAs was selected on the basis of the existing literature on human and canine different neoplasms [2, 3, 4]. Expression analysis was carried out using miRCURY LNA (Qiagen) technology; data analysis was performed by the CFX Maestro 2.2 (Biorad Laboratories) software, comparing miRNA expression profiles among biological groups (tumor vs healthy vs other pathologies) both in tissues and in plasma; miRNAs with log2 fold change (FC) ≥4 and p≤0.05 were considered. A statistical analysis was performed both in tissue and plasma by Wilcoxon test and Cox models in order to evaluate the existence of variables which may have an impact on survival, on the presence of metastases or relapses and the presence of genes that affect survival hazards. miRNAs expression profile highlighted 28 miRNAs significantly dysregulated in OSA tissues: 16 were under-expressed and 12 were over-expressed in tumors compared to healthy controls. Moreover, blood analysis revealed that 18 miRNAs were significantly dysregulated in OSA patients: 17 were over-expressed and only miR-29b-3p was under-expressed compared to healthy controls. Statistical analysis identified miR-148b-3p (up regulated) as a risk factor for survival and miR-191-5p, miR-26b, miR-30a (all down regulated) as protective factors for survival. Our results confirm the validity of the dog model to study the biological-molecular processes underlying human OSA. Statistical correlation between molecular data and clinical/follow-up data has highlighted markers worthy to be investigated in future studies in order to clarify whether these miRNAs may also have prognostic or therapeutic significance.

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GIVING SHAPE TO REAGENTS FOR NOVEL APPROACHES TO IMMUNOHISTOCHEMISTRY AND IMMUNOFLUORESCENCE

Claudia Rifici (1), Salvador D Aznar-Cernantez (2), A Abel Lozano-Pérez (2), José L Cenis (2), Alessandra Sfacteria (1), Giuseppe Mazzullo (1)

(1) Università degli Studi di Messina, Dipartimento di Scienze Veterinarie. (2) Department of Biotechnology, Instituto Murciano de Investigation y Desarrollo Agrario y Ambiental, Spain.
Corresponding author: C. Rifici (crifici@unime.it)

Immunohistochemistry (IHC) and immunofluorescence (IF) are important ancillary techniques for pathologists in routine diagnostic work, in basic research, and they allow the detection of target molecules in the diagnosis of tumors, sometimes essential before administering a targeted drug (1). Different critical points can influence the outcome of the final reaction, preventing a correct standardization and causing a high risk of suboptimal laboratory performance which leads to inferior diagnostics and a subjective interpretation of the staining results (2). The loss of affinity for the target epitope over time, the degradation or denaturation of proteins promoted by long-term storage, prolonged exposure to light of the antibody fluorophore-conjugated, and harsh treatments during the transport and preservation at the laboratory (i.e. freeze/thaw cycles) can alter the outcomes and consequently the correct clinical diagnosis (3).

Silk fibroin, is a protein obtained from the cocoons of *Bombyx mo*ri silkworms with a wide variety of known applications, ranging from textile use to biomedical uses. This versatile biomaterial, can be transformed into various formats such as gels, sponges, scaffolds, and films, as well as be successfully employed in different biomedical applications (4). Silk protein biomaterials have demonstrated good biocompatibility, presenting a slow and controllable degradation rate, and have a high capacity to adsorb biomolecules including proteins and drugs (5) without altering their biological activity. This stabilization can protect them, even during an eventual disruption in the pharmaceutical "cold chain"(6).

The aim of the study was to eliminate some of the critical points related to the use of antibodies (Abs) and reagents that can influence the outcome of the final reaction of IHC and IF, using the film at different concentrations of silk loaded whit different Abs in order to modulate their release and preserving their functionality. Therefore, the goal was to give a new pocket solid shape single-dose to the Abs and the reagents loaded in silk films making them more readily usable, reproducible and standardized for improving interlaboratory comparability in these diagnostic testing. These preliminary results showed a shiny positivity in the tissue, using different antibodies, even several months apart. The auto-fluorescent background was reduced in sections incubated with Ab-loaded silk eliminating the need of quenching steps on FFPE (formalin fixed, paraffin embedded) sections.

Moreover, thanks to the fibroin properties and the use of fluorescent-labeled Abs, a reduction in the times was possible, when compared to classic testing, which could improve intraoperative diagnostics of tumors and adapt operative procedures accordingly.

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PORCINE POLYSEROSITIS: WELL BEYOND GLASSER'S DISEASE

Jasmine Hattab, Abigail Rose Trachtman, Francesco Mosca, Pietro Giorgio Tiscar, Giuseppe Marruchella
Università degli Studi di Teramo, Facoltà di Medicina Veterinaria.
Corresponding author: G. Marruchella (gmarruchella@unite.it)

Polyserositis is a widespread and economically relevant disease condition, it most commonly affects 4-8 weeks old piglets and results from the hematogenous spreading of bacteria. Polyserositis has been long identified with Glasser's disease (GD) caused by *Glaesserella parasuis* (*Gp*). However, pathogens such as *Streptococcus suis* (*Ss*) and *Mycoplasma hyorhinis* (*Mh*) can induce similar lesions [1]. The present study aims to investigate prevalence, timing and etiology of polyserositis in a pig herd, under strict field conditions. The effect of GD vaccination of suckling piglets and sows was also assessed.

The investigation was carried out in a farrow-to-finish herd, where several cases of GD and streptococcosis had been previously diagnosed. Between September 2020 and March 2021, a total of 46 sows and 387 piglets were included in the present study. All piglets spontaneously dead between the 2nd and 16th week of age were necropsied on a weekly basis. In case of polyserositis, pericardium, fibrin clots or lung samples were collected and tested for *Gp*, *Mh* and *Ss* by PCR methods, following previously published protocols [2-4]. Whenever possible, bacteriological tests were additionally performed.

Mortality rate was 12.5% in suckling piglets (the vast majority observed during the first week of age) and 19.9% after weaning. Overall, polyserositis was detected in 44 out of 75 necropsied piglets, mainly at 8-9 weeks of age. PCR tests demonstrated the presence of *Mh*, *Ss* and *Gp* in 23, 13 and 6 piglets, respectively, while they yielded negative results in 12 cases. *Gp* was always detected in combination with other pathogens. On the contrary, *Mh* and *Ss* were detected as the only causative agent in 15 and 9 cases, respectively. Vaccinating sows (27/46) and piglets (101/346) did not significantly affect the mortality rate, the overall prevalence of polyserositis and the occurrence of GD (Fisher Exact test, $p>0.05$).

Salogni et al. have recently reported that *Mh* and *Gp* are the most common agents of porcine polyserositis and apparently occur at the same rate (55%) in Northern Italy. The present study highlights that each pig herd could represent a unique scenario, thus requiring a well-targeted diagnostic approach and management. In particular, our results support the emerging role of *Mh*, which reasonably explains the poor result of GD vaccination and/or antimicrobial therapy with beta-lactams. Last but not least, it should be carefully considered that polyserositis often results from the interaction of several factors (e.g., PRRSv infection), which should be properly checked to effectively control this health issue.

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FIRST INVESTIGATIONS ON A MOUSE MAMMARY TUMOUR VIRUS-LIKE ASSOCIATED WITH FELINE LYMPHOMAS

Francesca Parisi (1), Francesca Lessi (2), Michele Menicagli (2), Prospero Civita (3), Romano Liotti (2), Francesca Millanta (1), Giulia Freer (3), Mauro Pistello (3), Chiara Maria Mazzanti (2), Alessandro Poli (1)

(1) Università degli Studi di Pisa, Dipartimento di Scienze Veterinarie. (2) Fondazione Pisana per la Scienza, Pisa. (3) Cardiff University, School of Pharmacy and Pharmaceutical Sciences, College of Biomedical and Life Sciences. (4) Università degli Studi di Pisa, Ricerca Traslazionale e delle Nuove Tecnologie in Medicina e Chirurgia.
Corresponding author: F. Parisi (francesca.parisi@vet.unipi.it)

The Mouse Mammary Tumour Virus (MMTV) is implicated in the aetiology of murine mammary carcinomas and one of its variant, the type B leukemogenic virus, can cause murine thymic lymphomas. Furthermore, (i) a MMTV-like was suspected to be involved in human breast cancer [1, 2]; (ii) MMTV-like sequences were found in human lymphomas [3], and a common etiology between breast cancer and lymphoma was hypothesized [4]; (iii) MMTV-like sequences were amplificated from feline mammary carcinomas [5-7] and from the thymus of a kitten and the spleen of a cat [8]. The aim of this study was to investigate the presence of MMTV *env* like sequences (MMTVels) and protein 14 (p14) of a MMTV-like, respectively through molecular and immunohistochemical investigations, in fifty-three formalin fixed paraffin embedded feline lymphoma samples. Moreover, an epidemiological survey was conducted to investigate the hypothetical role of cats in MMTV epidemiology. Feline lymphomas were classified according to the National Cancer Institute Working Formulation (NCIWF, 1982) [9], the anatomical district and the immunophenotype [10] . MMTVels were detected in 5/53 tumours (9.4%): three were gastrointestinal lymphomas (one B-type diffuse large, one B-type small non-cleaved, and one T-type diffuse mixed lymphoma), and two nasal lymphomas (one B-type diffuse small cleaved lymphoma and one B-type diffuse mixed lymphoma). P14 expression was immunolocalized in the cytoplasm and rarely in nuclei exclusively of neoplastic cells from PCR-positive tumours. The presence of the MMTVels and p14 antigen was significantly correlated with the localization of neoplasms in nasal cavities. All cats with MMTVels-positive lymphoma had a history of contact with the outdoor environment and/or catteries and were FIV/FeLV negative. In conclusion, this study succeeds in demonstrating the presence of MMTVels and protein in feline lymphomas. Differently from murine and human literature, feline PCR positive lymphomas were mostly B-type, and this evidence should be further investigated to understand the mechanisms by which a MMTV-like virus could be involved in feline lymphoma tumorigenesis. The significant association between the presence of the viral sequences in lymphoid tumours and their nasal localization, together with the data collected through supplementary anamnesis, may support important information on the epidemiology of the virus.

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IMMUNOHISTOCHEMICAL CHARACTERIZATION OF IMMUNE SYSTEM CELLS IN LYMPHOID ORGANS FROM ROE AND FALLOW DEER

Niccolò Fonti (1), Francesca Parisi (1), Maria Irene Pacini (1), Francesca Millanta (1), Marcello Periccioli (2), Alessandro Poli (1)

(1) Università di Pisa, Dipartimento di Scienze Veterinarie. (2) Unità Funzionale di Sanità Pubblica Veterinaria e Sicurezza Alimentare Zona Distretto Grossetana, Dipartimento di Prevenzione, Azienda USL Toscana Sud Est, Amiata Grossetana e Colline Metallifere.

Corresponding author: N. Fonti (niccolo.fonti@phd.unipi.it)

Roe deer (*Capreolus capreolus*) and fallow deer (*Dama dama*) are common wild ruminants widely distributed in Italy, with a consistency of over 400 and 20 thousand heads for roe and fallow deer, respectively (1). In the recent decades, the interest in wildlife diseases has increased, together with the awareness of public health implications and the importance of active surveillance (2). Infectious diseases of this species can potentially pose health risks with economic and health repercussions on domestic animals and humans (3). Serological and molecular assays are often performed in wildlife investigations, but histology and immunohistochemistry (IHC) could provide complementary data to fully understand the pathogenesis of infection in these species, especially in chronic or subclinical diseases (4, 5). However, few studies have been conducted on haemal nodes and pathological tissues from roe (6, 7) and fallow deer (8) in which immune system cells have been phenotyped. Furthermore, no commercial antibodies specifically developed against these antigens are available. The aims of this study were (I) to determine the cross-reactivity and reliability of a wide anti-human panel of commercial antibodies on routine formalin-fixed and paraffin-embedded samples (FFPE), and (II) to describe the distribution and immunohistochemical localization of roe and fallow deer main immune cell subsets in the lymph nodes and spleen. Twenty retromandibular lymph nodes (RLNs) and spleen samples were collected from 10 roe deer and 10 fallow deer shot in the province of Grosseto during the 2020-2021 hunting season. Samples were fixed in formalin and routinely processed for histological and immunohistochemical evaluation. A panel of 12 commercial anti-human monoclonal and polyclonal antibodies against B-cells (CD79a, CD20), T-cells (CD3), T-reg (Foxp3), and macrophages (Iba-1, MAC387, AM-3K, CD68) was employed. Of these, eight succeeded in immunolabeling their specific target. The two anti-CD79a, the anti-Foxp3, and the anti-CD68 antibodies did not show suitable immunostaining, compared to control tissues. Antibodies known to recognize CD79a, CD20, CD3, Iba-1, Mac387, and AM-3K in human tissues, successfully labelled cells in cervine RLN and spleen with varying degrees of intensity. This study supplies the first immunohistochemical description of immune cell subpopulations in non-pathological spleen and RLNs from roe and fallow deer. The localization and distribution of the main immune cells of these species are those reported in domestic ruminants (9), and other cervids (10). Additionally, it provides a robust and easily repeatable manual IHC protocol suitable to immunolocalize cervine B-, T-cells, and macrophages in FFPE tissue samples. These results may support future diagnostic investigations on immune cell response and pathogenesis in roe and fallow deer diseases.

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MONITORING THE HEALTH STATUS OF HONEYBEE COLONIES OF THE CAMPANIA REGION IN PRE-WINTERING AND POST-WINTERING PERIODS

Karen Power, Manuela Martano, Gennaro Altamura, Anna Matrone, Anastasia Palumbo, Paola Maiolino

(1) Università degli Studi di Napoli, Dipartimento di Medicina Veterinaria e Produzioni Animali.
Corresponding author: K. Power (karen.power@unina.it)

Honeybee decline occurs all year round, but high rates of mortality take place especially during the winter season [1]. Among the risk factors held responsible and/or co-responsible for winter mortality, diseases affecting the hive such as varroasis, nosemiasis, virosis, represent a great threat for honeybee colonies. Many pathogens remain in honeybees and in the hive in a subclinical state and show clinical signs only when the colony is subjected to other factors which lower the individual and/or collective immune defenses [2]. Therefore, the aim of this study, founded by Sistema Nazionale INFEA- Regione Campania [3], was to monitor the health status of honeybee colonies before and after the wintering period to increase our knowledge about the presence of pathogens in the Campania region. For each province (Naples, Salerno, Caserta, Benevento, Avellino), 5 different apiaries were selected (total 25 apiaries) and from each apiary 2 different samples of 30 apparently healthy honeybees were collected, the first during the months of October-November (pre-wintering phase), the second during the months of April-May (post-wintering). For each sample (30/honeybees) *Varroa destructor* levels were assessed by icing-sugar technique [4], *Nosema* spp. infections were evaluated by smear of fecal suspension (10/honeybees) while the presence of six different viruses (Acute Bee Paralysis Virus (ABPV), Black Queen Cell Virus (BQCV), Chronic Bee Paralysis Virus (CBPV), Deformed Wing Virus (DWV), Kashmir Bee Virus (KBV), Sac Brood Virus (SBV)) was determined by Multiplex PCR (10/honeybees) [5]. According to the results obtained, specific Good Beekeeping Practices (GBP) were suggested. Pre-wintering samples showed the presence of low Varroa infection rates in 96% of apiaries, while 36% of samples were found positive for *Nosema* spp. Biomolecular analysis detected the presence of DWV (85%), BQCV (40%), ABPV (12%), SBV (12%), IAPV (4%). No sample was found positive for CBPV and KBV. During the wintering period no colony was loss. Analysis of the post-wintering samples confirmed low *Varroa* infection rates (100%) while less samples were found positive for *Nosema* spp. (13%) and DWV (17%). On the contrary more samples resulted infected with BQCV (48%) and ABPV (22%). No samples were found positive for CBPV, KBV as well as for IAPV and SBV. Our results are in line with previous studies [6,7], confirming the endemic nature of pathogens such as *Varroa*, *Nosema* spp. and different viruses and the association between pathogens, particularly between *Varroa*-DWV-ABPV, and BQCV-*Nosema*. However, conversely to what previously described, no sample was positive for CBPV, probably due to its seasonality. Moreover, during the post-wintering phase a decrease in prevalence of DWV, SBV, IAPV and *Nosema* spp. was observed, very likely after the application of GBP such as administration of acaricidal treatments and food supplements. Our study suggests the importance of monitoring the health status of honeybee colonies especially of those apparently healthy, to prevent winter mortality, and of applying GBPs to control, and possibly eliminate pathogens from the hives.

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HISTOLOGICAL AND IMMUNOHISTOCHEMICAL CHARACTERIZATION OF PULMONARY LESIONS IN CD40LKO MICE EXPERIMENTALLY INFECTED WITH PNEUMOCYSTIS MURINA

Andrea Cappelleri (1)(2), Simone Canesi (2), Luca Bertola (1)(2), Valentina Capo (3)(4), Anna Villa (3)(4), Camilla Recordati (1)(2), Eugenio Scanziani (1)(2)

(1) Università degli Studi di Milano, Dipartimento di Medicina Veterinaria e Scienze Animali. (2) Fondazione UniMi, Mouse and Animal Pathology Laboratory. (3) IRCCS San Raffaele Scientific Institute, San Raffaele Telethon Institute for Gene Therapy. (4) National Research Council, Institute of Genetic and Biomedical Research, Milan Unit.
Corresponding author: A. Cappelleri (andrea.cappelleri@unimi.it)

Pneumocystis spp. are fungal pulmonary opportunistic pathogens found in a wide range of mammalian species, including humans and mice. *Pneumocystis* spp. infection is particularly relevant in immunocompromised patients, like those affected by X-linked hyper-IgM syndrome, of which the CD40LKO mouse is a well-described model (3). Although pulmonary lesions due to *Pneumocystis* spp. infection have been widely described in SCID mice (1,4), little is known about the pulmonary inflammatory response in CD40LKO mice. In the present study we describe the histological and immunohistochemical features of pulmonary lesions in CD40LKO mice experimentally infected with *Pneumocystis murina*, in comparison to naturally infected SCID mice. All animal procedures were approved by the Animal Care and Use Committe of the San Raffaele Hospital (IACUC #749, #818). Twenty-six intranasally infected CD40LKO (C57BL/6 background) and 11 naturally infected CB17-SCID mice were examined, along with 5 uninfected CD40LKO mice used as controls. FFPE lungs were stained with H&E and IHC for: *P. murina,* CD3 (T-cells), B220 (B-cells), Iba-1 (macrophages), Ly6G (neutrophils), and Ym1 (M2 macrophages). No pulmonary lesions were found in uninfected CD40LKO mice. Eighteen out of 26 CD40LKO infected mice were positive for *P. murina*, which appeared as eosinophilic, foamy material containing punctate cysts in H&E, confirmed by IHC. Fourteen out of 18 *P. murina*+ CD40LKO mice had interstitial pneumonia, in which IHC revealed a predominance of interstitial macrophages. Interstitial pneumonia was not observed in *P. murina*- CD40LKO mice. In 22/26 infected CD40LKO mice, including 16/18 *P. murina*+ mice, multiple lymphocytic inflammatory aggregates were found around bronchi and blood vessels and in the pulmonary parenchyma. These aggregates were almost exclusively composed of B-cells. In 2/18 *P. murina*+ CD40LKO mice a T-cell lymphoma was diagnosed. Nineteen out of 26 infected CD40LKO mice, including 14/18 *P. murina*+ mice, had acidophilic macrophage pneumonia (AMP), characterized by the presence of alveolar macrophages or multinucleated giant cells laden with eosinophilic crystals that stained positive with Ym1 by IHC. All the 11 infected SCID mice were *P. murina*+ by IHC, but only 4/11 had interstitial pneumonia, characterized by more abundant neutrophils than CD40LKO mice. No lymphocytic aggregates nor AMP were found in SCID mice. Interstitial pneumonia has been classically associated to *Pneumocystis* spp. infection. (1,4) This was a consistent finding in *P. murina*+ CD40LKO mice in our study, but less common in SCID mice. Lymphocytic inflammatory aggregates were also exclusive of CD40LKO mice, with the predominance of B-cells in the aggregates being potentially peculiar of this model. Interestingly, this lesion was present in *P. murina*- CD40LKO mice as well. This could possibly be due to a resolution phase of the infection, with amount of the pathogen undetectable by IHC. Lymphocytic inflammatory aggregates have been previously reported in SCID mice experimentally infected with *Pneumocystis* spp., but not in course of natural infection (1,4). AMP is an idiopathic pulmonary disease of laboratory mice, frequently occurring in the C57BL/6 strain, although it was also reported to be enhanced in *Pneumocystis* spp. infection (2).

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SWINE PLEUROPNEUMONIA: LUNG SCORING AND Actinobacillus pleuropneumoniae PCR IDENTIFICATION IN PIEDMONT FARMS

Francesca Tiziana Cannizzo (1), Sara Divari (1), Matteo Cuccato (1), Silvia Ciaramita (1), Francesco Meliota (2), Davide Campelli (2), Bartolomeo Biolatti (3), Pier Giuseppe Biolatti(4), Enrico Bollo (1)

(1) Università degli Studi di Torino, Dipartimento di Scienze Veterinarie; (2) Fatro Spa; (3) Università di Scienze Gastronomiche di Pollenzo; (4) Libero professionista.

Corresponding author: FT Cannizzo (tiziana.cannizzo@unito.it)

Actinobacillus pleuropneumoniae is one of the major etiological agents of severe necrotising pleuropneumonia in pigs, leading to massive losses in the pig industry with high economic impact due to mortality, reduction in daily weight gain, lower feed efficiency, as well as veterinary expenses.

Prevention of swine pleuropneumonia is accomplished by vaccination plans. Isolates can be further differentiated into serovars, based mainly on capsule antigens, and 19 serovars are currently recognized [1]. Differences in the virulence potential of different serovars can be partly explained by the serovar-specific production of Apx toxins [2]. The typing of isolates is important to identify changes in serovar prevalence that may occur over time, indicating alterations in virulence, or host and environmental factors, and to guide the choice of the vaccination plan.

The objective of this study was to determine the serovars of *A. pleuropneumoniae* strains isolated from pneumonic lesions in naturally infected dead pigs in Piedmont Region.

A further aim was to evaluate the lung lesion score of the pathological outcomes and to compare it with serovars identified by multiplex PCR.

A total of 31 animals were included in this study from vaccinated and not vaccinated farms. Lungs were collected and a gross pathology scoring sheet was developed to enumerate the grossly visible lesions [score 0-4]. Samples for histological, microbiological and molecular investigations were appropriately collected. Molecular serotyping was carried out according to previously described by Stringer et al. (2021).

The lung lesions were represented by edema, hemorrhages, infarction, and necrosis associated with fibrinous pleuritis. *A. pleuropneumoniae* was isolated in 7/31(23%) samples.

On the contrary, a specific amplification product was identified in 17/31 (55%) samples. In 1/17 (6%) sample the specific serovar was not identified. A more severe score lesion [score 4] was associated with serovars 9/11 (100%) and 7/2 (100%). Serovar 5 and 6 were associated with score 3 in 2/3 (67%) and 5/7 (71%) samples, respectively; association with score 4 was reported in the remaining samples. Only serovar 2 induced low-grade lesions [score 1] in 2/3 (67%) cases.

Our results highlight the need of a multidisciplinary approach for detecting serovars of *A. pleuropneumonia* responsible for severe disease.

Further investigations are needed for better understanding the epidemiology swine pleuropneumonia.

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NON-CONVENTIONAL PAPILLOMA VIRAL GENE EXPRESSION IN A DOMESTIC CAT WITH A PULMONARY SQUAMOUS CELL CARCINOMA AND MULTIPLE DISTANT METASTASES

Kevin Pascal Spindler (1), Benedetta Passeri (1), Marzia Cino (1), Nicoletta Campanini (2), Chiara Guarnieri (1), Federico Armando (3), Elisabetta Razzuoli (4)

(1) Università degli Studi di Parma, Dipartimento di Scienze Medico Veterinarie. (2) Università degli Studi di Parma, Dipartimento di Medicina e Chirurgia. (3) Pathology Institute, University of Veterinary Medicine, Hannover, Germany (4) Istituto Zooprofilattico del Piemonte, Liguria e Valle d'Aosta.

Corresponding author: M. Cino (marzia.cino@unipr.it)

Papillomaviruses (PV) infect the skin and mucosal surfaces of many animal hosts causing highly malignant epithelial cancers. The role of PVs in squamous cell carcinoma (SCC) in cats has long been recognized (1). Primary pulmonary SCC in cats is a rare histologic diagnosis. Biologically, these neoplasms are reported to be locally aggressive and rare to metastasize (4). In this study we describe the highly metastatic behavior of SCC of probably pulmonary origin in a cat, with a non-conventional papilloma virus gene expressed in all metastatic lesion detected.

A 5 year-old neutered male European domestic short hair cat was referred for anorexia and vomiting. Multiple dermal nodular lesions were palpated and sampled during physical examination. Cytology evidenced multiple SCC. Chest radiographs revealed a right pulmonary mass. Due to the disseminated disease, the owners opted for euthanasia.

Tissue samples were collected from lungs, spleen, adrenal gland, mesenteric lymph node and dermal lesions and were FFPE prepared for routine histology and for polymeric system-immunohistochemistry (IHC) for cytokeratin AE1/AE3, 5/6, 7, 8/18, 17, 20, CKHMW. To confirm the squamous origin of the lesion, p40 (4) and TTF-1 antibodies (5) were applied (6).

DNA extraction, was performed to check PVs presence on SCC and metastasis. Total acid nucleic extraction was performed on four FFPE sections using AllPrep DNA FFPE Kit (Qiagen, Milano). β2-microglobulin gene amplification was performed to evaluate DNA amplificability. Feline-L1 DNA presence was tested by Real-Time protocols.

The right lung showed extensive epithelial neoformation diagnosed as SCC. The same cellular pattern was observed in all the lesions. Neoplastic cells were strongly positive for CKAE1/AE3, CK5/6, CK8/18 and p40, while TTF-1 was negative. PV was detected in all supposed primary and metastatic lesions.

These results hypothesize that pulmonary neoplasia may have been the primary site of SCC onset and the highly metastatic behavior. The detection of PV gene expression in all the lesions, suggests its potential role in the development of neoplastic and metastatic disease.

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DETERMINATION OF IMMUNE RESPONSE IN HEPATIC OVINE CYSTIC ECHINOCOCCOSIS

Davide De Biase (1), Paola Pepe (2), Francesco Prisco (2), Antonio Bosco (2), Giuseppe Piegari (2), Ilaria d'Aquino (2), Lorenzo Riccio (2), Serenella Papparella (2), Laura Rinaldi (2), Orlando Paciello (2)

(1) Università degli Studi di Salerno, Dipartimento di Farmacia. (2) Università degli Studi di Napoli, Dipartimento di Medicina Veterinaria e Produzioni Animali.

Corresponding author: Davide De Biase (ddebiase@unisa.it)

Cystic echinococcosis (CE) is a zoonotic infection caused by larval forms (metacestodes) of the tapeworm *Echinococcus granulosus*. The main hosts in the life cycle of this parasite are dogs and other carnivores whereas the intermediate stage known as hydatid cyst develops in the internal organs (mainly the liver and lungs) of humans and herbivores [1]. In most cases, hydatid cysts elicit mild inflammation, and the intermediate host may live with no symptoms for a very long time. However, the immune interplay between the parasite and the host is still to be fully elucidated. The aim of this study was to characterize the inflammatory phenotype in ovine liver cystic echinococcosis by the determination of different markers related to the local immune response. A total of 100 ovine livers were screened for the presence of CE and collected for histopathological analysis. Immunohistochemistry was performed using anti-Iba1, anti-MHC II, anti-CD3, anti-CD20 and anti-TGF antibodies. The quantitative concentrations of Tumor Necrosis Factor-α (TNFα), Interferon-γ (INF-γ), Interleukin (IL)-1 and IL-10 and Transforming Growth Factor (TGF)b were also determined by PCR. Out of 100 total cases, 15 were totally negative to all metacestods, 70 were positive to CE lesions, but negative for other parasites and 15 were positive exclusively to C. tenuicollis. Based on gross examination, samples were divided in three groups: 1) normal liver; 2) liver with fertile hydatid cyst and 3) liver with infertile hydatid cyst. Histologically, liver from group 1 didn't show any relevant alteration. In group 2, hepatocellular degeneration and cirrhosis were observed; the cyst wall consisted of three layers and the structural details of the protoscolices were clearly discernable. Inflammatory reaction around the cyst was mostly characterized by lymphocytes, plasma cells, macrophages and few giant cells. Admixed with inflammatory infiltrate, we observed loosely arranged fibroblasts and fibrous connective tissue. In group 3, a moderate to severe, multifocal to coalescing inflammatory infiltrate consisting mostly in palisading macrophages with foamy cytoplasm, neutrophils, multinucleated giant cells, lymphocytes and plasma cells centered necrotic debris and calcification. Moderate to severe fibroplasia was also observed. Immunohistochemical analysis revealed a diffuse immunolabelling of mononuclear cells for MHC II, Iba-1 and TGF-b in both group 2 and 3 and a predominant number of CD20+ B cells compared to CD3+ T cells. The results obtained from the molecular analyses showed absence of inflammatory markers in group 1. No significant statistical differences of the expression levels of Th-1 like immune cytokines INF-γ, TNF-α, and IL-12 were observed between group 2 and 3 ($p>0.05$). However, a significant increase in expression levels of Th-2 immune cytokines TGF-b and IL-10 was found in group 2 compared to group 3 ($p<0.05$). Our results suggest that 1) macrophages are mostly involved in the local immune response to hydatid cyst [3]; 2) Th-2 immune response may be prevalent, confirming that B cells are crucially important in the control of the immune response during parasite infection [3] and 3) the immunomodulatory role of IL-10 and TGF-b may ensure the persistence of the parasite within the host [3]. Further studies are needed to confirm these findings and better investigate the local inflammatory response involved in the different stages of ovine hydatidosis.

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FORENSIC PATHOLOGY CONTRIBUTIONS TO DIFFERENTIATE ACCIDENTAL AND NON-ACCIDENTAL INJURIES IN DOGS AND CATS

Ilaria d'Aquino (1), Giuseppe Piegari (1), Emanuela Vaccaro (1), Giovanni Valerio Salanti (1,2), Silvia Mariagiovanna Casciaro (1,2), Pierpaolo Coluccia (1), Leonardo Meomartino (1), Guido Rosato (2), Marina Pompameo (2), Orlando Paciello (1,2)

(1) Dipartimento di Medicina Veterinaria e Produzioni Animali, Università degli Studi di Napoli "Federico II". (2) Centro Regionale di Igiene Urbana Veterinaria-CRIUV – Polo Didattico Integrato Regione Campania.
Corresponding author: I. d'Aquino (ilaria.daquino@unina.it)

Several classifications of animal abuse have been reported in veterinary literature. They include physical abuse, emotional abuse, sexual abuse, and neglect [1]. Physical abuse is also known as nonaccidental injury (NAI) which is an injury that is purposefully inflicted upon an animal. In contrast, accidental injuries (AI) refer to traumatic lesions secondary to causal events, such as falls and collision with a high-speed object [2]. In the literature, only few reports have focused on post-mortem findings, and no studies have applied the post-mortem forensic examination in monitoring traumatic injuries. Therefore, the aim of this study was to evaluate the application of post-mortem examination in the identification and monitoring of accidental and non-accidental lesions in dogs and cats in Campania region. To this aim, we performed a forensic post-mortem examination of every cadaver of dog and cat who was referred to the Department of Veterinary Medicine and Animal Production, University of Naples between January 2020 and January 2022. All cadavers underwent a total body radiographic analysis, CT studies and a forensic post-mortem examination. In addition, tissue samples were collected for histopathologic examination. The joint evaluation of imaging, forensic macroscopic and microscopic examinations of the cadaver allowed us to assess the presence/absence of traumatic injuries. Inclusion criteria were as follow: 1) presence of trauma 2) absence of informations about the clinical history or witness testimony 3) outdoor crime scenes. Variables recorded were species, sex, age, and whether lesions were observed in the skull, thoracic, abdominal, or inguinal regions or if the trauma involved multiple regions. On the bases of these variables, the injuries were classified as accidental (AI) or non-accidental (NAI). A total of 100 animals met the inclusion criteria and were included in the study. In cats, we observed 30% of lesions localized in the head, 22% in the torax region, 11% in the inguinal region and no injury localized only in the abdominal region. Injuries localized in different regions of the body were seen in the 37% of cases. In dogs, imaging and post-mortem examination allowed us to observe that the areas most commonly injured were the torax region (43%) followed by the head (14%), the abdominal region (7%) and the inguinal region (7%). Multiple injuries were observed in 29% of cases. Based on the localization and type of lesion, it was possible to discriminate between non-accidental and accidental lesions. The frequency of nonaccidental lesions was 57% in the dog and 43,5% in the cat. A higher frequency of non-accidental trauma in the dog compared to the cat can be explained by the different ethology of the examined species. Dogs are highly social animals and submissive animals, more dependent on human interaction if compared to cats, and maintain these behaviors even when they are the victims of abuse [2,3]. In conclusion, this study highlights the usefulness of forensic post-mortem examination of a cadaver in identifying and monitoring accidental and nonaccidental lesions in cats and dogs when a body is recovered in outdoor crime scenes and no witness testimony is available. Moreover, our study laid the foundation for the development of a website titled "Unnamed animals" that aims to give an identity to the many unidentified animal cadavers found in the national territory and to and to reconcile them with the owners.

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LETHAL SEPSIS SHOCK AFTER DENTAL CLEANING IN A DOG: A CASE REPORT AND ITS FORENSIC-MEDICAL ASPECTS

Emanuela Vaccaro, Ilaria d'Aquino, Giuseppe Piegari, Francesca Paola Nocera, Luisa De Martino, Orlando Paciello

Università degli Studi di Napoli, Dipartimento di Medicina Veterinaria e Produzione Animale.
Corresponding author: E. Vaccaro (emanuelavaccaro3@gmail.com)

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 sepsis shock have been described in animals. 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 female cavalier king was subjected to dental cleaning procedures in a private veterinary clinic. 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 Unit of Forensic Pathology of the Department of Veterinary Medicine of Naples for further forensic necropsy and ancillary examinations. The necropsy was conducted using the forensic necropsy protocol. The gingival mucosae was hyperemic, edematous and hemorrhagic. The macroscopic examination revealed multifocal hemorrhages on the surface of the skin, myocardium, abdominal aorta and lung. 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 mild to moderate vascular ectasia. Finally, bacteriological examination allowed us to isolate *Staphylococcus pseduintermedius* 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 pseduintermedius* was made. Finally, "dental cleaning" was considered the cause of *Staphylococcus pseduintermedius* 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 suggested that sepsis shock could be a consequence not only of different types of surgical operations but also of mild non-surgical procedures; in particular, considering the normal colonization of animal's oral mucosa by saprophytic bacteria, dental procedures should always be performed using antibiotic prophylaxis to avoid massive spread of bacterial 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.

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THANATOLOGICAL AND TAPHONOMIC APPROACH ON ANIMAL REMAINS FOUND IN THE CITY OF REGGIO CALABRIA, ITALY

Giuseppe Piegari (1), Ilaria d'Aquino (1), Valerio Salanti (1), Leonardo Meomartino (2), Dario Costanza (2), Giovanni Federico (3), Giuseppe Lucifora (3), Orlando Paciello (1)

(1) Università degli Studi di Napoli "Federico II", Dipartimento di Medicina Veterinaria, Unità di Patologia. (2) Università degli studi di Napoli "Federico II", Dipartimento di Medicina veterinaria, Unità di Radiologia. (3) Istituto Zooprofilattico Sperimentale del Mezzogiorno, Italy.

Corresponding author: G. Piegari

Thanatology is a scientific discipline that investigates death from many different aspects. In forensic medicine, thanatology studies the post-mortem cadaveric changes in both human and animal cadavers [1]. Similarly, taphonomy is a sub-field of forensic anthropology that studies the vertebral remains and the variables involved in the cadaver decomposition [2]. Both forensic disciplines can provide law enforcement with useful information to correctly determine a temporal relationship between suspects and murder or to deny or confirm the testimony of a witness. Here we describe a thanatological and taphonomic approach on vertebrate remains found in 5 different pet carriers in the city of Reggio Calabria in Italy. In February 2020, 5 pet carriers were found abandoned in an urban area of Reggio Calabria. Pet carries and vertebrate remains were submitted to the Department of Veterinary Medicine of the University of Naples "Federico II" for subsequent forensic analysis. Macroscopic inspection of the carriers and the macroscopic, histopathological, and radiological analysis of the vertebral remains were performed in all 5 assessed cases. At the external inspection of the carriers, 2 out of 5 were entirely closed with adhesive tape. No signs of scratches were also observed at the internal inspection of the carriers. A complete small felid skeleton was detected in each assessed carrier by radiographic and macroscopic examinations. Cadavers were often in a horizontal resting position (head towards the bottom or entrance of the carrier and partially flexed limbs) and wrapped in dirty paper towels and cardboard sheets. As regard the post-mortem changes, cadavers detected in well-closed carriers showed a combination of mummification and decomposition, while a skeletonization or extreme decomposition was observed in other cases. Overall, forensic analysis showed evidence of post-mortem manipulation of the cadaver. Mummification of the animals has been attributed to the sealed environment, the paper towels and cardboard sheets in which the animals have been wrapped in and, finaly, the favorable temperatures. Based on international literature, it was estimated a minimum PMI of 4 months for mummificated animals and 6 months for animals in extreme decomposition phase [1-3]. This study reports an application of thanatological and taphonomic knowledge to veterinary forensic practice. In this study we identify two interesting cases of natural mummification. The mummification process results from the de-hydration of soft tissues in dry and warm environment. Furthermore, sealed environments can avoid or reduce the decomposition process due to insect activity [1-4]. Consequently, the permanence of the cadavers in well-closed carriers could be considered as a contributing factor to the mummification process observed in our study. Overall, mummification and complete skeletrization makes PMI difficult to estimate. Indeed, when the process is completed, animals remains can be preserved for years or even centuries. However, even in these cases, the thanatological and taphonomical analysis can provide valuable information to estimate the minimum PMI in forensic paractice. In conclusion, this study underlines the importance of a systematic and complete thanatological and taphonomical evaluation in all cadavers, even in those in advanced stage of decomposition or with special transformation processes.

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EARLY MACROPHAGE RECRUITMENT AND INCREASED NEOANGIOGENESIS IN THE YOLK SAC OF CHICKEN EMBRYOS INOCULATED WITH A PROBIOTIC BLEND

Lucia Biagini, Livio Galosi, Sara Berardi, Alessandra Roncarati, Subeide Mari, Valentina Grifantini, Giacomo Rossi

Università degli Studi di Camerino, Scuola di Bioscienze e Medicina Veterinaria.
Corresponding author: L. Biagini (lucia.biagini@unicam.it)

The use of the "in ovo" technique for administering substances, such as probiotic bacteria, to the chick before hatching has been widely demonstrated to be effective. Multiple studies observed how the early interaction of the chick with probiotics, through "in ovo" feeding, allows an improvement in growth performance and an increase in functionality of the immune and gastrointestinal system [1, 2]. The administration of probiotics in the amniotic fluid permits to obtain the direct contact with the gastroenteric and respiratory apparatus of the chick during hatching, when it ingests the residue of the amniotic fluid. It can be assumed that the effects observed after probiotic administration may also be linked to the influence on the structure and functionality of the yolk sac, a vital organ that performs multiple functions by acting as bone marrow for erythropoiesis, as intestine and liver for absorption and metabolism of nutrients, and as immune system for the transport of antibodies from hen to chick [3]. With the present study, we evaluate the effects of the administration of the probiotc mixture SLAB51® in the amniotic fluid of chicks at day 18 of incubation, with particular focus on the changes that occured in the yolk sac. Eighty Ross 308 eggs were incubated: half of the eggs (group P) received $1x10^5$ CFU of probiotic bacteria, diluted in 0.05 ml of saline sterile solution, while the remaining eggs, as control group (C), were inoculated exclusively with saline sterile solution. For each group, after 8, 12, 24 and 36 hours from administration, the yolk sac was collected in 10 eggs. Samples were subsequently processed for histological and immunohistochemical evaluation. The analysis of the yolk sacs collected at different timepoints showed a stronger presence of Iba1–positive macrophages in the treated eggs, compared to control samples. This trend was maintained for the different timepoints highlighting, as expected, that early probiotic administration strongly stimulates maturation and differentiation of yolk sac myelopoietic stem cells to differentiate into mature macrophages. Similarly, yolk sacs of group P at T8 showed isolated or grouped CD31+ cells; the same cells, at T36, were organized in small capillaries with a patent lumen as a marked induction of neoangiogenetic activity by the probiotic-enriched inoculum demonstrating an expansion of erythrocytic and granulocytic lineages from T8 to T36. These data confirm that yolk sac is the major intermediate erythropoietic and granulopoietic site where expansion and differentiation occur during chick development. To our knowledge, this is the first study highlighting which morphological changes are induced in the yolk sac by the "in ovo" administration of probiotics. Our results suggest that the improved growth and disease resistance shown by chicks inoculated with probiotics, as well as with pioneer colonizing bacteria, may be attributable to increased neoangiogenesis and early maturation of embryo macrophages, that promote chick metabolism, resulting in greater reactivity to environmental and pathogenic bacteria during the first weeks after hatching.

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FIREARM INJURY SUSPECT: DETERMINATION OF LEAD CONCENTRATION IN ANIMAL TISSUE BY ICP-MS

Ivana Pallante (1), Umberto Tizian (2), Giovanni Binato (3), Claudia Zanardello (4), Sonia Calderola (5), Massimo Nicolussi (2), Fiorenzo Signor (6), Franco Mutinelli (7), Nicola Pozzato (1)

(1) Istituto Zooprofilattico Sperimentale delle Venezie (IZSVe), SCT1 Laboratorio e Centro specialistico di Medicina Forense Veterinaria, (2) Medico Veterinario libero professionista, (3) IZSVe, SCS2 Laboratorio contaminanti e biomonitoraggio, (4) IZSVe, SCS3 Diagnostica specialistica Istopatologia e Parassitologia, (5) Regione del Veneto, Direzione Agroambiente, Programmazione e Gestione ittica e faunistico-venatoria, (6) Azienda ULSS 7 Pedemontana, (7) IZSVe, Dipartimento funzionale sperimentazione e benessere animale.

Corresponding author: N. Pozzato (npozzato@izsvenezie.it)

Firearm injuries represent a small amount of traumatic injuries in veterinary medicine but they play an important role in forensics because of the legal and criminal aspects associated, for instance, with suspected poaching cases against protected wildlife. These cases can be usually misdiagnosed with other traumas, such as accident or bite wounds, given the similarity in their anatomopathological lesions and because, the projectile and/or its fragments are not frequently detected during the autopsy. In addition, a wide expertise is expected by the pathologists in several fields, such as ballistic, to manage firearm injuries cases and to accurately evaluate entrance and exit wounds in the cadaver (1). In human medicine, digital radiography, computed tomography and nuclear magnetic resonance are commonly used to confirm the firearm injury suspects (2) but they are not always available, or their costs are not accessible in the veterinary counterpart. In this study, a transversal approach was adopted to overcome these limitations. The inductively coupled plasma-mass spectrometry (ICP-MS), a highly sensitive and accurate technique, has been applied here, for the first time, to detect lead concentrations in animal tissues and confirm firearm injury suspects. This technique is officially used in food safety to detect heavy metals residues in animal products. A total of 16 wild animals were conferred to IZSVe and a post-mortem examination was executed following the "National Guidelines for forensic autopsies" approved by the Italian Ministry of Health (3). In addition, samples from 12 hunted wild boars were included in the study. Two aliquots of muscle, bone or skin samples were taken from the investigated animals. One aliquot was sampled nearby the suspected lesion, while the other away from the lesioned area. The ICP-MS was performed to detect lead concentrations in both injured and healthy tissue of each animal to reduce inter-individual variability. Out of 16 animals autopsied, projectile or suspicious metallic fragments were found in only 8 cadavers. In all cases, the ICP-MS revealed lead concentrations of at least 15 times higher in injured tissue comparing with healthy tissue, including the 12 hunted wild boars. After log10 transformation, Paired Wilcoxon signed-rank test estimated a median difference of 3.072 log10 (IC 95% 3.041-3.103, p-value≤0.0001). These results strongly support the hypothesis that the bullet have left lead traces during its passage through the animal bodies, also in the absence of detectable metallic parts at gross pathology or imaging. It is noteworthy that in the case of one wolf with extensive post-mortem changes that showed a tibial fracture during the autopsy, we could attribute to firearm injuries the primary trigger of death. In conclusion, the ICP-MS application could firstly confirm firearm injury suspects and, secondarily, it could guarantee an accurate diagnosis of projectile injuries, reducing their underestimation, particularly in the case of the wolf where skeletal alterations were at first considered exitus of a non-forensic traumatic lesion. As a development of the approach hereby presented, the application of this technique to quantify other heavy metals, such as iron, copper, antimony and zinc, could allow determining the projectile composition fostering crimes investigations in prosecutions involving animals.

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AIVI

THE MICRO EPIDEMIC ONE HEALTH PROJECT: SHARE STORIES AND KNOWLEDGE ON ZOONOSES FOR THE SCIENTIFIC COMMUNITY AND CIVIL SOCIETY

Raoul Ciappelloni(1), Maria Luisa Marenzoni (2), Eros Rivosecchi (1), Anna Duranti (1), Carmen Maresca (1), Maria Paola Torlone (1), Monica Cagiola (1)

(1) Istituto Zooprofilattico Sperimentale dell'Umbria e delle Marche "Togo Rosati". (2) Università degli Studi di Perugia, Dipartimento di Medicina Veterinaria.

Corresponding author: R. Ciappelloni (raoul@ciappelloni.it)

Micro Epidemic One Health (MEOH) is a research project of the Italian Ministry of Health, dedicated to narrative medicine on zoonoses and their control. The project is conducted by the Experimental Zooprophylactic Institute of Umbria and Marche Regions and by the Department of Veterinary Medicine of Perugia University (Italy). The "Stories of Zoonosis" are offered to readers in the form of free narratives or interviews with privileged witnesses; they are accessible by personal computer or smartphone, through a plain Web interface. Narrations talk about real life experiences, carried out by veterinarians and healthcare professionals, who have faced these diseases directly. "Stories of Zoonosis" anthology , constantly updated, is made available to readers through an Open Access repository freely accessible on the Web (visible in beta version at the URL: http://195.231.3.150/). It is a full-responsive Web Platform developed in PHP, that uses Laravel as a Web framework, based on MVC (Model View Controller) architecture, and uses an Open Source PostgreSQL object relational Database. The scientific editorial board of the E-Journal SPVet.it (http://spvet.it - ISSN 1592-1581), manages the MEOH Platform like an ordinary Web paper, with infographics and multimedia attachments. Online "stories of zoonoses" are annotated (continuing reviewing of texts, in a context referable to crowdsourcing), by practitioners who want to communicate and share their experiences, in order to provide complementary information on issues of medical interest. All narratives (where possible) are equipped with contextual links, positioned in the text body, pointing to peer reviewed scientific literature. The Vet stories we are collecting represent useful literature on zoonotic diseases, helpful for Doctors and Veterinarians, as well as for common people, which may use these narrations to guide individual behavior and lifestyle.

FINE TUNING OF A SEQUENCING PROTOCOL FOR SPECIES IDENTIFICATION OF MYTILUS SPP. SPECIMENS

Virginia Filipello (1), Alice Giusti (2), Giulia Magagna (1), Chiara Malloggi (2), Michela Tilola (1), Andrea Armani (2)

(1) Istituto Zooprofilattico Sperimentale della Lombardia e dell'Emilia Romagna, Dipartimento di Sicurezza Alimentare. (2) Università di Pisa, Dipartimento di Scienze Veterinarie, FishLab.
Corresponding author: V. Filipello (virginia.filipello@izsler.it)

The genus *Mytilus* comprises eight species of mussels of commercial relevance, which have different geographic origin and distribution and can hybridize in case of coexistence (1). To date, events of habitat contamination with allochthonous species occur with increasing frequency, due mainly to anthropogenic transfer, posing threats to local ecosystems (2). Moreover, such events favour species mislabeling, which is reported as the most frequent issue affecting seafood products (3). To prevent these problems, EU Regulation 1379/2013 promotes the use of DNA-based methods. PCR-RFLP is to date one of the most efficient techniques used in *Mytilus* spp. species identification (4-5). However, it has some drawbacks linked to limited resolution and subjective interpretation of the results. To improve such aspects, an optimized protocol for the sequencing of short fragments was developed. A total of 50 samples were analyzed. Of them, 12 were DNA samples belonging to various *Mytilus* spp. that were already identified by PCR-RFLP in a previous study (5). The other 38 were tissue samples, of which 10 belonging to specimens directly collected from production sites in Chile, and 28 belonging to fresh and pre-cooked specimens collected from national market. For these samples, the total DNA was extracted using the DNeasy Mericon Food kit (Qiagen). The target PAP region was amplified from all the 50 samples (DNA and tissue) using the primer pair designed by Satto et al. (4). All PCR products were analyzed through capillary electrophoresis to verify the successful amplification, and then sequenced using Sanger technique. Twenty samples (41%), of which 10 from production site and 10 from market samples, were randomly selected to perform the RFLP analysis to be compared to the sequencing results. Ten out of the 12 species (83.3%) identified by PCR-RFLP in the previous study were confirmed by sequencing. The remaining 2 samples, identified as *M. chilensis* x *M. edulis* by PCR-RFLP were instead identified as *M. chilensis* x *M. trossulus* by capillary electrophoresis and confirmed by sequencing. For the remaining 38 samples, sequencing identified 20 tissue samples (52.6%) as *M. galloprovincialis,* 7 as *M. chilensis* (18.4%), 6 as hybrids *M. chilensis* x *M. galloprovincialis* (15.8%), 2 as *M. edulis* (5.3%), 2 as hybrids *M. chilensis* x *M. trossulus* (5.3%) and 1 as hybrid *M. galloprovincialis* x *M. edulis* (2.6%). The PCR-RFLP confirmed the sequencing results for 19 of the tested samples and failed the unambiguous identification of one sample which was identified as hybrid by sequencing. Overall, the species identification by PCR-RFLP failed in 3 out 32 (9.4%) tested samples (12 DNA and 20 tissue). These findings suggest that the optimized protocol relying on Sanger sequencing has some practical advantages. First, the use of capillary electrophoresis to visualize the PCR products allowed the clear distinction of *M. edulis* and *M. trossulus* which, having a difference in amplicon length of only 12 bp (177 bp *vs* 165 bp), are very hard to discriminate with a standard agarose gel electrophoresis. Moreover, the use of sequencing allowed the unambiguous identification of pure species and hybrids, especially in the case of *M. galloprovincialis* x *M. edulis,* in which a double peak at the SNP site is clearly visible. Considering the ever-dropping costs linked to sequence-based technology, we propose this sequencing protocol as a valid, consistent, and reliable alternative to the currently used methods.

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FOURIER-TRANSFORM INFRARED SPECTROSCOPY: RAPID SOURCE ATTRIBUTION OF Staphylococcus aureus DURING A FOOD POISONING OUTBREAK

Federica Savini (1), Federica Giacometti (1), Valentina Indio (1), Monica Pitti (2), Angelo Romano (2), Lucia Decastelli (2), Pietro Luigi Devalle (3), Ilaria Silvia Rossella Gorrasi (3), Sergio Miaglia (3), Andrea Serraino (1)

(1) Università degli Studi di Bologna, Dipartimento di Scienze Mediche Veterinarie. (2) Istituto Zooprofilattico Sperimentale del Piemonte, Liguria e Valle d'Aosta, SC Sicurezza e Qualità degli Alimenti. (3) Dipartimento di Prevenzione - Azienda Sanitaria Locale CN1, SC Igiene degli Alimenti e Nutrizione.
Corresponding author: F. Savini (federica.savini3@unibo.it)

Staphylococcus aureus represents a ubiquitous commensal that colonizes the anterior nares of healthy adults with percentages among the global population of 20 to 30% of intermittently and persistently infected respectively (1 2). Staphylococcal food poisoning (SFP) is one of the most prevalent causes of foodborne intoxication worldwide. Human food contamination by *S. aureus* associated with inadequate handling of cooked or processed foods is often associated with post-process contamination due to the responsibility of food handlers who carry entero-toxigenic staphylococci in their nares or on their skin (3, 4). On the evening of August 7th 2019, eleven persons in a nursing home for the elderly manifested gastrointestinal symptoms, nausea and headache. After notification to the Food Hygiene and Nutrition Service (Local Health Authority of Cuneo 1, Piedmont Region), an epidemiological investigation was carried out. A total of seven samples were collected, namely: one emesis sample from a symptomatic patient, four nasal swabs from the personnel involved in food handling that were on duty on the day before and the day the outbreak occurred and two food samples (chicken salad with and without mayonnaise). Samples were analysed for the presence of *S. aureus*, and further identification was performed by Matrix-Assisted Laser Desorption Ionization – Time of Flight (MALDI TOF) spectrometry. Besides Whole Genome Sequencing (WGS), Fourier-Transform Infrared Spectroscopy (FT-IR) was used for rapid species identification and for a preliminary determination of the degree of relatedness of the *S. aureus* isolates. *S. aureus* was isolated from all 7 analysed samples. Typing of *S. aureus* isolates by Multilocus Sequence Typing (MLST) identified the presence 4 sequence types (STs). Specifically, isolates originating from vomit, both chicken salads, and nasal swab of food handler #4 belonged to ST72. The maximum likelihood tree obtained using Single Nucleotide Polymorphisms (SNPs) analysis with CSI Phylogeny 1.2 revealed that the aforementioned samples clustered together. The results of the FT-IR confirmed the clusterization previously described, specifically food and food-handler #4 showed the same profiles when combining FTIR, SNPs and WGS, revealing the source of infection. We describe a strong microbiological and epidemiological evidence food poisoning outbreak, according to the EFSA nomenclature, due to *S. aureus*. The analyses performed with FTIR and WGS gave comparable results in terms of their ability to link different *S. aureus* isolates, but, while NGS provides more information, FTIR is a rapid and less expensive method. In addition, with FTIR it was possible to further discriminate between two isolates belonging to the same ST that were characterized by different genome size. Early identification of the source of infection during a food borne outbreak is of crucial importance for robust contact tracing, cohorting, and other infection control practices. This has a particular meaning during SFP outbreaks, where contamination of food can be caused by various factors such as cross-contamination by intermittent or persistent food-handlers.

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SURVEY OF RELEVANT FOODBORNE PATHOGENS IN FOODS AND THE ELDERLY IN THE ROMAGNA AREA BEFORE AND DURING THE COVID-19 PANDEMIC

Valentina Indio[1],*Federica Savini*[1], *Monica Cricca*[2,3], *Federica Giacometti*[1], *Michela Fantini*[3], *Vittorio Sambri*[2,3], *Andrea Serraino*[1], *Alessandra De Cesare*[1]

[1] Department Of Veterinary Medical Science, University of Bologna, Bologna, Italy; [2] Department of Experimental, Diagnostic and Specialty Medicine, University of Bologna, Bologna, Italy; [3] Microbiology Unit, The Great Romagna Area Hub Laboratory, Pievesestina, Cesena, Italy.

Corresponding Author: V. Indio (valentina.indio2@unibo.it)

A recent EFSA opinion [1] highlighted that 90% of the invasive listeriosis is due to the consumption of ready to eat foods containing more than 2000 colony forming units of *Listeria monocytogenes* per gr. Such high concentrations represent a serious risk for the elderly often representing the consumer group less prone to apply stringent hygienic practices in the domestic setting and consuming food even after the expiration date. The aim of this study was to cross check the data regarding food and human isolates in the Romagna area considering the elderly as target consumer group. Besides listeriosis, cases of salmonellosis and campylobacteriosis were also considered. The survey identified 112 human infections, including 40 listeriosis, 57 salmonellosis and 15 campylobacteriosis as well as 202 bacterial pathogens in foods (i.e., 171 *Salmonella*, 25 *Listeria monocytogenes* and 6 *Campylobacter*). The time period considered ranged between 2018 and 2021 in order to compare the data in the two years before the Coronavirus Disease (COVID-19) pandemic and the pandemic period. Univariate statistics were applied to test associations and correlations between variables adopting a suite of functions included within the R package *stats* [2] and a p-value<0.05 was considered as the significance threshold. Elderly subjects were divided into two age subgroups 60-75 (n=38) and >75 years (n=74) and normalized considering the age distribution in the Romagna area population (data from http://dati.istat.it/). The results showed no association with the different Romagna provinces, suggesting that the foodborne pathogens infections were equally widespread on the considered area. However, a discrete correlation was found between the number of human and food isolates throughout the time period and sites (*Salmonella* R^2=0.77; *Listeria monocytogenes* R^2=0.24; *Campylobacter* R^2=0.58). Interestingly, we found that salmonellosis cases were equally distributed between genders, while the other infections involved mainly males. Focusing on the distribution over the considered years, the data suggested that the infections (in terms of type and number) were equally distributed before and during the pandemic period. However, by stratifying the elderly by age we found that the incidence of infections in the over 75 years old subjects increased during the pandemic (p-value=0.046) especially for listeriosis (p-value=0.116) and campylobacteriosis (p-value=0.049). These data could be related to the changes of food purchase, preparation and storage habits during the lock down and suggest that gender and age-specific prevention strategies of foodborne pathogens should be adopted especially in emergency situations leading to isolation and behaviour changes in vulnerable consumer groups.

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IN SILICO EVALUATION OF SILVESTROL, ROCAGLAMIDE AND OTHER FLAVAGLINE COMPOUNDS AS POSSIBLE HEPATITIS E VIRUS INHIBITORS

Lorenzo Pedroni, Luca Dellafiora, Sergio Ghidini

University of Parma, Department of Food and Drug.
Corresponding author: L. Pedroni (lorenzo.pedroni@unipr.it)

Hepatitis E Virus (HEV) is a small positive sense single stranded RNA virus member of the *Hepiviridae* family, particularly of the Orthohepevirus genus. It leads to 20 million HEV estimated infections/year worldwide and 3.3 million symptomatic cases of Hepatitis E, according to the World Health Organization [1]. While the fatality rate usually ranges from 0.2% to 4%, it significantly increases for pregnant women [2]. The virus spreads via waterborne, zoonotic and foodborne transmission depending both on the considered country and on the considered HEV genotype [3, 4]. Pigs are the main reservoirs but even game and livestock, with all the related meat-based products, are a major source of infection [5-9].

Unfortunately, HEV in vitro culture and analysis are still difficult, resulting in a poor understanding of its molecular biology [10]. Moreover, HEV encodes for only one non-structural protein (ORF1): a multifunctional, multidomain, non-cleaved polyprotein which could be a potential druggable target but whose crystallization results unfeasible due to size-related reasons (185 kDa) [11].

In this context, an in-silico approach succeeded at overcoming the lack of HEV ORF1 structural data, allowing the identification of potential anti-viral compounds. Based on previous studies showing the inhibitory activity of the natural plant secondary metabolite silvestrol against HEV [12], our study provided a reliable model to: I) investigate the underpinning mechanisms; and II) provide a predictive framework to estimate the activity of silvestrol analogues for further dedicated investigations. The HEV RNA Helicase domain was modelled and refined via a homology modelling approach based on an innovative, crystallome-based strategy. Then the interaction with 9 silvestrol-related compounds, including rocaglamide and silvestrol, and 2 virtual decoys recovered from the DUD-E database, was computed via docking and molecular dynamics simulations.

Overall, our study presented mechanistic insights on HEV RNA Helicases and silvestrol dependent inhibition, expanded the current understanding of the structure-activity relationship for Silvestrol-related compounds and provided a blueprint for further analysis targeting the HEV RNA Helicase. Eventually, in-depth investigation is warmly sought to possibly extend the usage of either silvestrol, rocaglamide or some of the related natural compounds as feed additives in order to reduce the HEV diffusion in livestock. Notably, silvestrol has already been proven to be well tolerated in animals [13].

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USE OF FOOD GRADE ACETIC ORGANIC ACID TO PREVENT LISTERIA MONOCYTOGENES IN MOZZARELLA CHEESE

Erica Tirloni, Cristian Bernardi, Simone Stella

Università degli Studi di Milano, Dipartimento di Medicina Veterinaria e Scienze Animali
Corresponding author: E. Tirloni (erica.tirloni@unimi.it)

Listeria monocytogenes is a pathogenic microorganism that is a real challenge in dairy production plants as it is psychrotrophic, resistant to a wide range of pH and high salt content, ubiquitous and very persistent. In a previous study [1], prevalence of *L. monocytogenes* was 24.4% in mozzarella cheese in a dairy plant. Mozzarella cheese, thanks to its physical-chemical properties may represent a suitable substrate for the growth of *L. monocytogenes*. Previous studies investigated Minimal Inhibitory Concentration of lactic, acetic, citric and propionic acid for *L. monocytogenes* under conditions relevant to dairy products and some of these studies reported MIC calculated in the cheese matrix [2, 3]. Tirloni et al. [3] showed that 24.98 mM acetic acid in broth inoculated with *L. monocytogenes* did not determine the increase in absorbance through 7 days. When inoculated in primo sale cheese, *L. monocytogenes*, did not grow at 4°C in presence of 24.98 mM of acetic acid, while at 8°C, a concentration of 49.96 mM was needed to obtain a significantly lower growth. The objective of the present study was to evaluate the ability of acetic acid to inhibit the growth of *L. monocytogenes* when added to brine used for mozzarella storage. A strain of *L. monocytogenes*, isolated from a fresh dairy product was considered for this trial and specific challenge tests were performed on mozzarella cheese. After the inoculum, the packages were divided in ten series for the treatment with acetic acid at different concentrations: 6.25 mM (ac1), 12.49 mM (ac2), 18.375 mM (ac3), 24.98 mM (ac4), 49.96 mM (ac5), 75 mM (ac6), 99.92 mM (ac7), 133.2 mM (ac8) and 149.87 mM (ac9); moreover, a control series (CTRL, without acid addition) was prepared. All the samples were maintained at 8°C and analysed at t0 and after 2, 5 and 10 days in triplicate. When applied to brine, acetic acid resulted effective only when its concentration was above 24.98 mM (ac4): in this case no growth was recorded during the sampling sessions. At the concentrations of 6.25 mM (ac1), 12.49 mM(ac2) and 18.375 mM (ac3), a lower growth was recorded with final increases of 2.69, 1.50 and 1.02 Log CFU/g, respectively. With concentrations above 49.96 mM (ac5 series), a decrease in *L. monocytogenes* counts was observed, with the counts always below the detection limit of 1 Log CFU/g. The determination of pH of the samples showed the acidifying effect of treatment exerted, with a significant difference between the CTRL samples and all the other series ($P<0.01$). The concentrations of acetic acid (ac1, ac2, ac3, ac4 and ac5) did not have a repercussion on the sensorial characteristics of mozzarella cheese while concentrations above 75 mM (ac6) resulted to have a sensorial impact, determining a rejection of the product.

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EXPLORING THE RELATIONSHIP BETWEEN BRUISES ON CARCASSES OF BEEF CATTLE AND PRE-SLAUGHTER FACTORS

Silvio De Luca (1), Sergio Ghidini (1), Adriana Ianieri (1), Giovanni Di Florio (1), Maria Olga Varrà (1), Claudia Rosa Romeo (2), Emanuela Zanardi (1)

(1) University of Parma, Department of Food and Drug. (2) Istituto Zooprofilattico Sperimentale della Lombardia e dell'Emilia Romagna (IZSLER).

Corresponding author: S. De Luca (silvio.deluca@unipr.it)

The evaluation of lesions on carcasses at the slaughterhouse has been recognized as a valid method to assess animal welfare [1].While the body of research in this field is large toward pigs and poultry, little is known about the potential of abattoir-based measures in cattle [2]. Lately, the assessment of bruises on carcasses has been indicated as a potential indicator of welfare in cattle [2, 3]. The aim of this study was to determine whether bruises on cattle carcasses could reflect some pre-slaughter factors.

The study was conducted in a commercial abattoir located in the Northern Italy, which processed mainly beef cattle of different ages and breed. At the unloading, the transporter ID, travelling time, density of the animals per truck, number of floors in the truck, size and type of each truck were recorded as pre-slaughter factors. Bruises were assessed along the slaughter line after skinning and identification of carcasses but prior splitting. The lesions were evaluated in five anatomical sites (1=Front, 2=Rib, 3=Flank, 4=Loin, 5=Round) and classified according to their size (Small=0-8 cm; Medium=9-16 cm; Large≥16 cm), color (0= Red; 1= Purple; 2= Yellow), shape (0=Circular; 1=Linear; 2=Tramline; 3=Mottled), and number per each site. In the event of multiple bruises per each site, only the bruise presenting maximum severity of the size was recorded. The probability and the severity of bruising were examined through a mixed logistic regression, with pre-slaughter factors as explanatory variables. Differences between factors with more than two levels were tested through t-tests on differences of least square means, applying Tukey adjustment for multiple comparisons.

Overall, 1265 animals were examined for bruises post-slaughter, with 273 animals (21.6%; 95% Confidence Interval: 19.3%–23.8%) showing at least one bruise, of which 193 (70.7%) showed mild bruising, 59 (21.6%) medium bruising, and 21 (7.7%) severe bruising. Most of the bruised animals (50.2%) resulted with bruising at a single location on their body, 38.5% at two locations, 9.2% at three locations, and only 6 individuals (2.2%) showed bruising at four locations. Occurrence of bruising varied significantly depending on body parts, with the Front (10,8%) and the Round (7.9%) sites showing bruises more frequently than the other locations (χ^2_4=60.3; p<0.0001). The probability of bruising varied significantly among individual categories (χ^2_2=35.1; p<0.0001) and was negatively affected by the density of animals in the truck (χ^2_1=23.1; p<0.0001). In fact, steers had a lower probability of showing bruises than both heifers (t_{1244}=5.7; p<0.0001) and veals (t_{1244}=3.4; p=0.002), while the higher was the density of transported animals, the lower was the probability of presenting bruising (parameter estimate=-2.74 ± 0.57).

These results have shown that the presence of bruises on carcasses of beef cattle can be affected by some pre-slaughter factors, such as the density of the animals per truck during the transport, the gender and the age. In conclusion, this preliminary study supports the use of a scheme based on bruises on carcasses to evaluate welfare in cattle prior to slaughter.

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INCIDENCE OF MICROPLASTICS IN SEA BASS AND SEA BREAM

Giacomo Mosconi (1), Sara Panseri (1), Camilla Della Torre (2), Andrea Binelli (2), Stefano Magni (2)

(1) Università degli Studi di Milano, Dipartimento di Medicina Veterinaria e Scienze Animali. (2) Università degli Studi di Milano, Dipartimento di Bioscienze.

Corresponding author: G. Mosconi (giacomo.mosconi@unimi.it)

Plastic contamination is a widespread problem in all ecosystems. Extensive production and especially incorrect disposal are leading to a dramatic release of plastics into freshwater and marine aquatic environments [1]. To date, the adverse impacts of plastic and microplastic contamination have been extensively investigated in marine organisms [2]; while the effects on humans are still to be defined [3].

A controversial aspect of plastic contamination concerns the potential risks for humans from consuming contaminated fish species [4]. Therefore, the objective of this work was to characterise the presence of plastics in 20 samples of *Dicentrarchus labrax* and 20 samples of *Sparus aurata*, two of the most widely consumed fish species in the Mediterranean area [5], retrieved from off-shore aquaculture facilities located in different sites of the Mediterranean Sea, namely Italy, Greece, Turkey, Croatia and, Malta.

Three different districts of each fish were analysed: intestine, liver and, fillet. The samples were primarily homogenized in hypersaline solution with a blender and then digested with hydrogen peroxide; the resulting suspensions were then filtered through a cellulose nitrate filter. The qualitative and quantitative characterisation of the plastics was carried out using a Fourier Transform Infrared Spectrophotometer (μFTIR), which made it possible to identify not only the number of plastics in each sample but also characteristics such as size, shape, colour and polymer.

The results showed that only 27% of fishes analysed were affected by the presence of plastics in the gut. Moreover, a reduced number of plastic debris were detected in the fillet and liver, suggesting low translocation from the gut to these organs. This suggests a low incidence in human food consumption, for which a plastic input of 20.5 debris per year per capita was quantified. Different levels of contamination could be observed depending on the geographical region of origin of the plants: the most contaminated fish were from Turkey, where plastic debris was detected in 60% of the samples. Plastics were detected in 35% of the samples from Greek plants. Only 20% of the samples from Italian plants were contaminated, while no plastics were detected in samples from Croatia or Malta.

This is in line with current observations concerning the presence of surface plastics in the Mediterranean basin. The data obtained showed a prevalence of polyester and polyamide microfibres, of which the former, as already mentioned, is strongly associated with the production of clothing, while the latter could originate from the release of different fishing and aquaculture tools, such as fishing nets. The results of this study confirm the bioavailability of plastics to marine fish, highlighting the need to thoroughly monitor this kind of contamination, to predict the real risk to organisms at different levels of the trophic network, up to humans, and consequently plan effective solutions to this problem.

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RAPID TRACEABILITY METHOD FOR THE ON-SITE CONTROL AND INSPECTION IN THE CLAM SUPPLY CHAIN

Sarah Currò, Stefania Balzan, Luca Fasolato, Federico Fontana, Luca Bargelloni, Morgan Smits, Enrico Novelli.

Università degli Studi di Padova, Dipartimento di Biomedicina Comparata e Alimentazione.
Corresponding author: S. Currò (sarah.curro@unipd.it)

Clams are a high commercial value seafood product, highly appreciated for the peculiar taste and nutritional features. In particular, clams show excellent amounts of n-3 fatty acids which have significant beneficial effects in the prevention of human cardiovascular diseases [1]. Althought this seafood product is worldwide distributed, Italy is the main European producer. However, due to the high vulnerability, clams traceability represents a critical challenge for governments, trades, and consumers. The origin mislabelling in clam can hide (i) lower quality product; (ii) illegal, unreported and unregulated fishing activities; and (iii) may convey health risk in consumer [2]. Currently, due to the numerouses frauds identified in fishery sector, a strategic approach is required to reveal and prevent mislabeling in short time due to the easy perishable nature of the seafood product. Commonly, seafood bromatology and authenticity are investigated using traditional and specific analytical techniques based on chemical or biomolecular/isotopes methods that require long analytical times, the use of solvents and the intervention of specialized personnel [3]. In contrast, near-infrared spectroscopy (NIRS) is an innovative system that allows qualitative and quantitative parameters to be defined simultaneously to define the characteristics of the product. Moreover, NIRS is a rapid, green, economic tool applicable on-site to assess the origins of product in support of the authorities and food business operator during the authenticity control activities [4]. Thus, this study aimed to develop qualitative and quantitative models to evaluate the NIRS feasibility to trace the product and predict the fatty acids amount being affected by the environment and by trophic availability, in the clams reared in two farms located in the Chioggia lagoon. In detail, from August to December 2021, 246 individual samples were collected from two harvesting sites that differ in seabed composition and in the sea currents. First, a portable NIRS tool that operated in reflectance mode from 900 to 1680 nm was assessed to collect spectral data for authentication purpose on sample as-is and after homogenation. Second, from a subset of homogenated samples, fats were extracted [5] and then analysed using gas-chromatography method to define the respective fatty acids profile for the prediction of quantitative analysis [6]. The authentication models was developed using the machine learning approach by dividing the whole dataset in training set (n=173) and testing set (n=73) to test the model performance with an external validation (hold-out). Whereas, the prediction models (n = 60) of individual fatty acids were performed using the WinISI III (version 1.6) software to develop the modified partial least squares regression method using a cross-validation. The model for the origin authentication showed an 86% of accuracy. On the other hand, fatty acids models showed promising coefficient of determinations (R2cv) in cross-validation for C16:0 (60%), C16:2 n4 (52%), C20:5 n3 (61%) and C21:5 n3 (55%). These preliminary results showed an encouraging system in the labelling check and for the seafood inspection, becoming a possible tool to contrast clams mislabelling and safeguarding the traceability in the supply chain.

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COMPARISON OF PERFLUOROALKYL SUBSTANCES CONTAMINATION IN SEA AND LAKE FISH AND THEIR IMPACT ON FOOD SAFETY

Maria Nobile, Sara Panseri, Federica Di Cesare, Giacomo Mosconi, Villa Roberto, Francesco Arioli, Luca Chiesa

Università degli Studi di Milano, Dipartimento di Medicina Veterinaria e Scienze Animali.
Corresponding author: M. Nobile (maria.nobile1@unimi.it)

Perfluoroalkyl substances (PFASs) are fluorinated compounds with a wide range of industrial and commercial applications and they have become a global concern because of the toxicity and bioaccumulative properties [1]. The primary source of PFASs exposure is the diet, and mainly seafood and fish contaminated by water bodies [2]. Regard fish, sea bass (*Dicentrarchus labrax*) and sea bream (*Sparus aurata*) are the most commonly consumed in EU in different food plans, starting from infants with baby food, and indicated for all other age groups for their nutritional properties. On the other hand, within continental waters, also lakes are particular interesting for bioaccumulation of PFASs, for their hydro-morphological properties as the long residence time, and anthropological stress due to the local human activities [3]. The aim of this study was to investigate the occurrence of PFASs in the most consumed farmed sea fish (sea bass and sea bream) of the Mediterranean Sea in comparison with common lake fish from the major lakes of the Northern Italy. A total of 170 samples was collected in retail stores of Lombardy. In particular, 68 farmed sea fish divided in 34 sea basses (*Dicentrarchus labrax*) and 34 sea breams (*Sparus aurata*) from Italy, Greece, Croatia and Turkey and 102 caught lake fishes including 34 European whitefishes (*Coregonus lavaretus*), 34 perches (*Perca fluviatilis*) and 34 Mediterranean shades (*Alosa agone*) collected from Lake Garda, Lake Como and Lake Iseo.

Five grams of homogenized sample were added of 10 mL of acetonitrile for compounds extraction and protein precipitation. The exctract was then purified by STRATA PFAS cartridges and the instrumental analysis was carried out by an ultra-performance liquid chromatography-high resolution mass spectrometry (UPLC-HRMS) system. Of all 17 searched PFASs, only perfluorobutanoic acid (PFBA) and perfluorooctanesulfonic acid (PFOS) were found both in sea and lake fish, with higher concentration in the second ones. Moreover, perfluorobutanesulfonic acid (PFBS) was found only in lake fish. In particular Mediterranean shades (Alosa agone) from Lake Garda and Lake Como were the more contaminated species. In particular, the higher concentrations of PFBA (8.07±7.92 ppb) and PFBS (1.10±2.22 ppb) were detected in Mediterranean shades from Lake Garda, instead the higher average concentration of PFOS (9.90±6.46 ppb) in those from Lake Como. Regarding farmed sea fish, PFBA average concentration were comparable between sea bass (4.96±2.46 ppb) and sea bream (4.75±1.50 ppb), while PFOS was dectected at higher frequency and concentrations (0.15±0.20 ppb) in sea bass, especially those from Turkey.

A risk assessment based on a conservative approach, was estimated, and both for lake and sea fish did not exceed the tolerable weekly intake (TWI) of 4.4 ng/kg bw per week for the sum of 4 PFASs (perfluorooctanoic acid (PFOA), perfluorononanoic acid (PFNA), perfluorohexanesulfonic acid (PFHxS) and PFOS), estabilished by last EFSA note [4].

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OCCURRENCE AND SAFETY ASSESSMENT OF HEAVY METALS IN MUSCLES AND LIVERS OF ITALIAN HEAVY PIGS

Maria Olga Varrà (1), Lenka Husáková (2), Sergio Ghidini (1), Jan Patočka (2), Adriana Ianieri (1), Emanuela Zanardi (1)

(1) University of Parma, Department of Food and Drug. (2) University of Pardubice, Department of Analytical Chemistry (CZ).

Corresponding author: E. Zanardi (emanuela.zanardi@unipr.it)

The specific breeding and feeding strategies adopted in the context of the Italian heavy pig production system have repercussions on the hygienic and safety aspects of the related meat and meat products, including the occurrence of metals of toxicological significance [1].

The aims of this study were to determine the levels of some toxic metals in pig muscles and livers and estimate the dietary exposure of the Italian population to these contaminants through meat and liver consumption.

A total of 80 heavy pigs from 16 different farms were randomly selected for this study. For each animal, the diaphragm muscle and right lateral lobe of the liver were collected in a large slaughterhouse of northern Italy (Emilia-Romagna region), sub-portioned, and freeze-dried. Both tissues were analyzed via a single-purpose atomic absorption spectroscopy (AAS) for total Hg and inductively coupled plasma-mass spectrometry (ICP-MS) for As, Al, Cd, Cr, Cu, Fe, Ni, Pb, Sn, U, and Zn quantification. The dietary intakes of these metals by different age groups of the Italian population (children, adolescents, adults) through the consumption of pig fresh meat and liver were estimated taking into account both mean and 95th percentile (P95) chronic consumption data [2], and then compared with the relative health-based guidance values set up by the European Food Safety Authority and the Joint FAO/WHO Expert Committee on Food Additives.

Although all the samples were in line with the maximum limits for toxic heavy metals established by European Union [3], significant higher levels of Fe, Zn, Cd, Sn, Pb, U, Cu, and Hg were found in liver compared to muscles samples (p<0.001), especially concerning Cd concentrations which varied two orders of magnitude (43.3±19.6 vs. 0.77±0.42 µg/kg wet weight). When estimating the average dietary intake of heavy metals through meat and liver consumption, no severe risk for health was found, since heavy metals intakes were all below the toxicological health-based guidance values. Besides, the estimated levels of exposure to Cd through the consumption of mean liver amounts by children, adolescents, and adults were found to contribute to 24, 12, and 9% of the total tolerable weekly intake (TWI) of 2.5 µg/kg bw/week, respectively. In the calculation for P95 consumers (i.e., worst case scenario), the contribution of liver to TWI value of Cd was found to be higher (12%) only for adults.

These results warn about the high probability for the younger population of exceeding the TWI of Cd though the whole diet, considering that many other foods may be contaminated by significant Cd levels and that the same pig liver can be used as ingredients for the preparation of other meat products. In conclusion, since pig liver can be also employed as the primary ingredient for the formulation of pet foods, our data may provide some insights about the potential negative impact of Cd exposure on both human and animal health, hence reminding of the importance of targeted intervention measures aimed to reduce its occurrence into the food chain.

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IMPACT OF INFRARED RADIATION ON MILK SAFETY AND QUALITY TRAITS

Luigi Danesi, Sara Panseri, Federica Di Cesare, Erica Tirloni, Roberto Villa

Università degli Studi di Milano, Dipartimento di Medicina Veterinaria e Scienze Animali.
Corresponding author: L. Danesi (luigi.danesi@unimi.it)

Pasteurization is an established technique in food industry, it allows obtaining safe products thanks to the thermal inactivation of pathogenic microorganisms [1]. It is necessary for the treatment of many different food matrices, but despite its necessity, pasteurization has also vulnerabilities that are encouraging to apply new methods. In the case of milk, the first weakness is represented by the loss of nutritional and aromatic traits of the product after the heat treatment [2]. The other great limit is associated with the environmental impact (energy, water, and methane consumption). Considering the growing demand for high-quality milk products and the increased interest of companies in the topic of sustainability, a wide range of new techniques of food sanitation were developed (e.g. infrared radiation, radio frequency, microwave, etc.). Among them, Infrared Radiation (IR) presents very interesting advantages: reducing treatment time, higher properties preservation, high heating uniformity and energy efficiency [3]. This study aims to evaluate in two steps the efficiency of a sanitation system IR based on terms of safety, quality, and energy-saving on milk samples. In the first step of the work, the Total Bacterial Count (TBC) of 15 samples of raw milk was evaluated before and after the IR treatment. Moreover, to investigate the ability to maintain aromatic characteristics and nutritional elements of raw milk, all samples underwent HS-SPME and GC/MS analyses to determine and compare the volatile profile and the total fats, fatty acids, proteins, and vitamins profile before and after the IR treatment. In the second step of the study, the energy and water consumption of the IR process was modelled and compared (calculated on a projection of 1500 L/h of milk) with that of the conventional pasteurization (CP), to determine the improvement of energy efficiency. All raw milk samples showed comparable volatile and nutritional profiles before and after the IR treatment. Moreover, in all samples, a reduction of bacteria load was detected for each bacterial species considered after the IR treatment compared to baseline. In particular, *Enterobacteriaceae* were reduced under the level of 100 ufc/g in 13 samples, but the most relevant reduction was detected in the other two samples, initially characterized by the higher contamination (2500 and 2000 ufc/g), reduced to a value <200 ufc/g. Regarding Coliforms, all samples showed a value of <10 ufc/g after the IR treatment. Focusing on the most contaminated milk, characterized by a higher microbial load (300 ufc/g), the IR treatment demonstrated promising results. Considering *Bacillus spp*., they were detected only in three samples. All three samples presented a pre-treatment count of 4000 ufc/g, while after the process the value found was lower than 100 ufc/g. In all samples after the IR treatment, *S. aureus* and *L. monocytogenes* showed a final value of <10 ufc/g, even in those characterized by the highest level of contamination (respectively 3000 ufc/g for *S. aureus* and 2000 ufc/g for *L. monocytogenes*). Regarding the energy efficiency assessment, a decrease in energy consumption from 80 kw/h in the CP system to 58 kw/h in the IR process was registered. Also, the reduction of water consumption was relevant, moving from an average of 5000 L/h to 65 L/h for washing and from 100 L/h to 0 L/h for steam production, in CP and IR processes, respectively. Based on the preliminary results of this study, IR treatment proved to be a promoting alternative method to pasteurization for milk sanitization. It allows obtaining a product that could be safely consumed considering its ability to inactivate microorganisms. Moreover, the ability of IR to preserve the beginner characteristics of raw milk allows it to provide a plus value on the product in terms of quality. The sustainability performances were promising regarding energy and water consumption giving particular interest to this new technique in terms of environmental impact and economic saving.

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EFFECT OF A NEW FASTER RIPENING SYSTEM TO PRODUCE SEMI-HARD BUFFALO CHEESE

Marika Di Paolo, Rosa Luisa Ambrosio, Roberta Mazzocca, Valeria Vuoso, Lucia Vollano, Roberta Matera, Raffaele Marrone

Università degli Studi di Napoli Federico II, Dipartimento di Medicina Veterinaria e Produzioni Animali.
Corresponding author: M. Di Paolo (marika.dipaolo@unina.it)

Dairy industry is the driving force behind the Italian agri-food sector and represents a significant share of the total national food turnover [1] due to the assortment of cheeses and their high nutritional value appreciated by consumers. Buffalo milk contributes more than 12% to total milk production in the world [2] and offers excellent opportunities to produce cheeses characterized by noble nutritional benefits [3]. However, it is almost exclusively used for cheese-making of mozzarella, not being suitable for the preparation of ripened cheese due to high buffering capacity, low acid development, excessive syneresis, low lipolysis which lead to slow ripening and affect process costs [4]. For this reason, the choice of the ripening method plays a crucial rule in the production of ripened buffalo cheese [5]. The aim of this study was to evaluate the effect of experimental climatic parameters in industrial ripening plant (Stagionello® - European Patented Device and controlled pH – n. EP 2769276B1) on semi-hard buffalo cheese to reduce the ripening time by comparing it with that traditionally adopted. Semi-hard cheese was prepared from thermalised milk coagulated with commercial starter and rennet paste in 60 min. The coagulum was cut to medium size grains. After extracting whey, curd was steamed for 30 min, cheeses of flat cylindrical shape were pressed and refrigerated at 4°C for 48 h, immersed in brine (salt, 20% w/v) for 1 h and ripened. For each cheese-making session (n=3), samples of raw milk (L), curd (C), cheese on the first production day (T0), and ripened cheeses with traditional method (T1 = 40 days) and innovative method (T2 = 90 days) were collected. All samples were analyzed for the determination of physicochemical, rheological and microbiological profiles. Moisture content in all cheeses decreased, resulting in a relative increase in protein and fat values at the end of ripening. Texture profile analysis pointed out a positive correlation between ripening technologies and parameters such as hardness, gumminess, elasticity and chewiness which increased as result of the ripening process; however, no significant differences were found between traditional and innovative methods. Likewise, the colour was not differently conditioned by the different ripening methods adopted; in particular, in all ripened cheeses lightness (L*) decreased significantly ($p<0.01$) at the end of the ripening while redness (a*) and yellowness (b*) showed a slight increase. Nevertheless, it is worth highlighting the different contents in lipolysis and oxidation products between traditional and experimental aged cheeses: in T2 cheese their levels were higher than in T1 cheese. Despite the differences in duration between the two ripening methods, no significant differences in bacteria count were detected. Particularly, both methods slowed down the concentration of hygienic bacteria indicators (such as Coliforms and *Enterobacteriaceae*) as result of the decrease of pH and water activity [6]. The efficiency of the new experimental climatic parameters on microbiological aspects has been demonstrated by the absence of *Salmonella* spp. in T1 cheese, although these bacteria were found in curd and T0 cheese. In view of the economic and nutritional importance of buffalo dairy products, novel techniques should be investigated to improve the suitability of buffalo milk for manufacturing of cheese. To this purpose this research could be considered as pilot study that could do the groundwork and support the optimization of the ripening phase of buffalo cheese, providing useful information to improve the transformation of the buffalo milk.

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EFFECT OF BOVINE COLOSTRUM DIETARY SUPPLEMENTATION ONRABBIT MEAT QUALITY

Marta Castrica (1), Laura Menchetti (2), Stella Agradi (1), Giulio Curone (1) Daniele Vigo (1), Grazia Pastorelli (1), Alessia Di Giancamillo (1), Silvia C. Modina (1), Federica Riva (1), Valentina Serra (1), Dino Miraglia (3), Egon Andoni (4), Claudia M. Balzaretti (1), Gabriele Brecchia (1)

(1) Università degli Studi di Milano, Dipartimento di Medicina Veterinaria e Scienze Animali. (2) Università di Bologna, Dipartimento di Science e Tecnologie Agro-alimentari. Dipartimento di Scienze Veterinarie. (3) Università degli Studi di Perugia, Dipartimento di Medicina Veterinaria. (4) University of Tirana, Department of Public Health, Agricultural.

Corresponding author: M. Castrica (marta.castrica@unimi.it)

Bovine colostrum is a wealthy resource of biologically active compounds, antimicrobials, antioxidants, essential nutrients, and immune-regulating factors [1-3].

Beneficial immune effects of bovine colostrum have been demonstrated in human studies focused on benefits in health and diseases and in animals such as pigs [4, 5].

To date, studies on the use of bovine colostrum in animals, especially rabbits, are limited mainly with regard to possible positive effects on meat quality in terms of microbiological, chemical, and sensory profile. For this reason, in this first study in the global scientific panorama, the quality of meat from New Zealand White (NZW) rabbits (n=13) fed on a diet containing 5% w/w of bovine colostrum (bovine colostrum group; BC) was compared to meat from NZW rabbits (n=13) receiving a control diet, not enriched with BC, (Control group; C).

After slaughtering, the effect of dietary supplementation on microbiological and chemical characteristics of the rabbit loins, packed in an oxygen-permeable package, was evaluated at 48 h post mortem (day 0; D0), after 3 (D3) and 8 (D8) days of refrigerated storage. Data were analyzed at each time point by Mann-Whitney tests.

No difference was found in the microbiological parameters. After 8 days of storage, the concentrations of *Staphylococcus aureus*, *Enterobacteriaceae*, *E. coli*, and total coliforms in the C group were 0.838±0.312, 3.048±0.614, 0.23±0.23, and 2.97±0.59 Log CFU/g, respectively, while in the BC group were 0.452±0.243, 3.331±0.693, 0.17±0.17, and 2.70±0.64 Log CFU/g.

Conversely, the bovine colostrum dietary supplementation had effects by reducing thiobarbituric acid reactive substances (TBARS) values at all-time points ($p<0.001$) in BC compared with C group; while total volatile basic Nitrogen (TVBN) values were higher in BC than in the C group at D3 and D8 ($p<0.01$).

The preliminary findings in this study lead the authors to investigate further several aspects such as dietary supplementation with different BC percentages and also the evaluation of the BC rabbit's meat sensory aspects. However, it is interesting to note, in this study, the significant antioxidant activity against lipid oxidation in meat, expressed by bovine colostrum.

In conclusion, bovine colostrum as well as other by-products rich in bioactive substances, which exert an antioxidant power [6, 7] and other beneficial effects, are nowadays highly explored within the agro-food chain because they represent a sustainable strategy for enhancing food quality through the re-use of by-products otherwise destined for disposal.

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THE RESISTOME OF BULK TANK MILK FILTERS AS PREDICTED BY WHOLE METAGENOME SEQUENCING

Selene Rubiola (1), Guerrino Macori (2), Felice Panebianco (1), Tiziana Civera (1), Séamus Fanning (2), Francesco Chiesa (1).

(1) Università degli Studi di Torino, Dipartimento di Scienze Veterinarie. (2) University College Dublin, Centre for Food Safety.

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

In cattle production systems, antibiotics are extensively used to treat bacterial infections, including bovine mastitis; this latter is recognized as the root cause of antimicrobial compound use in dairy farms worldwide, leading to the potential selection and introduction of antimicrobial resistance (AMR) genes and bacteria in milk production environments and dairy products (1). Among the indicators of intramammary gland infection, increased somatic cell counts (SCCs) in milk are considered prognostic indicators. In the present context of growing concern about AMR, understanding the distribution of AMR determinants in an important food matrix such as milk has relevance in terms of protecting consumers and maintaining high food safety standards. Among the different approaches applied to characterize the ensemble of genes encoding AMR, that is the resistome, Next Generation Sequencing (NGS) technologies are regarded as promising techniques (2).

Herein, the resistome of dairy farms with history of different bulk tank SCCs was investigated through a whole metagenome sequencing approach, taking advantage of milk filters as promising tools. Milk filters were collected from the bulk tank of 5 dairy farms with high historical SCCs (>300,000 cells/mL) and 5 others with low SCCs (<150,000 cells/mL) in North-West Italy; sampling was repeated twice (N=20). A 10 g sample of each milk filter was added to 90 ml sterile ringer's solution and homogenized. DNA was extracted and whole metagenome sequencing was performed on an Illumina NovaSeq 6000 platform. High-quality, host-filtered reads were aligned to the MEGARes, CARD, ARG-ANNOT and Resfinder databases using BWA to perform a reads-based resistome characterization. Host-filtered reads were assembled *de novo* using IDBA-UD and ABRicate was used on generated scaffolds and metagenome assembled genomes (MAGs) to perform assembly-based resistome characterization. Spearman's correlation test was performed in order to assess any correlation between the presence of AMR genes and the SCCs herd group ($p<0.05$).

The assembly-based resistome characterization revealed the presence of 10 AMR classes, including aminoglycoside, antimicrobial peptides, β-lactam, fosfomycin, fluoroquinolone, macrolide, lincosamide and streptogramin (MLS), multidrug efflux pumps, phenicol, sulfonamide and tetracycline. Two more AMR classes were identified by the reads-based resistome characterization. Notably, most of the species harboring AMR genes were predicted to be Gram-negative genera, namely *Enterobacter*, *Acinetobacter*, *Escherichia*, and *Pseudomonas*, pointing out the role of these bacteria as reservoirs of AMR determinants. The application of both reads-based and assembly-based approaches, despite being computationally demanding, facilitated the comprehensive characterization of the resistome, while also allowing the construction of 8 high-quality MAGs. AMR classes were evenly distributed between the different farms; Spearman's correlation test did not highlight any significant correlation between the presence of AMR determinants and the SCCs herd group. Our findings suggest that milk filters can successfully be used to investigate the resistome of bulk tank milk through the application of whole metagenome sequencing. In accordance with our results, raw milk can be considered a source of antimicrobial resistant bacteria and genes; this points out the importance of properly informing food business operators about the risk associated with poor hygiene practices and consumers of the potential microbial food safety risks derived from raw milk products consumption.

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OCCURRENCE OF CHLORATE AND PERCHLORATE ANIONS IN RAW MILK

Radmila Pavlovic, Federica di Cesare, Maria Nobile, Giacomo Mosconi, Luigi Danesi, Sara Panseri, Luca Chiesa

Università degli Studi di Milano, Dipartimento di Medicina Veterinaria e Scienze Animali.
Corresponding author: R. Pavlovic (radmila.pavlovic1@unimi.it)

Chlorate (ClO_3^-) and perchlorate (ClO_4^-) are ubiquitous environmental contaminants present in various types of foodstuffs, milk dairy products included. ClO_3^- has emerged as a residue of concern, associated with chlorine sanitizer usage for disinfection of the equipment and water applied in food processing. Chlorate affects the thyroid and additionally the haematological system by oxidative damage of the red blood cells [1]. ClO_4^- is naturally occurring in the environment but also released from anthropogenic sources. The main potential sources of contamination are soil and groundwater contaminated as a result of industrial emissions or the use of certain natural fertilizers [2]. Perchlorate is well known to competitively inhibits the uptake of iodine in the thyroid, which may cause hypothyroidism, therefore breast-fed infants and young children with low iodine intake are particularly vulnerable to its exposure [3].

Milk and other dairy products are highly nutritious and provide many of the key nutrients required for the growth and development of infants and children, and they are beneficial for the maintenance of health in adults. The sanitation of dairy processing equipment is of major importance across the dairy supply chain, in order to prevent outbreaks of foodborne illness, but contemporary might bring the ClO_3^-. In order to protect consumers, European Commission set Maximum Residue Limit (MRL), for ClO_3^- in milk at 100µg/g (Commission Regulation (EU) 2020/749). Interestingly, MRLs of ClO_4^- has been established for certain food matrices (Commission Regulation (EU) 2020/685), but do not include the milk and dairy products.

Nevertheless, considering that data related to the content of ClO_3^- and ClO_4^- in diary foodstuffs remain limited, the main objective of this preliminary study was to evaluate the presence of these two anions in the raw milk samples. A total of 140 tank raw milk samples were randomly collected from dairy farms in Lombardy region. For this purpose, the analytical method based on ionic chromatography (IC) coupled with Exploris-Orbitrap®-High Resolution Mass Spectrometry (HRMS) was developed and validated. ClO_3^- was detected in 62% of analysed samples in the range of 2.5 to 18 µg/g with media ±SD value of 10.5±7.4. ClO_4^- was more frequently detected in 97% of samples. The concentration range was between 0.5 to 6µg/g, while media ±SD value was set at 4.2±2.4.

Although the concentrations of ClO_3^- and ClO_4^- were relatively low in the investigated raw milk, they did exhibit broad occurrences. It is reasonable to speculate that ClO_3^- enters the supply chain almost exclusively as a disinfection by-product, either through contact of the milk with chlorinated water or as a residue from cleaning processes present on equipment surfaces [4]. Our data demonstrated that the possible entry point for ClO_3^- starts on-farm practices. Water usage in all aspects of dairy production remains a critical step for introduction of ClO_3^- and ClO_4^- into the supply chain. The almost permanent presence of ClO_4^- indicate that diary caws are exposed to the latent dietary intake through the feed and water consumption [5]. Therefore, definition of MRL for perchlorate in milk becomes mandatory.

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MICROBIOLOGICAL ASSESSMENT OF DRY-AGED RAINBOW TROUT (*Oncorhynchus mykiss*)

Felice Panebianco, Giorgio Pasinetti, Selene Rubiola, Daniele Pattono, Francesco Chiesa, Pierluigi Aldo Di Ciccio, Tiziana Civera

Università degli Studi di Torino, Dipartimento di Scienze Veterinarie.
Corresponding author: F. Panebianco (felice.panebianco@unito.it)

Dry aging is a method of maturing meat that consists of a long period of refrigeration at monitored humidity. In 2019, the Australian chef Josh Niland published a book in which explained his innovative concept of fish curing, based on dry aging of whole individuals [1]. Since then, other high-class restaurants worldwide have started to use dry aging for the treatment of fish products. During dry aging, the fish skin becomes drier, there is a general loss of fluid and a substantial change in texture of muscle; the result is a product with a more concentrated flavour. Since dry-aged fish is becoming so popular, it would be opportune to evaluate the quality and safety of these products to provide useful data for the risk assessment process. The aim of this work was to evaluate the microbiological quality of rainbow trout subjected to dry aging in a restaurant located in Turin (Italy).

Rainbow trout (*Oncorhynchus mykiss*) from a local fish farm reached the restaurant in vacuum packaging (0-2°C) within 48 h after slaughter. After the removal of gills and scales and a preliminary washing in a saline solution (15% NaCl) for 40-60 s, the fish were suspended upside down in a dry aging cell at 2°C with relative humidity below 70%. At day 0, 3, 6, 10 (maximum aging period usually used by the restaurant), and 14 the following microbiological enumerations were performed on hypaxial muscle (HM), epaxial muscle (EM), and skin: i) total mesophilic bacteria; ii) total psychrophilic bacteria; iii) *Pseudomonas* spp.; iv) *Enterobacteriaceae*; v) lactic acid bacteria; vi) coagulase-positive staphylococci (suspected *Staphylococcus aureus* colonies were identified by MALDI-TOF MS); vii) yeasts and moulds. At each sampling point, detection of *Listeria monocytogenes* according to the UNI ISO 11290-1:2017 was performed on muscle and skin samples, while pH and a_w were measured on muscle samples.

From day 0 to day 6, almost all the microbial groups were under the limit of quantification in HM and EM samples, while the loads were higher for skin samples but always within acceptable levels. At day 10, *Pseudomonas* spp. (4.4±0.0 Log CFU/g), total mesophilic bacteria (2.8±0.1 Log CFU/g) and total psychrophilic bacteria (3.0±0.2 Log CFU/g) were quantifiable only in HM samples. The loads at day 14 were significantly higher especially for *Pseudomonas* spp. (6.1±0.8 Log CFU), total mesophilic bacteria (5.3±0.7 Log CFU/g) and total psychrophilic bacteria (5.4±1.0 Log CFU/g) in HM samples. Detection of *L. monocytogenes* and *S. aureus* was negative during the experiment, while pH and a_w values remained stable from day 0 until day 14.

These preliminary results showed that dry-aged rainbow trout was microbiologically safe for consumers, also considering that this product is usually cooked before consumption. Bacterial loads in muscle and skin samples were acceptable, especially up to day 10 of aging, and the foodborne pathogens *L. monocytogenes* and *S. aureus* were never detected. Further studies are needed to provide robust data for microbiological and chemical risk assessment of dry-aged fish of different species.

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PREVALENCE OF *Salmonella enterica* AND *Yersinia enterocolitica* IN HUNTED WILD BOAR IN SARDINIA AND EVALUATION OF CARCASS HYGIENE

Giuliana Siddi, Francesca Piras, Rita Sanna, Maria Pina Meloni, De Santis Enrico Pietro Luigi, Christian Scarano

Università degli Studi di Sassari, Dipartimento di Medicina Veterinaria
Corresponding author: Giuliana Siddi (giuliana.siddi@gmail.com)

The aim of the study was to assess the presence of *Salmonella enterica* and *Yersinia enterocolitica* in hunted wild boar in Sardinia. Samples collected from 66 wild boars harvested during 2020-2021 and 2021-2022 hunting seasons were analysed. Ileocaecal lymph nodes and colon content samples were collected. Moreover, in order to evaluate the hygiene of the slaughtering process, non-destructive sampling was performed in a dedicated structure where slaughtering operations were performed at the end of the hunting day; sampling was performed by sponge on carcasses at ham, back, belly and cheek sites (ISO 17604:2015). All the samples were tested for the presence of *Salmonella* (ISO 6579:2020) and *Y. enterocolitica* (ISO 10273:2017); on sponge samples, mean level (log_{10} CFU/cm^2) of aerobic colony count (ACC, ISO 4833: 2013) and *Enterobacteriaceae* (ISO 21528-2:2017) were determined. Presumptive colonies of *Salmonella* and *Y. enterocolitica* were confirmed by species-specific PCR analysis. On confirmed *Y. enterocolitica* isolates, the presence of *ystA*, *ystB* and *inv* genes was investigated. Results showed that 4.5% (3/66) and 43.9% (29/66) of the wild boar were carriers for *Salmonella* and *Y. enterocolitica*, respectively. Overall, 45% (29/66) of wild boar carried at least one of the investigated pathogens and 6.1% (3/66) carried both microorganisms. With regards to *Y. enterocolitica* virulence genes, *ystA* was found in 1/46 (2.2%) strains, *ystB* in 39/46 (84.8%) strains, and *inv* in 38/46 (82.6%) strains; therefore, among isolates, 86.9% (40/46) had at least one of the principal virulence genes. The mean levels of ACC detected on wild boar carcasses were ($\bar{x}$±SD) 2.46±0.97 log_{10} CFU/cm^2; as regards *Enterobacteriaceae*, mean levels were ($\bar{x}$± SD) 1.07±1.18 log_{10} CFU/cm^2. In a previous research carried out on wild boar coming from the Asinara island National Park in Sardinia, a *Salmonella* prevalence of 46.7% had been observed, thus much higher than that of the present study. A possible explanation could be that wild boar object of the previous study were periodically trapped inside the Park by mobile cage traps and afterwards transferred by boat and by truck to a slaughterhouse, thus undergoing the effect of the same stressful factors, such as caging and transport, that are well known to increase *Salmonella* susceptibility and shedding in slaughtered pigs. On the other hand, *Y. enterocolitica* was never detected in wild boar from the National Park; this finding probably reflects the origin of the animals from the geographical closed environment of Asinara island, as opposed to free-roaming wild boar, which could more easily come into contact with contaminated food and, most of all, pigs that represent the main reservoir of the pathogen. As regards microbiological contamination of wild boar carcasses, the mean ACC and *Enterobacteriaceae* values indicate a good hygiene level of slaughtering. Overall, results of our study demonstrate that enteropathogens are widely present in wild boar populations that may act as reservoir and spreaders. The zoonotic risk from infected wildlife is linked indirectly to the contamination of vegetable products and to hunting and carcass manipulation and directly to the ingestion of contaminated meat or meat products.

AMV

NOVEL FINDINGS ON GENETICALLY DRIVEN ENTERIC NEUROPATHY: THE RAD21 KNOCK-IN MOUSE

Francesca Bianco (1), Giulia Lattanzio (1), Maurizio Mazzoni (1), Luca Lorenzini (2), Vito Antonio Baldassarro (2), Luciana Giardino (2), Laura Calzà (2), Elena Bonora (3), Roberto De Giorgio (4), Paolo Clavenzani (1)

[1]Department of Veterinary Medical Sciences (DIMEVET), University of Bologna, Bologna, Italy. [2]IRET Foundation, Ozzano Emilia, Italy. [3]Department of Medical and Surgical Sciences (DIMEC), University of Bologna, Bologna, Italy. [4]Department of Translational Medicine, University of Ferrara, Ferrara, Italy.

Corresponding author: F. Bianco (francesca.bianco5@unibo.it)

RAD21 is a double-strand-break repair protein of the cohesin complex exerting various pivotal functions including neuronal maintenance and survival[1]. In a previous study, we detected a novel causative RAD21 (Ala622Thr) missense mutation in a consanguineous family, whose main clinical phenotype was neurogenic chronic intestinal pseudo-obstruction (CIPO)[2]. CIPO is a very severe dysmotility disorder characterized by recurrent sub-obstructive episodes, in the absence of mechanical causes of gut occlusion[3]. So far, the management of CIPO remains largely unsatisfactory, leading to poor quality of life for the affected patients. 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 (mouse homolog of human mutation). Our purpose was to perform a qualitative, quantitative and functional characterization of the enteric nervous system of Rad21KI vs. wild type (WT) mice. Using immunohistochemistry and confocal microscopy analysis, we applied a panel of antibodies, i.e. the pan-neuronal marker HuC/D, choline acetyltransferase (ChAT, for cholinergic motor neurons), neuronal nitric oxide synthase (nNOS, for nitrergic motor neurons) and synaptophysin (Syn, for synaptic vesicles surrounding myenteric perikarya), to whole mount myenteric plexus preparations of the small and large intestine of both subsets of mice. Gut transit was evaluated by measuring the total GI transit time feeding a carmine-red solution to animals (n=11 Rad21KI; n=9 WT). The arrival time of coloured faeces after oral administration of carmine red solution determined the intestinal transit time. The total number of HuC/D and HuC/D/nNOS-immunoreactive (IR) myenteric neurons did not significantly change in the small and large intestine of Rad21KI vs. WT. However, in the small intestine of Rad21KI, we showed that HuC/D/ChAT-IR myenteric neurons/field were significantly lower vs. WT mice (18.9±1.4 vs. 32.4±2; P≤0.005); likewise, in the large intestine HuC/D/ChAT-IR myenteric neurons/field were 19.8±2.1 in Rad21KI vs. 32.1±3.8 in WT mice (P≤0.005). The synaptic vesicle analysis via confocal microscopy showed a significant reduction of Syn-IR in the Rad21KI large and small intestine vs. WT (P≤0.005). Carmine red dye in vivo study showed an increase of intestinal transit time (260.5±20.8 and 190±12.2) in Rad21KI vs. WT (P≤0.005). In conclusion, in Rad21KI mice there was a significant reduction of HuC/D/ChAT myenteric neurons along with a lower synaptic density (large and small intestine) and a delayed intestinal transit. These findings illustrating a cholinergic deficit can explain, at least in part, the impairment of motility in CIPO patients with the RAD21 mutation. This initial finding provides a basis to further investigate the full spectrum of abnormalities in this mouse model as a paradigm to CIPO in humans.

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RECRUITMENT OF SPINAL INHIBITORY NEURONS IN INFLAMMATORY PAIN

Emma Merlin, Chiara Salio, Francesco Ferrini

Università degli Studi di Torino, Dipartimento di Scienze Veterinarie
Corresponding author: F. Ferrini (francesco.ferrini@unito.it)

Pain is perceived by the integration and processing of sensory stimuli conveyed to the different areas of the central nervous system through nociceptive pathways. The involvement of both the spinal dorsal horn (DH) and supraspinal centers in pain transmission has been thoroughly investigated, however many questions remain open, especially about the intricate mechanisms of modulation of nociceptive pathways.

The spinal DH receives input from primary afferent axons, which terminate in a modality-specific pattern in different DH laminae (1). The incoming somatosensory information is modulated through complex synaptic circuits involving excitatory and inhibitory interneurons. Most of the past studies have provided evidence that inflammatory pain increases the excitatory drive onto the spinal dorsal horn (2) and cortex (3), while little has been done on the involvement of inhibitory circuits and their role in chronic pain.

Here we analyzed the recruitment of inhibitory neurons in the spinal dorsal horn (DH) and somatosensory cortex (SSC) in a murine model of inflammatory pain induced by zymosan injection in a left hind footpad. Activation of inhibitory neurons was assessed by analyzing the expression of a marker of neuronal activation (Fos) with standard phenotypic markers of inhibitory neurons, such as the paired box gene 2 (Pax2) in the DH and parvalbumin/calbindin in the SSC. The colocalization between markers was detected by immunofluorescence and confocal microscopy.

We observed a significant increase in Fos expression, both in the DH and in the SSC, on the side receiving nociceptive input from the injected footpad. About 15% of Fos+ neurons were inhibitory (Pax2+), both in control and in inflammatory pain. However, following the overall increase of Fos+ neurons, also the total number of Fos+/Pax2+neurons was higher ipsilaterally to the site of injection as compared to the untreated side. Interestingly, in the SSC only few FOS+ neurons were inhibitory and no increase was induced by the inflammatory insult.

Our results indicate that a constant barrage of inhibition is required in both control conditions and inflammatory pain and confirm the pivotal role of inhibition in the DH in constraining the spread of nociceptive information. Local inhibitory circuits in this area represent an attractive potential target for controlling chronic pain. Conversely, the role of inhibition in SSC remains elusive and further studies are required. All the experimental procedures were approved by the Ministry of Health (Authorization 581/2020-PR).

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EFFECT OF BIOACTIVE PEPTIDES FROM ATLANTIC SALMON PROCESSING BY-PRODUCTS ON THE EUROPEAN SEA BASS AND GILTHEAD SEA BREAM OXYNTOPEPTIC AND ENTEROENDOCRINE CELLS GASTRIC MUCOSA

Maurizio Mazzoni (1), Alessio Bonaldo (1), Giulia Lattanzio (1), Luca Parma (1), Serena Busti (1), Åge Oterhals (2), Odd Helge Romarheim (2), Tone Aspevik (2), Pier Paolo Gatta (1), Claudio Tagliavia (1), Giacomo Vidotto (1), Paolo Clavenzani (1)

(1) Università degli Studi di Bologna, Dipartimento di Scienze Mediche Veterinarie. (2) Nofima, Tromsø, Norway.

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

Every year a considerable amount of marine by-products including fisheries by-catch and seafood processing leftovers is discarded while these by-products contain valuable nutrients that can be utilized as functional ingredients in feed industry. Considering their low molecular weight compounds and well-balanced amino acid profile, hydrolysates have been used as chemoattractant and fishmeal replacer in aquafeed. State of the art techniques within peptidomics and bioinformatics (often referred to as the in-Silico approach) was used to identify peptides with predicted anti-inflammatory, immunostimulatory/anti-microbial properties in the different fractions of by-products. Food intake regulation strongly relies on the gut–brain axis, and numerous studies have pointed out the significant role played by gut hormones in response to food digestion. In fish, the oxyntopeptic cells (OPs), secretes both hydrochloric acid and pepsinogen into the lumen to initiate protein digestion. In Teleosts, ghrelin (GHR) and neuropeptide Y (NPY) increases food intake and energy balance, while somatostatin (SOM) inhibits gastric acid secretion and food intake. The aim of the present study was to evaluate the effects of dietary inclusion level of bioactive peptide (BP), obtained from by-products of salmon processing (in Silico approach), on the presence and distribution of OPs and enteroendocrine cells (EECs) that express GHR, NPY and SOM in the gastric mucosa of European sea bass and gilthead sea bream. For this study, 27 sea bass and 27 sea breams specimens were used, divided into three experimental groups: one control group (CTR) fed with control diet (0 % BP) and two groups fed with different levels of BP (5% BP, BP5%; 10% BP, BP10%) in substitution to fish meal. Experimental procedures were evaluated and approved by the Ethical-Scientific Committee (ID 113/2020-PR). At the end of the trial, for each fish, the stomach, was sampled and then processed for IHC methods. Some SOM, NPY and 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 feature with a round shape without cytoplasmic prolongations. In the sea bream gastric mucosa, the BP10% group (16.8±7.5) exhibited a significant change in the mean number of NPY-IR cells respect to the CTR (CTR 8.5±4.8) and BP5% groups (BP10% *vs* CTR $P<0.01$; BP10% *vs* BP5% $P<0.05$). The EECs expressing SOM in the BP 10% diet (16.8 ± 3.5) were significantly different from the CTR (12.5±5) (CTR *vs* BP 10% $P<0.05$) and BP 5% groups (12.9±2.5) (BP 5% *vs* BP 10% $P<0.01$). In the sea bass, EEC SOM-IR cells increased in the BP 10% (5.3±0.7) compared to BP 5% (4.4 ± 0.8) (BP 5% *vs* BP 10% $P<0.05$). In conclusion, data suggest that BP from salmon have a promising implication as circular ingredients for European seabass and gilthead seabream. The study also provides novel advances in the morphofunctional changes of the digestive tract of these species in response to feeding stimuli.

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FOOT MUSCLES AND BONES IN THE ASIAN ELEPHANT (Elephas maximus): HOW TO SUPPORT AND MOVE 1,500 KG PER LIMB

Claudio Tagliavia (1, 2), Marco Canova (1), Jacopo Tondini (1), Giulia Salamanca (1), Cristiano Bombardi (1), Annamaria Grandis (1)

(1) Università di Bologna, Dipartimento di Scienze Mediche Veterinarie. (2) Università di Teramo, Facoltà di Medicina Veterinaria.

Corresponding author: C. Tagliavia (claudio.tagliavia2@unibo.it; ctagliavia@unite.it)

The foot of elephants developed an extraordinary system capable to support 6,000 kg of an adult animal, 1,500 kg per foot in station, 2,000 during stepping and 3,000 kg during ambulation, when only two limbs rest on the ground at the same time [1].

The occasional arrival at the Department of Veterinary Medical Sciences of the University of Bologna of the distal end of a right pelvic limb of an adult Indian elephant stimulated the desire to study its musculoskeletal structure, in order to provide new information to better understand locomotion and the susceptibility to joint pathologies in this heavy terrestrial mammal.

The stratigraphic dissection of the foot allowed us to observe the following characteristics: the presence of thick muscle bellies suggests a flexion-extension function of the toes, despite the fact that the end of the elephant's foot appears, on external observation, as a single, compact trunk; the presence of large, strong terminal interlacing tendons to form a thick band that contains the bones and joints acts as a protection them from possible dislocation during loading on the limb; a preallux that originates mid-caudally from the first digit and supports the distribution of weight [1] is composed only of cartilage; the presence of morphological peculiarities of some muscles compared to the literature [2, 3] suggest the hypothesis that there is a certain degree of intraspecific variability, perhaps to be attributed to an evolution of the species that is not yet completely stabilized; the presence of conformational alterations of some metatarsal and phalangeal bones can be attributed to a load prevailing on the lateral part of the foot in an animal of advanced age [4].

Finally, the bones were treated to obtain a skeletal preparation to be displayed in the Domestic Animal Anatomy Collection of the University of Bologna.

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DIET INFLUENCE ON LEPTIN SYSTEM IN THE ABOMASUM OF THE SHEEP

Elisa Palmioli (1), Paola Scocco (2), Anna Fagotti (3), Francesca Simoncelli (3), Cecilia Dall'Aglio (1), Kamil Dobrzyn (4), 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. (3) Università degli Studi di Perugia, Dipartimento di Chimica, Biologia e Biotecnologie. (4) University of Warmia and Mazury in Olsztyn, Department of Animal Anatomy and Physiology.
Corresponding author: E. Palmioli (elisa.palmioli@studenti.unipg.it)

The adipokines are biologically active molecules with hormonal action, whose secretion is influenced by the distribution and amount of the adipose tissue [1]. They take place in several metabolic activities, performing their roles through an endocrine, paracrine, and autocrine mode of action on several organs and tissues [2], and they can be considered as possible biological markers of animal health status [3]. This study belongs to a wider project that aims to maintain and improve the productive performances of the flock reared on semi-natural pastures, which suffer from an impoverishment of their nutritional value because of the growing summer drought stress [4]. Here we evaluated the adipokine leptin (Ob), with its receptor (Ob-R), in the abomasum of 15 adult Comisana x Appenninica female sheep reared on Apennine pastures and subjected to different diets (No. of approval 95/2018-PR). Until the maximum pasture flowering (MxF group), the sheep fed on fresh forage; from that moment until the maximum pasture dryness (MxD group), the experimental group (Exp group) received a feed supplementation in addition to MxD group feeding. To perform the study, the Ob system was investigated by immunohistochemistry (IHC) and Real-time quantitative reverse transcription-PCR (RT-qPCR) in the abomasum samples. Immunofluorescent double- label localization of Ob system with different neuroendocrine hormones was conducted to distinguish the gland cell types. The IHC was performed on formalin-fixed and paraffin-embedded sections by using a mouse monoclonal anti-Ob and a rabbit polyclonal anti-Ob-R as primary antibodies. RT-qPCR analysis was performed by Sybr Green system employing specific primers. The relative quantification of target genes was determined by Delta Ct (ΔCT) method using three reference genes for normalization purposes. The analysis revealed the presence of positive cells to Ob and Ob-R, mainly labelled as chief cells, in the mucous layer of the abomasum, specifically in the lower half of the fundic glands. The immunostaining intensity did not point out significant differences for the Ob system among the three groups of sheep. This result was partly confirmed also by a RT-qPCR preliminary analysis that did not show statistically significant differences. This led us to hypothesize that food supplementation does not influence the Ob system. However, the abundant immunostaining observed in the abomasum, in accordance with previous studies in humans and rats, suggests that the Ob may intervene locally in the regulation of abomasum functions also in sheep [5]. A better understanding of the gastrointestinal system can contribute improving sheep management and optimizing the sustainability of livestock production.

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EVALUATION OF DECELLULARIZED CARTILAGE MATRIX AND SELP HYDROGEL FOR REGENERATIVE MEDICINE APPROACH

Matteo Zoboli (1), Pedro Veloso (1), Teresa Frattini (1), Roberta Ciccimarra (1), Elena De Angelis (1), Luisa Ragionieri (1), Antonio Cacchioli (1), Ferdinando Gazza (1), Roul Machado (2) Paolo Borghetti (1) Francesca Ravanetti (1)

(1) University of Parma - Italy, Dept. of Veterinary Sciences. (2) Unversity of Minho - Portugal, Dept. of Biology.
Corresponding authors: F. Ravanetti (francesca.ravanetti@unipr.it), L. Ragionieri (luisa.ragionieri@unipr.it)

The regeneration of articular cartilage is one of the main challenges in the treatment of traumatic, chronic and degenerative osteoarticular diseases, since its avascularity limits the infiltration of progenitor cells and subsequent self-regeneration. As alternatives to traditional surgical methods, new approaches based on regenerative medicine are being investigated. The present study aims to set up an *in vitro* cartilage regeneration model based on decellularized cartilage, as a biomatrix, seeded with primary chondrocytes or bone marrow derived MSCs and subjected to normoxia (19% O_2) or hypoxia (5% O_2), as an approach for future tissue engineering in animals and human. The low oxygen tension, in fact, is considered a more powerful promoter of chondrogenesis differentiation than dynamic compression, responsible for the induction of the Sox9 promoter activity and the following stimulation of Col2 and aggrecan, which favors chondroinductive mechanisms [1, 2]. As bioadhesive to attach and stabilize the construct into the anatomical site, a natural protein-based polymeric hydrogel, consisting of Silk-elastine-like protein (SELP) was considered.

Decellularized matrix was obtained from equine metacarpo/metatarsophalangeal, received from the slaughterhouse, after a suitable combination of enzymatical digestion, chemical and mechanical treatments. The matrix decellularization was verified by DAPI and DNA quantification. As cellular model both equine chondrocytes at two different stages of de-differentiation (P2 and P4) and murine MSCs were tested on the matrix and cultured in normoxic and hypoxic conditions. Morphological and molecular investigations for cartilage differentiation markers and extracellular matrix production have been performed through histological staining, immunodetection and gene expression of relevant chondrogenic markers. The results indicated that the culture onto the matrix in hypoxic conditions promotes and sustains MSCs chondrogenic differentiation. As regards primary cells, specific histological and immunofluorescent stainings demonstrated the maintenance of mature chondrocytes, also supported by the gene expression analysis of Collagen type II, Aggrecan and Sox9. Histological and SEM analysis confirmed that cells were actively producing cartilage matrix. During the culture, P2-stage chondrocytes maintained a greater proliferation capacity, whereas P4-stage chondrocytes better responded to hypoxic culture conditions.

Regarding SELP hydrogel, different conditions for the formation of the hydrogel were tested. The chemical structures of hydrogel were analyzed by Fourier transform infrared spectroscopy (FTIR). Preliminary adhesion test indicated the capacity of hydrogel to connect both organic and inorganic substrates under mechanical load.

Further adjustments on the hydrogel formation protocol and adhesion test will be needed, however, both the strong adhesive properties of SELP hydrogel and the ability of matrix-seeded cells to progress to a mature chondrocyte phenotype producing a cartilage matrix are encouraging results.

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HISTOLOGICAL AND IMMUNOHISTOCHEMICAL STUDY OF ANNUAL *Nothobranchius rachovii* DIGESTIVE TRACT

Marilena Briglia, Kamel Mhalhel, Giuseppe Montalbano, Rosaria Laurà, Lidia Pansera, Mirea Sicari, Maria Levanti

University of Messina, Department of Veterinary Sciences.

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

The peptide hormone cholecystokinin (*CCK*) is known to play a key role in regulating food intake and satiety in higher vertebrates. In humans, increased concentration of *CCK* in older people was described as the basis of anorexia associated with aging [1, 2]. In rats as well, *CCK* induced strong anorexia in young adults and old subjects [3]. However, the role of increased plasma *CCK* concentrations in mediating the age-related decrease in appetite remains to be established.

African annual fishes from the genus Nothobranchius are small teleosts that inhabit temporary water. They are emerging as a prominent model organism in biomedical research, evolution studies, and developmental biology due to their short captive lifespan, amenability to captive breeding, embryonic diapause, short generation time, and huge eggs, susceptible to microinjection therefore to genetic manipulations [4]. They are considered convenient experimental models for life-long investigations on the effects of dietary and environmental manipulations [4].

The present study aimed to investigate, for the first time, the morphology of the gastrointestinal tract and the expression of the *CCK* of the short lifespan *Nothobranchius rachiovii*. For this reason, we have investigated the histological features of *Nothobranchius rachovii* gastrointestinal tract using Masson's trichrome stain and alcian blue–PAS. The *CCK* expression pattern was studied through immunohistochemical labelling and quantitative RT-PCR. Indeed, *CCK* expression was detected in scattered intraepithelial cells, and the variation of the pattern of expression was recorded between the different segments of adult's digestive tract.

Our study introduces *Nothobranchius rachovii* as a potential model for anorexia of aging, giving the first bases on the gastrointestinal tract morphology and *cck* expression pattern. Future studies on young and elderly *Notobranchius* can divulge the contribution of *cck* in the mechanisms of anorexia associated with aging.

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CHANGES IN MORPHOLOGY AND GLYCAN EXPRESSION IN THE BABOON Papio hamadryas OVIDUCTAL EPITHELIUM DURING THE MENSTRUAL CYCLE

Salvatore Desantis (1), Mario Cinone (1), Luca Lacitignola (1), Pietro Laricchiuta (2), Maria Albrizio (1)

(1) Università degli Studi di Bari Aldo Moro, Dipartimento dell'Emergenza e dei Trapianti di Organi. (2) Zoo Safari, Fasano (Brindisi).

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

The mammalian oviduct is a highly specialized structure where fertilization and early embryonic development take place. Its mucosal epithelium is involved in maintaining and modulating a dynamic intraluminal fluid via secretory activity. The mucosal epithelium of the oviduct consists of ciliated and non-ciliated (secretory cells). The oviductal glycoproteins (OGPs) are involved in pre-and post-fertilization events [1]. As regards baboons, reports exist on the cyclic changes of the oviductal epithelium and the localization of OGP in *Papio anubis* [2], whereas studies on the oviduct of baboon *Papio hamadryas* are absent. In this study, we investigated for the first time the morphology and glycan composition of baboon *Papio hamadryas* oviductal epithelium during the menstrual cycle. Oviducts were laparoscopically removed from 14 healthy adult female *Papio hamadryas* at the Zoo Safari (Fasano, Italy). The stage of the menstrual cycle was based on the sex hormone levels and the vaginal cytology features. Histological investigations were carried out on fimbriae, infundibulum, ampulla, and isthmus separately fixed in 4% (v/v) paraformaldehyde, embedded in paraffin wax, and stained with hematoxylin-eosin for morphological analyses and by means of a panel of 9 fluorescent lectins for glycoconjugate characterization. During the menstrual cycle, the morphometric analysis revealed a significant change in the epithelium height of all oviductal segments with an increase from the follicular phase to the preovulatory phase and a decrease in the luteal phase. However, the oviducts of the luteal and follicular phases showed similar values of the epithelium height in the fimbriae, whereas the isthmus epithelium height was significant ($P<0.01$) lower in the luteal phase. The apical protrusions were a characteristic feature of non-ciliated cells in the preovulatory phase oviducts. Lectin histochemistry detected the presence of OGPs mainly in the apical zone and the luminal surface of the epithelium with a clear presence of 1) highly mannose N-linked glycans (Con A) in the oviducts during the entire menstrual cycle, 2) α2,6-linked sialic acids (MAL II) in the preovulatory phase oviducts, 3) α2,3-linked sialic acids (SNA) in the preovulatory and luteal phases oviducts, 4) GalNAc terminating glycans (HPA) from the infundibulum to the isthmus of preovulatory and luteal phases, 5) GalNAc-DBA binding sites in the infundibulum of the luteal phase, 6) α1,3-linked fucosylated glycans (LTA) in the ampulla of preovulatory phase, and 7) α1,2-linked fucosylated glycans (UEA I) in the fimbriae of the luteal phase. Apical protrusions of preovulatory non-ciliated cells displayed binding sites for MAL II in the fimbriae and infundibulum, and for Con A and LTA in the ampulla. Non-ciliated cell apical protrusions of luteal oviducts showed UEA positivity in the fimbriae and HPA reactivity from the infundibulum to the isthmus. These results demonstrated for the first time regional morphological and chemical differences in the baboon *Papio hamadryas* oviductal epithelium as well as that glycosylation pattern varies during the menstrual cycle. The observed changes could be related to the oviductal region-specific functions. These findings add new data on the baboons which due to their size and anatomical similarity to humans, make an excellent model for female reproduction studies [3].

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RABBIT DIETARY SUPPLEMENTATION WITH BOVINE COLOSTRUM: IMPACT ON GUT AND LIVER INTEGRITY

Lucia Aidos (1), Alessia Di Giancamillo (1), Gabriele Brecchia (2), Laura Menchetti (3), Federica Riva (2), Giulio Curone (2), Claudia Balzaretti (2), Marta Castrica (2), Valentina Serra (2), Stella Agradi (2), Grazia Pastorelli (2), Daniele Vigo (2), Silvia Modina (2)

(1) Università degli Studi di Milano, Dipartimento di Scienze Biomediche per la Salute. (2) Università degli Studi di Milano, Dipartimento di Medicina Veterinaria e Scienze Animali. (3) Università degli Studi di Bologna, Facoltà di Agraria, Dipartimento di Scienze e Tecnologie Agro-alimentari.

Corresponding author: L. Aidos (lucia.aidos@unimi.it)

Italy is the second largest producer of rabbit meat in the world, after China, and the first one in Europe (1). Infections of the gastroenteric and reproductive system are among the main causes that limit the profitability of rabbit breeding and welfare (2). Recent studies showed that bovine colostrum (BC) may have beneficial effects on the individual health, and in preventing and treating several animal diseases (3). The aim of this study was to evaluate the effects of supplementing the diet of rabbit with BC, in terms of productive performance and integrity and health of the gastroenteric system and liver.

After weaning (35 days), rabbits were fed three types of diets until slaughtering (105 days): CTR (control, fed a commercial feed); BC1and BC2 (colostrum fed 2.5% and 5% BC, respectively). Body weight (BW) was registered on a weekly basis throughout the experiment. At slaughtering, samples of stomach body (fundic mucosa), and liver of 5 animals for each experimental group were collected for histological and histochemical analyses: sections of the gut were stained with Hematoxylin–Eosin (HE) in order to establish structural details, while sections of the liver were stained with Periodic Acid Schiff (PAS), which reveals neutral glycoconjugates (purple stain) and Azan for demonstrating collagen fibres (blue staining). This study was approved by the Ethic Committee of the University of Milan (OPBA_42_2021).

At weaning, the BC2 showed the lowest BW (438±90 g; $p<0.01$), but, after weaning, it was higher than the BC1 BW (1294±17 g; $p=0.049$). Finally, no differences were found in carcass weight. The fundic mucosa of the stomach was regularly organized in all groups, but in the BC1 and BC2 it was possible to observe haemorrhagic infiltrates in the lamina propria towards the lumen, while the basal side revealed to be morphologically normal. Intestinal samples revealed normal morphological organization. PAS staining revealed the presence of intrahepatic glucogen, evident in all groups and Azan staining showed an increasing degree of collagen fibre staining from CTR to BC2.

Overall, samples presented a normal structural organization of the gut with no damage of the epithelium. The changes observed in the lamina propria of the stomach and the presence of glucogen and collagen fibers in the liver, apparently had no negative effect on the productive performance taking into account that at slaughtering there were no significant differences between treatments as well as in health and welfare of the animals.

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ACUTE STRESS RESPONSE AND IMMUNOHISTOCHEMICAL LOCALIZATION OF HSP70 AND IGF-I IN MEAGRE (Argyrosomus regius) JUVENILES EXPOSED TO CHANGES IN WATER QUALITY

Martina Bortoletti (1), Elisa Fonsatti (1), Stefano Caberlotto (2), Roberto Macaluso (1), Giuseppe Radaelli (1), Daniela Bertotto (1)

(1) Università degli Studi di Padova, Dipartimento di Biomedicina Comparata e Alimentazione. (2) Valle Cà Zuliani Società Agricola S.r.l.

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

In aquaculture, fish welfare strictly relies on water quality. In particular, temperature, salinity and ammonia concentration represent the most common water parameters affecting the physiological stress of fish. Variations in these parameters can activate the hypothalamic-pituitary-interrenal axis (HPI) causing the release of corticosteroids. In fish, cortisol is the key stress hormone since it is the primary corticosteroid produced by the HPI axis [1]. Moreover, at the cellular level, the stress response is mediated by the heat shock proteins (HSPs), a family of highly conserved proteins found in all cells of all life forms [2]. Variations in water quality parameters can also affect fish growth by influencing the expression of insulin-like growth factors (IGFs) [3] which play a key role in growth regulation [4]. In fish, IGF-I is mainly produced in the liver, although several other organs express this molecule as well [5]. The present work is aimed at evaluating the effect of changes in temperature, salinity and ammonia concentration, on the acute stress response and on the immunohistochemical localization of HSP70 and IGF-I in juveniles of meagre (*Argyrosomus regius*), an emerging species in Mediterranean aquaculture. To this purpose, we used Radioimmunoassay (RIA) to evaluate whole-body cortisol levels and immunohistochemistry to investigate the cellular localization of HSP70 and IGF-I. Cortisol levels significantly increased only in the thermal-stressed group with respect to the control group, whereas the salinity and ammonia groups did not experienced increases in cortisol levels. The immunohistochemical localization of HSP70 and IGF-I did not differ among treatments. Overall, HSP70 immunoreactivity was expressed in skin, gills, liver and digestive system of both control and stressed animals. The anti-IGF-I antibody revealed an immunostaining in skin, gills, liver and digestive system as well as in pancreas and kidney. In conclusion, cortisol results revealed that temperature changes negatively impacted on meagre stress status, in contrast to osmotic and chemical shocks, to which meagre juveniles exhibited low sensitivity. However, the tissue localization of HSP70 and IGF-I highlighted that the stress response did not affect protein expression.

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INTEGRATION OF MICROCT AND HISTOLOGY DATA FOR VASCULATURE MORPHO-FUNCTIONAL ANALYSIS IN TISSUE REGENERATION

Antonio Palladino (1), Aurelio Salerno (2), Antonio Crasto (1), Carla Lucini (3), Livia D'Angelo (3), Paolo Antonio Netti (2), Antonio Cacchioli (4), Francesca Ravanetti (4), Chiara Attanasio(3)

(1) Università degli Studi di Napoli Federico II, Dipartimento di Agraria. (2) Istituto Italiano di Tecnologia, Center for Advanced Biomaterials for HealthCare@CRIB. (3) Università degli Studi di Napoli Federico II, Dipartimento di Medicina Veterinaria e Produzioni Animali. (4) Università degli studi di Parma, Dipartimento di Scienze Medico-Veterinarie.
Corresponding author: A.Palladino (antonio.palladino@unina.it)

Artificial or bioartificial engineered tissues can partially or completely replace damaged tissues by restoring their physiological function. The demand for more sophisticated therapeutic options in veterinary medicine has tremendously increased particularly in equine regenerative medicine. Promising results have been achieved in the treatment of tendon and cartilage lesions, tendinopathies, and degenerative joint disorders [1]. Each engineered construct holds different properties depending on the tissue to be replicated. As for vascularized tissues, complex biocompatible structures, namely scaffolds, play a key role in supporting oxygen and nutrient supply, thus sustaining tissue neoformation and integration with the host. Scaffold architecture significantly impacts its regenerative potential [2] while preclinical trials are essential to define scaffold-host interactions. In compliance with the 3R principle, there is a clear need to optimize both the procedures to evaluate scaffold performance and the analysis methodology decreasing the number of animals required to gain consistent data. Current technologies used in preclinical research generate huge amounts of data that need to be elaborated and interpreted correctly. Therefore, we designed this study to evaluate the results of scaffold integration with the host tissue after implantation in a mouse subcutaneous pocket model. We evaluated the angiogenic response developed by the host and the degree of scaffold integration by using a combined morphometric approach based on both histological and µ-CT analyses. 6layer scaffolds, made of polycaprolactone (PCL) microspheres, with an ordered structure were produced by thermal sintering. Scaffolds were, then, implanted in 6 BALB/c mice (135/2019-PR) and retrieved 21 days post-implantation when animals were deeply anesthetized and perfused with Microfil, a contrast agent for µ-CT. Here, we describe a method to extract quantitative data from µ-CT reconstructions such as (i) total vessel volume; (ii) %of vessel penetration; (iii) distribution of vessel diameters. The general principle of this approach is the refinement of the region of interest (ROI), thus producing a volume of interest (VOI) that matches scaffold volume. This VOI serves as a dataset from which to extract volumetric information. Then VOIs are divided into 3 identical parts, -proximal, -medial, and -distal to follow vessel progression into the scaffold, thus obtaining their depth of penetration (DoP). In parallel, to evaluate tissue integration in detail, histological and immunofluorescent analyses were made to look at vessel distribution and collagen synthesis. By this methodology, we observed a vessel invasion for 1,38 mm^3 corresponding to the 1,53% of the scaffold volume. Regarding DoP, vessel distribution is 52,35%, 24,43%, and 23,04% in proximal, median, and distal zones respectively. We, then, looked at the diameter distribution being this value a key indicator of vessel maturity, highlighting that 55% of vessels fall into the range from 5,99 µm to 53.99 µm while the remaining 45% are distributed into intervals from 54 to 136 µm. Collectively, we propose a new method to track vessel formation by using a multi-modal approach posing the basis for: i) the fabrication of novel scaffolds for Tissue Engineering ii) the integration of detailed information for a wide range of morphological and functional analyses.

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THE HIGHLY CONSERVED PIVOTAL ROLE OF NEUROTROPHINS IN TASTE BUDS: EVIDENCE IN ZEBRAFISH

Claudia Gatta (1), Valentina Schiano (1), Chiara Attanasio (1), Carla Lucini (1), Antonio Palladino (2)*

(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.

Corresponding author: A.Palladino (antonio.palladino@unina.it)

Neurotrophin family is composed of nerve growth factor (NGF), brain derived neurotrophic factor (BDNF), Neurotrophin 3 (NT3) and NT4 [1]. These neurotrophins regulates several crucial functions through the activation of two types of transmembrane receptors, namely p75, which binds all neurotrophins with similar affinity, and tyrosine kinase (Trk) receptors [2]. Neurotrophins, besides the well-known pivotal role in the development and maintenance of the nervous system, also display the ability to regulate the development of taste buds in mammals [3]. Therefore, the aim of this study is to investigate if NGF, BDNF, NT3 and NT4 are also present in the taste buds of zebrafish (*Danio rerio*), a powerful vertebrate model organism. To this end zebrafish heads were fixed in Bouin's fluid and embedded in paraffin. Microtomic slices were incubated with rabbit polyclonal antibodies against NGF (H-20, sc-548 Santa Cruz Biotechnology, Inc, CA, USA), BDNF (N-20, sc-546 Santa Cruz Biotechnology, Inc, CA, USA), NT-3 (N-20, sc-547, Santa Cruz Biotechnologies, CA, USA) and NT-4 (N-20, sc-545, Santa Cruz Biotechnology, Inc, CA, USA) diluted 1:150; 1:200; 1:200 and 1:200; respectively. Immunolabelling was then detected by a consequent incubation with anti-rabbit EnVision+System-HRP and a solution of 3-3' diaminobenzidine tetrahydrocloride (Sigma, St. Louis, MO, USA). Positive cells for each antiserum were counted along serial sections of oropharyngeal cavity. The results showed the presence of neurotrophins in taste bud cells of the oropharyngeal cavity, also demonstrating that BDNF positive cells are the prevalent cell population in the posterior part of oropharyngeal region. In conclusion, our results suggest that all tested neurotrophins are present in zebrafish sensory cells and lead to the assumption that taste bud cells in this fish species contain the same homologous neurotrophins reported in mammals, further confirming the high impact of zebrafish model in translational research.

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CHARACTERIZATION OF LONGISSIMUS DORSI AND SEMIMEMBRANOSUS MUSCLE FIBRES IN NERO DI LOMELLINA AND COMMERCIAL HYBRID NEWBORN PIGLETS: PRELIMINARY DATA

Margherita Pallaoro (1), Silvia Modina (1), Mauro Di Giancamillo (1), Fabrizio De Luca (1), Carlo Rinaldi (1), Lucia Aidos (2), Silvia Mazzola (1), Annamaria Costa (1), Eleonora Buoio (1), Raffaella Rossi (1), Alessia Di Giancamillo (2)

(1) Università degli Studi di Milano, Dipartimento di Medicina Veterinaria e Scienze Animali. (2) Università degli Studi di Milano, Dipartimento di Scienze Biomediche per la Salute.

Corresponding author: Margherita Pallaoro (margherita.pallaoro@unimi.it)

Swine farming is widely developed in Italy, especially due to the importance of this species in Italian typical food industry. Although the most common bred pigs are the commercial hybrids, it is still possible to find some native breeds, especially where the local tradition is deeply felt [1]. Recently, the genetic program of the *Nero di Lomellina*, an autochthonous swine from the area of Pavia, has been recognised by the Italian Association of Pig Breeders. These animals have rustic traits and their meat is suitable for being processed into fine cured products.

The following study aimed to analyse the structure of the muscle tissue of *Nero di Lomellina* bred, with a focus on the *Longissimus Dorsi* and the *Semimembranosus* muscles of newborn piglets.

The research was carried out on five *Nero di Lomellina* that died during parturition and the data were compared with those of five commercial hybrids, dead under the same conditions. All animals were weighed and their sex reported. Samples of each muscle were partly paraformaldehyde-fixed and paraffin embedded and partly frozen. Haematoxylin-Eosin and Succinate Dehydrogenase stainings (SDH) were carried out to identify the morphology and the oxidative capacity (positive staining for red fibres and negative for white fibres) of the muscle fibres respectively [2, 3]. Histometric analyses were also performed: the area, the perimeter and the number of fibres in the two muscles of both breeds as well as the positive-SDH fibres were evaluated and data statistically analysed [2, 3].

Interestingly, the statistical analysis revealed different trends between the two muscles, though non-significant, except for the number of fibers in the *Longissimus dorsi*, which is significantly lower in Nero di Lomellina piglets ($P<0.05$). This could be an effect of the genetic selection that has been promoted in the past decades.

Moreover, already at birth, the SDH-staining highlighted the presence of both red and white fibres, but quantitatively the preliminary results did not show significant differences between the two breeds.

These preliminary data in newborns may suggest possible differences in the structure of adult muscles. Further studies investigating adult samples may lead to a different muscular structure between the adults of the two breeds, which may be translated into meat products with different characteristics [4].

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NEW INSIGHTS ON THE MORPHOLOGICAL ANALYSIS OF THE AGING COCHLEA

Federica Fioretto (1), Antonio Palladino (2), Daniela Giaquinto (2), Ferdinando Scavizzi (3-4), Marcello Raspa (3-4), Livia D'Angelo (2), Paolo de Girolamo (2), Elena De Felice (1), Chiara Attanasio (2)

(1) Università degli Studi di Camerino, Scuola di Bioscienze e Medicina Veterinaria. (2) Università degli Studi di Napoli, Dipartimento di Medicina Veterinaria e Produzioni Animali. (3) Consiglio Nazionale delle Ricerche (CNR) – Istituto di Biologia Cellulare e Neurobiologia. (4) Consiglio Nazionale delle Ricerche (CNR) – Istituto di Biochimica e Biologia Cellulare – International Campus of Development (EMMA-INFRAFRONTIER_IMPC).
Corresponding author: F. Fioretto (federica.fioretto@unicam.it)

Aging is physiologically associated with a sensory decline (Age Related Sensory Decline or ARSD). Among others, one of the sensory deficits contributing to a deterioration in quality of life is age-related hearing loss (ARHL), that affects half of adults over the age of 70. Therefore, ARHL represents a significant public health concern also according to the World Health Organization (WHO) which reports that by 2050 there will be about 1 billion people over the age of 65. ARHL is characterized by a combination of conductive, namely middle ear defects, and/or neurosensory hearing loss, namely loss of hair cells or neurons [1]. The cochlea, which contains the sensory organ of hearing, includes two types of sensory cells, inner and outer hair cells, as well as two types of primary auditory neurons, type I and type II spiral ganglion neurons. Neuronal degeneration is one of the hallmarks of ARHL resulting in difficulties in speech discrimination, particularly in noisy environments. Mouse is currently the gold standard among mammalian models in biogerontology, thanks to its relatively short lifespan along with some specific characteristics and age-related diseases similar to humans. For this study, we selected the inbred strain C57BL/6J which holds the allelic variant for the Cdh23 gene, one of the genes responsible for presbycusis. Therefore, the chosen strain physiologically develops hearing impairment with aging [2]. The research was approved with protocol 1177/2020-PR by the Ministry of Health. In this work we analyze the cochlear structures during aging, by focusing our attention on four timepoints: 2, 6, 12, and 18 months, aiming to follow the progressive degeneration of these structures over-time and to relate it to hearing loss. To this end, mice were perfused with 4% paraformaldehyde, the cochleae were retrieved, decalcified, and embedded in paraffin. Decalcification is a critical step in cochlea processing, therefore we tested different protocols prior to identify the most effective. Serial sections of 7 µm were cut parallel to the mid-modiolar plane and processed for histology and immunohistochemistry. We initially focused on type I and type II neurons of the spiral ganglion that innervate the inner and the outer hair cells, respectively. From a thorough analysis of the histological sections, we observed a consistent reduction in type I neurons corresponding to a decrease of 34.7% throughout the considered time points. This finding perfectly fits with a functional hearing loss being neural degeneration commonly linked to several inner ear defects, both in humans and animals. To corroborate these findings IHC studies are underway aiming to provide useful insights in age-related morpho-functional changes for translational applications.

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THE ROLE OF ZINC IN BOVINE OOGENESIS AND EARLY EMBRYOGENESIS

Valentina Lodde, Rodrigo Garcia Barros, Mara Pizzolo, Alberto M. Luciano, Federica Franciosi

Università degli Studi di Milano, Dipartimento di Medicina Veterinaria e Scienze Animali, Laboratorio di Biologia della Riproduzione e dello Sviluppo

Corresponding author: V. Lodde (valentina.lodde@unimi.it)

One of the unsolved questions in mammalian oogenesis and early embryogenesis relates to the morpho-functional and molecular processes that drive transcription in the growing oocyte, its silencing in the fully grown oocyte, and the reactivation of the embryonic genome after fertilization. In the last years, we are considering the hypothesis that Zinc, which is emerging as a critical component in several conserved processes that regulate female germ cell growth, fertility, and pregnancy (1), is a determinant of transcriptional regulation in bovine oogenesis (2) and early embryogenesis.

We first mined the EmbryoGENE transcriptomic dataset, revealing that physiological conditions impact several zinc transporters and metallothionein throughout the final phase of oocyte growth and differentiation and in the preimplantation embryo. We then used an optimized culture system that supports the ability of growing-transcriptionally active - bovine oocytes to undergo meiosis and early embryonic development (3), to test whether zinc supplementation is beneficial to the acquisition of meiotic competence when subsequently subjected to standard in vitro maturation. Our data indicated that Zinc supplementation induced a significantly higher percentage of mature oocytes and a lower percentage of oocytes at intermediated stages of meiotic progression compared to the control group. Furthermore, we confirmed the hypothesis that Zinc supplementation might support transcription in growing oocytes by directly measuring the transcription level using the Click-iT® RNA Imaging Kit (Invitrogen). Accordingly, we observed that Zinc sequestration with a Zinc chelator (TPEN) rapidly reduced global transcription in growing oocytes, which was reversed by Zinc supplementation in the culture medium. In addition, Zinc supplementation impacted the chromatin state by reducing the level of global DNA methylation as assessed by indirect immunofluorescence, which is consistent with the increased transcription. These studies suggest that altering Zinc availability by culture medium supplementation supports global transcription, ultimately enhancing meiotic competence. In the early embryo, preliminary results indicate that zinc supplementation supports embryonic development by increasing the percentage of embryos reaching the blastocyst stage of development. Studies are in progress to test how Zinc regulates the activation of the embryonic genome in the bovine embryo.

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UNDERSTANDING THE COMPLEX MICRO- AND MACRO-STRUCTURES OF THE MENISCUS VASCULARIZATION USING MULTIDISCIPLINARY APPROACH: HIGH-RESOLUTION MICRO-COMPUTED TOMOGRAPHY SUPPORTED BY HISTOLOGICAL ANALYSIS

Valentina R. Herrera M. (1), Ville-Pauli Karjalainen (2), Silvia Modina (3) Mikko Finnila (2), Simo Saarakkala (2), Ali Mobasheri (2), Alessia Di Giancamillo (1).

(1) University of Milan, Department of Biomedical Sciences for Health (SCIBIS). (2) University of Oulu, Research Unit of Medical Imaging, Physics and Technology (MIPT). (3) University of Milan, Department of Veterinary Medicine and Animal Sciences (DIVAS).

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

Knee osteoarthritis (OA) is associated with changes in meniscal structure and biomechanical function. In meniscal tissue engineering, it is crucial to find novel methods that allow the identification and support for the use of complex systems such as those employed in regenerative medicine. Understanding the complex micro- and macro-structures of the meniscus require multidisciplinary approaches - imaging, histological, molecular, biochemical, engineering, and other techniques - to increase the limited knowledge of the biology that characterizes tissue development. Three different areas can be distinguished in mature meniscal tissue depending on its vascularization, composition, and regenerative abilities: 1) an outer zone, completely vascularized, able to undergo regenerative mechanisms; 2) an intermediate zone, with transitional characteristics, and 3) an inner zone, avascularised, with very low healing capacity [1, 2]. In order to characterize the meniscal vascularization and microstructures, high-resolution micro-computed tomography (µCT) was used to image five new-born and five adult swine medial meniscus in 3D. For further validation, classical histological techniques were proposed as methods to observe meniscal development and vascularity. Five medial menisci for each group were imaged with µCT with following settings: 40 kV, 250 µA, 3.3 µm isotropic pixel size, 1515 ms exposure time, and without filter. New-born samples were collected in a local farm from piglets died immediately after birth crushed under mother weight, while adult samples were collected in a local slaughterhouse; no animals were sacrificed for experimental purposes. Menisci were isolated from joints, fixed in 10% buffered formalin, and dissected in three anatomical sections: 1) posterior horn, 2) central body, and 3) anterior horn. Posterior horn was chosen to be analysed because it is the portion that most expresses vascular factors [3], it is the most weight-bearing portion [4] and it seems to be the area that first develops the cartilage-like phenotype [2]. Adjacent radial sections of posterior horn were dissected for µCT imaging and histological analysis. Thickness, fiber orientation, blood vessels and calcification structures were obtained by µCT imaging and image analysis; validation were done by morphological staining (Hematoxylin & Eosin and Safranin-O) and vascular-related marker immunostaining. Our data suggested that µCT could be an excellent adjuvant in meniscal tissue engineering; it allows to visualize internal three-dimensional structures, to obtain quantitative data and – in addition to classic microscopical techniques - allows to localize microstructures within the tissues, such as the vascular network of the meniscus. Although the initial cost of the equipment is high, the cost of analyzing the samples is practically nothing considering the normal laboratory equipment.; the speed of techniques and analysis as well as promoting collaborations in scientific research represent further advantages. The only considerable disadvantage is the inability to reuse the sample for histological analysis if the soft tissue is irreversibly dehydrated in preparation for imaging (not valid for other tissue type).

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MALE GERM CELL PROLIFERATION AND APOPTOSIS IN SEXUALLY IMMATURE MEAGRE (ARGYROSOMUS REGIUS) TREATED WITH RECOMBINANT FOLLICLE STIMULATING HORMONE

Rosa Zupa (1), Neil Duncan (2), Ignacio Giménez (3), Chrysovalentinos Pousis (1), Letizia Passantino (1), Aldo Corriero (1)

(1) University of Bari Aldo Moro, Department of Emergency and Organ Transplantation. (2) IRTA-Institute of Agrifood Research and Technology, Sant Carles de la Ràpita, Spain. (3) Rara Avis Biotec, S. L., Valencia, Spain.
Corresponding author: A. Corriero (aldo.corriero@uniba.it)

The meagre *Argyrosomus regius* (Asso, 1801) is a promising emerging aquaculture species. The use of recombinant gonadotropins to induce gametogenesis in sexually immature fish has the potential to reduce the generation time for selective breeding programmes of aquaculture species that reach first maturity at large size, such as the meagre [1-3]. The aim of this work was to assess the effects that a six-week recombinant follicle stimulating hormone (rFsh) administration had on germ cell proliferation and apoptosis in sexually immature meagre.

Financial support for this study was provided by the European Union´s Programme H2020 (GA 862658, NewTechAqua). Sexually immature male meagre (18 months of age) reared in indoor tanks at the Institute of Agrifood Research and Technology (IRTA; Sant Carles de la Ràpita, Spain) underwent weekly treatments with increasing doses of rFsh (week 0: 6 µg/kg; week 1: 9 µg/kg; week 2 to week 6: 12 µg/kg). Control fish were treated with physiological saline solution. Fish were sacrificed at week 0 (6 control fish) and week 6 (9 control and 4 rFsh-treated fish). For each fish, body mass (BM) and testis mass (TM) were measured and relative testis mass (gonadosomatic index) was calculated as GSI=100×TM/BM. Testis samples were fixed in Bouin's solution, dehydrated in ethanol and embedded in paraffin wax. Proliferating and apoptotic germ cells were identified on histological sections through the immunohistochemical detection of the proliferating cell nuclear antigen (PCNA) and the terminal deoxynucleotidyl transferase-mediated 2'-deoxyuridine 5'-triphosphate nick end labeling (TUNEL) method, respectively. The density of anti-PCNA–positive single spermatogonia and anti-PCNA–positive spermatocysts (i.e., spermatogenic cysts containing spermatogonia or primary spermatocytes), as well as the surface occupied by TUNEL-positive apoptotic cells, were measured on randomly selected fields of testicular sections. Statistical differences for all the analysed parameters were evaluated by a 2-tailed Student's t test.

Fish treated with rFsh displayed significantly higher GSI, significantly lower surface occupied by apoptotic cells and significantly higher density of anti-PCNA positive spermatocysts compared to both controls. The present results suggest that, in sexually immature meagre, apoptosis plays a major role in removing spermatogonia that cannot proceed towards meiosis. Treatment with rFsh supported survival and proliferation of spermatogonia as well as spermatogonial entry into meiosis. In conclusion, the administration of increasing doses of rFsh proved to be effective as a first step of a treatment protocol to induce precocious puberty in meagre.

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BOVINE DIAPHRAGM FOR MUSCLE 3D ORGANOID

Tiziana Martinello (1), Gianluca Ventriglia (1), Eylem Emek Akyurek (2), Marco Vincenzo Patruno (2), Roberta Sacchetto (2)

(1) Università degli Studi di Bari, Dipartimento di Medicina Veterinaria. (2) Università degli Studi di Padova, Dipartimento di Biomedicina Comparata e Alimentazione.

Corresponding author: T. Martinello (tiziana.martinello@uniba.it)

A primary cell 3D organoid technology represents a physiologically very interesting model for basic research and drug screening, in fact this system can demonstrate effect and toxicity of drugs reducing the costs. Bioengineered scaffolds derived from the decellularized extracellular matrix (ECM) obtained from discarded animal organs and tissues are attractive candidates for 3D organoid production. The use of a natural decellularized scaffold preserves the physiological and mechanical properties and ECM proteins for attachment, migration, and proliferation of cells, reduces immunogenicity and preserves their histological structures.

Bovine diaphragm seems to be an adequate candidate to obtain a muscle 3D organoid.

Morphologically, the diaphragm is a flat muscle in which the peripheral muscle fibers are separated from the deeper ones by the interposition of a large aponeurotic lamina which constitutes the tendon centre. We have recently observed that bovine diaphragm is constituted by a multilayer in which muscle fibres layers are separated by connective tissue. Starting from diaphragm muscles obtained from animals euthanized at slaughterhouse, we evaluated three different points, costal caudal (12th rib), costal intermediate (9th rib), sternal. The caudal dorsal portion of diaphragm was used to produce the 3D muscle scaffold, being this part histologically the most appropriate for our purpose. A protocol to decellularize the diaphragm scaffold was tuned, evaluating the complete elimination of nuclei and the integrity of collage fibres by histological staining. To obtain preliminary data about biocompatibility of diaphragm scaffold, the decellularized samples were recellularized using primary cells from a fresh biopsy of bovine cutaneous trunci muscle, used as a representative bovine fast-twitch skeletal muscle, immediately after slaughter.

In conclusion, we demonstrated that decellularized caudal dorsal portion of bovine diaphragm can be a promise muscle 3D organoid, which maintain its structure after decellularization cycles and does not loss its biocompatibility.

MESENCHYMAL STROMAL CELLS FROM CANINE ADIPOSE TISSUE COMPRISE SUB-POPULATIONS CHARACTERIZED BY TIME-DEPENDENT BINDING AFFINITY TO CULTURE PLASTIC

Gabriele Scattini (1), Martina Pellegrini (2), Giulio Severi (2), Luisa Pascucci (1)

(1) Università degli Studi di Perugia, Dipartimento di Medicina Veterinaria , (2) Istituto Zooprofilattico Sperimentale dell'Umbria e delle Marche "Togo Rosati".

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

Mesenchymal Stromal Cells (MSC) are currently the most interesting cells used for advanced therapies both in human and in veterinary medicine [1]. Their ability to modulate inflammation/immunologic related disorders, their pro-regenerative potential, the tropism for injured sites, and the paracrine signaling make them "smart" therapeutic tools [2]. Over the last years, the interest in canine MSC has dramatically risen. Different sources are currently used to treat a range of diseases; adipose tissue (AT) is one of the most studied since it is abundant, easy to collect using minimally invasive procedures, and rich in MSC [3, 4]. When comparing different protocols published in the relevant literature, it is evident that the majority of studies describe a series of consolidated phases [5-8]: 1. Mincing tissue with scissors or scalpel; 2. Digestion by collagenase type I for 45 min to 1 h at 37°C by gently shaking in a water bath; 3. Centrifugation and removal of the floating lipid layer; 4. Filtration of the stromal vascular fraction (SVF) through 100 and 70, or 40 µm filters; 5. Washing and new centrifugation; 6. Removal of the supernatant, resuspension of the cell pellet, and seeding in a culture flask. 7. Removal of non-adherent cells from the culture 48 hours after seeding. In our multi-year experience in manipulating AT-derived MSC from animal species, we observed that vascular-stromal fragments, which are generally removed by filtration or discarded after 48h from seeding, release over time cells that are able to attach to the plastic and proliferate for successive passages. Two possible hypotheses were formulated: i) Cells adhere at different time points but share the same MSC biological identity; ii) Cells adhere at different time points because they belong to different tissue niches. To verify the right hypothesis, subcutaneous adipose tissue collected from 3 dogs was minced and digested with collagenase I. Three cell populations were obtained at different time points from isolation (48h, 96h, and 144h). They were expanded until passage 3 and characterized by flow cytometry for positive (CD90, CD44, CD29) and negative (CD14, MHC2, CD45) MSC markers as well as for CD31 (endothelial cell marker), CD146 (pericyte marker), alphaSMA (smooth muscle cell marker). At passage 3, cells were evaluated for viability (MTT assay), doubling time and trilineage differentiation ability. Additionally, non-adherent fractions were subjected to morphological investigations. No significant differences between the 3 subpopulations were observed. They were all characterized by the expression of MSC positive markers and by the absence of negative markers. CD31, CD146, and alpha-SMA were expressed by less than 5% of the cells in all the 3 sub-populations. No differences in differentiation ability and viability were detected. Doubling time ranged between 25 and 30 hours in all the 3 experimental groups. Morphological evaluation of non-adherent fractions revealed a reorganization of residual elements. In conclusion, the 3 cell subpopulations were similar in terms of immunophenotype, proliferation and differentiation potential. In this view, the procedure of sequential adhesions seems to be a useful method to efficiently improve MSC yield. Indeed, it allows to optimize cell recovery, reducing the amount of sampled tissue and shortening the time necessary to obtain an adequate number of cells for clinical applications. However, functional differences cannot be excluded and potency assays are required in order to explore possible distinct biological features.

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MORPHOLOGY STUDY OF Heterotis niloticus ALIMENTARY TRACT

Caterina Porcino, Marialuisa Aragona, Germana Patrizia Germanà, Francesco Abbate, Marzio Cometa, Antonino Germanà, Maria Cristina Guerrera

University of Messina, Department of Veterinary Sciences.
Corresponding author: M.C. Guerrera (mguerrera@unime.it)

Heterotis niloticus (Cuvier, 1829), also known as African bony tongue, is a large primitive freshwater teleost. Its basal position in the general fish phylogeny as osteoglossiforms put it in a clade of interest for evolutionary processes studies [1]. Additionally, its considerable growth performance makes it attractive in fish production, so it has a high aquaculture potential [2, 3].

In the present study, the morphology of the alimentary tract of *H. niloticus* was systematically investigated from the oral cavity to the rectum, including the associated glands. Gross anatomy and light microscope analysis were carried out to perform this investigation.

H. niloticus show peculiar morphological characteristics, and its digestive tract share similarity with both reptiles and birds. Inside the oral cavity, there are two tubular structures with digitiform ends alongside the triangular-shaped tongue. Taste buds upholster these structures. The oral cavity is connected to the stomach by a short esophagus. The stomach is adapted to mechanical trituration, and it is divided into a pars glandularis and a thick-walled pars muscularis. *H. niloticus* presents a gizzard flowing into the anterior intestine and two blind pyloric appendages. These latter exhibit specific functions such as immune defense, effectively, appendages present secondary lymphoid organs. The anterior intestine continues with the middle and posterior tracts up into the rectum. Histological investigation revealed that from the mouth to the anus, all the canal regions share similar structural features. These features are typical of hollow organs. On the other hand, differences found in mucosa structure reflect the different functions of the different parts of the apparatus.

In conclusion, this study could represent a starting point for further anatomical and physiological studies and contributes to enriching scientific literature regarding the morphology of the alimentary tract of *H. niloticus*. Knowledge about this digestive system may help optimize the feeding protocols. Matching the nutritional requirements and the digestive capacity of adult *H. niloticus* may help to improve its growth performance and ensure its conservation.

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DIET SUPPLEMENTATION INFLUENCES GHRELIN SYSTEM EXPRESSION IN THE SKIN OF THE SHEEP

Francesca Mercati (1), Elisa Palmioli (1), Paola Scocco (2), Polina Anipchenko (1), Sara Moscatelli (2), Cecilia Dall'Aglio (1), Daniele Marini (1), Margherita Maranesi (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: F. Mercati (francesca.mercati@unipg.it)

Ghrelin is an orexigenic hormone produced by the stomach and other tissues including the intestine, placenta and pituitary gland. Serum ghrelin levels are influenced by acute and chronic changes in nutritional state in humans (1). Besides, this hormone has many complex functions that cannot be limited to the regulation of systemic energy metabolism. In the integumentary system, ghrelin is involved in skin repair and diseases and it acts as a potential endogenous anti-inflammatory factor (2). However, there is little information regarding the skin so we investigate this hormone and its receptor in the skin of sheep fed with a different diet. A group of 10 Comisana x Appenninica adult female sheep were fed with fresh hay from June to the pasture maximum flowering. From this period to maximum dryness, the control group (Cnt) was fed with fresh hay while the experimental group (Exp) was fed with 600 gr/die/head of barley and corn (1: 1) in addition to the fresh forage. Skin samples were collected from the thoracic region and processed to perform PCR and immunohistochemistry. Samples for molecular biology were immediately frozen in liquid nitrogen and stored at −80°C. Five µg of total RNA were reverse transcribed with the random hexamer method. Serial experiments were carried out to optimize the quantitative reaction, efficiency and Ct values. The amplification fidelity of samples was verified by agarose gel electrophoresis. Samples for morphology procedures were fixed in 10% neutral-buffered formalin solution and included in paraffin wax. Immunohistochemistry was performed by using polyclonal anti-ghrelin and anti-ghrelin receptor antibodies (Abcam Cambridge UK). The immunological reaction was detected with the ABC kit and visualized with diaminobenzidine (Vector laboratories). The immunostaining showed the presence of the ghrelin system in the skin appendages and in particular in the hair follicle. The ligand was localized at the suprabulbar region level in a confined area of the hair follicles while the receptor showed a wider extension that is from the infundibular to the suprabulbar region. The staining was also observed in the sweat glands and in the smooth muscle cells. A significant difference for both ghrelin (3.6-fold) and its receptor (2.9-fold) was evidenced by Real-time PCR between the Exp and Cnt groups. Immunostaining suggests that the ghrelin system is involved in the biological regulation of hair follicles and sweat glands probably affecting epithelial turnover, follicular cycle and gland secretion. The abundant distribution of the receptor suggests that positive structures may be subjected to both a paracrine and endocrine action. Differences observed between two groups of sheep suggest that the dietary supplementation may have a positive modulating effect on the skin transcripts of ghrelin and its receptor.

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FROM THE MICROSCOPE TO THE DISH: ANATOMY APPLIED TO SUSTAINABILITY

Paola Scocco (1), Maurizio Canavari (2), Francesca Mercati (3), Elena De Felice (1), Elisa Palmioli (3), Sara Moscatelli (1), Laura Del Gobbo (1), Andrea Catorci (1)

(1) Università degli Studi di Camerino, Scuola di Bioscienze e Medicina Veterinaria. (2) Università degli Studi di Bologna, Dipartimento di Scienze e Tecnologie Agro-Alimentari. (3) Università degli Studi di Perugia, Dipartimento di Medicina Veterinaria.

Corresponding author: P. Scocco (paola.scocco@unicam.it)

Sustainability is an overused term whose true meaning is often unknown. Sustainability means the condition of a development able of ensuring the satisfaction of the present generation needs without compromising the possibility of future generations to realize their own. In this view, the conservative management of grassland ecosystems is able to maintain their ecological processes and biodiversity. Indeed, grasslands represent a feed source for grazers and, at the same time, grazing activity helps to preserve natural grassland biodiversity. However, the environmental sustainability has to be balanced with the economic sustainability of the farmers. Due to climatic change, enhancing summer drought stress, the moment of maximum flowering of the pasture tends to be anticipated and the period between the maximum flowering and the maximum dryness of the pasture to be reduced. So, summer aridity decreases the grassland pastoral value, consequently affecting the morpho-functional features and physiology of different organ tissues of ovines. This, negatively reflects on both animal's production and farm income. But the farmers that cannot have adequate income will stop bringing their flocks to the mountain pastures and, this inevitable choice, will lead to a loss of biodiversity. How the anatomy can aid to solve this problem? First, a comparative analysis of the modifications induced by those due to the vegetative cycle of the forage on organs closely related to animal production (rumen, abomasum, reproductive system, mammary glands) must be performed in tandem with the body state of the animal and product quality tests. Then, buffer actions towards worsening in forage pastoral value have to be carried out, and a comparative analysis between control and experimental animal groups of the previously investigated parameters must be carried out. At the same time, an economical analysis of cost/benefit of buffering actions in order to evaluate if they could mitigate the productive loss induced by summer aridity preserving the farm economic sustainability must be performed. Anatomical competences were applied to investigate the morphology of above mentioned organs (Approval 95/2018-PR) in different pasture vegetative cycle time, and to assess the expression of the apelinergic system in mammary gland, abomasum and female reproductive apparatus, in order to contribute to plan useful buffer actions and to improve the scientifc knowledge about apelinergic system. Results demonstrated that a diet supplementation during the period between maximum pasture flowering and dryness allows to a better organ's functionality and to an higher milk quality and quantity reflecting also on organoleptic and chemical composition of cheese [1-3]. The cheese properties were recognized by consumers which were willing to pay an higher price for the cheese, so, enhancing the farm income. Farmers and consumers need to be trained on these issues; so, we started with an ambitious scientific dissemination project to raise awareness among children about environmental sustainability and the social usefulness of research, in order to train aware future consumers.

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ANIV

OUTBREAK OF Leptospira borgpetersenii SEROGROUP SEJROE INFECTION IN KENNEL DOGS

Andrea Balboni (1), Elisa Mazzotta (2), Maria Beatrice Boniotti (3), Cristina Bertasio (3), Laura Bellinati (2), Laura Lucchese (2), Mara Battilani (1), Letizia Ceglie (2), Silvia Marchione (2), Giulio Esposito (4), Alda Natale (2)

(1) Alma Mater Studiorum – Università di Bologna, Dipartimento di Scienze Mediche Veterinarie. (2) Istituto Zooprofilattico Sperimentale delle Venezie. (3) Istituto Zooprofilattico Sperimentale della Lombardia e dell'Emilia Romagna "Bruno Ubertini". (4) Azienda USL di Bologna, Unità Operativa Complessa Veterinaria A e C Sanità Animale e Igiene degli Allevamenti e delle Produzioni Zootecniche.

Corresponding author: A. Balboni (a.balboni@unibo.it)

Leptospirosis is a neglected zoonosis of worldwide distribution and maintenance or reservoir hosts play a very important epidemiological role. Canine leptospirosis is caused by several *Leptospira* serovars with variable geographic distribution and reporting a wide range of clinical manifestations from sub-clinical to severe (1, 2). Kennels may represent high-risk environments for the diffusion of *Leptospira* infection in dogs and consequently a threat to public health (3). This study describes an outbreak of *Leptospira* infection in a kennel in Italy in 2020, both with clinically ill and asymptomatic dogs. Fifty-nine dogs, including three ill dogs, were tested for *Leptospira* spp. infection by microscopic agglutination test (MAT) and real-time qPCR. Multi-locus sequence typing (MLST) analysis was used to genotype the identified leptospires. Thirty of 59 (50.9%) dogs had MAT titer and/or molecular positivity indicative of *Leptospira* infection. Twenty-two of 59 (37.3%) dogs exhibited seropositivity against at least one serovar belonging to the Sejroe serogroup and MLST analysis identified *L. borgpetersenii* serogroup Sejroe (*Leptospira* ST155) as the responsible for the outbreak. Up to now, Sejroe serogroup infection was sporadically reported in dogs. The extension of the MAT antigen panel to several serovars belonging to the serogroup Sejroe could be useful in the diagnosis of canine leptospirosis. The detection of *Leptospira* DNA in seronegative dogs confirmed the usefulness of the molecular analysis, both on blood and urine samples, to identify infected subjects. Dogs may serve as sentinel of leptospires in specific environments and surveillance of *Leptospira* infection in kennels is strongly recommended even when the correct vaccine prophylaxis is administered, because of the vaccines currently available are not able to protect for all the serogroups.

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MODELING FOOT AND MOUTH DISEASE CONTROL SCENARIOS IN AREAS WITH DIFFERENT LIVESTOCK DENSITY IN ITALY

Alessandro Mannelli (1), Francesco Valentini (1), Lucrezia Dellepiane (2), Alessandro Bellato (1), Alessandra Scaburri (3), Marco Tironi (3), Stefano Muò (1), Claudio Caruso (4), Silvia Bellini (3)

(1) Università degli Studi di Torino, Dipartimento di Scienze Veterinarie. (2) Istituto Zooprofilattico Sperimentale del Piemonte, Liguria e Valle d'Aosta, Torino. (3) Istituto Zooprofilattico Sperimentale della Lombardia e dell'Emilia Romagna, Brescia. (4) Azienda Sanitaria Locale Cuneo 1 - Sede di Racconigi.

Corresponding author: A. Mannelli (alessandro.mannelli@unito.it)

Foot and mouth disease (FMD) outbreaks in previously free areas have very severe consequences. Based upon current legislation, FMD control measures include destruction of infected, and of epidemiologically connected herds. If justified by risk assessment and economic evaluation, further intervention can be implemented, including pre-emptive slaughter of at-risk herds, as well as emergency vaccination [1]. Vaccination is favoured when outbreaks occur in densely populated livestock areas, and when the airborne spread of the virus is probable. The Italian livestock production is characterised by a heterogeneous geographic distribution. In fact, some of the most densely populated livestock areas in Europe can be found in plains in northern Italy. On the other hand, certain areas are characterised by herds of different species, reared by traditional husbandry systems, which may hamper disease control. In this study, we built an agent-based, stochastic, computational model to simulate FMD control scenarios, in different Italian geographic areas. FMD transmission parameters were based upon kernel functions of distance among herds, and upon herd attributes, such as animal species and herd size [2]. Furthermore, information on potential FMD transmission routes was collected on a sample of farms in northern Italy. The uncertainty of between-herd transmission and of vaccine efficacy was modelled by beta distributions. Simulation showed that the introduction of FMD virus in densely populated livestock areas in northern Italy would result into the rapid spread of the infection, which could not be arrested by mandatory disease control measures alone. Based on the results of 100 simulation runs, after two weeks from the first case of FMD, the median number of infected herds was 66, first quartile = 46, third quartile = 143. The adoption of further control measures was simulated. Fourteen days after vaccination of susceptible herds, the median number of infected herds was 11, first quartile = 8, third quartile = 26. Furthermore, even in a scenario where vaccination was applied when 100 herds were already infected, the epidemic was controlled in the majority of simulations, and the number of new infections gradually declined. In comparison with vaccination, pre-emptive slaughter of herds neighbouring infected farms was less effective, unless it was carried out on a very large number of farms. In geographic areas, which were not classified as densely populated, early disease detection and stamping out were effective in controlling an FMD outbreak, without the need for vaccination. The possibility to include herd attributes, and their effects on infectivity and susceptibility, as well as the effects of different transmission routes makes the model very flexible. Optimization of intervention, such as culling or vaccination, can be simulated by using graph-based criteria, such as betweenness centrality and degrees, as indicators of the potential role of each herd in FMD transmission dynamics [3].

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SEMI-QUANTITATIVE RISK ASSESSMENT OF AFRICAN SWINE FEVER VIRUS INTRODUCTION IN PIG FARMS, IN ITALY

Annalisa Scollo (1), Francesco Valentini (1), Giorgio Franceschini (1), Alessia Rusinà (1), Stefania Calò (2), Veronica Cappa (2), Alessandro Bellato (1), Alessandro Mannelli (1), Silvia Bellini (2)

(1) Università degli Studi di Torino, Dipartimento di Scienze Veterinarie. (2) Istituto Zooprofilattico Sperimentale della Lombardia e dell'Emilia-Romagna, Brescia.

Corresponding author: A. Scollo (annalisa.scollo@unito.it)

We applied a semi-quantitative risk assessment to classify pig farms in terms of the probability of introduction of African swine fevers virus (ASFV) [1]. Following on-farm data collection via a specific checklist, we applied a modified Failure Mode and Effect Analysis (FMEA) to calculate risk priority codes (RPC), indicating increasing risk levels ranging from 1 to 5. In the calculation of RPC, we included the importance of biosecurity measures, as attributed by experts. To consider geographic risk factors, we classified pig farms based on distance from wooded areas, as a proxy of exposure to wild boars. Furthermore, we calculated an index of local spatial clustering to classify farm locations in terms of pig population density. We visited 62 commercial pig farms, mainly in the northern regions Lombardy, Emilia-Romagna, and Piedmont, where most of the Italian pig production is concentrated. Moreover, we included 12 non-commercial pig farms in an Apennine area of northern Tuscany. In commercial farms, highest risk levels (RPC=5) were obtained from the evaluation of biosecurity measures associated with farm structure and with personnel practices. In fact, separation of premises from the external environment was often incomplete, and contacts of personnel with pigs from other farms occurred. RPC=4 was most frequently obtained for biosecurity measures related to the management of vehicles for the transport of animals, and for the management of materials such as feed and slurry. Better results were obtained for pig management inside the farm (RPC=3). In non-commercial farms, vehicles were not effectively washed and disinfected, and feed, fodder and slurry were not properly managed (RPC=5). Moreover, there was not a clear separation between the farms and the external environment, procedures for cleaning and disinfecting premises at the end of each production cycle were absent, and rodent control was poor. On the other hand, swill feeding to pigs was rarely reported. Among visited pig farms, those on the Apennines were the most exposed to the risk of contact with wild boars, given the proximity to wooded areas. Indeed, ASF cases were reported in Italy, starting on January 2022, on the northern Apennines. Wild boar movements along an uninterrupted wood cover might lead to the spread of ASFV to our study area [2]. Visited farms in the plains of the Po river valley were in densely populated livestock areas. Under these circumstances, the introduction of ASFV may result in the transmission among neighbouring farms, especially when biosecurity measures are not properly applied [3]. Our results were useful to identify critical biosecurity issues, and to provide the basis for intervention. Improvement of biosecurity associated with farm structure requires economic investment. On the other hand, the improvement of daily practices in farm management relies upon information of farmers on the risk of ASFV introduction. Furthermore, specific biosecurity measures, if identified with the participation of farmers, are more likely to be accepted and implemented.

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ROLE OF OUTER MEMBRANE VESICLES (OMVS) FROM S. Infantis IN ANTIMICROBIAL RESISTANCE: EVALUATION OF B-LACTAMASE ACTIVITY

Toppi Valeria, Scattini Gabriele, Musa Laura, Pascucci Luisa, Chiaradia Elisabetta, Tognoloni Alessia, Franciosini Maria Pia, Casagrande Proietti Patrizia

Università degli Studi di Perugia, Dipartimento di Medicina Veterinaria.
Corresponding author: V. Toppi (toppivaleria@gmail.com)

Outer membrane vesicles (OMVs) are spherically bilayered nanoparticles, released into extracellular milieu by Gram-negative bacteria. OMVs contain different cargo molecules and mediate several biological processes as horizontal gene transfer, export of metabolites, cell-to-cell communication and biofilm formation [1]. Recent studies have shown that OMVs are involved in antibiotic resistance (AR) mechanisms by including β-lactamase enzymes, which degrade β-lactam antibiotics, in their lumen. Recently, bacterial strains of *S*. Infantis resistant to different classes of antibiotics and producers of extended-spectrum β-lactamases (ESBLs) have spread widely in Europe [2-4]. Since no studies have been conducted on *Salmonella* Infantis' OMVs yet, the aim of the work was to collect OMVs from *Salmonella* Infantis β-lactam resistant and susceptible strains and to evaluate the β-lactamase activity in the OMVs. OMVs were isolated through filtration and ultrafiltration of culture supernatants. To evaluate and compare the presence of β-lactamase enzymes into OMVs, β-lactamase activity was quantified by Nitrocefin test in OMVs concentrates, in the eluted samples and in the filtered supernatants. Transmission electron microscopy (TEM) and Dynamic Light Scattering (DLS) were used to investigate OMVs morphology. Proteins from all samples were quantified by Bradford assay and separated by SDS-PAGE. Anti-β-lactamase antibody was used to confirm the presence of β-lactamase enzymes into OMVs. In the study we found that both β-lactam and susceptible strains release OMVs. TEM images showed round shaped vesicles, mainly organized in clusters, with an electrodense appearance. No β-lactamase activity value was detected in OMVs from susceptible strains. A very low value was measured in filtered supernatants and a higher value was measured in OMVs concentrates. Anti-β-lactamase antibody confirmed the presence of β-lactamase enzymes in the lumen of the OMVs. These results suggest that β-lactamase enzymes get packaged into OMVs from bacterial periplasm during OMVs biogenesis. A possible explanation could be that enzymes are loaded into OMVs to be protect from proteases degradative action. Therefore, the role of OMVs from *S*. Infantis in AR seems to be confirmed by our data.

The possibility of investigating and understanding whether OMVs play a role in the mechanisms of AR opens the way towards the chance of developing new therapeutic strategies to fight antibiotic resistance in the future.

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AUTOPHAGY UP-REGULATION UPON FHV-1 INFECTION ON CRFK CELLS

Gianmarco Ferrara (1), Consiglia Longobardi (2), Erminia Romano (1), Sara Damiano (1), Roberto Ciarcia (1), Giuseppe Iovane (1), Ugo Pagnini (1), Serena Montagnaro (1)

(1) Università degli Studi di Napoli Federico II, Dipartimento di Medicina Veterinaria e produzioni animali. (2) Università della Campania "Luigi Vanvitelli", Dipartimento di Salute Mentale e Fisica e Medicina Preventiva.
Corresponding author: G. Ferrara (gianmarco.ferrara@unina.it)

Autophagy is a highly conserved cellular pathway that contributes to the maintenance of cellular homeostasis through degradation of cytosolic material. The autophagy network is also involved during host-virus interactions, representing a cellular defense mechanism with an antiviral activity [1]. Thanks to millennia of coevolution, several viruses are able to modulate the autophagy machinery to their own advantage. It has previously been observed that different members of the Herpesvirus family (such as Human herpes simplex, HSV, and Kaposi'sarcoma human herpesvirus, KSHV) can evade autophagy through the action of virally encoded inhibitors [1]. Other Herpersvirus members, such as Varicella-zoster virus (VZV), Pseudorabies virus (PRV), Duck enteritis virus (DEV), and Bovine herpesvirus type 4 (BoHV-4) induce increased autophagic flux in order to accomplish the secondary envelopment and successfully complete their life cycle [2-5]. Very little is currently known about the interaction between feline herpesvirus (FeHV-1) and autophagy. FeHV-1 is a widespread virus responsible for feline viral rhinotracheitis (FVR), which includes upper respiratory and ocular disease. The aim of this work is to investigate the relationship between this virus and autophagy and to evaluate its proviral or antiviral role. Monolayers of Crandell-Rees Feline Kidney Cell (CRFK) were infected with FeHV-1 strain Ba/91 at various time points (3, 6, 12, 24, 48, and 72 hours post infection) using MOI 1. Cell lysates were collected and electro-transferred onto polyvinylidene fluoride (PVDF) membranes after being run in SDS-page gels. Autophagy-related signaling pathways were examined by incubating each membrane with primary antibodies against specific marker involved in the autophagy process. Western blot analyses found that the level of LC3-II was significantly higher since 12 hours post infection and increased until 72 hours post infection. The conversion of LC3-I into LC3-II and the degradation of p62/SQSTM1 indicated complete autophagy flux. In the second experiment, autophagy was pharmacologically inhibited by exposing the cells to bafilomycin (BAF) for 3 hours before infection. BAF has the property of impeding acidification during the late stage of autophagic flux, thereby avoiding the fusion of autophagosomes and lysosomes. Cells viablility was assessed by the MTT assay. This showed that cytotoxicity was lower in Crfk treated with BAF before infection. The viral titer of surnatants was determinated with TCID50 assay while cell lysate was used for western blot analysis. The FeHV titers (TCID50/mL) were significantly lower in the BAF treated cells than in the mock-treated cells after 24 hours of infection. Western blot analysis revealed lower levels of LC3I conversion and p62 degradation in BAF treated cells than mock-treated cells. In addition, densitometric analysis showed a decrease in the expression of glycoprotein D and an increase of glycoprotein B in the infected cells. We hypothesize that the decrease in FeHV titers and glycoprotein sintesis is due to the apparent failure of secondary envelopment caused by BAF treatment as described for VZV infection [6]. These findings contribute to a better understanding of the biology and pathogenesis of FeHV infection, as well as new insights into the development of effective therapeutic strategies.

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THE PRESENCE OF MASTITIS PATHOGENS IN DIFFERENT HUSBANDRY SYSTEMS IN MOUNTAIN DAIRY HERDS

Alessandro Bellato (1), Stefania Bergagna (2), Federica Traverso (1), Manuel Furlan (1), Patrizia Robino (1), Patrizia Nebbia(1), Stefano Guazzetti (3), Alessandro Mannelli (1)

(1) Università degli Studi di Torino, Dipartimento di Scienze Veterinarie. (2) Istituto Zooprofilattico Sperimentale del Piemonte, Liguria e Valle d'Aosta. (3) Azienda Unità Sanitaria Locale di Reggio Emilia.
Corresponding author: A. Bellato (alessandro.bellato@unito.it)

Mastitis is a major concern in dairy herds, due to the economic costs and the threat to cow health. Subclinical mastitis is less harmful to the cow but costlier for the breeder, and albeit more frequent it is often under-reported. Some of the most frequent causative agents are Gram-positive bacteria ubiquitous in dairy farms. In the mountains, farming techniques are less homogeneous than in the plains. Due to the lack of transport infrastructures, they depend heavily on the local marketing of the products. This study aimed to evaluate the presence of mastitis pathogens in clinically healthy cows reared in three different mountain areas of the northern Apennines (Italy).

Between April and September 2021, we collected milk samples of 246 clinically healthy cows from 16 small- and medium-sized mountain herds, situated between 270 and 800 m a.s.l. In the province of Lucca (LU) and Alessandria (AL), small farms process milk on-site and sell it locally, while medium-sized farms supply milk directly to the consumer or to industrial dairies. In Reggio Emilia province (RE), the production of Parmigiano Reggiano is organized in social dairies owned by the breeders. Information on husbandry systems was gathered by interviewing the breeders. Milk samples were cultured to identify latent infection by contagious or environmental/opportunistic pathogens [1]. Isolates were analysed via MALDI-TOF mass spectrometry following the manufacturer's indication (Bruker).

We isolated contagious agents – namely *Staphylococcus aureus*, *Streptococcus agalactiae*, and *Corynebacterium bovis* – from 37 cows of 9 herds. Their prevalence was twenty times higher in AL (60.6%) than RE (3.0%). Environmental bacteria were isolated in 58 cows from 14 herds, non-aureus staphylococci (NAS) in 111 from 14, other uncommon mastitis pathogens in 5 cows from 3 herds. NAS prevalence differed among geographic areas. The risk of infection with contagious agents increased if milking was done with a milking cart but decreased if the farmer had attended training courses. Environmental bacteria were favoured by grazing activity, NAS by tie-stall, while contagious agents were not affected by the type of housing.

Rapid and accurate identification of the pathogen of mastitis is of paramount importance in dairy herds. Latent infections can severely damage the herd, especially if caused by contagious agents. Implementing rapid diagnostic techniques helps monitor udder health and prevent transmission to other cows. MALDI-TOF has high accuracy in diagnosing the most common mastitis pathogens [2] and provides useful information for the epidemiology of mastitis in remote mountain herds.

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STUDENT COMPETITION "MICROBIOLOGY CAN BE…2021": HOW A STUDENT CAN ACQUIRE COMMUNICATION SKILLS IN MICROBIOLOGY

Team 1- G. Bulfone, M. Casotto, S. Dafrawi, T.M. Ferranti, M. Nicolosi, E. Reucci (1), Team 4-B. Barzagli, B. Brenna, P. Bolognini, S. Carra, M. Negherbon, F. Pepe (1), Team 5-M. Becchetti, M. Golino, E. Sorgon, M. Taddei, E. Terna, V. Vella (1), Team 7-G. Belli, C. Bulgarello, M. Campagna, A. Cartechini, C. Chiarini, N. Del Bianco, (1) Team 8-C. Barbetti, I. Berselli, E. Illuminati, V. Mattioli, E. Rosi, B. Serni (1), Team 9-S. Allegri, S. Attura, C. Cecchini, L. Rillo, E. Vescovi, E. Zavagli (1), Team 10-A. Baglioni, J. Bardus, T. Coppola, M. Mietto, C. Tocchi (1), Team 11-S. Brondolo, A. Celli, S. Fantoni, M. Larini, F. Settimi (1), Raoul Ciappelloni (2), Maria Luisa Marenzoni (1)

(1) Università degli Studi di Perugia, Dipartimento di Medicina Veterinaria. (2) Istituto Zooprofilattico Sperimentale Umbria e Marche "Togo Rosati"

Corresponding author: ML. Marenzoni (marialuisa.marenzoni@unipg.it)

A day one competence required in graduates in veterinary medicine is the communication skill [1]. Application of this competence to the microbiology is relevant because microbiology literacy has even considered life-saving for people after the SARS CoV-2 pandemic [2, 3]. Aim of project is to work with collaborative groups of students to create and communicate didactic messages on general microbiology and infectious diseases of domestic animals in a fun way to promote and make students aware of their future role as science communicator.

Partecipants were students that were divided into groups according to the clinical rotation practical activities (5-6 students/group, defined as "Team"); they were asked to produce entertaining or educational material, of short duration, which allows them to spread concepts of microbiology or infectious diseases of domestic animals, like infographics, cartoons/comics, videos, jingles or any other original material that can be used to disseminate useful information on this topic. Rules of the game (respect, ethics, absence of plagiarism, etc.) were explained during the course of infectious diseases, and deposited on the platform dedicated to the students' teaching material (unistudium). The time that the students dedicated to the project was that spent during the waiting times of the clinical rotations, while for the actual creation they are asked to dedicate part of their free time (estimated time: 30 minutes-two hours, depending on the clarity of the project ideas and the technical capacity). The created material, after signing the informed consent to the dissemination of the product, was placed on the unistudium page to make it visible to all Teams, who then voted the project that they liked it more. Voting was done through telematic functionality. The third, second and first team classified with a symbolic prize were awarded: sweets and chocolate, typical of the Umbrian territory. Despite the Covid pandemic, which made meetings difficult, the first edition ended in 2021 with a production of documents by 8 out of 11 Teams, who showed competence in the topic, lightness in the way of dealing with it and ability to communicate these concepts, even complex and often considered boring. The material produced was published, in full or partial format, as a Team work, in the electronic magazine SPVet (http://www.spvet.it/) of the Experimental Zooprophylactic Institute of Umbria and Marche "Togo Rosati". This initiative was included also in a Current Research project of the Institute's Ministry of Health Experimental Zooprophylactic Umbria and Marche "Togo Rosati" (IZS UM 04/20 RC).

The project permits students to work in team group, share objectives, acquire communication skills. Moreover, they should have understood the relevance of their role in disseminating scientific information. This activity produced a certificate of participation and a publication in which they appear as authors and this should encourage them to start writing their curriculum vitae.

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GEOGRAPHIC DISTRIBUTION OF HEALTH INDICATORS OF DAIRY HERDS IN PIEDMONT (NORTHWESTERN ITALY)

Alessandro Bellato (1), Alessia Tondo (2), Lucrezia Dellepiane (3), Giuseppe Ru (3), Alessandro Dondo (3), Alessandro Mannelli (1), Stefania Bergagna (3)

(1) Dipartimento di Scienze Veterinarie, Università di Torino. (2) Associazione Italiana Allevatori. (3) Istituto Zooprofilattico Sperimentale del Piemonte, Liguria e Valle d'Aosta.

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

When studying the health of dairy cows, the most attention is paid to mastitis. However, also ketosis and inter-calving intervals are monitored in herds to evaluate performance and assess the health and wellbeing of the cows. The present study aimed to evaluate cows' health using herd-level health indicators (HIs) and assess whether their distribution was geographically homogeneous.

Using the test-day records of the Italian Breeders Association (AIA), we estimated four herd-level HIs, namely the incidence of mastitis, ketosis, reproductive disorders, and fresh-cow removal. We produced individual estimates for most of the dairy herds in Piedmont (n=1233) analysing them for five years (2015-2020). We compared the herds based on the distance from the population mean. We mapped the geographic distribution of each HI, using Bayesian smoothing to avoid overweighting small herds' estimates and assessed the local aggregation with the Getis G-statistic.

HIs' estimates were not correlated with each other, yet no more than 5.2% of cases strongly disagreed. Herd size was an acceptable proxy for each HI since approx. Fifty lactating cows were the size beyond which their trends changed slope. Ketosis and fresh-cow removal increased with herd size, while mastitis and reproductive disorder decreased. Consistently, mastitis incidence was higher in hilly and mountainous areas, where it aggregated in clusters. Ketosis was prevalent in the plain, concentrated in a large focus between the cities of Torino and Cuneo. Reproductive disorders were more dispersed.

Wide-scale comparison of HI helps benchmark dairy herds and define population-adapted goals that every farm can sensibly achieve. The scarce disagreement among HI estimates suggested that a herd can hardly be excellent in one HI and poor in another and confirmed that breeder skills represent an unmeasured risk factor affecting cow health in many ways. We confirmed our hypothesis that some health problems tend to aggregate locally. Starting from test-day records, it is impossible to investigate the factors that determine the geographic distribution. However, it is sensible to assume that transport infrastructures, husbandry systems and the availability of veterinary services are among the most important drivers. The usefulness of geographic analysis for veterinary services lies in its ability to identify areas where problems are prevalent and herds at risk of poor health. Using test-day records, it is possible to calibrate the intervention thresholds on the territorial epidemiological situation in real-time.

NATURE IDENTICAL COMPOUNDS AS POTENTIAL ALTERNATIVES TO ANTIBIOTICS IN FOOD-PRODUCING ANIMALS

Costanza Spadini, Mattia Iannarelli, Alicia Maria Carrillo Heredero, Nicolò Mezzasalma, Marica Simoni, Federico Righi, Simone Bertini, Clotilde Silvia Cabassi

Università di Parma, Dipartimento di Scienze Medico-Veterinarie.
Corresponding author: C. Spadini (costanza.spadini@unipr.it)

Botanicals have recently gained attention as alternatives to antibiotics importance in veterinary medicine, especially for the treatment and prevention of infections in food-producing animals, according to the last guidelines and legislation in force in the EU (2015/C 299/04, Commission Notice; Reg. EU 2003/1831; Reg. EU 2019/06) [1].

Nature Identical Compounds (NICs) are chemically synthesized pure bioactive compounds, which occur naturally in essential oils (EOs) of different plants at various concentrations. Their application in the current post-antibiotic era can be of interest in the veterinary field, also considering their potential multiple biological activities (antimicrobial, antioxidant, anti-inflammatory and immunomodulatory) and their safety (GRAS by FDA) [2, 3].

The present work aimed to evaluate the antimicrobial activity and stability over time of NIC salone or in combination with the emulsifier Tween 20. The antimicrobial activity of five different NICs – carvacrol (CAR), cinnamic aldehyde (CINN), menthol (MEN), terpineol (TER) and thymol (THM) – were evaluated against four reference bacterial strains – two Gram negative (*E. coli* - EC and *Salmonella* Typhimurium - ST) and two Gram positive (*Staphylococcus aureus* - SA and Methicillin-Resistant *S. aureus* - MRSA). The NICs antimicrobial activity was evaluated alone and in association with Tween 20 as emulsifier at time T0 and T1 (after 18 months of preservation at +4°C in DMSO solution). The broth microdilution method was used to assess the Minimal Inhibitory Concentration (MIC) of each compound and the checkerboard assay was employed to evaluate the association between NICs and Tween 20, whose interaction was calculated by the Fractional Inhibitory Concentration (FIC) Index [4, 5].

At T0 the lowest MIC value (128 µg/ml) was obtained for CINN against SA, whereas on the other bacteria CINN MIC was 256 µg/ml, also obtained for CAR and THM against all the tested bacteria. The highest MIC values were obtained for MEN on ST (4096µg/ml) and TER against all the tested bacteria (2048 µg/ml). After 18 months of NICs storage, MIC values decreased in the majority of the cases. The MICs of CAR, THM and TER on Gram negatives were halved, similarly to CAR on SA. Concerning Gram positive strains, MICs reduced by half also for MEN on SA and by 1,8 folds for CINN on MRSA. The results obtained by the checkerboard assays demonstrate that the association between NICs and Tween 20, at T0, resulted indifferent for all the tested bacteria and compounds, except for TER and MEN on EC, and CINN on MRSA, which resulted additives. At T1 the associations worsened, resulting in indifference for each combination, except for TER-Tween 20 against MRSA, which resulted additive.

In conclusion, NICs – particularly CAR, CINN and THM – can be considered good antimicrobial alternatives against the most representative bacteria in husbandry infections, also considering their stability over time. Furthermore, we have ascertained that Tween 20 as an emulsifier does not impair the antimicrobial activity of these compounds. Further studies on NICs application as feed additives are needed to implement their suitability in vivo on livestock animals, thus tackling the overuse of antibiotics in the veterinary field. This project has received funding from the European Union's Horizon 2020 research and innovation programme, under the grant agreement No 774340.

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MINT, OREGANO, ROSEMARY AND TEA TREE ESSENTIAL OILS: NATURAL STRATEGY TO REDUCE ANTIBIOTIC USE IN VETERINARY FIELD

Costanza Spadini, Mattia Iannarelli, Alicia Maria Carrillo Heredero, Nicolò Mezzasalma, Federico Righi, Marica Simoni, Simone Bertini, Clotilde Silvia Cabassi

(1) Università di Parma, Dipartimento di Scienze Medico-Veterinarie.
Corresponding author: M. Iannarelli (mattia.iannarelli@studenti.unipr.it)

Nowadays, antibiotic resistance has become increasingly important, both in human and veterinary fields [1].

A promising alternative to antibiotic molecules are essential oils (OEs), volatile compounds synthetized by aromatic plants as secondary metabolites, which can be obtained by distillation or squeezing, and generally combine antioxidant, anti-inflammatory, analgesic and antimicrobial properties [2, 3].

The aim of this work was to evaluate the antimicrobial activity of four different EOs: mint (MEO), oregano (OEO), rosemary (REO) and tea tree (TEO), either alone or emulsified with Tween 20 (TW20), whose function is to create a suspension with the essential oil, against four different bacterial strains (*E. coli*-EC, *Salmonella Typhimurium*-ST, *Staphylococcus aureus*-SA, Methicillin-Resistant *S. aureus*-MRSA). The antimicrobial activity of each EO towards each bacterial strain was tested using the in vitro microdilution method to evaluate the minimal inhibitory concentration (MIC), whilst the checkerboard assay was employed to evaluate the association between EOs and TW20 [4].

The persistence of the antimicrobial activity over time was also evaluated, both on pure EOs or on their TW20 emulsion, at time T0 and T1 (after storage at 4°C for 10 months) by checkerboard assay and at time T0 and T2 (after storage at 4°C for 22 months) by MIC test.

At time T0 the best results were obtained with OEO, which had a MIC against EC, ST and SA of 0.05% and against MRSA of 0.098% and with TEO which had a MIC of 0.2% against EC and ST. The worst results were obtained for MEO and REO against ST having a MIC of 2.1%. At T2, the antimicrobial activity on average has become weaker against EC, ST and SA bacterial strains, whereas was stable against MRSA, except for OEO, where the MIC value lowered 3 times (0,032%). The stability of antimicrobial activity of the combination between EOs and TW20, tested with the checkerboard assay highlighted indifference of action at T0 in most of cases, except for REO which showed a synergistic interaction on EC. Conversely, MEO (against EC, SA, MRSA), REO (against SA) and TEO (against all bacterial strains) showed an additive interaction with TW20, whereas OEO resulted in antagonist with TW20 against SA. At T1 most of the associations between OEs and TW20 remained stable or worsened, resulting in an indifferent activity except for REO against MRSA, which resulted in additivity with TW20.

In conclusion, OEs showed good antimicrobial activity even at low concentrations and a good spectrum of action on different bacterial strains of veterinary interest, especially OEO and TEO. Thus, they could represent a good alternative to antibiotics in the veterinary field. However, it should be highlighted that their antimicrobial activity can deteriorate over time. Additionally, TW20 could be considered a good carrier for EOs, since it does not impair the antimicrobial action of EOs.

This project has received funding from the European Union's Horizon 2020 research and innovation programme, under grant agreement No 774340.

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BUILDING UP EFFLUX-MEDIATED ANTIMICROBIAL RESISTANCE IN Staphylococcus pseudintermedius

Elisa Rampacci (1), Tommaso Felicetti (2), Donatella Pietrella (3), Stefano Sabatini (2), Fabrizio Passamonti (1)

(1) Università degli Studi di Perugia, Dipartimento di Medicina Veterinaria. (2) Università degli Studi di Perugia, Dipartimento di Scienze Farmaceutiche. (3) Università degli Studi di Perugia, Dipartimento di Medicina e Chirurgia.
Corresponding author: E. Rampacci (elisa.rampacci@gmail.com)

Last of the resistance mechanisms to be identified, the overexpression of membrane efflux pumps (EPs) allows bacteria to extrude noxious compounds, including antimicrobial agents, influencing multiple stages of resistance development [1]. The study of EPs in *Staphylococcus pseudintermedius* has been neglected to date. This microorganism is among the most relevant antimicrobial-resistant bacteria worldwide due to its frequent implication in clinical diseases of dogs and cats, increasing adaptation to the human organism, and the high levels of resistance to clinically relevant antibiotics [2, 3].

Here, we performed an in silico prediction of drug EPs in *S. pseudintermedius*, and we investigated their role in conferring resistance to antibiotic and biocidal agents and biofilm formation.

A *S. pseudintermedius* efflux mutant was obtained by stimulating the isogenic strain ATCC 49444 with increasing concentrations of the efflux system substrate ethidium bromide (EtBr). The efflux activity of *S. pseudintermedius* ATCC 49444 and its derivative was quantified by fluorometry. Changes in antimicrobial susceptibility were evaluated in the presence/absence of the EP inhibitors thioridazine (TZ) and reserpine (RES) by checkerboard assays. The biofilm-forming capability of the original strain and its derivative and the biofilm inhibition ability of TZ and RES were evaluated by microtiter-plate test. Homologues of EPs of *Staphylococcus aureus* and *Staphylococcus epidermidis* were searched by exploratory GenBank investigations. Gene expression analyses and sequencing were then conducted on selected genes.

S. pseudintermedius efflux mutant showed a higher efflux activity than the parent strain and increased resistance to gentamicin, 2% chlorhexidine digluconate and ciprofloxacin, which is the major active metabolite of the veterinary fluoroquinolone enrofloxacin [4]. On the contrary, enrofloxacin efficacy was only slightly affected by the augmented efflux. TZ restored the bacterial susceptibility to gentamicin and ciprofloxacin. Biofilm production was greatly increased in *S. pseudintermedius* efflux mutant and it was significantly inhibited by TZ and RES at MIC/2 ($p=0.04$). EtBr pressure induced a transient overexpression of multiple transporters of the Major Facilitator Superfamily (MFS). However, only the multidrug EP gene norA remained highly expressed in *S. pseudintermedius* efflux mutant, which had an 11 bp-deletion in norA promoter region. icaA gene, encoding for extracellular polymeric substances of staphylococcal biofilm, was up-regulated as well.

How the overexpression of MFS EPs influences biofilm production at the transcript level is not clear. However, the physiological role of MFS EPs, particularly NorA, appears to be far more complex than merely that of an antibiotic export protein. Combinations composed of an antibiotic EP substrate and an EP inhibitor might be an attractive strategy to combat staphylococcal infections in the context of veterinary and human medicine.

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COVID-19 AND COMPANION ANIMALS: SOURCES AND LEVEL OF INFORMATION OF ITALIAN CITIZENS

Andrea Laconi (1), Alessandra Piccirillo (1), Barbara Saracino (2), Eliana Fattorini (3), Giuseppe Pellegrini (3), Massimiano Bucchi (3), Lucia Bailoni (1)

(1) Università degli Studi di Padova, Dipartimento di Biomedicina Comparata e Alimentazione. (2) Università degli Studi di Bologna, Dipartimento di Scienze Politiche e Sociali. (3) Università degli Studi di Trento, Dipartimento di Sociologia e Ricerca Sociale.

Corresponding author: A. Laconi (andrea.laconi@unipd.it)

This study aimed to collect and analyse data on the sources and level of Italian public information on the risk of infection from COVID-19 in the humans-companion animals' relationships. Data were collected within the FISR-COMIS project (FISR2020IP_01119) through a survey-type investigation with questionnaire. The survey was conducted on a national sample, proportional and representative by gender, age, and home area of Italian populations aged over 15 years, between 19 November and 8 December 2021. Data were collected using the Computer Assisted Telephone Interview (CATI) technique for 30% of the sample and the Computer Assisted Web Interviewing (CAWI) technique for the remaining 70%. A total of 1,008 subjects were interviewed; after weighting, the total cases were reduced to 985 to have a sample structure identical to the Italian population with regard to gender, age, and level of education.

Analyses have shown that 45.1% of the interviewed Italians is aware that companion animals can be infected with Sars-CoV-2. However, only 29.8% is familiar with the preventive measures to adopt to avoid viral transmission between infected/suspected humans and companion animals, and only 20.7% knows which pets could be at risk of infection. Higher awareness regarding the risk of Sars-CoV-2 transmission between animals and humans (51.7%) and the measures to prevent it (33.3%) has been detected in Italians owing one or more companion animals at home in comparison to those not owing any pet. However, people working with animals have the highest level of knowledge: 48.1%, 49.4% and 37% are aware of companion animal's susceptibility to Sars-CoV-2, animal species at risk of infection and measures to prevent human to animal transmission, respectively. Notably, 40.4% of Italian citizens declares to have failed to inform themselves. While news broadcasts (26.4%) represent the main source of information, only 3.5% of the interviewees rely on veterinarian's advice, even though 31.9% consider this source of information as the most trustworthy. Overall, 72.4% of Italians recognize that the communication campaign on COVID-19 and companion animals has been very inadequate.

In conclusion, our survey-type investigation highlights the need for increasing awareness on the risk of companion animals to be infected with Sars-CoV-2 and potentially be involved in the transmission cycle of this human virus. Furthermore, a more adequate communication strategy should be implemented directly involving professionals, who are perceived as the most scientifically reliable source of information.

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INTRAMAMMARY INFECTIONS AND SOMATIC CELL COUNT IN COWS WITHOUT ANTIBIOTIC TREATMENT AT DRY-OFF: A CASE-CONTROL STUDY

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

Università degli Studi di Milano, Dipartimento di Medicina Veterinaria e Scienze Animali.
Corresponding author: L. Filippone Pavesi (laura.filippone97@gmail.com)

Antibiotics were widely used to prevent or treat bacterial infectious diseases. In recent years, antibiotic drugs have been 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 about antimicrobial usage in dairy farms is the use of the blanket dry cow therapy, which consists of treating all quarters of all cows with antimicrobials at the dry off to cure existing and prevent new intramammary infections (IMI). Moreover, considering customer fears and, on the other hand, the development of antimicrobial resistance, a European Commission regulation banned the use in a preventive way of antimicrobials drugs. Alternatively, to blanket dry cow therapy, selective dry cow therapy has been introduced, which consists in not treating with antimicrobial drugs cows with low SCC and without IMI at dry-off (2). This study reports results regarding a selective dry cow therapy approach on a commercial dairy farm in northern Italy. The selected herd comprises 460 milking cows and is free from contagious udder pathogens. The selective dry therapy approach started in October 2020 and ended in September 2021, aiming to reduce antibiotics usage during the dry period by 40%. One hundred fifty cows were dried off during this period, and 46 cows were enrolled. Enrolling criteria were average SCC <200.000 cell/ml, current lactation, and no IMI from major udder pathogens. Cows were sampled at dry-off and 10 days after calving to assess SCC and IMI status; moreover, they will be monitored for the first 100 days of lactation to record any clinical mastitis cases. After sampling at dry-off, cows were randomly assigned to the following treatment, only internal teat sealant (ITS) (24 cows) or intramammary antibiotic treatment and internal teat sealant (A+ITS) (22 cows). Results showed no significant differences in IMI distribution between quarters belonging to two groups at dry-off (ITS 23%; A+ITS 39%) and after calving (ITS 31%; A+ITS 33%). $\chi 2$ test showed a non-significant slight increase in IMI in post-partum in ITS cows, and it showed a significant decrease (p=0,033) in IMI in post-partum A+ITS cows. In 3 post-partum milk samples from A+ITS cows have been isolated major pathogens such as S*trep. dysgalactiae* and *Strep. uberis.* SCC values have been compared with the U-Mann Whitney test, and it showed no significant difference for both treatment groups at the dry off (ITS 83x10^3 cells/ml; A+ITS 127,65x10^3 cell/ml) and post-partum (ITS 68,95 x10^3 cells/ml; A+ITS 199,65 cell/ml). No clinical mastitis was observed in both groups during first 100 days in milk. This study confirms that it is possible to apply selective dry therapy, with a low risk of new infection or SCC increase at calving, considering cows without IMI from major pathogens and SCC values <200.000 cell/ml in the previous lactation period. The use of selective dry cow therapy is a valuable method for reducing and more responsible usage of antibiotics in dairy farms in a One Health perspective (3).

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DETECTION AND MOLECULAR CHARACTERIZATION OF CANINE CORONAVIRUS TYPE I AND II STRAINS FROM DOMESTIC DOGS IN SOUTHERN ITALY, 2019-2021

Francesco Mira (1), Gabriele Chiaramonte (1), Gianvito Lanave (2), Giorgia Schirò (1), Marta Canuti (3), Paolo Capozza (2), Vincenzo Randazzo (1), Francesco Antoci (1), Domenico Vicari (1), Annalisa Guercio (1), Nicola Decaro (2), Giuseppa Purpari (1)

(1) Istituto Zooprofilattico Sperimentale della Sicilia "A. Mirri". (2) Università degli Studi di Bari, Dipartimento di Medicina Veterinaria. (3) Università degli Studi di Milano, Dipartimento di Fisiopatologia Medico-Chirurgica e dei Trapianti.
Corresponding author: G. Schirò (giorgia.schiro91@gmail.com)

Canine coronavirus (CCoV) (genus *Alphacoronavirus*) is the causative agent of mild gastroenteritis in dogs, although some mutants are associated with systemic, often fatal disease [1]. Two distinct genotypes with worldwide distribution (CCoV-I and CCoV-II) and two sub-genotypes (CCoV-IIa and CCoV-IIb) have been reported. In Italy, CCoV infections have been repeatedly reported [1, 2], but information on the molecular epidemiology and the genomic features of strains circulating in recent years are still limited. Aim of this study was to provide an in-depth update on the epidemiological and molecular features of CCoVs identified in domestic dogs. A cross-sectional study was conducted on 285 dogs suspected of having infectious gastrointestinal disease. Rectal swabs, faeces, and tissue samples were collected from dogs between 2019 and 2021 and the presence of CCoV and other canine viral enteropathogens (canine parvovirus type 2, CPV-2; canine adenoviruses type 1 and 2, CAdV-1 and -2; canine distemper virus, CDV; norovirus, NoV; rotavirus, RoV) was investigated by molecular assays. CCoV typing and M and S genes sequence and phylogenetic analyses were performed [2-4]. CCoV was detected in 39 dogs (13.68%), alone (in 5 dogs) or in co-infection with other viral pathogens (CPV-2, CAdV-1, NoV). Among co-infections, CPV-2 was the most frequently detected agent (33 dogs, 84.6%). Single (20 CCoV-I and 8 CCoV-IIa) or mixed (9 CCoV-I/CCoV-IIa) coronaviral infections were evidenced and CCoV-IIb subtype was not detected. CCoV strains were evidenced in intestine samples and rectal swabs, while CCoV-IIa strains were also detected in four dogs also in lung, liver, and kidney samples. According to the phylogenetic analysis based on the M gene, Italian and reference CCoV strains segregate within two separate clades corresponding to the two genotypes. According to the sequence and phylogenetic analyses of the 5'-end of the S gene, CCoV-I strains showed the highest nucleotide identities with CCoV-I strains from Greece and segregated with either Elmo/02 or 23/03 reference strains; CCoV-IIa strains showed the highest nucleotide identities with CCoV-IIa strains from Italy and Greece and segregated with CB/05 reference strain. As CCoV remains a widespread enteric virus of dogs, in this study we identified and characterized the viral strains recently circulating in Southern Italy. Our data provide evidence for CCoV-I and CCoV-IIa single or mixed infection in the rectal swabs/enteric tract and CCoV-IIa extraintestinal infections. CCoV exhibited a high co-infection rate with CPV-2. The description of the genetic diversity of CCoV updates the current knowledge about viral strains circulating in Italy and provides useful data for future studies aimed at acquiring an in-depth knowledge of the epidemiology and evolution of canine coronaviruses.

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IDENTIFICATION AND MOLECULAR CHARACTERIZATION OF A DIVERGENT ASIAN-LIKE CANINE PARVOVIRUS TYPE 2B (CPV-2B) STRAIN IN SOUTHERN ITALY

Giorgia Schirò (1), Francesco Mira (1), Marta Canuti (2), Stefano Vullo (1), Giuseppa Purpari (1), Gabriele Chiaramonte (1), Santina Di Bella (1), Vincenza Cannella (1), Vincenzo Randazzo (1), Calogero Castronovo (1), Domenico Vicari (1), Annalisa Guercio (1)

(1) Istituto Zooprofilattico Sperimentale della Sicilia "A. Mirri". (2) Università degli Studi di Milano, Dipartimento di Fisiopatologia Medico-Chirurgica e dei Trapianti.

Corresponding author: G. Schirò (giorgia.schiro91@gmail.com)

Canine parvovirus type 2 (CPV-2) emerged in the late 1970s and nowadays, it is still a relevant infectious agent, responsible for a severe and often fatal disease in domestic and wild carnivores. In the last decades, several studies analysed the spread of CPV-2 in Italy [1, 2] and documented the introduction and spread of CPV-2 strains from other geographic areas in the Italian canine population [3, 4]. This study reports the genomic characterization of a CPV-2b strain related to Asian strains that was recently detected in a stray puppy in Italy. Tissue samples (lung, heart, intestine, spleen, liver, kidney) were collected during necropsy in February 2022 and then submitted for virological analyses: the presence of canine viral enteropathogens (CPV-2; canine coronavirus, CCoV; canine adenoviruses type 1 and 2, CAdV-1 and -2; canine distemper virus, CDV; norovirus, NoV; rotavirus, RoV) was investigated [4, 5]. The near complete genominc sequence, encompassing the full coding region, was obtained [4]. Sequence, phylogenetic, and recombination analyses were performed [4, 6]. At necropsy, hyperaemia of the gastric mucosa, catarrhal fluids in the stomach, haemorrhage of the serous membrane of the small intestine, congestion and enlargement of mesenteric lymph nodes, pulmonary oedema with marginal petechiae and necrosis were observed. The virus was detected in all tissue samples and typed as CPV-2b. The sequence was related to strains of Asian origins and was unrelated to CPV-2b strains previously reported in Europe. Sequence analysis revealed genetic signatures typical of Asian strains (NS1: 60Val, 545Val, 630Pro; VP2: 5Gly, 267Tyr, 324Ile) and mutations rarely reported in Asian CPV-2b strains (NS1: 588N; VP2: 370Arg). Phylogenetic analyses placed this strain in well-supported clades including also Asian CPV-2 sequences, but always as a basal, single-sequence long branch. No recombination was observed for this strain. The introduction and spread in Europe of CPV-2c strains of Asian origins have been described in domestic and wild carnivores [2, 3, 4, 7]. Additionally, this study reports the first evidence and molecular analysis of an Asian-like CPV-2b strain in the Italian canine population, confirming the continuous changes in the global spread of CPV-2. The evidence for this virus in a puppy from a stray litter suggests local viral circulation and the need for further molecular epidemiological surveys. The full-length genome analysis presented in this study could help to better trace the spread of CPV-2 with different geographical origins.

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Brucella melitensis IMMUNOPROTEOMICS REVEAL STRAIN-SPECIFIC ANTIGENS SUITABLE FOR THE UNBIASED DIAGNOSIS IN A DIVA STRATEGY

Bruno Tilocca (1), Alessio Soggiu (2), Viviana Greco (3,4), Luigi Bonizzi (2), Andrea Urbani (3,4) Flavio Sacchini (5) Giuliano Garofolo (5), Manuela Tittarelli (6), Paola Roncada (1)

(1) Università degli Studi "Magna Graecia" di Catanzaro, Dipartimento di Scienze della Salute. (2) Università degli studi di Milano, Dipartimento di Scienze biomediche, chirurgiche e odontoiatriche. (3) Università Cattolica del Sacro Cuore, Dipartimento di Scienze biotecnologiche di base, cliniche intensivologiche e perioperatorie. (4) Fondazione Policlinico Universitario Agostino Gemelli. (5) Istituto Zooprofilattico Sperimentale dell'Abruzzo e del Molise, Centro di Referenza Nazionale per le brucellosi animali.

Corresponding author: P. Roncada (roncada@unicz.it)

Brucellosis is a worldwide relevant zoonosis caused by the Gram-negative bacteria *Brucella* spp. In the Mediterranean area, *Brucella melitensis* is among the most common species with sheep and goats being the major reservoir of the pathogen [1,2]. Diagnostic and prophylactic measures adopted for the control of brucellosis suffer from a lack of specificity and cross-reactivity, respectively. Also, live-attenuated *B. melitensis* Rev.1 administration in goat and sheep showed residual virulence and cross-reactivity with the current diagnostics, raising the need for studies to amend both the vaccinal and diagnostic strategies. In our study, we employed an immunoproteomic approach to underline the specific immunogenic proteins of both *B. melitensis* Rev.1 and *B. melitensis* 16M strains, meant respectively as the reference of the vaccinal and field strains, and discriminate their antigens from each other and from those of the most cross-reactive specimens (i.e. *Escherichia coli* O157:H7 and *Yersinia enterocolitica* O:9) [3]. Following the optimized resolution of the brucellae proteomes, independent incubation of the bacterial protein profiles with properly depleted sera from either Rev. 1 or 16M infected animals revealed a distinct map of immunogenic proteins for the assayed bacteria. Differential immunogenic proteins have been identified both computationally and analytically via MS/MS, yielding a list of suitable candidates to be potentially employed in the design of novel diagnostics and/or prophylactic strategies. Universal stress protein and related nucleotide-binding proteins (Uspa), nucleoside diphosphate kinase and putrescine utilization regulator were uniquely identified as immunogenic and predictive for the exclusive presence of the vaccinal strain. On the other hand, ABC transporter-substrate-binding protein (cluster4_ leucine/isoleucine/valine/benzoate, broad-specificity amino acid ABC transporter-substrate-binding protein) and ABC transporter-substrate-binding protein (cluster1maltose/g3p/polyamine/iron) were identified as putative biomarkers of *B. melitensis* 16M, free from cross reactivity with other cross-reactive specimens and the *B. melitensis* Rev. 1 employed at vaccinal purposes. The independent identification of the immunogenic proteins through our optimized workflow, confirms a previous in-silico bioinformatic prediction performed on the same bacterial strains [4]. Sorting the major immunogenic proteins of both the vaccinal and field strains is of pivotal importance for the design of unbiased serodiagnostic strategies crowning the conventional diagnostics for brucella. Also, identification of independent antigen patterns may serve as the starting point for drawing optimized prophylactic strategies targeting other molecules than those already addressed by the diagnostic tests; thus, differentiating infected from vaccinated animals. This, in turn, improves monitoring campaigns which currently represents one of the keystones for the adoption of fair and efficient eradication and control plans against brucellosis in animals and humans.

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BIOINFORMATICS-BASED PREDICTION OF EPITOPE CANDIDATES SUITABLE FOR RAPID IN-FIELD SCREENING OF AFRICAN SWINE FEVER

Bruno Tilocca (1), Alessio Soggiu (2), Luigi Bonizzi (2), Domenico Britti (1), Paola Roncada (1)

(1) Università degli Studi "Magna Graecia" di Catanzaro, Dipartimento di Scienze della Salute. (2) Università degli studi di Milano, Dipartimento di Scienze biomediche, chirurgiche e odontoiatriche.

Corresponding author: B. Tilocca (tilocca@unicz.it)

African Swine Fever (ASF) is an acute hemorrhagic fever affecting wild suids and domestic pigs with dramatically high mortality and morbidity rate. The causal agent of ASF, the African Swine Fever Virus (ASFV), is an icosahedral virus of 200nm diameter, composed of an outer envelope layer of host derivation and a linear 170–190 kb long dsDNA molecule encoding 150-200 viral proteins; of these, 68 have structural functions whilst more than 100 proteins are associated to replicative/virulence functions. Although ASFV is a non-zoonotic pathogen, its environmental resistance, persistence and diffusion make ASF a notifiable disease by the World Organization for Animal Health (OIE) and the enactment of targeted measures to treat, reduce or eradicate the pathogen circulation. Nevertheless, as of today, no efficient therapeutic intervention nor prophylactic measures exist to fight ASFV diffusion, underlining the importance of the early diagnosis and the need for efficient in-field screening of ASF [1]. Recommended guidelines for the diagnosis of ASF are rather laborious and expert personnel demanding, making them impracticable in the desirable context of the rapid in-farm screening. In this scenario, various attempts are performed to optimize the early detection of ASFV through diverse cutting-edge technologies relying antibody-antigen specificity. Although, the stability and sensitivity of these new methods still need to be further improved to reach, or overcome, the performances of the OIE-recommended diagnostics [2]. To our knowledge, diagnostic innovations so far designed target single epitopes and suffer from specific limitations hindering their suitability for the early in-field diagnosis. In this view, the design of innovative diagnostics based on a panel of multiple ASFV epitopes would amend versatility and the analytical performances of the deliverable, ensuring high quality and accuracy standards worth of implementation in rapid in-field monitoring programs. Pursuing this view, we performed epitope prediction from the major AFSV structural proteins holding the potential to be targeted in innovative rapid diagnostic tests. Specifically, selected ASFV structural protein sequences were retrieved from data repositories and their tridimensional structure was computed ad hoc via SWISSMOL. Altogether, linear and 3D protein structures were subjected to the prediction of the epitope sequences, that are likely to elicit antibody production, by three commonly employed bioinformatic tools, each of which relies on a peculiar algorithm and gives emphasis to specific features of the potential epitopic sequences. The list of both linear and discontinuous peptide epitopes resulting from the above calculations was further quality-checked and filtered, avoiding short peptides epitopes and the inclusion of cross-reactive epitopes against the taxonomically-related specimens.

Bioinformatics and in-silico prediction of the epitopes is among the last frontiers in the biomedical field as enable large-scale screening of the biomarkers of several infectious diseases in a safe and reproducible manner, reduces the animal testing and enables tailoring of the experimental variables to reproduce a variety of real in-field conditions [3, 4]. The present study provides a list of candidate biomarkers whose batch employment held the potential suitability for the unbiased rapid in-field diagnosis and, in turn, might be implemented in screening programs, crowing the current monitoring and control campaigns that are currently running in the diverse countries of the globe.

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DETECTION OF HUMAN AND FISH VIRUSES IN MARINE GASTROPODS

Sara Ciulli, Francesca Errani, Patrizia Serratore, Enrico Volpe

Alma Mater Studiorum, Università degli Studi di Bologna, Dipartimento di Scienze Mediche Veterinarie.
Corresponding author: S. Ciulli (sara.ciulli@unibo.it)

Gastropoda include more than 75,000 existing species; they are highly abundant in marine ecosystems, playing important ecological roles as grazers, predators and major food sources for higher trophic levels. Marine gastropods are also an important source of animal protein to humans [1]. Some gastropod species, such as *Tritia mutabilis*, is an important resource for the small-scale fishery in the Adriatic Sea [2]. Similar to bivalves, gastropods are very susceptible to environmental contaminants and have been shown to accumulate metals, marine biotoxins and viruses [1]. However, so far only few studies investigated the presence of viruses in gastropods [3, 4].

As part of the project PO FEAMP ITALIA 2014-2020 "Pilot action for the sustainability of small-scale fishery in Emilia-Romagna Region" an investigation was conducted to assess the presence of human and fish viruses in gastropods collected in the Adriatic Sea between 2017 and 2021. Particularly, viruses usually accumulated in bivalve molluscs such as the Hepatitis A virus (HAV) and the Noroviruses (NoV) genogroup I and II were investigated. Furthermore, the presence of one of the most threatening viral pathogens for aquatic animals was investigated: the nervous necrosis virus (NNV). A total of 34 samples, of which 27 were *T. mutabilis,* were processed for virological analyses by using previously optimized protocols [5-8]. For comparison, human virus presence was also investigated in 54 clam samples (*Ruditapes philippinarum*) collected in the Adriatic Sea in 2017-2021; indeed, this bivalve mollusc species is frequently contaminated by human viruses [9].

HAV was detected only in one gastropod sample out of 34 (2.9%) while NoV was not detected in any of the tested gastropod specimens. On the other hand, in clam samples HAV and NoV were detected in 5.6 % and 37% of samples respectively. HAV detected in *T. mutabilis* clustered with HAV IA strains, whereas HAV detected in clams clustered within both subtypes IA and IB. Subtype HAV IA is the most common in human infections in Southern Italy [10]. Concerning fish virus, NNV was detected in 9 gastropod samples out of 34 (26.5%) and particularly in 5 specimens of *T. mutabilis* out of 27 (18.5%). All the detected NNV clustered with RGNNV genotype, the most widespread in the Mediterranean Sea; no reassortant strains were detected in gastropods.

Summarizing, this investigation pointed out the presence of viruses in marine gastropods, however most of them were fish viruses. The NNV role in gastropods needs further analysis. As a whole, investigated gastropods showed a lower contamination by human viruses compared to bivalve molluscs posing minor concern to human health.

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ADVANCEMENT OF BOHV-4 BASED VECTOR FOR OVINE AND SWINE IMMUNIZATION

Valentina Franceschi (1), Luca Russo (1), Francesca Macchi (1), Noemı Sevilla (2), Miriam Pedrera (3), Simon P. Graham (3), Veronica Martın (2), Gaetano Donofrio (1)

(1) Università degli Studi di Parma, Dipartimento di Scienze Medico-Veterinarie. (2) Centro di Investigazione in Salute Animale, Istituto Nazionale di Investigazione Scientifica (CISA-INIA), Valdeolmos, Madrid, Spain. (3) The Pirbright Institute, Pirbright, UK.

Corresponding author: G. Donofrio (gaetano.donofrio@unipr.it)

Bovine herpesvirus 4 (BoHV-4) potential as a gene delivery vector for immuno-prophylaxis and gene therapy has been already well documented in mice, sheep, rabbits, goats and pigs. Its peculiar molecular and biological characteristics such as little or no pathogenicity, absence of oncogenicity; capability to accommodate large amounts of foreign genetic material; ability to establish persistent infection and to be maintained in an episomal state in dividing cells, combined with the possibility to be manipulated using infectious BoHV-4-derived bacterial artificial chromosome (BAC) genomes make this virus a perfect candidate for vectorialized vaccination strategies [1]. In these last years, we have made several advancements in the study and proposal of new vaccines against two animal viruses, of serious economic and health concern, in particular Morbillivirus peste des petits ruminants virus (PPRV), and Nipah Virus (NiV), that affect respectively sheep and pigs.

In regards to PPRV, there is an effective need of a DIVA vaccine, able to discriminate between natural infected and vaccinated animals. For this reason, we generated a recombinant BoHV-4 based vector able to deliver PPRV hemagglutinin H antigen (BoHV-4-A-PPRV-H-ΔTK) [2]. Vaccination with BoHV-4-A-PPRV-H-ΔTK protected sheep from virulent PPRV challenge and prevented virus shedding. Protection was correlated with anti-PPRV IgGs, neutralizing antibodies and IFN-g producing cells induced by the vaccine. Detection of antibodies exclusively against H-PPRV in animal sera and not against other PPRV viral proteins such as F or N could serve as a DIVA diagnostic test when using BoHV-4-A-PPRV-H-ΔTK as vaccine, suggesting that BoHV-4-A-PPRV-H-ΔTK could be a promising new tool for PPRV eradication programs.

NiV is an emergent pathogen capable of causing acute respiratory illness and fatal encephalitis in pigs and humans. We tested the immunogenicity of bovine herpesvirus (BoHV-4) vectors expressing either NiV attachment (G) or fusion (F) glycoproteins, BoHV-4-A-CMV-NiV-GΔTK or BoHV-4-A-CMV-NiV-FΔTK, respectively in pigs [3]. These recombinant vaccines were compared with a canarypox (ALVAC) vector expressing NiV G, previously demonstrated to induce protective immunity in pigs. Both BoHV-4 vectors induced robust antigen-specific antibody responses. BoHV-4-A-CMV-NiV-GΔTK stimulated NiV-neutralizing antibody titers comparable to ALVAC NiV G and greater than those induced by BoHV-4-A-CMV-NiV-FΔTK. In contrast, only BoHV-4-A-CMV-NiV-FΔTK immunized pigs had antibodies capable of significantly neutralizing NiV G and F-mediated cell fusion. All three vectored vaccines evoked antigen-specific CD4 and CD8 T cell responses, which were particularly strong in BoHV-4-A-CMV-NiV-GΔTK immunized pigs and to a lesser extent BoHV-4-A-CMV-NiV-FΔTK. These findings emphasize the potential of BoHV-4 vectors for inducing antibody and cell-mediated immunity in pigs and provide a solid basis for the further evaluation of these vectored NiV vaccine candidates.

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GENERATION OF A LENTIVIRAL VECTOR-BASED PSEUDOVIRUS NEUTRALIZATION ASSAY FOR SARS-COV-2 AND ITS VARIANTS

Valentina Franceschi, Luca Russo, Gaetano Donofrio

Università degli Studi di Parma, Dipartimento di Scienze Medico-Veterinarie.
Corresponding author: G. Donofrio (gaetano.donofrio@unipr.it)

COVID-19 is an ongoing pandemic caused by the highly infectious coronavirus SARS-CoV-2 that is engaging worldwide scientific research to find a timely and effective eradication strategy. Great efforts have been put into anti-COVID-19 vaccine generation in an effort to protect the world population and block SARS-CoV-2 spread. Many approaches have been employed to develop prophylactic and therapeutic measures against SARS-CoV-2, including whole inactivated vaccines, subunit vaccines, RNA-based vaccines, viral vectored vaccines [1], monoclonal neutralizing antibodies, and fusion inhibitors, most of which were designed to target the SARS-CoV-2 spike glycoprotein (S). To validate the protective efficacy of the vaccination campaign and effectively control the pandemic, it is necessary to quantify the induction of neutralizing antibodies by vaccination, as they have been established to be a correlate of protection [2]. Natural SARS-CoV-2 infections have been reported in domestic animals as dog, cat, and ferret, in captive animals but also in wild and farmed minks. Human-to-animal and domestic-to-wild susceptible animal transmission could be possible, generating also potential animal virus reservoir [3]. It is therefore very important to have available a test to quantify neutralizing antibodies even in animal species, most of all considering the possibility to have human-to-animal spreading of this virus.

We described a new SARS-CoV-2 pseudovirus neutralization assay, based on a replication-incompetent lentivirus expressing an adapted form of SARS-CoV-2 S protein and an ACE2/TMPRSS2 stably expressing cell line has been minimized in terms of protocol steps without loss of accuracy. This simplified neutralization system is to improve SARS-CoV-2 vaccination campaign by means of an easy and accessible approach to be performed in any medical laboratory, maintaining the sensitivity and quantitative reliability of classical serum neutralization assays. Further, this assay can be easily adapted to all the new emerging different coronavirus variants by simply modifying the pseudotyping vector. Considering that the new SARS-CoV-2 variants has gained the capability to expand species tropism to animals, such as murines [4], the possibility to have an effective neutralization assay, easily adaptable on all the new emerging variants should be regarded toward the possibility to facilitate the control of the ongoing pandemic.

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BOHV-4 ORF45 IS AN INDISPENSABLE GENE FOR BOHV-4 LYTIC REPLICATION AND ITS PROTEIN PRODUCT IS ASSOCIATED TO THE VIRION

Luca Russo, Valentina Franceschi, Gaetano Donofrio

Università degli Studi di Parma, Dipartimento di Scienze Medico-Veterinarie.
Corresponding author: G. Donofrio (gaetano.donofrio@unipr.it)

Bovine herpesvirus 4 (BoHV-4) is a gammaherpesvirus, belonging to Rhadinovirus genus, with no strict clear association with disease, even if increasing evidence of its secondary pathogenic role in cases of post-partum metritis in cattle are reported. BoHV-4 potential as a gene delivery vector for immuno-prophylaxis and gene therapy has been already well documented, thanks to its favorable molecular and biological characteristics, such as little or no pathogenicity, absence of oncogenicity; capability to accommodate large amounts of foreign genetic material and the possibility to be manipulated using infectious BoHV-4-derived bacterial artificial chromosome (BAC) genomes [1]. Molecular studies on its Open Reading Frames (ORFs) and gene products are on-going, in order to better clarify the interaction mechanisms between the viral particles and the host cells and also to deeper understand its application as aviral vector. BoHV-4 ORF45 codifies for a protein (orf45) of unknown function. To better understand the role of ORF45 gene product during BoHV-4 lytic replication, a recombinant BoHV-4 was generated by homologous recombination from a BoHV-4 genome cloned as a Bacterial artificial chromosome (BAC) (pBAC-BoHV-4-A), in which most of the BoHV-4 ORF45 was substituted by the insertion of a selectable expression cassette, containing the gene for kanamicina resistance and galactokinase expression. The resulting recombinant BoHV-4 genome (pBAC-BoHV-4-AΔORF45-KanaGalK) was completely unable to reconstitute Infectious Replicating Viral Particles (IRVPs) and to replicate when transfected in permissive cell lines in comparison to its revertant clone (pBAC-BoHV-4-ΔORF45-Revertant), where an ORF45 expression cassette, driven by an heterologous promoter was positioned in opposite direction respect to the natural ORF45 in the pBAC-BoHV-4-AΔORF45-KanaGalK genome backgroung. Since ORF45 was tagged with an HA epitope (YPYDVPDYA) we were able to demonstrate that ORF45 gene product is associated to the virion particles. This work demonstrates that BoHV-4 replicating cycle is dependent on ORF45 gene product and provides direct evidence that ORF45 gene product is necessary for BoHV-4 lytic replication.

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PULSED ACTIVE SURVEILLANCE FOR THE EVALUATION OF COMMENSAL ANTIMICROBIC RESISTANT BACTERIA IN A SMALL ANIMAL VETERINARY UNIVERSITY HOSPITAL: PRELIMINARY RESULTS

Raffaele Scarpellini, Elisabetta Mondo, Federica Giacometti, Federica Savini, Francesco Dondi, Roberta Troia, Massimo Giunti, Federico Tomasello, Silvia Piva.

Alma Mater Studiorum - Università di Bologna, Dipartimento di Scienze Mediche Veterinarie;
Corresponding author: R. Scarpellini (raffaele.scarpellini@unibo.it)

Emergence of multidrug-resistant (MDR) bacteria is a massive threat to both human and animal health. Veterinary University Hospitals (VUHs) are considered high-risk environments for the selection and transmission of MDR agents (1) due to the high-density of referred patients and the presence of students. Some of the most frequently isolated MDR bacteria in veterinary medicine are extended-spectrum betalactamase (ESBL) and carbapenemase (CPE)-producing Enterobacteriaceae and methicillin-resistant Staphylococci (MRS). Those MDR bacteria are frequently involved in healthcare-associated infections (HAIs) and outbreaks (2). Like in human settings, a microbiological surveillance system is an important tool to estimate MDR bacteria rates, as well as to prevent HAIs and to serve as a database for infection control. Since November 2020, a surveillance plan has started at the Small Animal Clinic of the Bologna VUH. This plan includes a pulsed active surveillance with periodic sessions of one month, performed every four-months, that aimed at monitoring asymptomatic MDR bacteria carriers rate at the time of hospital admission and at discharge, MDR bacteria-associated risk factors, the % of MDR bacteria acquisition and its trend over time. For every session, 25 patients, hospitalized for at least 48 hours, were sampled at the time of hospital admission and before discharge. Rectal and oral swabs were respectively cultured into selective media for ESBL and CPE-producing Enterobacteriaceae, and for MRS. Owners of included pets were asked to fill a consent form, as well as to fill a survey to investigate potential risk factors for MDR exposure (e.g., comorbidities, previous antimicrobial treatments, ...). Preliminary results obtained from 3 sessions of pulsed active surveillance and a total of 75 investigated patients indicated a % of MDR bacteria carriage of 45,3% (95% CI, 34-56,6) at the admission and of 65,3% (95% CI, 54,5-76,1) at discharge, with a % of MDR acquisition of 38,3% (95% CI, 27,3-49,3). Risk factors for acquisition were >6 days of hospitalization (p=0.017844) and antimicrobial treatment during hospital stay (p=0.01357). ESBL-producing *Escherichia coli* was the most frequently isolated species. Those preliminary results need to be extended in terms of number of patients and further analyzed by genotypic and phenotypic characterizations of isolates to better define resistance patterns and perform an epidemiologic evaluation.

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IDENTIFICATION OF A RECOMBINANT BOVINE TOROVIRUS

Francesco Pellegrini, Francesca Caringella, Paolo Capozza, Roberta Cardone, Georgia Diakoudi, Michele Camero, Gabriella Elia, Nicola Decaro, Vito Martella, Gianvito Lanave

Università degli Studi di Bari, Dipartimento di Medicina Veterinaria.
Corresponding author: F. Pellegrini (francesco.pellegrini@uniba.it)

Torovirus (ToV) is a distinct genus of enveloped, single-stranded, positive-sense RNA viruses belonging to the order *Nidovirales*, family *Tobaniviridae*, subfamily *Torovirinae*, formerly classified into the family *Coronaviridae* [1]. The genome of ToV is 25–30 kb in length and contains two large overlapping open reading frames (ORFs), ORF1a and ORF1ab, that encode two nonstructural proteins, and four small ORFs (ORF2-5) coding for the structural proteins of the spike (S), membrane (M), haemagglutinin-esterase (HE), and nucleocapsid (N) [1]. Of the four ToV species recognized, equine torovirus (EToV), bovine torovirus (BToV), and porcine torovirus (PToV) are associated with enteric infections and diarrhea in horses, cattle, and pigs, respectively, whilst human torovirus (HToV) is associated with gastroenteritis, especially in individuals with suppressed immune functions. Although ToV generally cause subclinical infection or mild diarrheal disease in adults, they can be deadly when naïve young animals and children are involved [1]. Using a metagenomic approach we identified a recombinant BToV, from a stool sample of a 20-day old calf, with clinical signs of enteritis (depression and diarrhea with blood). A total of 38 stool samples, 17 from healthy and 21 from cattle with enteric signs, were investigated using the Illumina NextSeq 500 platform. The data were analyzed with the online platform: Genome Detective Viral Tool, version 1.136 (https://www.genomedetective.com). In parallel, bioinformatic analysis on the reads obtained was carried out using the package of softwares of Geneious Prime v.2022.1.1. Sample #572/19-5 yielded a total of 8665052 sequence reads of which 7259990 reads (83%) did not appear to be viral and were removed. De novo assembly started from 1405062 reads, of which 79% were assembled into viral contigs. A total of 103676 reads were mapped to ToV and a genome of 28007 nucleotide (nt) in length was obtained, with a depth of coverage of 474X. The consensus sequence was analyzed using the web-based tools Basic Logic Alignment Search Tool (Basic Logic Alignment Search Tool (BLAST), https://blast.ncbi.nlm.nih.gov/Blast.cgi) and FASTA (https://www.ebi.ac.uk/Tools/sss/fasta/), using the default values to find homologous hits, yielding the highest nt identity (97.59%) to the strain BToV SC-1 Sichuan/2018 (GenBank accession MN073058) [2], whilst nt identity to the prototype BToV Breda/1979 [3] (accession AY427798) was 81.6%. Also, the BToV sequence obtained in this study shared high sequence identities (96.56-96.34%) with the strains BToV Ishikawa/2010 (accession LC088094) and BToV Kagoshima/2014 (accession LC088095) respectively. These viruses were characterized as recombinant viruses between BToV Breda/1979 and PToV, displaying two recombination breakpoints mapped to the 3′ end of the ORF1b coding region and the 3′ end of HE coding region. As a result, the nearly 76% of the genome is similar to PToV genome whilst the complete S gene is of bovine origin [4]. Accordingly, the virus #572/19-5 is a porcine-bovine recombinant ToV, like viruses described previously in the Japan and China [3,4]. This finding highlights the diversity of bovine ToVs. Diagnostics able to detect and distinguish between the two different BToV types could be helpful to assess better the epidemiology of these enteric viruses and to understand if there are relevant phenotypical differences.

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SEROLOGICAL SURVEY FOR HEPADNAVIRUS IN HOUSEHOLD DOGS

Paola Fruci (1), Andrea Palombieri (1), Federica Di Profio (1), Vittorio Sarchese (1), Francesco Pellegrini (2), Paolo Capozza (2), Georgia Diakoudi (2), Gianvito Lanave (2), Vito Martella (2), Barbara Di Martino (1)

(1) Università degli Studi di Teramo, Dipartimento di Medicina Veterinaria. (2) Università degli Studi di Bari, Dipartimento di Medicina Veterinaria.

Corresponding author: P. Fruci (pfruci@unite.it)

In 2018, a novel orthohepadnavirus (Domestic Cat Hepadnavirus, DCH), similar to hepatitis B virus (HBV), was identified through transcriptomics studies in a domestic cat with large cell lymphoma [1]. Subsequent molecular investigations revealed positivity rates ranging from 6.5% to 12.5% in cat blood samples and up to 14.0% in liver tissue [1-4]. Also, antibodies specific to recombinant core DCH (DCHc Abs) have been found in 25.0% of feline sera [5], remarking the results of direct epidemiologic investigations. The potential clinical impact of DCH on feline health is currently under investigation [6, 7]. A positive correlation between an increased level of markers indicative of structural or functional liver damage and high serum viral loads (>10^4 genome copies per ml) has been observed [6]. In addition, histological lesions associated with inflammation and neoplasia mirroring the features observed in HBV-associated hepatopathies, have been identified in cats with DCH-associated chronic hepatitis or hepatocarcinomas (HCC) [7]. More recently, hepadnavirus DNA was identified in 6.3% of canine serum samples with altered hepatic markers [8]. When testing in Western blot (WB) a subset (n=20) of the hepadnavirus-positive (viremic) sera, a total of 13/20 (65.0%) reacted with the recombinant DCHc antigen (Ag). On full genome sequencing, the novel hepadnavirus, designated domestic dog hepadnavirus (DDH), revealed high nucleotide identity (about 98%) and similar organization to DCH [4]. Herein, we report the results of a serological investigation performed on age-stratified collection of canine sera (n = 600) by using an antibody detection ELISA assay based on core-like particles of the DCH strain ITA/2018/165-83 [3], expressed in baculovirus system [5]. For the development of the ELISA, mock infected *Sf9* insect cells lysate and DCHcAg, both at final concentrations of 1 µg/ml, were coated onto 96 well EIA plates (Costar, Italy) at 100 µl per well in carbonate-bicarbonate buffer (0.05 M, pH 9.6). The wells were washed five times with 0.1% PBS-T and then blocked with 200 µl of SuperBlock™ Blocking Buffer (Invitrogen, Ltd, Paisley, UK) at room temperature for two hours. To determine the optimal working dilutions, 2-fold dilutions of dog sera positive for IgG in WB were prepared, starting with a dilution of 1:25 until 1:400. Each serum dilution was added to wells coated with either the DCHcAg or mock infected *Sf9* cells. The sample dilution was considered optimal when for each serum tested was obtained a positive/negative ratio (OD_{405} of DCHcAg /OD_{405} of mock infected cells) ≥2.0 and OD_{405} value close to 1.0 for IgG. Out of 600 canine serum samples tested, a total of 144 (24.0%) were positive for IgG with an OD_{405} ranging from 0.4 to 1,2 (mean OD_{405} of 0,7). To further investigate the possible viremic status, each positive serum was assessed molecularly, using a consensus PCR with pan-hepadnavirus primers targeting the polymerase ORF [4]. Viral DNA was found with a detection rate of 5.5% (8/144). Direct sequencing of PCR-positive samples revealed the highest nucleotide identity (98–99% at nt level) to DCH, suggesting the possibility of a free circulation of hepadnaviruses among domestic carnivores (cat and dog), rather than different viral species with a specific host range.

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GEOGRAPHICAL ASSOCIATION OF THE SPREAD OF EFFLUENTS FROM PIG FARMING WITH THE INCIDENCE OF URINOCULTURE PRESCRIPTIONS AS A PROXY OF ANTIMICROBIAL RESISTANCE IN PIEDMONT IN THE PERIOD 2015-2019

Walter Martelli (1), Rosanna Desiato (1), Andrea Rocchetti (2), Marco Dalmasso (3), Eva Pagano(4), Daniela Marchis (1), Simona Zoppi (1), Giuseppe Ru (1)

(1) Experimental Zooprophylactic Institute for Piedmont, Liguria and Valle d'Aosta. (2) Department of Microbiology, SS Antonio e Biagio Hospital, Alessandria, Italy. (3) Epidemiology Unit, Azienda Sanitaria Locale TO3 , Regione Piemonte, Grugliasco, Italy. (4) Clinical Epidemiology, Città della Salute e della Scienza Hospital, Torino, Italy.
Corresponding author: W. Martelli (walter.martelli@izsto.it)

Antibiotic resistance is a major threat to global health. In the European Union (EU) almost 400,000 cases of antibiotic-resistant infections occur each year and around 25,000 people die because of lack of effective drug treatment (EMA, 2021). In animal husbandry, group therapy is a common practice: about 87% of antimicrobials are administered to farming animals via medicated feed. It has been estimated that about 75% of the dose of antibiotics administered is not absorbed by the animal and is excreted in the faeces (Massé et al., 2014). Kumar and coll. (2005) showed that sewage spreading close to residential areas can increase antibiotic-resistant infections risk in the general population. Moreover, a strong association with methicillin-resistant *Staphylococcus aureus* (MRSA) infections was identified for inhabitants of areas near pig farms and especially in areas sprinkled with pig farm effluents (Casey et al., 2016). In Italy similar epidemiological studies evaluating the exposure of the general population to animal husbandry associated risk factors are lacking despite their potential relevance for public health. For this purpose, the aim of this work was to describe the spatial distribution of antimicrobial resistance (AMR) in the general population of the Piedmont region taking into account determinants linked to pig farming and their potential association to human health. The number of urine culture tests (urinoculture) prescriptions in women in the 18-40 age group as proxy of antibiotic resistance events along with the population size have been collected for each regional municipality. Regional georeferenced data of pig farms and of farming wastewater spreading were used as exposure factors. To investigate the association a negative binomial regression model was fitted at municipality level using as dependent variable the incidence of prescription events in humans and as explanatory variables the sum of the slurry estimated in UBA/km2 (adult livestock units/km2) and the number of pig farm. Assuming a potential delay between the exposure and the effects, two different reference period were used: 2011-2015 for the animal-based data and 2015-2019 for the human urinoculture data. An overall excess risk for urinoculture prescription (IRR 1.07, CI95% [1.029-1.106]) was statistically associated with the exposure to effluents whereas no association was detected with animal density per municipality (IRR 1.01, CI95% [0.973-1.053]). A clear linear dose-response effect (Cochran–Armitage test for trend, $p<0.001$) was detected for increasing levels of amount of effluents spread. In this study, we have been able to show a clear dose-dependent association between geographical human exposure to pig manure spreading and a proxy of health outcome in humans. Further investigations will be necessary to better understand the pathways of the spread of antibiotic resistance from animal husbandry to the exposed human populations.

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MULTI-LOCUS SEQUENCE TYPING AND ANTIMICROBIAL RESISTANCE PROFILES OF EQUINE Streptococcus equi SUBSPECIES zooepidemicus ISOLATES

Francesca Paola Nocera (1), Domenico Simone (2), Loredana Capozzi (2), Laura Del Sambro (2), Giorgia Barbieri(2), Carmelinda Trisolini (2), Vincenzo Laera (2), Claudia Cerracchio (1), Filomena Fiorito (1), Antonio Parisi (2), Luisa De Martino (1)

(1) Università degli Studi di Napoli "Federico II", Dipartimento di Medicina Veterinaria e Produzioni Animali, Italia. (2) Istituto Zooprofilattico Sperimentale di Puglia e Basilicata, Italia.
Corresponding author: F.P. Nocera (francescapaola.nocera@unina.it)

Streptococcus equi subsp. *zooepidemicus* (*S. zooepidemicus*) is one of the most important veterinary pathogens frequently associated with equine endometritis, which is one of the main causes of infertility in mares [1]. In this study, we surveyed 22 strains of *S. zooepidemicus* collected during 2021 from cervico-uterine swabs of mares affected by infertility. The variability of the isolated strains was assessed with multi-locus sequence typing (MLST) from whole-genome sequencing (WGS) data. Sequencing libraries were prepared as reported by Bianco *et al.* [2]. An average of 446.061 reads (95% CI: 426.208–465.914) were produced for each isolate and quality-checked, *de novo* assembled and annotated as described by Trotta *et al.* [3]. The average length of reconstructed genomes was 2.078064 bp (95% CI: 2.055839-2.100289), which was expected for genomes of *S. zooepidemicus.* The assembled genomes were assigned to STs using the *S. zooepidemicus* scheme targeting seven loci (*arcC*, *nrdE*, *proS*, *spi*, *tdk*, *tpi*, *yqiL*) available in the PubMLST database. Isolates were also tested for antimicrobial resistance using the Kirby-Bauer disk diffusion method on Mueller-Hinton plates (Mueller-Hinton with 5% sheep blood for *Streptococcus* spp.). MLST revealed a wide variability of STs with six (27.3%) novel STs, 4/22 (18.2%) isolates assigned to ST92, 3/22 (13.6%) to ST205, and one strain (4.5%) for each of the following STs: ST10, ST30, ST39, ST49, ST101, ST132, ST147, ST314, ST369. The antibiotic resistance pattern of the three ST205 strains (resistance to amoxicillin-clavulanate, ampicillin, amikacin, gentamicin, streptomycin, enrofloxacin, sulfamethoxazole-trimethoprim, tetracycline, oxytetracycline) was the most common profile observed in half of the studied *S. zooepidemicus* strains. In addition, antibiotic-resistant phenotypes of the four ST92 isolates resulted to be conserved only across two of them. However, high resistance rates to tested antibiotics were always observed, enough to classify all the strains as multidrug-resistant. Precisely, a very high proportion (>95%) of the isolates was resistant to penicillins, fluoroquinolones, aminoglycosides, sulfonamides and tetracyclines. Our study provides a comprehensive insight into the epidemiology, antibiotic resistance profile and ST diversity of *S. zooepidemicus* strains causing ipofertility problems in mares. To our knowledge, this report represents the first equine ST identification of *S. zooepidemicus* in Italy. Thus, considering that little information is currently available on the genetic strains and clonal spread of equine

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EVIDENCE FOR GENETIC HETEROGENEITY OF CRESS DNA VIRUSES IN CATS

Violetta Vasinioti, Francesco Pellegrini, Paolo Capozza, Roberta Cardone, Georgia Diakoudi, Costantina Desario, Cristiana Catella, Eleonora Lorusso, Linda A. Ndiana, Michele Camero, Nicola Decaro, Vito Martella, Gianvito Lanave

Università degli Studi di Bari Aldo Moro, Dipartimento di Medicina Veterinaria.
Corresponding author: V. Vasinioti (violetta.vasinioti@uniba.it)

Rep-encoding single-stranded (CRESS) DNA viruses (phylum *Cressdnaviricota*) are abundant in nature and use a replication mechanism (rolling circle replication), relying on a conserved replicase (Rep). CRESS DNA viruses encompass several viral families including *Circoviridae*. Circoviruses (CVs) are nonenveloped, spherical viruses with a circular DNA genome of approximately 2 kb length and they are classified into two genera, *Circovirus* and *Cyclovirus.* CVs are able to cause severe disease in birds and pigs. CVs have also been identified in human stools, blood, and cerebrospinal fluid (CSF), as well as in various wild and domestic vertebrates. A canine CV (CaCV) was discovered in 2012 and associated, although not firmly, with respiratory and gastrointestinal diseases and, in some cases, with systemic disease involving vasculitis (1). In cats, a feline cyclovirus was reported in pooled fecal samples collected from sheltered cats in a 2014 study on feline virome (2). Moreover, a novel unclassified feline CRESS DNA virus was retrieved in 2018 from fecal samples of cats with diarrhea (3).

A total of 530 archived samples (361 sera, 131 stools and 38 respiratory swabs) collected at the Department of Veterinary Medicine, University of Bari, Italy, during 2011-2021 were screened for the presence of CV DNA using a nested PCR protocol with broadly reactive primers (4) able to amplify a partial (~400bp) Rep sequence. Overall, CV DNA was detected in 47 (8.9%) out of 530 samples. CV DNA was identified in 9/361 (2.5%) sera, 32/131 (24.4%) stool samples and 6/38 (15.8%) respiratory swabs. Positive samples were subjected to direct sequencing, yielding 29 (61.7%) sequences of good quality. Based on interrogation of the NCBI and EBI sequence databases using BLASTN and FASTA Nucleotide, the rep sequences were characterized. Also, a nucleotide alignment, using a selection of CV sequences retrieved from the databases, was used to analyze the data set with Geneious Prime vs 2021.1. On sequence and phylogenetic analysis in the partial rep-gene, the sequences formed separate clusters, along with other CV strains. A large group of sequences (n=10) were tightly grouped together and resembled viruses detected in bats, whilst a spare sequence was similar to another bat CV. Sequences similar to CV detected in bird (n=2), in fish (n=1), in pig (PCV3) (n=2), in dog (CaCV) (n=3) and in mollusks (Avon-like) (n=2) were also identified. Interestingly, we also identified sequences similar to human-associated cycloviruses NG12 (n=2), TN9 (n=2), TN12 (n=2) and PK2111 (n=2),

The CV sequences (n=24) detected in the stools likely reflected dietary habits and environmental contamination, due to the large range of animals preyed by cats. Interestingly, the large cluster of bat-like sequences, highly homogenous genetically (>98.3% nt identity), could hint to a common dietary source of cats or, to a cat-adapted virus. In addition, the CV sequences detected in the sera might imply virus replication able to sustain viremia in the animals. This included CaCV (n=3), human cyclovirus TN12 (n=1) and Avon-like CRESS DNA virus (n=1). Overall, these findings indicate a vast genetic heterogeneity of CRESS DNA viruses in cats and raises several interrogatives that are worth investigating more in depth.

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DETECTION BY NGS AND GENOME CHARACTERIZATION OF BOVINE KOBUVIRUS (SPECIES AICHIVIRUS B, GENUS KOBUVIRUS) IN A DAIRY HERD OF CENTRAL ITALY

Stefano Petrini (1), Valentina Curini (2), Cecilia Righi (1), Valeria di Lollo (2), Claudia Torresi (1), Silvia Pirani (1), Paola Gobbi (1), Monica Giammarioli (1), Viola Giulio (3), Francesco Feliziani (1) and Cesare Cammà (2)

(1) Istituto Zooprofilattico Sperimentale dell'Umbria e delle Marche, "Togo Rosati", Perugia. (2) Istituto Zooprofilattico Sperimentale dell'Abruzzo e del Molise, Teramo. (3) Veterinario libero professionista.
Corresponding author: S. Petrini (s.petrini@izsum.it)

Neonatal calf diarrhoea (NCD) is the most important cause of economic losses to dairy and beef cattle herds worldwide (1). Among the different infectious agents associated with NCD is present the Bovine Kobuvirus (BKV), which causes neonatal calf diarrhoea (2). This study describes the detection and genome characterisation of a BKV in calves in a dairy herd located in central Italy. During the period from January to December 2021, 66 cases of calves with severe neonatal calf diarrhoea were reported in a cattle farm comprising 120 heads located in central Italy. The herd was regularly immunised using different commercial vaccines against *Bovine alphaherpesvirus 1* (BoHV-1), *Bovine respiratory syncytial virus* (BRSV), *Bovine Parainfluenza-3* (BPI-3), *Bovine Viral Diarrhea Virus* (BVDV), *Bovine Coronavirus* (BCoV), *Bovine Rotavirus* and *E. coli* (K99). Clinical signs were observed in calves from 3 to 20 days of age. They progressively showed diarrhoea, anorexia, lethargy, dehydration, loss of appetite, poor mobility and sensory depression. No fever was detected during the clinical symptoms. In most cases, the animals died within 12-24 hours after the onset of clinical symptoms. Nasal and rectal swabs were carried out from 66 calves with severe neonatal calf diarrhoea for BoCV, BVDV, and major bacterial agents that could have caused diarrhoea, namely *E. coli spp*., and *Salmonella spp.* In addition, 5 pools of environmental faeces were used to detect *Cryptosporidium spp*. and *Eimeria spp*. In the 66 cases clinical, BKV was detected by RT-PCR from 21 rectal and 16 nasal swabs, respectively. In 13 animals, co-infection with BKV and BCoV was detected from 10 nasal and 5 rectal samples. No other virological positivities were detected. Bacteriological examinations revealed several non-pathogenic *E. coli* resistant to different antimicrobial drugs. Also, the protozoa were not detected. Nucleic acid extracted from a rectal swab was processed by NGS using the Illumina platform. Total RNA was reverse transcribed and sequenced using Illumina DNA Prep kit (3). BKV genome was detected and a complete sequence was obtained after a *de novo* assembly. It showed the highest nucleotide sequence identity (90.34%) with BKV KY407744.1 identified in Egypt in 2014. Instead, the best match (88.76% of nt identity) of only VP1 sequence is with BKV KY024562 identified in Scotland in 2013. This is the first report describing the whole genome sequence of BKV from Italy. In this regard, it is worth to mention that only seven complete genome sequences of BKV are available on NCBI database, therefore there is a compelling need for further studies assessing the whole genome constellation of BKV circulating strains. Moreover, viral isolation and animal studies are required in order to evaluate the pathogenicity of BKV and its role in neonatal calf diarrhoea.

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DEVELOPMENT OF ANTIGENIC AND MOLECULAR RAPID TEST DEVICES FOR DETECTION OF AFRICAN SWINE FEVER VIRUS

Barbara Colitti (1), Simone Cavalera (2), Silvia Dei Giudici (3), Federica D'Errico (4), Pier Paolo Angioi (3), Stefano Petrini (4), Anna Maria Sechi (3), Annalisa Oggiano (3), Francesco Feliziani (4), Laura Anfossi (2), Sergio Rosati (1)

(1) Università degli Studi di Torino, Dipartimento di Scienze Veterinarie. (2) Università degli Studi di Torino, Dipartimento di Chimica, (3) Istituto Zooprofilattico della Sardegna, Sassari, (4) Istituto Zooprofilattico di Umbria e Marche, Perugia.

Corresponding author: B. Colitti (barbara.colitti@unito.it)

African Swine Fever (ASF) is one of the most important viral diseases in swine industry, due to its contagiousness and impact on animal trade and animal productions. In 2007, a highly virulent genotype II ASFV strain emerged in the Caucasus region and spread into the Russian Federation and Europe through territorial contiguity by wild boar movement or jumping from infected Countries by transport and accidental release of contaminated pork-based food [1]. The rapid and on-field detection of the virus represents an urgent demand to efficiently control the diffusion in the wild cycle.

Here we describe the development and preliminary characterization of two rapid tests with the aim of prompt identification of ASFV in passive and active surveillance.

A colorimetric lateral flow immunoassay (LFIA) was developed, due to the extreme simplicity, cost-effectiveness, and easy on-field operation. The LFIA relied on the sandwich-type immunoassay using a single Mab directed against viral p30 and used as capture and linked to gold nanoparticles (GNP) as detector. The device was adapted to work with EDTA blood as well as different target tissues (i.e. spleen, lymph nodes, bone marrow). For these latter biological matrices, a single-use tool for tissue homogenization was also developed.

A second test was designed to detect viral DNA using a Recombinase Polymerase Amplification (RPA) based assay. This isothermal nucleic acid amplification employs a set of primer pairs targeting the highly conserved region [2] B646L gene, encoding the capsid protein p72, and a fluorescent internal exo-probe capable to generate a real time read out detected by the ISO-T8 fluorometer (Axxin- Australia).

Both methods were optimized using inactivated culture virus and viral DNA, kindly provided by the Italian National Reference Laboratory for Pestivirus and Asfivirus (IZSUM). The methods were then validated using archival samples from naturally and experimentally infected swine or wild boar tissues, kindly provided by the Istituto Zooprofilattico Sperimentale della Sardegna. Diagnostic sensitivity (DSe) was evaluated using a real time PCR as reference test. Diagnostic specificity (DSp) was conducted with a set of wild boar tissues, collected during the 2019 hunting season in Piedmont.

Preliminary results suggest that LFIA is highly specific, with DSe reaching 100% in samples with a Ct value <30 and 80% with a Ct value <35. Evaluation of RPA-based approach is still under evaluation but preliminary results suggest a sensitivity comparable to RT-PCR but the method is less affected by may known PCR inhibitors.

The combination of the simple extraction protocol, a portable lab setup and rapid test results, allows the on-site use of the assay, enabling the faster detection of ASF cases. This combined approach could represent a valid support for control measures and evidence-based on-field biosecurity decisions.

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PRELIMINARY INVESTIGATION ON THE ROLE DISPLAYED BY WILD BIRDS IN ANTIBIOTIC-RESISTANCE CIRCUIT

Laura Musa (1), Patrizia Casagrande Proietti (1), Valentina Stefanetti (1), Valeria Toppi (1), Marco Gobbi (2), Federico Aisa (3), Isabella Costantino (3), Martina Brescia (3), Maria Pia Franciosini (1)

(1) Università degli Studi di Perugia, Dipartimento di Medicina Veterinaria. (2) Istituto Zooprofilattico Sperimentale dell'Umbria e delle Marche "Togo Rosati". (3) Università degli Studi di Perugia, Dipartimento di Medicina Veterinaria, Ospedale Veterinario Universitario Didattico.

Corresponding author: L. Musa (laura.musa94@hotmail.com)

Recently wild birds are matter of interest from the scientific community as biological indicators of environmental contamination in every kind of habitat, including urban and suburban areas. In this scenario a progressive increase of studies has focused on wild animals as carriers of resistant bacteria in the environment [1] since they could act as efficient AMR (antimicrobial resistance) source and epidemiological links among human, livestock and natural environments. The aim of this work was to evaluate the antibiotic- susceptibility of commensal *Escherichia coli*, and the possible presence of ESBL *E. coli* and *Salmonella* spp. in some species of wild birds admitted to the Veterinary Hospital (Department of Veterinary Medicine, University of Study, Perugia). The most of the birds, represented by nocturnal and diurnal raptors, came from the WildUmbria, a wildlife rescue center, showed traumatic lesions (hunting injuries, traumatic shock and impacts etc.) similarly to what occurs in other rescue centers [2].

Common buzzards (*Buteo Buteo*) and kestrels (*Falco tinnunculus*) were the most investigated species among the diurnal raptors and tawny owl (*Strix aluco*) and scops owl (*Otus scops*) among the nocturnal ones. Cloacal swabs were collected before eventual antimicrobial treatments. A total of 70 resistant isolates were obtained from 70 *E. coli* (100%) while *Salmonella* spp. was not detected unlike other works documenting the presence of this microorganism in magpies, crows, gulls [3]. However, it should be reported that the prevalence of *Salmonella* spp. in birds of prey it is not remarkable if compared to what occurs in other bird species [4]. Ampicillin, (61.42%); ($P<0.001$), amoxicillin with clavulanic acid (42.85%); ($P<0.001$) and ciprofloxacin (18.57%), were found to be the antibiotics against which bacterial strains displayed the highest prevalence of resistance. This is not surprising since these molecules are widely used in both the veterinary and human medicine. It should also be reported that 10 out of 70 isolates (14.28%) showed MDR profile with the constant presence of beta lactam category. Five out of 70 *E. coli* isolates (7.14%) were ESBL and 4 showed a multi-resistance profile and 2 included resistance to imipenem, often considered of last choice against human infections by "super resistant" bacteria. Despite of the ban on the use of cephalosporins, resistance occurrence is reported in various species of zootechnical interest such as grazing cattle [5] but also in industrially and / or organic chickens. In last case, the vertical transmission of resistance genes by the poultry breeders could be hypothesized, because of the pyramid production system, characterizing this sector [6]. The role of the soil, as possible source of contamination of resistant bacteria for wild birds was speculated for wastewater presence from urban centers, for livestock manure and / or for the elimination of "contaminated" faeces by other wild animals, also in relation to their eating habits.

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USE OF ATOVAQUONE/PROGUANIL HYDROCHLORIDE FOR THE TREATMENT OF AVIAN MALARIA IN SNOWY OWLS (Bubo scandiacus)

Rossella Samarelli (1), Nicola Pugliese (1), Antonella Schiavone (1), Roberto Lombardi (1, 2), Natalizia Palazzo (1, 2), Valeria Campobasso (1, 2), Medhat S. Saleh Antonio Camarda (1, 2).

(1) Università degli Studi di Bari "Aldo Moro", Dipartimento di Medicina Veterinaria, (Italy). (2) Osservatorio Faunistico Regionale della Puglia, Bitetto, Italy.

Corresponding authors: A. Camarda (antonio.camarda@uniba.it); N. Pugliese (nicola.pugliese@uniba.it)

Avian malaria is a ubiquitous disease affecting avian species worldwide, caused by parasites belonging to the genus *Plasmodium* (*Apicomplexa*: *Haemosporida*), and transmitted by *Culicidae* mosquitoes (1). Within the *Plasmodium* genus, 55 species are currently known to infect avian hosts (2). Among those, *Plasmodium relictum* is one of the most diffused species (3).

The disease is characterized by great variability in the effects on the infected birds, which range from the absence of clinical signs to death, according to the host species and other still unknown factors (4-5). Despite the increasing detection of *Plasmodium* spp. infection in birds, and the potentially deleterious effects it may generate in hosts, little knowledge about treatments is available to date.

Therefore, the present study is aimed to report the efficacy of treatment with atovaquone/proguanil hydrochloride (AV/PG) administered to three captive-bred snowy owls (*Bubo scandiacus*) housed in a wildlife rescue center in Apulia, Italy. The study was authorized by the Ethic Committee of the Department of Veterinary Medicine with the number 12/2022.

In September 2021, the three birds, one female and two males, aged between 5 and 10 years, presented evidence of clinical concerns such as anorexia, loss of body weight and prolonged bleeding after accidental injury. Due to the clinical signs, the veterinary staff performed a blood screening and tests for the detection of the infectious diseases potentially causing the exhibited clinical signs. After testing positive in nested PCR for the detection of haemoprotozoa (6), subsequent sequencing revealed *Plasmodium relictum* as the responsible parasite for the disease in the three birds. In addition, the following qPCR revealed a high parasitic load in each bird. The AV/PG combination at a dosage of 10.5/4.2 mg/kg was orally administered once a day for three days. After a break of one week, another 3 days treatment course was administered. Blood was collected from the birds at 7, 30, and 60 days after the last AV/PG administration (T7, T30, and T60, respectively), to assess the effectiveness of the treatment and disclose potential relapse. The count of blood cells and the main biochemical parameters for hepatic, splenic, pancreatic, and renal functionality were measured before treatments and concurrently with the pathogen search. No positivity to the parasite was detected until 60 days after the end of the treatment, and no deleterious effect was observed during the observation period.

The presented results suggest that the combination of AV/PG can be used effectively to treat avian malaria by *P. relictum*. Additionally, they provide a dosage that can be safely administered to such valuable birds.

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OCCURRENCE OF NEGLECTED VECTOR-BORNE PATHOGENS IN DOGS FROM NUO, NIGERIA

Aya Attia Koraney Zarea (1, 2), Linda A. Ndiana (1), Maria Tempesta (1), Daniela Mrenoshki(1), Nicola Decaro (1), Francesco Pellegrini (1), Vito Martella (1), Grazia Greco (1)

(1)Department of Veterinary Medicine, University of Bari Aldo Moro, Bari, Italy. (2) Department of Microbiology and Immunology, Veterinary Research Institute, National Research Centre, Cairo, Egypt.
Corresponding author: G. Greco (grazia.greco@uniba.it)

Bartonellosis, Q fever and anaplasmosis are vector-borne diseases of veterinary and medical concern, caused by small, pleomorphic, Gram-negative, fastidious intracellular bacteria, still neglected in some countries, with dogs and their ectoparasites often involved in the transmission. Nigeria is one of the most densely populated countries in Africa where dogs are mainly used for herding and security purposes thus potentially representing a reservoir.

This study aimed at molecular detection of neglected vector-borne infections in dogs from Nigeria. A total of 100 blood samples were collected from dogs in Nuo, the capital city of Akwa-Ibom state, Nigeria. DNA of each sample was extracted and screened by qPCR or cPCR assays for *Bartonella* ssrA gene (1), haemoplasma 16S rRNA (2), *Anaplasma*/*Ehrlichia*16S rRNA (3) the repetitive element IS1111a of *Coxiella burnetii* (4), and ompB gene of Spotted fever group of rickettsia. *Bartonella* and haemoplasma species identifications were completed by sequence analyses of ITS and 16S rRNA amplicons. Moreover, *Anaplasma*/*Ehrlichia* positive samples were subjected to specific cPCR for *Ehrlichia canis* and *A. platys* identification.

Overall, 37% (n=37, CI: 27.54 to 46.46%) of examined dogs were positive for at least one pathogen, with 13% (n=13, CI: 6.41 to 19.59%) being co-infected. Haemoplasma species were dominant (34%, n=34 CI: 24.72 to 43.28%) with *Mycoplasma haemocanis* (16%, CI: 8.81 to 23.19%) and *Candidatus* Mycoplasma haematoparvum (18%, CI: 10.47 to 25.53%) equally distributed. Furthermore, *C. burnetii* (5%, CI: 0.73 to 9.27%), *Candidatus* B. merieuxii (3%, CI: 0.00 to 6.34%), and *A. platys* (2%, CI: 0.00 to 4.74%) were also recorded. Neither *Ehrlichia canis* or Spotted fever group rickettsioses were detected.

This study is the first detection of *Candidatus* B. merieuxii in dogs from Nigeria along with *C. burnetii* and haemoplasmas. In conclusion, dogs living in Nigeria are exposed to vector borne pathogens, some of them displaying risk of infections for humans that share the same environment. Dog vector-borne disease surveillance and control is a useful and complementary component for human health.

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CHARACTERIZATION OF CATALASE-NEGATIVE GRAM-POSITIVE COCCI FROM SHEEP AND GOAT FORESKIN BY CULTUROMIC APPROACH

Mrenoshki D. (1), Zarea A.A.K. (1, 2), Grassi A. (3), Argiolas G. (4), Lucente M.S. (1), Lacalandra G.M. (1), Tempesta M. (1), Greco G. (1)

(1) Department of Veterinary Medicine, University of Bari Aldo Moro, Bari, Italy. (1 ,2) Vet. Res. Inst., National Research Centre, Cairo, Egypt. (3) I-Vet, Veterinary Diagnostic Laboratory, Flero-Brescia, Italy. (4) Veterinarian Freelancer, Sementusa Tech, Cagliari, Italy.

Corresponding author Greco G. (grazia.greco@uniba.it)

The genital microbiota of animals is currently under investigations. Although several studies characterized the vaginal microbiota composition of sheep and goats, the foreskin microbiota has not yet been fully characterized. The identification of catalase-negative, gram-positive cocci, that are significant components of the genital microbiota, is still a challenge. New species are constantly discovered and rapid testing systems do not include them in their databases so testing these bacteria can fail or result in erroneous identifications. Culturomics is a testing approach based on multiple culture conditions, MALDI-TOF mass spectrometry and 16S rRNA sequencing for the identification of bacterial species. The aim of this study was to detect and characterize the catalase-negative, gram-positive cocci detected from foreskin of sheep and goats. This study protocol was approved by the Animal Ethics Committee of the Veterinary Medicine Department of the University of Bari "Aldo Moro", n. 11/2021. From February to April 2021, 46 foreskin swabs were collected from 35 rams and 11 bucks from 12 different herdsof Basilicata region, Italy. Samples were stored ad +4C° and analysed within 24 hours. For the detection of the target bacteria, each swab was seed on Edwards modified medium (TKT) plates supplemented with 5% defibrinated sheep blood and incubated microarofilically at 37°C for 48-72 h. Small (≤3 mm diameter), circular, and translucent colonies were subcultured on sheep blood agar (TSA) for emolysis evaluation and submitted for further analysis. Five colonies for each isolate were analysed for the identification by MALDI-TOF (Bruker MALDI Biotyper Server Version: 4.1.100 (PYTH)) (1). Additionally, for each isolate cPCR was performed on 16S rDNA with the universal primer pairs 27F/1492R, and the products were analyzed with BLAST from NCBI (https://blast.ncbi.nlm.nih.gov/). Out of 46 isolates, 36.96% (17) were *Streptococcus* spp. [i.e.: *Str. ovis* (13.04%, 6), *Str. pluranimalium* (10.87%, 5); *Str. lutetiensis* (8.70%, 4); *Str. uberis* (2.17%, 1); *Str. equinus* (2.17%,1)], followed by *Aerococcus viridans* (21.74%, 10), *Facklamia sourekii* (26.09%, 12), *Enterococcus* spp. (10.87%, 5) and other minor species (2/46, 4.35%). Out of 46 isolates, 33 (71.74%) and 46 (100%) were identified by MALDI-TOF and 16S rRNA, respectively, with moderate agreement between the tests (k Cohen's=0.44) linked to the missing identification of *Facklamia sourekii* by MALDI-TOF thus supporting for the need of costant updates of the library when new species are descovered. The culturomic approach herein used allowed the fast and efficient characterization of all the Gram-positive, catalase-negative cocci isolated from foreskin (Se=100%). In conclusion, this study expands the knowledge on the small ruminant foreskin microbiota. providing bases for further investigations for possible links with animal and human health.

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SERO-EPIDEMIOLOGICAL SURVEY OF RESPIROVIRUS IN ALPINE CHAMOIS AND DOMESTIC GOATS

Daniele Bonato (1), Joel Filipe (1), Maria Lucia Mandola (2), Oriana Anna Sparasci (2), Roberto Viganò (3), Camilla Luzzago (1)

(1) Università degli Studi di Milano, Dipartimento di Medicina Veterinaria e Scienze Animali. (2) Istituto Zooprofilattico Sperimentale del Piemonte, Liguria e Valle d'Aosta, S. S. Diagnostica virologica specialistica. (3) Studio Associato AlpVet.
Corresponding author: D. Bonato (bonato.daniele23@gmail.com)

In recent years, novel respiroviruses have been identified in goats, suffering respiratory syndrome in China [1] and in Alpine chamois (*Rupicapra rupicapra rupicapra*) in Italy [2], showing interstitial pneumonia associated with catarrhal bronchopneumonia. Full genome analysis indicated that chamois respirovirus is a putative novel member of the genus *Respirovirus* [2], distinct from already defined species, namely Bovine Respirovirus 3 (BRV3, former Bovine Parainfluenza Virus 3) and Caprine Respirovirus 3 (CRV3, former Caprine Parainfluenza Virus 3) identified in China. Due to the impact on chamois' populations of pneumonia complex, the investigation on respiratory pathogens is of some interest. Moreover, major outbreaks reported in chamois in Europe have been triggered by pathogens initially cross-transmitted at the interface with livestock [3].

A sero-epidemiological survey was carried out to assess how widespread the novel respirovirus infection is in chamois and goats in distinct study areas in Italy between 2010 and 2015. Sampling was carried out in the north-western Italian Alps, province of Verbano-Cusio-Ossola (VCO), where chamois respirovirus has previously been detected and goats shared pasture in summer season, and in two separated control areas, Lecco alpine area and Alessandria, for chamois and goats respectively.

Sera (189 chamois and 237 goats) were tested by virus neutralization test against the chamois respirovirus strain, named ChamoisRV/IT2014 and BRV3, strain Abbott. A high prevalence of ChamoisRV/IT2014 antibodies was observed in the chamois population of VCO, showing a seroprevalence of 34.5% (95% CI 27.1-42.8), while for the BRV3 it was of 12.9 % (95% CI 8.4-19.5). Interestingly, the antibody titer geometric mean for the chamois respirovirus (47.3) was statistically higher than BRV3 (13.7) (p-value =0.046), suggesting a cross-reaction between the tested viruses. On the other populations (chamois from Lecco, and goats from Alessandria and VCO), none to almost zero prevalence was found for both viruses.

We report the circulation of the novel respirovirus in the VCO chamois population at least from the beginning of the 2010s, but it still didn't spread into other regions nearby either in chamois or goat populations. The origin and evolution of respiroviruses will be further investigated trough phylogenetic approaches.

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DETECTION OF DOMESTIC CAT HEPADNAVIRUS IN CATS WITH CAVITARY EFFUSIONS

Gabriele Ratti, Angelica Stranieri, Donatella Scavone, Sara Meazzi, Alessia Giordano, Saverio Paltrinieri, Stefania Lauzi

Università degli Studi di Milano, Dipartimento di Medicina Veterinaria e Scienze Animali.
Corresponding author: G. Ratti (gabriele.ratti@unimi.it)

A novel member of the genus Orthohepadnavirus, named Domestic Cat Hepadnavirus (DCH) has been identified in cats from Australia in 2016 [1]. Thereafter, DCH has been reported in cats from different countries, including Italy, with prevalence ranging from 1.9% to 10.8% [2, 3]. A potential DCH pathogenetic role in feline hepatic diseases has been suggested but a correlation between DCH infection and liver disease still needs to be established [4]. In November 2022, DCH was detected by qPCR [2] in the serum of a cat admitted to the Veterinary Teaching Hospital (VTH) of Lodi with a history of hyperthermia, diffuse oedema, suspected liver disease and cavitary effusion. Following this finding, aim of this study was to retrospectively investigate the correlation between DCH and the presence of cavitary effusions in cats. Cats with and without effusions were selected from the caseload of the VTH laboratory diagnostic routine activity between 2020 and 2022. The DCH-positive cat was included in the group of cats with cavitary effusions. Cavitary effusions were classified on etiological basis, according to laboratory findings or post-mortem examinations. Cats were further classified as absence, low, medium or high likelihood of liver disease according to hematobiochemical analysis results. Serum and effusion samples were tested for DCH presence by qPCR [2] and positive samples were further subjected to whole genome sequencing [1].

A total of 75 cats were enrolled. Among those, 34 cats presented cavitary effusion associated with feline infectious peritonitis (n=8), inflammatory (n=9), neoplastic (n=6), septic (n=5), hemorrhagic (n=3), transudative (n=2) and chylous (n=1) etiology. Cats were categorized as absent (n=50), low (n=7), intermediate (n=9) or high (n=6) likelihood of liver disease. Overall, DCH was detected in 2/75 cats (2.7%) in both blood and abdominal effusions, with a higher viral load in blood samples. Both DCH positive cats were negative for feline immunodeficiency virus antibodies and feline leukemia virus antigens, had an intermediate likelihood of liver disease and showed an inflammatory effusion with similar cytologic features, such as mixed inflammatory cells including a noticeable number of eosinophils. DCH sequence analysis revealed the presence of the same DCH strain in serum and effusion samples. The phylogenetic analysis showed that the DCH sequences from this study belonged to the putative genotype A and clustered with the Australian strain but were not included in the clade with other Italian DCH sequences. These results suggest high genetic variability of DCH circulating in Italy. The low number of positive animals does not allow to define if DCH is associated with liver disease or with cavitary effusions in cats. However, given the similar features between the two DCH-positive cats, further studies on a wider caseload are recommended to confirm the pathogenic role of DCH in cats and its relationship with cavitary effusion.

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PREVALENCE AND RISK FACTORS ASSOCIATED WITH EXTENDED-SPECTRUM B-LACTAMASE- AND AMPC-PRODUCING Escherichia coli IN DOGS

Alessia Facchin (1), Gabriele Ratti (2), Martina Penati (1), Alessia L. Gazzonis (1), Greta Masiero (1), Paola Scarpa (1), Paola Dall'Ara (1), Camilla Luzzago (1), Giovanni L. Alborali (2), Stefania Lauzi (1)

(1) Università degli Studi di Milano, Dipartimento di Medicina Veterinaria e Scienze Animali. (2) Istituto Zooprofilattico Sperimentale della Lombardia e dell'Emilia Romagna "Bruno Umbertini".

Corresponding author: A. Facchin (alessia.facchin@unimi.it)

Dogs and cats share the same environment and live in close contact with humans. Pets may be potential reservoir for antimicrobial resistance (AMR) [1] and dogs in particular may transmit resistant microorganisms to their owners [2]. The aims of this study were to evaluate the prevalence of extended-spectrum β-lactamase (ESBL)- and AmpC-producing *Escherichia coli* (*E. coli*) in dogs, to investigate the presence of resistant genes and to identify risks factors associated with ESBL/AmpC–producing *E. coli* presence. Rectal swabs were collected between May 2020 and February 2022 from dogs admitted to the Veterinary Teaching Hospital of Lodi, University of Milan and to veterinary private clinics in the provinces of Monza Brianza and Pavia, in Lombardy region. Anamnestic information was registered for all the animals. ESBL-producing *E. coli* were screened using McConkey agar supplemented with cefotaxime (1 mg/L). Species identification of single colonies was accomplished via matrix-assisted laser desorption/ionization-time-of-flight mass spectrometry (MALDI-TOF MS) using the direct transfer method [3]. Phenotypically confirmed isolates were tested to PCR to investigate the presence of bla_{CTX-M}, bla_{TEM}, bla_{CMY} and bla_{SHV} genes [4, 5]. PCR analysis was performed for *E. coli* phylogroup identification [6]. Statistical analysis was performed to identify risk factors associated with ESBL/AmpC-producing *E. coli* presence. Out of the 82 total rectal swabs, 13 (15.9 %) samples were positive for ESBL/AmpC-producing *E. coli* supported by bla_{TEM} (69.2%), bla_{CTX-M} (61.5%) and bla_{CMY} (30.8%) gene detection. No positivity was found for bla_{SHV} gene. In general, a single or two resistance genes were detected in six strains while one strain only harbored three genes. The pathogenic phylogroup B2 was the most frequently detected (30.8%). Statistical analysis showed that antimicrobial therapy was significantly associated with ESBL/AmpC-producing *E. coli* (OR=6.08 [95 % CI: 1.19– 1.02], *P*=0.0416) whereas sex, age, hospitalization, type of ownership, season and geographical origin of dogs were not identified as risk factors. Our results confirm that domestic dogs may play a role as hosts of ESBL/AmpC-producing *E. coli* highlighting the need of surveillance programs and antimicrobial stewardship to reduce the emergence and spread of resistant bacteria. Further investigations are needed to genetically characterize the ESBL/AmpC-producing *E. coli* isolates in order to understand the potential zoonotic role.

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VIROLOGICAL INVESTIGATION IN WILDLIFE ADMITTED TO THE VETERINARY TEACHING HOSPITAL IN PISA

Maria Irene Pacini, Micaela Sgorbini, Mario Forzan, Maurizio Mazzei

Università degli Studi di Pisa, Dipartimento di Scienze Veterinarie.
Corresponding author: MI. Pacini (mariairene.pacini@phd.unipi.it)

Research on wildlife diseases has been recognized as a crucial aspect of wildlife conservation projects, such as reintroduction and translocation programmes, and also of disease surveillance programs for domestic animals and humans [1]. Likewise, it has been widely accepted that wildlife health can be considered an indicator of ecosystem health. Wildlife admitted at wildlife rescue centers may play a sentinel key role representing an often unexploited source of crucial information about pathogens circulating in ecosystems, mainly in wildlife/domestic interface [2]. Furthermore, the wildlife rescue centers represent a system in which multiple injured, diseased, and stressed animals interact and where the risk of pathogen introduction, amplification, and spread is remarkable. Therefore, the knowledge of the sanitary status of wild animals circulating in the area of the rescue activity helps to improve the knowledge of the circulating pathogens. Moreover, the release of the recovered animals in wild habitats can lead to the spread of pathogens on the territory, representing a health risk for the populations that live there.

The Veterinary Teaching Hospital (VTH) of the Department of Veterinary Sciences of the University of Pisa provides a 24-hour emergency service for injured wildlife ungulates, large carnivores, mustelids, and porcupines.

The present study was carried out on injured wildlife animals admitted to the VTH from September 2020 to September 2021. Animals older than 12 months, of both sexes, belonging to roe deer, fallow deer, fox, badger, porcupine, and pine marten species, were included. At admission, each animal was submitted to a complete clinical exam, and fecal sample collection. Fecal samples were stored at −20°C until analysis. A species-specific molecular analysis panel was performed on the basis on previous literature.

A total of 47 animals were included in the study: 21/47 roe deer, 3/47 fallow deer, 12/47 foxes, 6/47 badgers, 1/47 pine martens and 4/47 porcupines. Three out of 12 foxes (25%) and 2/6 badgers (33%) tested positive for canine adenovirus (CAdV) type1; 1/21 roe deer (5%), 2/12 foxes (17%) and 2/4 porcupine (50%) tested positive for kobuvirus (KoV); 3/21 roe deer (14%), and 5/12 foxes (42%) tested positive for astrovirus (AstV).

Results from the molecular investigations are in line with what has been already reported in Italy or other European countries and confirm the presence of various viral pathogens in wildlife animals in the studied area [3-6].

Noteworthy, some of these positives represent the first detection in the studied species, underlining the importance of research and health monitoring in wild species as a method for the identification of emerging or re-emerging pathogens. Moreover, our results underline the important role of wildlife rescue centers in the disease surveillance and that hospitalized wildlife animals must be considered, and consequently managed, as a source of transmissible pathogens, although most of them are asymptomatic.

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USE OF MOLECULAR ASSAYS FOR THE IDENTIFICATION AND GENETIC CHARACTERISATION OF FELINE IMMUNODEFICIENCY VIRUS (FIV) IN SEROPOSITIVE CATS

Veronica Facile (1), Laura Gallina (1), Maria Chiara Sabetti (2), Alessia Terrusi (1), Lorenza Urbani (1), Francesco Dondi (1), Andrea Balboni (1), Mara Battilani (1)

(1) Alma Mater Studiorum – Università di Bologna, Dipartimento di Scienze Mediche Veterinarie. (2) Università di Parma, Dipartimento di Scienze Medico-Veterinarie.

Corresponding author: A. Balboni (a.balboni@unibo.it)

Feline immunodeficiency virus (FIV), genus *Lentivirus*, is responsible for feline immunodeficiency syndrome in domestic cats (1). The prevention and control of disease caused by FIV are primarily based to direct measures, in particular to the identification and isolation of infected subjects. Indirect diagnostic methods, which are the most widely used in clinical practices, showed limitations in the early and terminal stages of infection: in these cases, the use of direct molecular tests is recommended (2). In this study, different molecular assays targeting the Long Terminal Repeats (LTR) region or the *env* gene (3) were used and compared to each other to detect FIV proviral DNA in blood samples from seropositive cats (SNAP Combo Plus FeLV Ag/FIV Ab, IDEXX, USA) referred to the University Veterinary Hospital of the Department of Veterinary Medical Sciences (University of Bologna) in 2018-2021 to confirm their infection status. The identified viruses were genetically characterized analyzing the hypervariable regions V3-V4 and V3-V5 of the FIV *env* gene. Fifty FIV seropositive cats were included in the study: 98% domestic short-haired cats, 80% males and with median age of 9 years and 3 months. Fourty-six of 50 cats tested positive by PCR assay targeting the LTR region and 43/50 cats tested positive by at least one of the five different PCR assays targeting the *env* gene used. Two cats were negative in all the used PCR assays, suggesting a false positive serological test result. The nucleotide sequences of the FIV hypervariable regions V3-V4 or V3-V5 were obtained for 28/50 positive cats. From phylogenetic analysis, all FIVs sequenced in this study clustered with subtype B reference viruses, except one FIV which differed from all currently known subtypes and which showed a low percentage of identity with all FIV sequences analyzed, suggesting that it could belong to a to date unreported viral subtype. Furthermore, sequence variability was lower in viruses identified in symptomatic cats than in those identified in healthy cats and no recombination sites were detected. The variability of circulating viruses emerged in this study, confirms the need to an integrated approach, adopting different tests, to accurately diagnose the infection status of cats. Furthermore, acquiring information regarding the epidemiology of the territory and the viral evolution of circulating subtypes is essential for the development and use of adequate diagnostic methods.

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ARNA

ELLAGIC ACID AND GALLIC ACID CAN MITIGATE METHANE PRODUCTION AND AMMONIA FORMATION IN AN IN VITRO MODEL OF SHORT-TERM RUMEN FERMENTATION

Michele Manoni (1), Melissa Terranova (2), Sergej Amelchanka (2), Luciano Pinotti (1, 3), Paolo Silacci (4), Marco Tretola (1, 4)

(1) Università degli Studi di Milano, Dipartimento di Medicina Veterinaria e Scienze Animali. (2) ETH Zurich, Agrovet-Strickhof. (3) Università degli Studi di Milano, Coordinating Research Centre: Innovation for Well-Being and Environment. (4) Agroscope (Posieux), Institute for Livestock Sciences.

Corresponding author: M. Manoni (michele.manoni@unimi.it)

Livestock production is of particular importance for human nutrition, but it has a high environmental impact. In particular, ruminants are the major contributors to the total livestock greenhouse gas emissions [1-2]. Several feeding strategies aimed to decrease the environmental footprint of ruminants were and are currently investigated. One of the most promising strategies is based on the dietary supplementation with tannins, that can mitigate methane production and ammonia formation, but also affect feed digestibility in ruminants [3]. The aim of this study was to assess the effect of dietary supplementation with two metabolites of hydrolysable tannins, ellagic acid (EA) and gallic acid (GA), on gas production using an in vitro rumen fermentation system. Rumen fluid was collected from one of four fistulated, lactating cows in each of the four runs. Fermenters were fed with a standard diet (200mg DM hay). Five different supplementation conditions were investigated (% of DM): i) EA 7.5, ii) EA 15, iii) GA 7.5, iv) GA 15, v) EA 7.5 + GA 7.5. After an incubation of 24 h at 39°C, several parameters were measured with the rumen samples: pH, ammonia, total SCFA production, microbial count (bacteria and protozoa) and in vitro dry matter digestibility (IVDMD). After gas phase collection, total gas production, methane and CO_2 were evaluated using gas chromatography. The treatments did not affect microbial count and pH ($P>0.05$). Total SCFA production decreased by 10% after all treatments ($P<0.001$). Total gas production decreased by 10% after all treatments ($P<0.05$). Methane production was significantly decreased by EA 15 (-20%, $P< 0.001$) and EA+GA (-25%, $P< 0.001$) treatments. CO_2 production was also decreased ($P<0.001$), but to a lesser extent as compared to methane. Ammonia production was significantly decreased by EA 15 (-13%, $P<0.001$) and EA+GA (-20%, $P<0.001$). All the treatments except GA 7.5 caused a slight but significant 10% reduction of IVDMD ($P<0.001$). These results showed that EA and GA can decrease gas production but affect digestibility of dry matter. The high doses of EA and GA were used to mimic a diet including natural extracts rich in hydrolysable tannins. Indeed, further work will concentrate on the dosage of tannin-secondary metabolites from rumen samples and on long-term in vitro incubation screening also with lower doses of metabolites, in order to see whether the decrease in gas production and decrease digestibility can be dissociated.

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SWEET VS. SALTY FORMER FOOD PRODUCTS IN PIGLETS SLIGHTLY AFFECT ADIPOSE TISSUE COMPOSITION

Alice Luciano (1), Marco Tretola (1, 2), Sharon Mazzoleni (1), Francesca Fumagalli (1), Luca Ferrari (1), Luciano Pinotti (1)

(1) Università degli Studi di Milano Dipartimento di Medicina Veterinarie e Scienze Animali. (2) Agroscope, Institute for Livestock Sciences.

Corresponding author: A. Luciano (alice.luciano@unimi.it)

The aim of this study is to investigate the possibility to partially replace standard ingredients with two different types of former food products sugary from confectionary production (FFPs-C), and salty from bakery production (FFPs-B). Their effects were evaluated on fatty acid profile (Saturated fatty acids, SFA; Monounsaturated fatty acids, MUFA; Polyunsaturated fatty acids, PUFA; and ω-6/ω-3 ratio) of subcutaneous adipose tissue and on selected metabolites (choline, and two metabolites associated with FFPs consumption, namely theobromine and caffeine) in post-weaning piglets. Thirty-six post-weaning female piglets were randomly assigned to a standard diet (CTR), or two diets in which conventional ingredients were partially replaced by 30% (w/w) of FFPs-C or FFPs-B for 42 days. Growth performance, feeding behavior and diets composition were reported in (1). Blood samples were collected at day 42. All samples have been analyzed at UNITECH OMICs (UNIMI, Italy) using ExionLC™AD system, connected to TripleTOF™6600 System (SCIEX) equipped with Turbo V™Ion Source with ESI-Probe. Untargeted metabolomics analysis was performed in positive and negative mode. Subcutaneous abdominal fat samples were collected from 12 piglets after slaughtering. Fatty acids analysis was performed in both feed and subcutaneous abdominal fat samples by gas-chromatography. Data were analysed using IBM SPSS Statistics software using one-way analysis of variance (ANOVA) to compare means, and Pearson's correlation was performed to study the association between the fatty acids in subcutaneous-fat samples and blood-metabolites. In the CTR diet, PUFA content is 47,95% of fatty acids, while SFA is 28,02% and MUFA content is 24,03%. In the two experimental diets (FFPs-C and FFPs-B) SFA, MUFA and PUFA were equally distributed: in FFPs-C diet, SFA content is 35.32%, MUFA is 37.97% and PUFA is 26.70%; while in FFPs-B diet the SFA content is 27.68%, MUFA is 37.40 and PUFA is 34.92%. When ω-6 and ω-3 ratio was considered, the ratio was higher in FPPs-C compared to CTR and FFPs-B (10.17%, 8.87% and 9.81%, respectively). Fatty acid profile of subcutaneous adipose tissue samples has shown no significant differences ($p<0.05$) between groups for SFA, with the only exception for tendency ($p>0.15$) observed for SFA between CTR and FFPs-B tissue samples. A lower percentage of MUFA were observed in CTR group compared to FFPs-C and FFPs-B group. In the case of PUFA, the FFPs-C group shows the lowest value compared to FFPs-B and CTR groups. When ω-6 and ω-3 ratio was considered, a slight decrease in the ratio ($p<0.05$) was recorded in subcutaneous adipose tissue of animals fed with FFPs-C diet. High positive correlation ($r>0.80$) between choline, and two metabolites associated with FFPs consumption, namely theobromine and caffeine, and MUFA was found. The results indicated that piglets were able to rebalance the fatty acids profile. The main differences were observed for MUFA and PUFA in adipose tissue of CTR animals compared to FFPs animals. Indeed, CTR animals showed lower MUFA compared to FFPs animals, while PUFA were higher in CTR animals vs. FFPs-C animals. Furthermore, MUFA has shown high and positive correlation with choline, theobromine and caffeine, associated with FFPs consumption. Future studies will unravel the synergisms among ex-food-based diets, adipogenesis, lipogenesis, and body fat accumulation in pigs. This study is part of the project: "Susfeed" funded by Fondazione Cariplo (Italy, Ref: 2018-0887). This study was approved by OPBA (UNIMI) and received the authorization by Italian Ministry of Health (N° 405/2019-PR).

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IN VITRO DRY MATTER AND CRUDE PROTEIN DIGESTIBILITY IN DOG BONES AND RAW FOOD (BARF) DIETS

Nicoletta Rovere, Matteo Ottoboni, Eleonora Fusi, Alice Luciano, Luciano Pinotti

Università degli Studi di Milano, Dipartimento di Medicina Veterinaria e Scienze Animali
Corresponding author: N. Rovere (nicoletta.rovere@unimi.it)

Feeding a bones and raw food (BARF) diet has become an increasing trend in canine nutrition. These diets try to imitate the feeding behaviour of the wolf. Thus, BARF diets contain a high amount of animal components like meat, offal, and raw meaty bones, (representing the wolf's prey) combined with comparatively small amounts of vegetable ingredients [1].

This study evaluated the in vitro dry matter and crude protein digestibility in BARF diets. For these purpose eight samples of dogs BARF diets and two commercial dog food, used as reference materials, were analysed and tested in the assay. The BARF diets were based on raw beef and poultry by-products, while the commercial pet food were one dry and one wet. All samples were analysed for dry matter (DM), crude protein (CP), ether extract (EE) and ash content. Furthermore, using an in vitro assay, simulating gastric and small intestinal digestion, both dry matter digestibility (IVD-DM) and crude protein digestibility (IVD-CP) have been measured. Briefly, after the pepsin (39°C for 6h) and pancreatin (39°C for 18h) incubation in the IVD-DM and IVD-CP test, the undigested residues was dried at 105°C overnight. The IVD-DM was calculated from the difference between dry matter in the sample and the undigested residue. The IVD-CP was calculated from the difference between the nitrogen content in the original sample and the nitrogen content undigested residue measured by the Kjeldahl method.

All BARF diets and wet pet food were characterized by high moisture content (DM 380 g·kg-1), while in the case of dry pet food DM content was 920 g·kg-1. On average BARF diets and commercial diets were characterised by the following values, on dry matter basis: CP 368 g·kg-1; EE 442 g·kg-1; ash 52 g·kg-1. All BARF samples and reference materials were characterized by high digestibility values. Both IVD-DM and IVD-CP reached values higher than 80%. Of note in the case of IVD-DM a substantial variability within samples has been observed (Standard Deviation SD ±5.5). While in the case of IVD-CP value observed presented less variability (SD ±1.4). In light of these results, it can be concluded that proposed IDV method has some potential in determining protein digestibility in BARF diets, while the assay seems to be limited for measuring DM digestibility, as indicate by the large SD recorded in the BARF diets. The reason of that it is unclear and merit further investigations.

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THE INCLUSION OF HEMP CAKE IN VEAL CALVES' DIET: EFFECTS ON THE PHYSICAL AND CHEMICAL CHARACTERISTICS OF MEAT

Sheyla Arango, Nadia Guzzo, Lucia Bailoni

Università degli Studi di Padova, Dipartimento di Biomedicina Comparata e Alimentazione.
Corresponding author: S. Arango (sheylajohannashumyko.arangoquispe@phd.unipd.it)

Hemp (*Cannabis sativa* L.) is a rich source of polyunsaturated fatty acids like linoleic and alpha-linolenic acids. Hemp cake, derived from the cold mechanical extraction of oil, can be used in animal feeding to improve the nutritional properties of milk, meat and eggs, as reported in literature [1-3]. The aim of the study was to determine the effect of hemp cake inclusion in the diet of veal calves on the physical properties and chemical composition of meat. Fifty-two male Holstein calves (25±13days of age) were housed in one room of ten pens, divided in two homogeneous groups and fed two concentrates with the same lipid (2.58%DM) and protein (13.38%DM) content. An inclusion of 0% (CTR) and 3% (HM) of hemp cake in solid feed was used. The hemp cake used had 8.70%MS of ether extract (13.3, 17.5 and 69.2% of total FA for saturated (SFA), monounsaturated (MUFA) and polyunsaturated (PUFA) FA, resp.) and derived from seeds of Futura variety. Feeding hemp cake did not affect (P>0.05) the average daily gain (1.50 kg/d) and the concentrate feed intake (2.69 kg DM/d). At slaughter, after 171 days of fattening period, meat samples were taken from the fifth and sixth ribs (*Longissimus dorsi* muscle) of 24 carcasses (12 per treatment) after 24 h post-morten. The samples were vacuum-packaged and stored in a controled room at 2 to 4°C for 6 d. Then, the values of pH, color, cooking loss, shear force, chemical and fatty acid (FA) composition were determined, using the official procedures. No differences on pH (5.73 on average) and colour parameters (L, lightness: 46.6; a, redness: 7.8; b, yellowness:14.9) were observed between the two experimental groups. Cooking weight losses (29.5 vs. 31.1%; P<0.05) and shear force (25.79 vs 36.18 N; P<0.01) values were higher in meat of HM group. The chemical composition of veal meat was similar in the two groups (74.2, 3.7 and 22.7% of water content, intramuscular fat content, and protein content resp. and 2.9 mg/kg of iron). The FA composition of veal meat showed an average of 45.28, 47.18, and 7.54% of total FA respectively for SFA, MUFA, and PUFA. The oleic acid was more important in veal calf's meat and the percentage was higher in HM group (40.27 vs. 38.94% of total FA; P<0.05). The palmitic and the stearic acids were also high but no differences were observed in two groups (C16:0, 26.45 and C18:0, 12.26% of total FA, resp.). An unexpected lower value of alpha-linolenic acid (C18:3 n3) was found in the group with the inclusion of hemp cake in the diet (0.36 vs 0.45% of total FA; P<0.05). Due to the similar values of linoleic acid in the two groups (C18:2 n6, 7.10% of total FA), the ratio between n-6 and n-3 fatty acids was higher in the HM group (16.8 vs. 19.9; P<0.05). To conclude, the inclusion of hemp cake in the diet of veal calves did not changed the meat color, that remains pale (low values of redness and yellowness) and, for this reason, still well accepted by the consumers. Unfortunately, the weight loss and the tenderness after cooking appear to be negatively affected by the hemp cake inclusion. The enrichment of n-3 fatty acids of meat, obtained by the calves receiving hemp cake, was not successful due to a possible low percentage of hemp inclusion in the diet. Further experiments using higher doses are suggested to improve the fatty acid profile of meat.

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CONSUMPTION OF ANIMAL SOURCE FOODS: SURVEY ON THE BEHAVIOR OF ITALIAN CITIZENS DURING THE COVID-19 PANDEMIC

Lucia Bailoni (1), Barbara Saracino (2), Eliana Fattorini (3), Giuseppe Pellegrini (3), Massimiano Bucchi (3), Andrea Laconi (1), Alessandra Piccirillo (1)

(1) Università degli Studi di Padova, Dipartimento di Biomedicina Comparata e Alimentazione. (2) Università degli Studi di Bologna, Dipartimento di Scienze Politiche e Sociali. (3) Università degli Studi di Trento, Dipartimento di Sociologia e Ricerca Sociale.

Corresponding author: L. Bailoni (lucia.bailoni@unipd.it)

This study aimed to collect and analyse data on the behaviour of Italian citizens during the COVID-19 pandemic on the consumption of food of animal origin and the sources and level of information on the risk of infection of eating raw products.

Data were collected within the FISR-COMIS project (FISR2020IP_01119) through a survey-type investigation with questionnaire. The survey was conducted on a national sample, proportional and representative by gender, age, and home area of Italian populations aged over 15 years, between 19 November and 8 December 2021. Data were collected using the Computer Assisted Telephone Interview (CATI) technique for 30% of the sample and the Computer Assisted Web Interviewing (CAWI) technique for the remaining 70%. A total of 1,008 subjects were interviewed; after weighting, the total cases were reduced to 985 to have a sample structure identical to the Italian population with regard to gender, age, and level of education.

Analyses have shown that 84.1% of the respondents consume foods of animal origin in domestic settings at least once a week, but this value dramatically decreases (18.1%) when meal is not consumed at home. In Italy the consumption of raw products is not very widespread: nearly 60% of citizens have never consumed raw meat or raw fish in the last 12 months. About half of the interviewed citizens purchased products of animal origin mainly at the supermarket (50.5%) or in specialized shops (41.4%), whereas direct sale from producers to consumers is uncommon (4.3%). When choosing products of animal origin, Italians consider relevant the following aspects: appearance (64%), origin (55.9%), cost (34.6%), and quality brand or label (31.4%). Only 10.6% of consumers regard as important the environmental, social or ethical aspects. Italian citizens have high or good confidence (79.7% in total) in the control chain of animal-source foods. Only a quarter of the interviewees searched for information on the possible risk of contracting SARS-CoV-2 by consuming foods of animal origin. Notably, news broadcasts have been the main source of information for these citizens. Over three quarters (78.3%) of citizens agree that foods of animal origin may not be a source of virus transmission and over 80% have not changed their consumption habits during the COVID-19 pandemic. Finally, 70.9% of Italians recognizes that the communication on the relationship between COVID-19 and consumption of animal source food has been very inadequate.

Based on the results of the survey and after a public debate mediated by experts, the research group of the FISR_COMIS project has proposed guidelines to promote a better communication considering: sources, cultural background and communication style. The document is available to all public and private organizations at https://www.progettocomis.it/.

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EVALUATION OF THE INCLUSION OF *Ascophyllum nodosum* IN MILK REPLACER FED TO DAIRY CALVES IN PRE-WEANING PERIOD

Scaglia Elena, Serena Reggi, Irene Ferri, Sara Frazzini, Matteo Dell'Anno, Silvia Grossi, Carlo Angelo Sgoifo Rossi, Luciana Rossi.

Università degli Studi di Milano, Dipartimento di Medicina Veterinaria e Scienze Animali (DIVAS.)
Corresponding author: E. Scaglia (elena.scaglia@unimi.it)

The neonatal period represents a crucial phase in dairy cattle, in fact, calf is subjected to a wide variety of challenges and stressors that can affect their health and performance and can lead to increased mortality and morbidity rates. In this contest, neonatal diarrhea is a commonly reported disease in young animals with a negative economic impact on the animal production worldwide. The economic losses can be attributed to the direct costs related to the mortality, treatments and labor, but also to long-term effects on performance [1-3]. For these reasons, the maintenance of the gut health in young animals was prioritized as one of the most important issues in the recent years, and a priority in line with the global strategies to tackle the antibiotic resistance. The close relationship between diet and the gut health underlines the pivotal role of the nutrition in the prevention of enteric disorders. In particular, nutrients and bioactive compounds can modulate the gut microbiota and its metabolic activity, reduce the oxidative stress and inflammation [4]. Actually, many feed ingredients have shown functional activities, among them Ascophyllum *nodosum,* a brown alga, have shown an important antioxidant and antibacterial activity demonstrated through *in vivo* and *in vitro* studies [4]. Therefore, the aim of this study was to investigate the inclusion of *Ascophyllum nodosum* in milk replacer fed to dairy calves in pre-weaning period (ethical authorization number 129_2021). Twelve calves were divided into two groups by individual weight randomly: control group (CTRL, n=6) fed milk replacer; treatment group (TRT, n=6) fed milk replacer including 10 g of *Ascophyllum nodosum*. At the birth, the calves received the same good-quality colostrum (IgG concentration >50mg/mL), afterwards, from the time the animals entered the trial they were fed the same feeding plan for 42 days: animals received 4 L/day at the beginning of the trial (T0) by a feeding bottle twice daily, by a one-liter increment per animal every week. *Ascophyllum nodosum* was administered once a day by dissolving it in milk. The individual body weight was evaluated weekly. Fecal score and fecal color were evaluated daily to monitor the incidence of diarrhea [5]. Every 2 weeks individual fecal samples were collected for microbiological analysis. Briefly, the bacteria count was carried out on each sample by serial dilutions and plating in order to estimate the number of colony-forming bacterial units (CFU) per gram of feces. In particular, total bacteria, *Lactobacillus* spp. and coliform bacteria were evaluated by selective medium (PCA, MRS and VRBL respectively). At T0 and day 42 (T6) individual blood samples, derived from routine operations, were collected by jugular vein. The integration with *Ascophyllum nodosum* did not affect palatability as well as it did not provide any significant differences in terms of growth performance. Conversely, the incidence of moderate diarrhea (fecal score 2; scale 0-3) in treated calves was reduced by 50,66% (p<0.0113), confirmed by the microbiological analysis. In conclusion, this study, although limited to a few animals, can be considered a good starting point to study the benefits brought by the inclusion of *Ascophyllum nodosum* within the pre-weaned calves' diet. In fact, it was seen that its supplementation led to a decrease in the incidence of diarrhea resulting in improved gut health.

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TRIBUTYRIN SUPPLEMENTATION IN MILK REPLACER AS FUNCTIONAL FEED ADDITIVE FOR NEWBORN HOLSTEIN CALVES

Matteo Dell'Anno, Elena Scaglia, Serena Reggi, Silvia Grossi, Carlo Angelo Sgoifo Rossi, Luciana Rossi

Università degli Studi di Milano, Dipartimento di Medicina Veterinaria e Scienze Animali.
Corresponding author: M. Dell'Anno (matteo.dellanno@unimi.it)

Neonatal calf diarrhoea (NCD) is a well-known and globally diffused disease in dairy farms, which negatively impacts profitability and animal welfare. NCD can be the reason of 75% of calf mortality during the pre-weaning period [1]. To limit the incidence and detrimental effects of NCD, antibiotics are used to treat young animals [2]. Antimicrobial resistance is one of the most important threat worldwide. In line with European policies and recent limitations on veterinary drugs (Reg. UE 6/2019), valuable alternatives are urgently needed to cope with this issue [3]. Functional feed additives contain bioactive compounds that can improve the animals' health status and immune defence [4]. In this scenario, tributyrin is composed of three butyric acid moieties esterified to a glycerol backbone, with antibacterial activity. Moreover, it promotes both gut health maintenance and nutrient absorption [5].

The aim of this study was to evaluate the effect of tributyrin supplementation in pre-weaning Holstein calves on NCD incidence, gut integrity, and performance. At birth, 12 Holstein calves were randomly allotted in two experimental groups that differed for the inclusion of 0.3% of liquid tributyrin in milk replacer on milk powder (CTRL and TRIB group) for 42 days (OPBA authorization number 79_2020). Individual body weight, feed intake, faecal score (0-3; considering diarrhoea ≥ 2) were recorded to assess the zootechnical performance and NCD occurrence. Blood samples were obtained on day 42 to evaluate the serum metabolic profile, diamine oxidase (DAO), glucagon-like peptide 2 (GLP-2), serum antioxidant barrier (Oxy-Adsorbent test) levels by immunoenzymatic and colorimetric assays. TRIB group showed no differences in zootechnical performance, while a statistically significant reduction in NCD occurrence was observed (38.37% for CTRL and 27.91% for TRIB group; $p < 0.01$) considering the whole experimental period compared to CTRL group. In particular, a statistically significant decrease in the frequency of moderate NCD (faecal score = 2) was observed in TRIB group compared to CTRL (27.13% for CTRL and 17.05% for TRIB group; $p<0.01$). Serum metabolites, GLP-2 and antioxidant barrier status did not show differences among groups. Calves from TRIB group revealed a lower serum levels of DAO compared to CTRL (10.37±0.55 and 12.73±0.55 ng/L respectively; $p<0.01$). The supplementation of tributyrin in milk replacer for pre-weaning calves did not influence feed palatability as highlighted by the similar growth performance in the two groups. Obtained results indicate that tributyrin supplementation could prevent the NCD occurrence and support the intestinal integrity. Tributyirn supplementation in calf's milk replacer could be considered a valuable functional feed additive to limit the detrimental effect of NCD during the pre-weaning period. Further studies will be necessary to investigate the effect of tributyrin on gut microbiota in pre-weaning calves.

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WELFARE EVALUATION IN BUFFALO SPECIES BY CLASSYFARM SYSTEM

Domenico Vecchio (1), Giovanna Cappelli (1), Gabriele Di Vuolo (1), Esterina De Carlo (1), Francesca Fusi (2), Valentina Lorenzi(2), Giuseppe De Rosa (3), Fabio Napolitano(4), Luigi Bertocchi(2)

(1) IZSM, CReNBuf, Portici, Italy. (2) IZSLER, CReNBa, Brescia, Italy. (3) Università degli Studi di Napoli Federico II, Agraria, Portici, Italy. (4) Università degli Studi della Basilicata, Scienze Agrarie, Forestali, Alimentari ed Ambientali, Potenza, Italy.

Corresponding author: D. Vecchio (domenico.vecchio@izsmportici.it)

ClassyFarm is an integrated system for the categorization of the farms according to the risk assessment methodology (RA) [1]. It is an Italian innovation for facilitating and improving the collaboration and the dialogue between the breeders and the competent authority, in order to raise the level of safety and quality of food of animal origin. ClassyFarm collects, gather and process data referred to the following evaluation areas: biosecurity, animal welfare, health, antimicrobial usage. It can be applied to several livestock species, included buffaloes. On request of the Italian Ministry of Health, CreNbuf, in collaboration with CReNBa, has developed a checklist (CL) for buffalo welfare assessment, based on RA and included in the ClassyFarm system [4]. The multiple-choice CL consists of 80 items. Each item is scored according to 3 categories: "insufficient", "acceptable" and "excellent" [2].

The assessment system includes non-animal based (N-ABMs) and animal-based measures (ABMs). N-ABMs are divided into 2 macro-areas: Area A (28 items) "Management factors"; Area B (30 items) "Housing factors". ABMs [3] are assessed in Area C (14 items). The CL has been tested in 341 farms, with an average of 353 head (min 10, max 2,240). The average overall welfare value was 61.27% (on a scale 0 to 100%). The values of the specific areas were: A, 60.20%; B, 41.34%; C, 71.55%. At least one potential legislative non-compliance was recorded in 39.80% of the farms. These CL represent a functional and smart tool to assign animal welfare index to each farm, and to improve farm management and housing conditions by using the data collected in each Area, giving answers to consumers and add value to farmers' good practices. The Italian Ministry of Health is promoting the application of this system at European and international levels.

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CHARACTERIZATION OF ANTIOXIDANT AND ANTIMICROBIAL PROPERTIES OF ALGAL EXTRACTS

Sara Frazzini, Irene Ferri, Sara Panseri, Matteo Dell'Anno, Elena Scaglia, Luciana Rossi

Department of Veterinary Medicine and Animal Sciences – DIVAS; Università degli Studi di Milano
Corresponding author: Irene Ferri (irene.ferri@unimi.it)

In animal farming, where antibiotic drugs are often used to cope with critical phases of the pig's life [1-3], new sustainable feed additives or ingredients that can reduce the use of antibiotics are needed. Since antimicrobial resistance (AMR) is one of the most important threats worldwide, according to EFSA guidelines [4, 5], is necessary to investigate new alternative strategies to reduce the problem of AMR. Among all possible feed ingredients proposed, algae, given their composition, could be valuable as functional compound. In addition to their nutritional qualities, algae are a rich source of many biologically active compounds and one of the richest sources of natural antioxidants and antimicrobial compounds [6]. To this end, this study aimed to evaluate *in vitro* the antioxidant and antimicrobial capacity against porcine O138 *E. coli* of four different algae species (*Arthrospira platensis; Ascophyllum nodosum; Chlorella vulgaris; Lithotamnium calcareum*) and a species of cyanobacteria (*Schizochytrium* spp.). Firstly, extracts were characterized for a metabolomic profile by High-Performance Liquid Chromatography (HPLC), using a Restek RP column with programmed gradient flow of 0.1% HCOOH in water and methanol, followed by an analysis of raw data, using Compound Discoverer (CD) 3.3 software. The antioxidant capacity was determined by the ABTS Radical Cation Decolorization Assay testing three different concentrations (100%; 75%; 50%), and the total antioxidant capacity, after six minutes of reaction, was expressed as the percentage of inhibition (PI%). The antimicrobial effect of the extracts against porcine O138 *E. coli* was evaluated following the microdilution bacterial growth method [7]. The growth rate of *E. coli* was estimated, measuring the absorbance, every hour for six hours. The measured OD was converted in $\log_{10}$ of the number of cells/mL. All assays were performed in technical triplicate and three biological replicates that are meant to verify the replicability of the experiment. Data were analyzed using analysis of variance (ANOVA), in particular, one-way ANOVA was used for the antioxidant activity analysis, while for growth inhibition two-way ANOVA, including the effect of treatment, time and their interaction was used. Post-hoc pairwise comparisons were performed through the Bonferroni Sidak's test, and differences were considered statistically significant for $p<0.05$. The results disclose that all extracts showed antioxidant abilities, in particular, *Ascophyllum nodosum* extract exhibited the highest antioxidant effect (57.75±1.44 percentage of inhibition; $p<0,001$) compared to other extracts. Regarding the antimicrobial activity, *Ascophyllum nodosum* was the most effective showing a significant inhibitory activity, in particular, the $\log_{10}$ cells/mL of *E. coli* used as control resulted significantly higher compared to algal extract at a concentration of 25%, and 12.5% after 4 hours (8.45±0.036 and 7.22±0.025 $\log_{10}$ cells/mL, respectively; $p<0.005$). The results obtained from this study disclose that algal and cyanobacteria extracts, and in particular, the extract of *Ascophyllum nodosum* could be valid functional feed ingredients that can be introduced into the pig's nutrition. Its addition would bring nutritional benefits and could be considered a valid alternative for the reduction of antibiotics. Further *in vivo* studies will be conducted to confirm the encouraging results observed *in vitro* also in breeding conditions.

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EFFECT OF DIETARY HEMP SEEDS SUPPLEMENTATION ON NUTRIENT DIGESTIBILITY AND INTESTINAL HEALTH OF ADULT DOGS

Sara Frazzini (1), Elena Scaglia (1), Bianca Castiglioni (2), Paola Cremonesi (2), Filippo Biscarini (2), Valeria Besana (3), Luciana Rossi (1)

(1) Università degli Studi di Milano, Dipartimento di Medicina Veterinaria e Scienze Animali. (2) Consiglio Naziona le delle Ricerche, Istituto di Biologia e Biotecnologia Agraria. (3) Medico veterinario libero professionista, Cavenago di Brianza.
Corresponding author: S. Frazzini (sara.frazzini@unimi.it)

In animals, as well as in humans, a good general state of health is also favored by a healthy gastrointestinal system and consequently by a correct balance of the microbiota present in it [1]. The composition of the microbiota is influenced by several factors, but undoubtedly diet is one of the most influential [2]. Functional ingredients have gained renewed interest in pet nutrition due to their important role in providing health benefits. Due to their quality and nutritional composition and their high content of biologically active compounds, hemp seeds have been used as ingredients to improve the nutritional and health grade of feeds [3]. The aim of this study (ethical authorization n° 142_2021) was to evaluate the effect of dietary inclusion of dehulled hemp seeds on nutrient digestibility and intestinal microbiota of adult dogs. For this purpose, twelve adult dogs, housed at the Cave Canem boarding house, were divided into two experimental groups balanced for weight, sex, age, and body condition score (BCS, assessed on a 0-9 scale): control group (CTRL, n=6) and treated group (HEMP, n=6). Animals were fed twice a day with two isoenergetic, isoproteic, balanced, and complete homemade diets. HEMP diet differed from CTRL diet for the inclusion of 4% in 1 Kg of feed of decorticated *Cannabis sativa* L. seeds with a negligible level of tetrahydrocannabinol according to Regulation (CE) n. 1782/2003. The rations were formulated to cover the day's energy and protein needs (70 g/1000 kcal metabolizable energy); for each animal administered weight of prepared diets considered reproductive status, activity level, age, and BCS. The dogs were observed for 21 days. At the beginning (T0), and at the end of the nutritional trial (T2), fresh fecal samples were collected for digestibility evaluation, rectal swabs were obtained for the evaluation of the gut microbiota and blood samples were collected for evaluation of oxidative stress. The digestibility analysis, carried out by the indirect method of insoluble acid ash [4], showed that, even if there is no significant change between the control diet and the treated one, the percentage of total digestibility increases at T2 ($p<0.05$). Likewise, an increase in the percentage of protein digestibility ($p<0.05$) was also observed. Since the dogs in the study were, prior to the start of the trial, fed a commercial diet the results obtained support the fact that homemade diets are more digestible than commercial ones, this leads to a greater benefit for the animal because the nutritional amount that we administer is more assimilated, allowing greater respect for the daily needs. Regarding the intestinal microbiota, the sequencing analysis revealed a modification in the principal phyla that composed the dog intestinal microbiota. The data obtained highlight that the administration of and homemade diet, with the addition of functional ingredients, can be useful to enrich the microbiota diversity. Finally, analyses conducted on serum showed that the antioxidative barrier strength increased at T2 in the HEMP group compared with CTRL, emphasizing that the addition of hemp seed leads to an increase in endogenous antioxidant ability.

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INTRODUCTION OF A ROBOTIC MILKING SYSTEM IN A DAIRY FARM: EFFECTS ON BEHAVIOR, WELFARE, PRODUCTION AND HEALTH

Giovanni Buonaiuto (1), Damiano Cavallini (1), Julio De Matos Vettori (2), Ludovica Mammi (1), Sara Speroni (1), Loucine Attarian (1), Alberto Palmonari (1), Giorgia Canestrari (1), Francesca Ghiaccio (1), Giulio Visentin (1), Andrea Formigoni (1)

(1) Università degli Studi di Bologna, Dipartimento di Scienze Mediche Veterinarie. (2) Università degli Studi di Teramo, Facoltà di Medicina Veterinaria.

Corresponding author: D. Cavallini (damiano.cavallini2@unibo.it)

Over the last few years, there has been a decrease in dairy farms and an expansion of the remaining ones [1]. In this context, the introduction of new precision technologies makes it possible to help farmers in their daily farm operations [2]. Since the end of the last century, automatic milking systems (AMS) have spread all over the world, allowing animals to go milking without operator conduction [3]. The aim of this research was to analyze how the transition from a traditional milking parlor (TMP) system to a AMS affects the behavior, well-being, production and health of dairy cows. The observational trial was carried out at the teaching dairy barn of the University of Bologna where there was a 5 + 5 herringbone milking parlor and in which two milking robots were installed (Fullwood, Packo, UK). Data collection covered two consecutive periods (of 9 months each) in which milking took place in the parlor (period 1) or by AMS (period 2).

The results show that the introduction of the AMS, as expected, generates an increase in the number of daily milkings (from 2 to a range of 2.5-3.2 milkings/day in the TMP to AMS). This greater number of milkings led to a rise in milk production (+11%, $p<.01$) and unexpected also in milk components (+20% fat and +16% protein, $p<.01$). Also, the ration was changed, a quota of the concentrates shifted from the TMR to the supplementary feed delivered by AMS distributor. This change resulted in greater efficiency in feed utilization (+8% feed efficiency and +5% nitrogen efficiency $p<.01$). Indeed, the supplementary concentrate started to be delivered according to the cattle productivity. From the point of view of animal welfare, a decrease in the state of restlessness of animals was noted and comparable rest time and cycles. The reason is to be found in the greater tranquillity of the cows that can go to milking at the moment they deem most appropriate without changing their routine. Following the transition to the milking robot, the health of the udder at the farm level undergoes a significant improvement (reduction in SCC and mastitis cases), this improvement is due to the milking of the single quarter by the robot, which is less stressful for the tissues of the teats.

In conclusion, the change from the TMP to the AMS increased productivity, feed efficiency, welfare and health of the dairy cows at the University of Bologna dairy barn.

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AN INNOVATIVE WATER REMEDIATION SYSTEM FOR SUSTAINABLE O. mykiss FARMING

Eleonora Buoio (1), Chiara Cialini (1) Simone Livolsi (2), Chiara Bazzocchi (1), Alessia Di Giancamillo (2), Vittorio Maria Moretti (1), Gian Luca Chiarello (2), Annamaria Costa(1)

(1) Department of Veterinary Medicine and Animal Sciences, University of Milan. (2) Department of Chemistry, University of Milan. (3) Department of Biomedical Sciences for Health, University of Milan.
Corresponding author: Annamaria Costa (annamaria.costa@unimi.it)

In aquaculture, the main problems concern water pollution and waste generation [1], which facilitates the emergence of diseases among the reared species, with possible health risk for biodiversity and for humans. The bacterial nitrification process used in biological filtration systems is considered to be the most effective. However, due to its numerous drawbacks, a new strategy capable of enhancing water quality should be developed. Photocatalysis is a low-cost and sustainable process that has been shown to oxidize ammonia in water to harmless molecular nitrogen compounds [2].

The purpose of this study is to assess the influence of photoelectrocatalysis (PEC) in a rainbow trout (*Oncorhynchus mykiss*) circulating water system on the conversion of ammonia into non-toxic molecular nitrogen (gaseous N2).

A multidisciplinary approach was employed to assess the effect of treated water on the health of O. mykiss reared in recirculating aquaculture system (RAS), by comparing three tanks equipped with the biological filter and the photocatalytic-electrophotocatalytic system (treatment) to three tanks equipped only with the biological purification system (control).

The plant has operated as follows: the first stage of 30 days (T0-T1) established the typical aquaculture bacteria in biofilters. In the second stage (T1-T2), from day 30 to day 32, the fish were housed in tanks and the system was switched to photocatalysis. Then, at the beginning of the third phase until the end of the experimental study (day 40), the system was switched to PEC to assess the possible positive effects on water quality and fish health.

The results obtained in the second phase, with the reactor operating in photocatalysis, demonstrated that total ammonia nitrogen (TAN) generated in the tanks was exposed to photoinduced oxidation on the photocatalyst surface, as well as ammonia oxidation up to nitrate via nitrite production as an intermediate species. During the PEC phase, the experimental group's water had a faster abatement and a lower accumulation of NH3 and NO2-, perhaps due to TAN oxidation by photoelectrocatalysis, which resulted in gaseous nitrogen. The decreased concentration of nitrites, which are very harmful to fish life, in the treated tanks is a promising conclusion. The increased nitrate content in the PEC system is irrelevant since fish are not exposed to toxic concentrations of NO3. To evaluate environmental stress response in the six tanks, both physiological and molecular aspects were monitored. There were no differences in growth performance across groups, and no gill changes or abnormalities were discovered.

In addition, the expression of the oxidative stress markers SOD1, GPx1 and GR in the liver of fish reared in the control group tanks were significantly higher with respect to PEC-test group at T3. This suggested a higher oxidative stress exposure in the control group [3]. Finally, TNFα gene in gills showed a significant higher expression in the control group at both T2 and T3 when compared to treated groups. These results could be explained since TNFα is an important component of early inflammatory events in fish [4]. Based on these findings, we can conclude that the electro-photocatalytic filter had a beneficial influence on the welfare of O. mykiss when compared to the traditional biological purification method now in use. Further research should be conducted to assess the long-term efficacy of this novel water filtering device.

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TLR-2 AGONISTS PRIME MACROPHAGES FOR ENHANCED CYTOKINE RESPONSES AND SUSCEPTIBILITY TO AFRICAN SWINE FEVER INFECTION

Giulia Franzoni (1), Susanna Zinellu (1), Elisabetta Razzuoli (2), Lorena Mura (1), Chiara G. De Ciucis (2), Antonio G. Anfossi (3), Simon P. Graham (4), Bernardo Chessa (3), Silvia Dei Giudici (1), Annalisa Oggiano (1)

(1) Istituto Zooprofilattico Sperimentale della Sardegna, Dipartimento di Sanità Animale. (2) Istituto Zooprofilattico Sperimentale del Piemonte, della Liguria e della Valle d'Aosta. (3) Università degli Studi di Sassari, Dipartimento di Medicina Veterinaria. (4) The Pirbright Institute, Porcine Reproductive and Respiratory Syndrome Immunology Group.
Corresponding author: G. Franzoni (giulia.franzoni@izs-sardegna.it)

Toll-like receptor 2 (TLR2) ligands are attracting significant attention as prophylactic and immunopotentiator agents against several viruses. We previously observed that a synthetic diacylated lipopeptide (Mag-Pam2Cys_P48) polarized porcine macrophages towards a proinflammatory antimicrobial phenotype [1]. In this study, we investigated its role in modulating porcine macrophage responses against African swine fever virus (ASFV), the aetiological agent of one of the greatest threats to the global pig industry [2]. Macrophages are the main target of ASFV, and the virus has evolved mechanisms to counteract their responses [3]. Particularly, we analyzed the impact of Mag-Pam2Cys_P48 on porcine monocyte-derived macrophages' (moMΦ) susceptibility to infection and responses to two ASFV isolates of diverse virulence: the attenuated NH/P68 and the Sardinian virulent 26544/OG10. Untreated and Mag-Pam2Cys_P48-treated moMΦ were infected using a multiplicity of infection (MOI) of 1, and our data revelated that Mag-Pam2Cys_P48 did not influence virus infection nor its modulation of surface markers expression (MHC I, MHC II DR, CD14, CD16, CD163) 21 h post-infection. However, Mag-Pam2Cys_P48 treated moMΦ released higher levels of IL-1α, IL-1β, IL-1Ra, and IL-18 in response to infection with the attenuated NH/P68 ASFV compared to mock-infected controls. Higher levels of IL-1β were also observed in supernatants of 26544/OG10-infected compared to mock-infected Mag-Pam2Cys_P48-treated moMΦ (moM(Mag-Pam2Cys_P48)). The effect of this diacylated lipopeptide to influence ASFV ability to grow in moMΦ was also investigated. When infected using a MOI of 0.01, Mag-Pam2Cys_P48 enhanced the ability of both NH/P68 and 26544/OG10 strains to replicate in moMΦ: higher levels of infectious virus particles were detected in culture supernatants of moM(Mag-Pam2Cys_P48) compared to moMΦ at 48 h and 72 h post-infection. Then, we investigated whether other TLR2 agonists similarly modulated moMΦ responses to ASFV. The impact of Mag-Pam2Cys_P48 was compared to other two synthetic diacylated lipopeptides: Mag-Pam2Cys_P80 and Mag-Pam2Cys_Mag1000, which also efficiently polarized porcine macrophages towards a proinflammatory antimicrobial phenotype. Our data reveled that all three TLR2 agonists promoted release of IL-1α, IL-1Ra, and IL-18 in response to the attenuated NH/P68, but not the virulent 26544/OG10, highlighting differences between these ASFV strains. Nevertheless, all the tested diacylated lipopeptides enhanced the ability of both strains to replicate in moMΦ. These data indicate that ASFV developed mechanisms not only to covertly replicate in these cells, but to enhance replication in macrophages in a pro-inflammatory state. Our data suggest caution in the use of TLR2 agonists as prophylactic or immunopotentiator agents against ASFV, and a better understating of how the virus elude**s** host defenses is needed to aid the rational design of therapeutic strategies against ASFV.

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TOXICOLOGICAL AND IMMUNOLOGICAL IMPACT OF GRAPHENE PRISTINE VERSUS GRAPHENE OXIDE IN A CLOSE-TO-HUMAN ANIMAL MODEL: A PILOT STUDY IN SWINE

Giulia Franzoni (1), Paola Nicolussi (1)*, Giovannantonio Pilo (1), Maria Giovanna Cancedda (1), Guotao Peng (2), Alejandro De La Cadena (3), Renzo Vanna (4), Jeremiah Marcellino (5), Ngoc Do Quyen Chau (6), Marco Orecchioni (7/8), Giuseppe Tedde (1), Simona Macciocu (1), Federica Loi (1), Stefania Poddighe (1), Gavina Carta (1), Loredana Secchi (1), Dario Polli (3/4), Yuta Nishina (9/10), Giulio Cerullo (3/4), Ciriaco Ligios (1), Alberto Bianco (6), Andrea Ferrari (5), Bengt Fadeel (2), Lucia Delogu (8/11)*

(1) Istituto Zooprofilattico Sperimentale della Sardegna, Italy. (2) Institute of Environmental Medicine, Karolinska Institutet, Stockholm, Sweden. (3) Dipartimento di Fisica, Politecnico di Milano, Italy. (4) Istituto di Fotonica e Nanotecnologie - CNR, Milan, Italy. (5) Cambridge Graphene Centre, University of Cambridge, United Kingdom. (6) CNRS, University of Strasbourg, Strasbourg, France. (7) La Jolla Institute for Allergy and Immunology San Diego, California, USA. (8) University of Sassari, Faculty of Chemistry, Italy. (9) Precursory Research for Embryonic Science and Technology, Japan Science and Technology Agency, Japan. (10) Research Core for Interdisciplinary Sciences, Okayama University, Japan. (11) Nano-immune lab, University of Padua, Padua, Italy.

Corresponding author: G. Franzoni (giulia.franzoni@izs-sardegna.it)

Graphene-based materials (GBMs) are of considerable interest for biomedical applications, and a through analysis of their biocompatibility is required for their safe clinical translation [1 ,2]. Most of the previous biocompatibility studies on GBMs were carried out in rodents, nevertheless they are not necessarily a good representation of the anatomical and physiological characteristics of humans. The porcine model is unique in terms of its immunological and anatomical similarities to humans, especially with respect to the cardiovascular, urinary, integumentary, and digestive systems [3, 4], thus we carried out a pilot study of GBMs biocompatibility using swine as a model. The toxicological and immunological impact of pristine graphene (GR) and graphene oxide (GO) were investigated through an integrative analytical approach. First, *ex vivo* experiments using peripheral blood mononuclear cells (PBMCs) and macrophages obtained from pigs indicated that GR and GO were biocompatible and triggered the release of pro-inflammatory cytokines. Then, GBMs were injected intraperitoneally (i.p.) into 12-week-old animals. Haematological and biochemical data obtained at 1, 7, 14, and 21 days post-injection showed that neither GO or GR caused any systemic inflammatory or pro-coagulant responses, nor did they trigger any signs of renal or hepatic dysfunction. Moreover, the analysis of a panel of 84 immune-related genes revealed that only a limited number of genes were modulated by the two different GBMs. Animals were sacrificed 21 days post-injection, and transient absorption imaging as well as Raman mapping showed the presence of GO and GR in the mesentery, but not in the liver, spleen, or kidneys of exposed animals. Histological evaluation revealed no signs of alterations in these organs, but clusters of both materials were detected in the mesentery and the GO aggregates (up to 30 mm) were surrounded mainly by macrophages with formation of granulomas, whereas modest local reactions were observed around the GR clusters (up to 0.2 mm), with T cells being the main population recruited. Overall, these results reveal that i.p. injection of GBMs resulted in a modest local tissue reaction without any systemic toxicity. Our study carried out in a large animal model provides important guidance for future biomedical applications of these materials.

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PERIPHERAL BLOOD BIOMARKERS MAY ASSIST IN THE EARLY DETECTION OF ENDOMETRITIS IN THE TRANSITION DAIRY COW. A PRELIMINARY STUDY

Bisconti M. (1), Barberio A. (1), Granato A. (1), Zulian L. (1), Toson M. (2), Gabai G. (2), Dacasto M. (2), Giantin M. (2), Stefani A. (1)

Istituto Zooprofilattico Sperimentale delle Venezie, Legnaro PD. (2) Dipartimento di Biomedicina Comparata e Alimentazione – BCA, Università di Padova.

Corresponding author: M. Bisconti (mbisconti@izsvenezie.it)

Clinical (CE) and subclinical endometritis (SCE) are diseases that affect dairy cows during the puerperium, producing negative effects on fertility and milk production (1). In this preliminary study, we assessed the association between the occurrence of endometritis and isolation of bacteria in the uterus in order to promote a rational use of intrauterine antibiotics in postpartum. Additionally, we evaluated the expression of target genes involved in the inflammatory and immune response, as well as the variation of serum biochemical parameters, i.e. acute phase proteins and markers of oxidative stress in healthy and diseased (CE or SCE) cows in order to identify potential early biomarkers of disease.

A longitudinal study was performed following 50 cows during the transition period. Cows were randomly selected every week according to parity and transition management out of about 400 cows scheduled to calving. For each cow, blood samples were collected on day 7 before calving, and 7 and 21 days after it. Complete blood cell count and lymphotypization were performed on EDTA tubes; biochemical profile, including haptoglobin, reactive oxygen metabolites and OXY test, were evaluated in serum samples. Moreover, total RNA from whole blood sampled in PAXgene Blood RNA Tubes (Becton Dickinson, Heidelberg, Germany) was used to carry out the gene expression analysis by qPCR of some factors involved in the inflammatory and immune response (cytokines, L-selectin, complement factors and haptoglobin) as well as in the antioxidant response (CAT, SOD, NQO1, GPX1, GPX3, GSTA1, GSTM1, GSTP1 and Nrf2). Twenty-one days after calving, uterine swabs were collected to perform bacteriological culture and uterine cytological examination (cytobrush) and an ultrasound gynecological examination was carried out, too.

Based on the results of the ultrasound examination and cytobrush polymorphonuclear (PMN) leukocytes count, cows were classified ex post in three groups: healthy (H), affected by SCE or CE.

The microorganisms most often recovered from the endometrium have been classified into two groups: primary pathogenic bacteria and opportunistic bacteria (2). In this study, a correlation between bacteria isolation and endometritis was obtained only when CE and primary pathogens were considered.

In cows affected by CE and SCE, an increase in total leukocyte counts (WBCs) was observed ($P<0.05$). Moreover, a decrease in cholesterol, glucose, and microelements together with a simultaneous increase in parameters of muscle stress and liver metabolism (CK and AST) were observed.

The flow cytometric lymphotypization of the peripheral blood highlighted a decrease in CD4+ T lymphocytes before calving ($P<0.05$) and an increase of CD21+ B lymphocytes before and immediately after parturition ($P<0.05$) in both CE and SCE affected cows.

Among the selected target genes, known to be involved in the modulation of the inflammatory and immunological response, only a significant increase ($P<0.05$) of interleukin-10 and haptoglobin in mRNA levels was observed 7 days after calving and merely in cows affected by CE.

As a whole this study suggests that only CE is associated with a uterine infection caused by primary bacteria.

On the other hand, the expression of IL-10 and haptoglobin genes in the blood at 7 days postpartum may assist in the early detection of CE and, therefore, be potentially included in a panel of blood biomarkers for the diagnosis of endometritis in cows, whose usefulness clearly needs validation studies on a higher number of dairy.

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COW AND GOAT MILK EXTRACELLULAR VESICLE EFFECTS ON TWO IN VITRO MODELS OF HUMAN INTESTINAL INFLAMMATION

Samanta Mecocci (1), Alessio Ottaviani (2), Elisabetta Razzuoli (3), Livia de Paolis (3), Floriana Fruscione (3), Giulia Franzoni (4), Silvia Dei Giudici (4), Paola Fiorani (2), Daniele Pietrucci (5), Giovanni Chillemi (5), Katia Cappelli (1)

(1) Università degli studi di Perugia, Dipartimento di Medicina Veterinaria. (2) Università di Roma Tor Vergata, Dipartimento di Biologia. (3) Istituto Zooprofilattico Sperimentale del Piemonte, Liguria e Valle d'Aosta, Oncologia Veterinaria e Comparata - Centro Di Referenza Nazionale (Cerovec). (4) Istituto Zooprofilattico Sperimentale della Sardegna, Dipartimento di Sanità Animale. (5) Università della Tuscia, Dipartimento per la Innovazione nei sistemi biologici, agroalimentari e forestali (DIBAF).

Corresponding author: K. Cappelli (katia.cappelli@unipg.it)

Among their known functions, Extracellular Vesicles (EVs) can modulate immunity and inflammation. An EV role in the pathogenesis of chronic inflammatory diseases such as inflammatory bowel disease (IBD) has been found. IBD, mainly composed of Crohn's Disease and Ulcerative Colitis, have a dramatically rising incidence in western countries and lacks a resolutive therapy. Several factors seem to contribute to its onset, but IBD are certainly characterized by immune dysregulation and barrier function disruption. Milk-derived EVs (mEVs) can have immunomodulatory and anti-inflammatory effects and milk is one of the most promising food sources of EVs. In this context, the aim of this study was to evaluate bovine and goat mEV anti-inflammatory and immunomodulating effects on two in vitro models of human bowel disease: a co-culture of Caco-2 and THP-1 cells, and IPEC-J2 cells, an accepted swine model.

To this purpose, mEVs were isolated and characterized following the methods used in our previous study [1], a proinflammatory environment was induced through IFN-γ and LPS stimuli, cell gene expression was evaluated through RT-qPCR in SYBR Green chemistry and an ELISA test was applied to confirmed cytokine release.

The pro-inflammatory environment instauration was assessed through the significant up-regulation of the proinflammatory cytokines IL17A, CXCL8, IL1B, TNFA, IL12A, IL23A, as well as TGFB1, and the cellular damage indicators NOS2 and MMP9 in inflamed cells compared to those in basal culture conditions. The administration of mEVs led to a partial restoration of initial homeostatic conditions highlighted by a statistically significant decrease of most of the tested cytokines together with a significant down-regulation of MMP9 and the up-regulation of MUC2 and TJP1.

These results indicate, in addition to the clear effect on inflammation which is reduced following the mEV administration, beneficial effects of mEVs on the intestinal barrier in an attempt to restore cellular homeostasis and mucosal functions.

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A PRELIMINARY STUDY OF PTX3 EXPRESSION IN AMNIOTIC FLUID AND PLACENTA IN DOGS

Alessia Inglesi, Joel Filipe, Paola Dall'Ara, Paola Roccabianca, Silvia Dell'Aere, Debora Groppetti, Federica Riva

Università degli Studi di Milano, Dipartimento di Medicina Veterinaria e Scienze Animali.
Corresponding author: A. Inglesi (alessia.inglesi@unimi.it)

The long pentraxin 3 (PTX3) is a soluble pattern recognition molecule expressed in response to inflammatory stimuli [1]. It acts as a humoral component of the immune response against pathogens, but also as a modulator of sterile inflammation, playing a key role in female fertility [2-4]. Our study aimed to characterize the expression pattern of PTX3 by qPCR and immunohistochemical (IHC) analysis in dog placenta and amniotic fluid (AF) of eighteen mothers. This study, performed by Italian Law, was approved by the Ethical Committee of the Università degli Studi di Milano (OPBA_77_2017). Placenta samples with necrotic or other lesions were PTX3 positive. PTX3 was intensely expressed by the amnios, the cells of glandular chambers, and the marginal hematoma, while moderately expressed by fibroblasts in the connective tissue, the cytotrophoblast, and the endothelium of maternal vessels. PTX3 expression was not observed in the stroma of villi, despite the presence of villus infarcts or fibrosis. Gestational age was positively correlated to PTX3 mRNA expression ($p<0.05$). Amniotic PTX3 mRNA expression showed a higher tendency ($p<0.1$) in pups with longer gestational age. Placental PTX3 expression varied between healthy and pathological puppies with a gradual significant increase from score 0 to 2 ($p=0.0005$). Our results support a link between amniotic PTX3 and foetal development and gestational age in dogs as previously described in humans. Therefore, PTX3 expression can be investigated as a diagnostic inflammation marker in a larger canine cohort and amniotic fluid could represent a potential prognostic substrate to assess neonatal maturity.

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EVALUATION OF ECPV2 PREVALENCE IN ITALY

Livia De Paolis (1), Chiara Grazia De Ciucis (1), Katia Cappelli (2), Samanta Mecocci (2), Tiziana Nervo (3), Maria Ines Crescio (4), Marco Pepe (2), Rodolfo Gialletti (2), Laura Migone (1), Luca Mechelli (2), Gian Guido Donato (3), Katia Varello (1), Paola Modesto (1), Alessandro Ghelardi (4), Elisabetta Razzuoli (1)

(1) Istituto Zooprofilattico Sperimentale del Piemonte, Liguria e Valle d'Aosta, Centro Di Referenza Nazionale oer l'Oncologia Veterinaria e Comparata (CEROVEC). (2) Università degli Studi di Perugia, Dipartimento di Medicina Veterinaria. (3) Università degli Studi di Torino, Dipartimento di Scienze Veterinarie. (4) Istituto Zooprofilattico del Piemonte, Liguria e Valle d'Aosta, Sede Centrale di Torino. (5) Azienda Usl Toscana Nord-Ovest, UOC Ostetricia e Ginecologia, Ospedale Apuane.

Corresponding author: L. De Paolis (livia.depaolis@izsto.it)

Equine squamous cell carcinomas (eSCCs) represent the most common epithelial malignant tumor arising from non-pigmented skin and muco-cutaneous junctions, such as eyelids and external genitalia of both male and female horses [1]. To date, *Equus caballus* Papillomavirus type 2 (EcPV2) has been suggested as the etiological agent of genital eSCCs in horses [1, 2]. Most recently, studies provided evidence of similarities between human and eSCCs PVs-induced, thus proposing the horse as a spontaneous model for human disease [2, 3]. Few studies reporting the EcPV2 prevalence among healthy horses are present, with none of them including italian horses [4,5]; actually, that lack of data does not permit to identify at risk populations and, thus, to develop screening protocols aimed to the early detection of the infection, as for humans [6]. The aim of our study was to estimate the genoprevalence of EcPV2 in clinically healthy horses resident in Italy and to evaluate innate immune response. To this purpose, following the breeding season (2020-2021), 234 healthy horses (200 mares and 34 males) from different regions of Italy were observed at two Veterinary Teaching Hospitals, Perugia (OVUD) and Turin (OVU). Penile and vulvar swabs were collected through sampling with sterile cytobrushes from those horses displaying no sign of neoplastic lesion or PVs infection. DNA and RNA was extracted from cytobrush using QIAamp DNA/RNeasy Mini Kit (Qiagen) according to manufacturer's instructions and EcPV2-*L1* presence and expression was checked by RT-qPCR as well as host gene expression to evaluate the innate immune response. Our results confirmed EcPV-2-*L1* DNA presence in 71/234 (30.3%) samples and *L1* expression in 48% of positive samples. Overall, positive samples were tested for *E2*, *E6* and *E7* oncogene expression: all samples resulted positive for EcPV2-*E6* and *E7*, whereas only 76% (54 out of 71) was positive to EcPV2-*E2*. No statistical significant differences were found in genoprevalence associated with sex, age and origin. English Thoroughbred resulted the breed with major risk of infection compared to other breeds (OR 3.3, 95% CI, 1.8-6.2). Concerning the mares, the 40.2% (51 out of 129) of animals resulted positive to EcPV2; our findings showed a major positivity in pluriparous (p=0.0111) and mares under natural mating (p=0.0037). A significant difference in EcPV2-genoprevalence between mares with and without foal was highlighted (p=0.0452). These findings suggested a sexual transmission of PVs in horses as in men; indeed, according to our results, the asymptomatic EcPV-infection appeared to be more common in breeding horses and responsible for hypofertility, thus leading to significant economic losses. Concerning the host innate immune response, samples expressing L1 showed an increased expression of *IL1B* (p=0.0139) and *IL12p40* (p=0.0133) and a decreased expression of *RANKL* (p=0.0229) and *TGFB* (p=0.0177). These findings could suggest the presence of an effective immune response, which could eventually explain the low eSCCs incidence in positive horses, despite a high EcPV2-genoprevalence (30%).

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ENVIRONMENTAL EXPOSURE OF WILD BOAR TO CADMIUM AND ITS IMPACT ON MACROPHAGES

Chiara Grazia De Ciucis (1), Valentina Ciccotelli (1), Lucia Masiello (1), Antonio Giovanni Anfossi (2, 3), Barbara Vivaldi (1), Mauro Ledda (2), Susanna Zinellu (4), Silvia Dei Giudici (4), Enrica Berio (5), Andreoli Tiziana (6), Monica Dellepiane (6), Simona Zoppi (7), Chiara Masotti (7), Maria Ines Crescio (8), Annalisa Oggiano (4), Carlo Ercolini (1), Giulia Franzoni (4), Elisabetta Razzuoli (1)

(1) National Reference Center of Veterinary and Comparative (CEROVEC), Istituto Zooprofilattico Sperimentale del Piemonte Liguria e Valle d'Aosta. (2) Department of Veterinary Medicine, University of Sassari. (3) Mediterranean Center for Disease Control (MCDC), University of Sassari. (4) Department of Animal Health, Istituto Zooprofilattico Sperimentale della Sardegna. (5) Department of Imperia, Department of Istituto Zooprofilattico Sperimentale del Piemonte, Liguria e Valle d'Aosta. (6) Department of Savona, Istituto Zooprofilattico Sperimentale del Piemonte Liguria e Valle d'Aosta. (7) Laboratory of Veterinary Pathology, Istituto Zooprofilattico Sperimentale del Piemonte Liguria e Valle d'Aosta. (8) Biostatistic, Epidemiology and Risk Analysis, Istituto Zooprofilattico Sperimentale del Piemonte Liguria e Valle d'Aosta.
Corresponding author: C.G. De Ciucis (chiaragrazia.deciucis@izsto.it)

Cadmium ($Cd2^+$) is one of the most toxic heavy metals [1], and through environmental contamination enter the food chain enhancing the bioaccumulation [1]. In Liguria, wild boar (*Sus scrofa*) were utilised as a bioindicator for organic pollutants [2] during the hunting seasons between 2016 and 2020. One-thousand-two-hundreds-seventy-one kidney and liver samples were analyzed and the levels of cadmium were determinated with an atomic absorption spectrometer; this quantification showed higher prevalence of contamination in the eastern part of the Region [3]. In one of these polluted areas, 21 wild boar were additionally sampled and the ratio between hepatic and renal $Cd2^+$ concentration was monitored, since it could represent a valid index of intoxication degree (Chronic <1/Acute>1). In particular, our data showed that most animals presented chronic intoxication of this heavy metal. Moreover, $Cd2^+$ is able to induce a strong immunosuppression and decrease of resistance to infections [4]. In the past, we investigated the impact of 2 or 20 μM of $Cd2^+$ on porcine enterocytes (IPEC-J2 cells), using doses previously defined as 'low' (2 μM) or 'moderate' (20 μM) [5]. According to our results, IPEC-J2 cells were able to absorb $Cd2^+$, with subsequent decreased in their viability and modulation several pro-inflammatory molecules [6]. In this context, to investigate the role of cadmium in immunomodulation, we utilized five healthy wild boar as blood donors for in vitro experiments [4, 7]. We investigated the effects of scalar doses of $Cd2^+$ on wild boar macrophages (moMϕ). MoMϕ viability was evaluated with a LDH assay. This showed a reduction of macrophages vitality after treatment with 2 and 20 μM Cd2+, but not accompanied by a release of IFN-β, antiinflammatory or pro-inflammatory cytokines determined by ELISA and multiplex method, respectively. After 24h treatment with 20 μM of Cd2+, RNA was extracted from macrophages by RNeasy Mini Kit (Qiagen) according to manufacturer's instructions. The exposure to the heavy metal determined a down-regulation of gene expression of TNF-α, IL-12p40, TLR3, TLR4, TLR7, TLR8, TLR9, CD14, MD2, BD2, MyD88, p65, and NOS2. The results of 2016-2020 monitoring suggested that wild boar can be useful as bioindicator of environmental contamination in highly urbanized areas and can help understand the type of contamination. In addition, *in vitro* experiments on macrophages revealed that Cd2 negatively affected the immune function of these cells, likely leading to increased susceptibility to infection.

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TOBACCO SEEDS AS A MODEL OF MULTIVALENT EDIBLE VACCINES FOR WEANED PIGS

Serena Reggi (1), Matteo Dell'Anno (1), Eric Cox (2), Antonella Baldi (1), Luciana Rossi (1)

(1) Università Degli Studi di Milano, Dipartimento di Medicina Veterinaria e Scienze Animali (DIVAS). (2) Ghent University, Laboratory of Immunology, Department of Translational Physiology, Infectiology and Public Health, Faculty of Veterinary Medicine. Belgium.

Corresponding author: S. Reggi (serena.reggi@unimi.it)

Post-weaning diarrhea (PWD) and edema disease (ED) are the major problem in piggeries worldwide and result in significant economic losses due to mortality, weight loss, growth retardation, and treatment cost [1]. Verocytotoxic and enterotoxigenic *E. coli* strains (VTEC and ETEC) are the major pathotypes involved [2]. Main current control strategies require the use of antimicrobials, however, the emergence of resistance among *E. coli* isolates and new European recommendations about the therapeutic use of ZnO highlight the need for alternative control measures. Plant-based oral vaccines provide an economically feasible platform for the large-scale production of vaccine antigens for animal health, and edible seeds provide a natural encapsulation of recombinant protein, ensuring stability in planta and after harvest in the final feed formulation [3, 4]. Based on previous results [5], the aim of this study was to develop tobacco plants for the seed-specific expression of antigens of major *E. coli* pathotypes of piglets. The gene sequences of F4 adhesive fimbriae (FaeG), F18 adhesive fimbriae (FedF), and the B subunit of the verocytotoxin-e (VT2e-B), optimized from plant expression, were cloned into a binary vector under the control of soybean β-conglycinin promoter for the seed-specific expression (pBI-Congl). Tobacco has been extensively used as an edible vaccine candidate for its fast growth and large number of seeds [6]. Tobacco leaf disks were agroinfected with three clones of *Agrobacterium tumefaciens* EHA105 carrying pBI-Congl-FaeG, PBI-Congl-FedF, and pBI-Congl-Vte2-B respectively. Ten different lines of transformed and regenerated tobacco plants were obtained for each antigen. PCR and Southern analysis, carried out on genomic DNA extracted from leaves, confirmed the stable integration of the genes in R0 and R1 generation. The western blotting analysis, carried out on total soluble proteins of seeds, confirmed the specific expression of FedF, FaeG, and Vt2e-B genes. On the contrary, western blotting carried out on the leaf, culm, and root did not show any specific signal. The use of engineered plants was an interesting alternative for the production and seed-based delivery of vaccines. The simultaneous inclusion in the animal diet of the seeds from the FaeG, FedF, and Vt2e-B tobacco lines can be considered as interesting multivalent oral vaccine candidates against the major virulence factor of the main *E. coli* pathotype in the weaning period. This research is under patent control (Patent Filling 102021000006461). Obtained data encourage further *in vivo* evaluations of the engineered tobacco seeds' efficacy against challenges with pathogenic *E. coli* strains.

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IMMUNOMODULATORY EFFECTS OF GOAT MILK EXTRACELLULAR VESICLES (MEVS) ON SWINE MACROPHAGES FROM EX VIVO PRELIMINARY EXPERIMENTS

Giulia Franzoni (1), Livia De Paolis (2), Chiara Grazia De Ciucis (2), Samanta Mecocci (3), Annalisa Oggiano (1), Katia Cappelli (3), Elisabetta Razzuoli (2)

(1) Istituto Zooprofilattico della Sardegna. (2) Istituto Zooprofilattico Sperimentale del Piemonte, Liguria e Valle d'Aosta, Centro Di Referenza Nazionale oer l'Oncologia Veterinaria e Comparata (CEROVEC). (3) Università degli Studi di Perugia, Dipartimento di Medicina Veterinaria.

Corresponding author: E. Razzuoli (elisabetta.razzuoli@izsto.it)

Extracellular vesicles (EVs) are lipid-bilayer nanometric structures playing a pivotal role in cell to cell communication and being present in every biological fluid including blood, urine, bronchoalveolar lavage, fluid, saliva and bile [1]; in particular, EVs are able to modulate immune response and inflammation of targeted cells by transporting bioactive molecules, such DNAs, mRNAs, microRNAs, lipids, and proteins [2]. Most recently, there has been a growing interest in milk EVs (mEVs) due to its cost effectiveness and large-scale production; indeed, it has been demonstrated mEVs ability of modulating immunity [3]. Since macrophages played a crucial role in innate immune response [4], also in cancer, our study aimed to evaluate mEVs immunomodulatory effects in swine macrophages from ex vivo preliminary experiments. mEVs were extracted from goat bulk tank milk through preliminary differential centrifugation followed by ultracentrifugation step [3]. Monocytes were isolated from swine PBMC, differentiated into macrophages and splitted in 4 groups. Two groups were left untreated (Mφ), while other two were exposed to IFN-γ and LPS in order to achieve M1 polarization, classically activated macrophages. Then, cells were treated with $5*10^8$ mEV; untreated cells were used as controls. Following the treatment, total RNA was extracted at selected time points (24 h and 48 h) and gene expression was assessed using primers of targets involved in innate immune response and reference genes.

Our findings highlighted in Mφ treated at 24h a significative upregulation of IL6, IL1Beta, TNFalfa, IL12p35, EBI3, TLR2, TLR7 and TLR9 accompanied by a downregulation of NFkBp65 and CD14. At 48 h treated Mφ showed a significative increase of IL6, TNFalfa, Il12p40, BD1, TLR9. Concerning the polarized M1 cells, mEVs treatment at 24 h led to an upregulation of IL12p40, IL10, IL18, EBI3, TLR5, TLR9. At 48 h mEVs treatment in M1 determined an increase of IL12p40, IFNbeta, NFKBp65, CD14, EBI3, TLR2, TLR8, TLR9. Our results outline a pro-inflammatory effect of mEVs in swine macrophages. In particular, the cells phenotypes could be defined as is antimicrobial and antitumoral.

Our data outline the ability of these vesicles to modulate innate immunity also in swine macrophages. This data requires further studies to be confirmed; however, they provide an important basis for the possibility to use them as antimicrobial and anticancer molecules.

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IN VITRO IMMUNOMODULATORY EFFECTS OF GOAT MILK EXTRACELLULAR VESICLES (MEVS) IN SWINE ENTEROCYTES

Chiara Grazia De Ciucis (1), Livia De Paolis (1), Samanta Mecocci (2, 3), Daniele Pietrucci (4, 5), Giovanni Chillemi (5), Floriana Fruscione (1), Katia Cappelli (2, 3), Elisabetta Razzuoli (1)

(1) Istituto ZooprofilatticoSperimentale del Piemonte, Liguria e Valle d'Aosta, Centro di Referenza Nazionale per l'Oncologia veterinaria e comparata (CEROVEC). (2) Università degli Studi di Perugia, Dipartimento di Medicina Veterinaria. (3) Università degli Studi di Perugia, Sports Horse Research Center (CRCS). (4) CNR, IBIOM, Institute of Biomembranes, Bioenergetics and Molecular Biotechnologies. (5) Università della Tuscia, Dipartimento per l'Innovazione nei Sistemi Biologici, Agro-alimentari e Forestali (DIBAF).

Corresponding author: C.G. De Ciucis(chiaragrazia.deciucis@izsto.it)

Extracellular vesicles (EVs) are nanometric spherical structures, enclosed in a lipid bilayer membrane and secreted by multiple cell types under specific physiological and pathological conditions [1]. In particular, their complex cargo has been proved able to modulate immune cells within an inflammatory microenvironment [2]. Among the most promising food sources of EVs, milk extracellular vesicles (mEVs) have increasingly drawn interest, with their immunomodulatory potential being confirmed by several studies [3, 4] and anti-inflammatory effects *in vitro* [5, 6] In this context, aim of this study was to characterize goat mEVs immunomodulating activities on porcine intestinal epithelial cells (IPEC-J2) after establishing a proinflammatory environment due to LPS or H_2O_2 stimuli. EVs were harvested from goat bulk tank milk through several differential ultracentrifugations, as previously described [5]. IPEC-J2 were exposed to 10^8 concentration of mEVs for 24h and 48h, respectively. IPEC-J2 were exposed to purified lipopolysaccharides (LPS; 1 µg/mL from Escherichia coli 0111:B4) or 200 µM H_2O_2; after 2 hours of LPS or H_2O_2 stimuli, cells were exposed to mEVs at a concentration of 10^8/mL. Total RNA was extracted (RNeasy mini Kit, Qiagen) at selected time points (24h and 48h) and gene expression was tested by RT-PCR using primers of targets involved in innate immune response and reference genes (GAPDH). Cell exposure to LPS or H_2O_2 caused a pro-inflammatory response characterized by increase of IL-6, TNFA, IL8 and NOS2 gene expression. The exposure to 10^8 mEVs in LPS model determined down regulation of TGFB and IL12p35. At the same time, we observed an increased expression of TLR1, TLR2, MUC2 and EBI3. Concerning H_2O_2 stimuli, we observed an increase of TLR2, 7, 8, IL8 and NOS2 gene expression after exposure to 10^8 mEVs. Our data outline the ability of these vesicles to modulate innate immunity also in swine enterocytes.

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SICLIMVET

COMPLEMENTARY FEED FOR THE CONTROL OF PRURITUS IN ATOPIC DERMATITIS IN DOGS

Andrea Marchegiani (1), Alessandro Fruganti (1), Elena Dalle Vedove (2), Benedetta Bachetti (2), Marcella Massimini (2), Cataldo Ribecco (2), Matteo Cerquetella (1), Andrea Spaterna (1)

(1) Università degli Studi di Camerino, Scuola di Bioscienze e Medicina Veterinaria. (2) Research and Development Unit (NIL), C.I.A.M. srl.

Corresponding author: A. Marchegiani (andrea.marchegiani@unicam.it)

Pruritus is a common manifestation in dogs with allergic skin diseases and itching can significantly affect the quality of life of both affected animals and their owners, with even severe repercussions [1]. Pharmacological treatments and complementary feeds that are able to control itching quickly and in the long run are in great demand and attract the attention of many researchers and companies. The aim of this study was to assess the effectiveness of a complementary feed containing flavonoids, stilbenes, and cannabinoids (obtained from vegetable/botanical by-products/vegetable/botanical source) in the control of itching in dogs suffering from atopic dermatitis. Such complementary feed has been shown to be able to reduce the gene expression of ccl2, ccl17, il31ra and tslp in an experimental in vitro model of atopic dermatitis [3]. The primary efficacy endpoint was the reduction of CADESI-04 and pruritus visual analogue scale (pVas) scores. The study protocol was successfully submitted to the Animal Welfare Body of the University of Camerino (protocol code 10/2021). Ten dogs affected by atopic dermatitis, diagnosed according to current guidelines [1, 2], received a hypoallergenic food for the duration of the study. Once enrolled, in the first 6 weeks dogs received the administration of oclacitinib (Apoquel®, Zoetis) twice daily for two weeks and then once daily for 4 weeks. Starting from the fifth week, the administration of complementary feed began, according to the following dosage: twice daily for two weeks and then once daily for 8 weeks. Administration of oclacitinib was discontinued at week 6 in all dogs enrolled in the study, who received the complementary feed up to week 12. In all dogs there was a marked reduction in both CADESI-04 and owner-reported pVas for pruritus in the first four weeks of oclacitinib administration. In the fifth and sixth week of the study (oclacitinib + complementary feed) the trend of CADESI-04 and pVas was the same, as well as from the seventh week onwards for all dogs enrolled in the study. Although data collected are only preliminary, it is possible to highlight that the complementary feed effectively control itching in supplemented dogs, which did not show any adverse event. This study further confirm the ability of selected complementary feed to control dermatological disease manifestation in dogs [4].

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FIRST EVIDENCE OF LACK OF ASSOCIATION BETWEEN BLOOD PHENOTYPES AND *Bartonella* INFECTION IN CATS

Eva Spada (1), Daniela Proverbio (1), Roberta Perego (1), Luciana Baggiani (1), Maria Grazia Pennisi (2), Valeria Blanda (3), Alessandra Torina (3), Francesca Grippi (3), Paola Galluzzo (3)

(1) Università degli Studi di Milano, Dipartimento di Medicina Veterinaria e Scienze Animali. (2) Università di Messina, Dipartimento di Scienze Veterinarie. (3) Istituto Zooprofilattico Sperimentale della Sicilia "Adelmo Mirri".
Corresponding author: E. Spada (eva.spada@unimi.it)

In people, blood types have been associated with both decreased and increased rates of a variety of infections. Little is known about this phenomenon in veterinary medicine. The hypothesis of this study is that blood types B and AB might predispose cats to *Bartonella* spp. infection as the N-acetylneuraminic acid present on their erythrocytes could serve as an attachment site for pathogen colonization. Therefore, 138 feline archived frozen whole-blood samples from a previous blood type epidemiological study [1] were analyzed. Samples had been collected at the University of Milan and Messina between November 2014 and December 2015 and breed, sex, age, region of origin and lifestyle data recorded. Blood typing was previously determined by tube agglutination technique [1]. To increase the number of rare blood types, all frozen type-B and type-AB blood samples were included and matched with a type-A sample from a cat with the same demographic characteristics. If more than one matched frozen type A samples was available, this was also included in the sample population. The final population was 71 type-A cats, 34 type-B cats and 33 type-AB cats. Samples were screened for *Bartonella* spp. DNA using real-time PCR (RT-PCR) [2]. Associations between blood phenotype, demographic factors and positive and negative RT-PCR *Bartonella* spp. status were investigated by Chi-square analysis. Significant factors from univariate analysis were tested with logistic regression. Odds Ratio (OR) with 95% confidence intervals (CI) were calculated. Blood samples were from 55 (39.9%) stray colony cats, 23 (16.7%) shelter cats and 60 (43.5%) owned cats, 81 (58.7%) from northern Italy and 57 (41.3%) from southern Italy. Most, 115 (83.3%), were from domestic shorthair cats, 69 (50.0%) from males and 63 (45.7%) from females. Age was available for 126 cats, with a resultant median age of 2 years (range: four months-16 years); about half (67, 53.2%) were young (≤2 years), 49 (38.9%) were adult (3 to 10 years) and 10 (7.9%) were senior (≥11 years). On RT-PCR analysis 15/138 (10.9%) samples were *Bartonella* spp. positive. There was no significant difference in prevalence (P=0.6857) of RT-PCR positive (46.7%%) and negative (52.0%) type-A cats. There were twice as many *Bartonella* spp. RT-PCR positive type-B cats (40.0%) as RT-PCR negative type-B cats (22.8%), but this result was not statistically significant (P=0.1450). The number of RT-PCR negative type AB cats (25.2%) was not significantly higher (P=0.3107) than type-AB RT-PCR positive cats (13.3%). Univariate analysis showed origination from southern Italy (P=0.0353) and being from a colony (P=0.0269) were significantly associated with RT-PCR *Bartonella* spp. positive status, but logistic regression confirmed only the latter as a risk factor for infection (OR=3.5, 95%CI: 1.12-10.78, P=0.0317). The hypothesis that *Bartonella* infection is associated with feline blood phenotypes was not supported by this preliminary study. Further studies investigating a larger number of infected cats and more infectious diseases will increase our understanding of the role of blood type antigens as pathogen receptors in cats.

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EFFECTS OF EQUINE GASTRIC ULCER SYNDROME (EGUS) ON FITNESS PARAMETERS MEASURED BY INCREMENTAL TREADMILL TEST IN STANDARDBRED RACEHORSES

Chiara Maria Lo Feudo, Luca Stucchi, Giovanni Stancari, Bianca Conturba, Enrica Zucca, Francesco Ferrucci

Università degli Studi di Milano, Dipartimento di Medicina Veterinaria e Scienze Animali.
Corresponding author: C.M. Lo Feudo (chiara.lofeudo@unimi.it)

Equine Gastric Ulcer Syndrome (EGUS) is a highly prevalent clinical disease in horses, which can be classified, based on the localization of lesions, as Equine Squamous Gastric Disease (ESGD) or Equine Glandular Gastric Disease (EGGD). Although EGUS is recognized as a common cause of poor performance in racehorses, its direct effects on athletic capacity have been scarcely investigated, and most reports are based on trainers' expectations [1]. In a study, EGUS was identified as the only cause of poor performance in four racehorses, and treatment with omeprazole was effective in improving performance [2]; other authors reported decreased aerobic capacity, stride length and time to fatigue in racehorses with experimentally induced ESGD, during a treadmill test [3]. Objective investigations about the effects of naturally occurring EGUS on performance quality are lacking. Therefore, the present study aims to evaluate the associations between EGUS severity and some fitness parameters measured during an incremental treadmill test in Standardbred racehorses in training. The records of Standardbreds referred to the University of Milan for poor performance between 2002 and 2021 were retrospectively reviewed. Horses underwent a complete diagnostic protocol, including clinical examination, laboratory analyses, incremental treadmill test, high-speed treadmill endoscopy, lower airway endoscopy, bronchoalveolar lavage cytology, gastroscopy. Those showing other possible causes of decreased performance were excluded from the study, except for lower airway inflammation; in fact, this would have reduced dramatically the population size. However, no association between bronchoalveolar lavage cytology and gastroscopic findings was observed, suggesting that the contribution of airway inflammation in impairing fitness parameters was equal among horses with different EGUS severity. During gastroscopic examination, a 0-4 score was assigned to ESGD, while EGGD was evaluated for absence/presence [1]; a total EGUS score was obtained by adding 1 point to ESGD score in horses showing concomitant EGGD. Fitness parameters obtained during incremental treadmill test included speed at a heart rate of 200 bpm (V200), speed at a blood lactate of 4 mmol/L (VLa4), heart rate at a blood lactate of 4 mmol/L (HRLa4), peak lactate, maximum heart rate (HRmax), minimum pH and maximum hematocrit. The associations between fitness parameters and EGUS and ESGD scores were evaluated by Spearman correlation, while Mann-Whitney test was used to compare them between horses with ot without EGGD. Statistical significance was set at $p<0.05$. EGUS grade was inversely correlated with V200 ($p=0.0025$) and minimum pH ($p=0.0469$), and positively correlated with HRmax ($p=0.0004$); ESGD grade was inversely correlated with V200 ($p=0.0025$) and VLa4 ($p=0.0363$), and positively correlated with HRmax ($p=0.0407$). Although a trend was observed, no significant differences in V200 were observed between horses with or without EGGD ($p=0.073$); in horses with EGGD, HRmax was significantly higher ($p=0.0027$), and minimum pH was lower ($p=0.0087$) compared to horses without EGGD. These results show a negative association between aerobic capacity and EGUS, in particular ESGD; similarly, human athletes affected by gastroesophageal reflux disease show an impaired athletic capacity [4]. Although different hypotheses have been proposed, including abdominal pain and decreased appetite due to lactate accumulation, the underlying mechanisms are still unknown, and it is not clear whether EGUS represents a cause or a consequence of an early lactate accumulation and post-exercise acidosis.

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NEUTROPHIL-TO-LYMPHOCYTE RATIO, MONOCYTE-TO-LYMPHOCYTE RATIO, PLATELET-TO-LYMPHOCYTE RATIO, ALBUMIN/GLOBULIN RATIO AND C-REACTIVE PROTEIN/ALBUMIN RATIO IN DOGS WITH INFLAMMATORY PROTEIN-LOSING ENTEROPATHY

Federica Cagnasso (1), Cristiana Maurella (2), Elena Benvenuti (3), Antonio Borrelli (1), Enrico Bottero (3), Barbara Bruno (1), Riccardo Ferriani (3), Piero Ruggiero (3), Veronica Marchetti (4), Paola Gianella (1)

(1) Università degli Studi di Torino, Dipartimento di Scienze Veterinarie. (2) Istituto Zooprofilattico Sperimentale del Piemonte, Liguria e Valle d'Aosta, Torino. (3) Endovet Associazione Professionale, Roma. (4) Università degli Studi di Pisa, Dipartimento di Scienze Veterinarie

Corresponding author: F. Cagnasso (federica.cagnasso@unito.it)

The neutrophil-to-lymphocyte ratio (NLR) and platelet-to-lymphocyte ratio (PLR) have been recently explored as diagnostic markers in canine chronic enteropathy.[1-3] In the human counterpart, a relationship between C-reactive protein/albumin ratio (CRP/ALB) and the inflammatory bowel disease activity has been found;[4] while a reduced albumin/globulin ratio (AGR) has been evaluated as a prognostic aid in human patients with cancer.[5] However, there is scattered information on the NLR, monocyte-to-lymphocyte ratio (MLR), PLR, CRP/ALB and AGR in canine inflammatory protein-losing enteropathy (PLE). The aims of this study were to compare NLR, MLR and PLR between healthy and PLE dogs, to evaluate NLR, MLR, PLR, serum CRP/ALB and AGR in dogs with PLE at diagnosis (T0) and after 1-month therapy (T1), and their correlation with canine chronic enteropathy clinical activity index (CCECAI). A total of 53 dogs with PLE were retrospectively enrolled. Age, sex, CCECAI, complete blood count, albumin, globulin and CRP were obtained from electronic medical records at T0 and T1. A control group of 63 healthy dogs was built. PLE dogs showed higher NLR, MLR and PLR compared to healthy dogs. At T0, AGR and CRP/ALB correlated negatively and positively with CCECAI, respectively. No correlation was found between NLR, MLR, PLR and CCECAI. A significant difference was found between T0 and T1 for the following parameters: NLR, MLR, PLR, CRP/ALB and AGR. The results suggested that NLR, MLR and PLR are elevated in dogs with PLE and that CRP/ALB and AGR could be used as novel potential markers of disease activity.

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PULMONARY ARTERY STIFFNESS AND RIGHT VENTRICULAR SYSTOLIC TIME INTERVALS IN ASTHMATIC HORSES

Elena Alberti (1), Chiara Bozzola (1), Luca Stucchi (2), Chiara Maria Lo Feudo (1), Giovanni Stancari (1), Bianca Conturba (1), Francesco Ferrucci (1), Enrica Zucca (1)

(1) Department of Veterinary Medicine and Animal Sciences, Università degli Studi di Milano. (2) Veterinary Hospital, Università degli Studi di Milano.

Corresponding author: E. Alberti (elena.alberti@unimi.it)

In human medicine, pulmonary artery stiffness (PAS) and right ventricular systolic time intervals (RVSTIs), such as acceleration time (AT), ejection time (ET) and acceleration time index (AT/ET), are pulsed wave Doppler (PWD) echocardiographic parameters useful to evaluate pulmonary vascular bed. In patients with chronic respiratory diseases, such as asthma, PAS increases while RVSTIs decrease due to remodeling of the vessel wall and consequent increase in pulmonary pressure [1, 2].

As human asthma, equine asthma cause remodeling of the pulmonary artery wall, leading to a decreased pulmonary artery elasticity and consequently to pulmonary hypertension [3]. Therefore, it was hypothesized that evaluation of PAS and RVSTIs can be useful even in asthmatic horses. Recently, a study established the feasibility of PAS in healthy horses [4]. Moreover, only two studies reported RVSTIs in horses with increased pulmonary pressure [3, 5].

The present research aimed to evaluate differences in PAS and RVSTIs among healthy, mild/moderate (MEA) and severe (SEA) equine asthma affected horses and to investigate possible correlation between PAS or RVSTIs and ratio of pulmonary artery to aorta diameter (PAD/AOD) that is an indirect marker of pulmonary hypertension [6]. Finally, PAS and AT cut-off values for diagnosis of SEA were established.

This prospective clinical study was approved by the Institutional Animal Care Committee (OPBA_27_2020) of the University of Milan. Echocardiographic examination and PWD of the pulmonary flow were performed in 23 MEA affected horses and 15 SEA affected horses and the measurements were compared to those of 15 healthy horses [4]. Severe asthmatic horses had a significant higher PAS and lower RVSTIs compared to healthy subjects and MEA affected ones. In addition, heart rate (HR) was significantly higher in SEA affected horses, with HR above the normal range in 7/15 subjects, than healthy and mild asthmatic horses. In order to evaluate possible influence of HR on these measurements, differences between groups were evaluated also for parameters adjusted for HR. Significant differences between SEA horses and others subjects were confirmed also for PASHR and ATHR, indicating that PAS and AT were not influenced by HR variations. Moreover, PAD/AOD resulted positively correlated to PAS and negatively correlated to AT and AT/ET, suggesting that they may be useful as PWD indicators of pulmonary hypertension. Finally, a PAS of 8.18 kHz/sec and a AT of 0.202 sec showed a very high sensitivity (PAS 93.33%; AT 86.67%) and specificity (PAS 86.84%; AT 89.47%) for the diagnosis of SEA.

Given the similarities between equine and human asthma, the results of the present research suggest that modification of the pulmonary vascular bed and pulmonary hypertension, due to severe equine asthma, can induce pulmonary artery stiffness even in horses.

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STRAIN AND SHEAR WAVE ELASTOGRAPHY OF PARENCHYMATOUS AND GLANDULAR ORGANS IN HORSES

Chiara Bozzola (1), Elena Alberti (1), Luca Stucchi (2), Chiara Maria Lo Feudo (1), Giovanni Stancari (1), Bianca Conturba (1), Francesco Ferrucci (1), Enrica Zucca (1)

(1) Università degli Studi di Milano, Department of Veterinary Medicine and Animal Sciences. (2) Veterinary Hospital, Università degli studi di Milano.

Corresponding author: C. Bozzola (chiara.bozzola@gmail.com)

Elastography is a non-invasive diagnostic technique, based on ultrasound, which provides real time information about tissues elasticity [1]. Studies on small animals reported the use of elastography to evaluate spleen and mammary gland and to differentiate malignant and benign lesions [2, 3]. There are only few studies on its use in horses to evaluate ligaments and tendons elasticity [4, 5]. The aim of this preliminary study was to verify the applicability of Strain Elastography (SE), Point Shear Wave Elastography (pSWE) and Shear Wave 2D Elastography (2D-SWE) for the evaluation of spleen, liver, kidney and parotid gland in horses. This study was approved by the Institutional Animal Care Committee of the University of Milan (OPBA_55_2021). A MyLab 9VET machine (Esaote Genova, Italy) was used to perform all the techniques in five healthy horses in order to evaluate, qualitatively and quantitatively, the elasticity of the mentioned parenchymatous and glandular organs. SE showed a medium elasticity of liver and spleen parenchyma, while the kidney and the parotid gland were stiffer. Moreover, the kidney was not evaluated with SE in one horse with BCS 7/9. pSWE measurements of spleen and kidney were fairly repeatable in all subjects, with an average value of 1.6 kPa and 37.8 kPa respectively, while the elasticity values obtained in the liver and parotid gland showed grater variability. Finally, 2D-SWE mean value of spleen elasticity was 2 kPa and that of the liver was 3.1 kPa, while we were unable to acquire repeated measurements of the kidney and the parotid gland with this method.

This study revealed some useful information about possible technical limitations in horses. First of all, the presence of a high amount of subcutaneous fat, which increases the organs depth and attenuates the ultrasound signals, reducing the applicability of SE [6]. Movement of the organs synchronous with breathing, particularly of the liver due to its nearness to the diaphragm, is the principal limitation with all the elastographic techniques [6]. As established in human medicine, 2D-SWE measurement is not feasible in very hypoechoic or anechoic organs, such as the parotid gland [7]. The acquisition capacity of 2D-SWE was also limited by highly stiff and deep organs like the kidney in equines [7]. Despite these limitations, the results suggested a good diagnostic potential for all methods particularly for the evaluation of the spleen and the liver, and for SE and pSWE of kidney and parotid gland. However, this in the first attempt to evaluate parenchymatous and glandular organs in horses with three elastographic methods, and further studies are needed to validate their diagnostic use in equine patients.

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PROGNOSTIC INDICATORS FOR CANINE PARVOVIRAL ENTERITIS: A RETROSPECTIVE OF 76 CASES (2017–2021)

Giulia Maggi, Chiara Ceccarelli, Maria Luisa Marenzoni, Domenico Caivano, Francesco Porciello, Maria Chiara Marchesi

Università degli Studi di Perugia, Dipartimento di Medicina Veterinaria.
Corresponding author: G. Maggi (giulia.maggi@studenti.unipg.it)

Canine hemorrhagic enteritis caused by canine Parvovirus is one of the most common cause of morbidity and mortality in dogs, especially if younger than 6 months. Survival rates for canine parvoviral enteritis (CPE) have been reported to be <9.1 % in dogs without treatment and >60% in dogs with treatment [1]. The treatment for CPE is symptom-based and involves use of anti-emetics, fluid therapy and antibiotics for vomiting, dehydration and secondary bacterial infection [2]. The identification of risk factors for survival of dogs affected by CPE can be useful as prognostic indicators for clinical outcome in these dogs. Potential prognostic indicators have been reported to be associated with decreased survival as body weight, sex and numerous clinico-pathological parameters on hematologic and serum biochemistry profiles [3]. To the best of our knowledge, few studies reported the predisposing factors to CPE in Italy; therefore, the aim of this study was identify prognostic factors associated with survival of dogs admitted to the Veterinary Teaching Hospital of Perugia University with naturally canine parvovirus infection.

Medical records of dogs with CPE admitted to the Veterinary Teaching Hospital of Perugia University from 2017 to 2021 have been reviewed. Seventy-six dogs with clinical signs suggestive of CPE and diagnosis of parvovirosis confirmed by CPV-specific real-time polymerase chain reaction (RT-PCR) from fecal swab were included in the study. From medical records were extrapolated data on signalment, history, clinical examination, hematology, serum biochemistry, diagnostic tests, progression of clinical signs during hospitalization and outcome.

The differences in continuous and categorical variables were separately compared using the t-test and chi-squared test, as appropriate. Variables scoring $p \leq 0.20$ in univariate model or considered to be biologically relevant were included in the multivariable model. Odds ratios and corresponding 95% confidence intervals were obtained by means of logistic regression. Survival of the parvovirus infected dogs was analyzed using the Kaplan-Meier survival analysis. Finally, survival curves were plotted, and the difference between groups were compared using the Log-rank test.

Our model showed the following positive prognostic factors: winter season, male sex, dog ownership and normal heart rate. According to the survival model developed using the Cox curve, it was found that parameters such as male sex, small size, and normal heart rate increased the survival rate during hospitalization.

The identification of prognostic indicators is useful to help the clinicians in the management decision and to provide scientifically based data when making decision regarding hospitalization approaches.

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INHALED MINERAL PARTICLES IN EQUINE ASTHMA: INNOCENT BYSTANDER OR CONCURRENT CAUSE OF DISEASE?

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

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

Corresponding author: M. Bullone (Michela.bullone@unito.it)

Equine asthma is one of the commonest cause of consultation in equine practice, characterized by non-septic inflammation of the lower airways in presence of chronic relevant respiratory signs. Equine asthma is a multifactorial syndrome [1] that can manifest as different disease entities/phenotypes, based on severity and recurrence of clinical signs [2]. Due to their nature and activities, joined to the increased use of synthetical and artificial sand for riding arenas [3], horses are frequently exposed to dusts originating from soil or footing surfaces which may affect respiratory health. High loads of aerosolised inorganic particles, such as respirable crystalline silica (RCS), may play a role in the pathogenesis of equine asthma as, if inhaled, silica induces alveolar inflammation. Giant multinucleated cells (GMCs), resulting from macrophage fusion, are considered a hallmark of the inflammatory response representing a specialization for improved phagocytosis [4,5]. The current work aimed at investigating the implication of inhaled RCS in equine asthma. To do so, we performed a cross-sectional study on mild/moderate and severe asthmatic horses (MEA and SEA, respectively) and control cases. Ethical approval was obtained by the Local Animal Ethical Committee (DVM, UniTO; Prot. N. 711, 17/03/2021). Ten horses per group were studied, for a total of 30 horses. Enrolled horses underwent bronchoalveolar lavage under sedation. Cytospin slides were obtained using 200 µl of unfiltered fresh samples, centrifugated at 500 RPM for 5 min at room temperature and stained with May Grunwald-Giemsa. Differential cell counts were performed for neutrophils, lymphocytes, macrophages, mast cells and eosinophils at 100x magnification on a minimum of 400 cells/5 high power fields (HPF), blinded to the animal ID. The same operator also performed GMC counts on 15 randomly selected HPF per cytospin slide (40x). Lastly, intra- and extracellular putative RCS particle counts (based on morphological and colorimetric standards) were performed on a total of 30 randomly selected HPF per cytospin slide (40x) by polarized light microscopy. Group differences in mean particle load were assessed with Kruskal Wallis test. Association of mean particle load with other variables was assessed with generalized linear models. RCS load was significantly higher in SEA vs. MEA and controls. No significant difference was observed between MEA and controls to this regard. Increased RCS in SEA horses was associated with a statistically significant increase in GMCs in this group, suggesting inhaled RCS as one of the possible causes of disease chronicity in SEA. Conversely, extracellular RCS was significantly higher in MEA and SEA vs. control horses. This is in agreement with a defective mucociliary clearance in both diseased groups. Given the inability of the immune system to effectively process and neutralize RCS once in the lungs, its accumulation in time is invariably linked with chronic inflammatory stimulation. In conclusion, our data support a possible role of silica particles in equine asthma pathophysiology, with a positive association observed between the burden of inhaled RCS and disease severity. Due to the high number of variables and biases that should be considered to this aim, large-scale work is warranted to gain meaningful insights and applicable results in the future.

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A FIRST LOOK AT THE PREVALENCE AND CLINICAL SIGNIFICANCE OF MACROPHAGE FUSION IN EQUINE ASTHMA

Alessandra Romolo (1), Ilaria Basano (1), Giulia Iamone (1), Guilia Memoli (1), Jean-Pierre Lavoie (2), Barbara Miniscalco (1), Michela Bullone (1)

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

Corresponding author: M. Bullone (michela.bullone@unito.it)

Equine asthma is one of the commonest conditions encountered in equine respiratory medicine. Cytological assessment of bronchoalveolar lavage fluid (BALF) is the gold standard technique for the diagnosis of equine asthma. BALF of asthmatic horses is most characterized by an increase in neutrophilic granulocyte differential cell count, defined as greater than 5% in the mild form, and over 25% in the severe one. Increases in the differential cell counts of mast cell (>2%) and/or eosinophil (>1%) can also be observed, concomitantly or alone [1]. Alveolar macrophages are normal resident of the lower airways, primarily in the alveoli, and their fusion produces giant multinucleated cells (GMC). The molecular mechanisms regulating macrophage fusion are ill-defined [2]. In BALF of asthmatic horses, GMC are occasionally observed but completely disregarded. This study focuses on the role of GMC in equine asthma, with the aim of providing further tools to classify equine asthma phenotypes. Our hypothesis was that GMCs are increased in equine asthma compared to control horses, and their number is associated with disease chronicity. We performed a retrospective study on data from asthmatic and respiratory control horses referred to the Veterinary Teaching Hospitals of the University of Turin and Montreal with complete clinical data and still available BALF cytology slides for re-assessment. A total of 34 asthmatic and 10 control horses were studied. There were 21 horses with mild to moderate asthma and 13 with severe asthma. Signalment and clinical data were retrieved from medical files. Cytological data were obtained by re-assessment of all cytospins slides by the same operator, blinded to the animal ID. Differential cell counts and GMC quantification were performed separately. GMC were defined as macrophage-like cells with ≥2 nuclei and their number expressed as GMC:single macrophages (GMC:M) ratio. The mean values of GMC in asthmatic (moderate and severe) vs. control horses were compared with Kruskal Wallis test. Any significant association among the variables studied was investigated by means of multiparameteric linear models. GMC were observed in 70% control BALF samples and almost all asthmatic samples. The presence of GMC was higher in horses affected by the severe phenotype of the disease, in line with its chronicity. We also observed a negative independent association of GMC with mast cell differential counts (percentage), whose interpretation is more challenging. The negative nature of the relationship observed could be due to the fact that relative (percentage) rather than absolute counts were employed. A possible role of mast cells and GMC in equine asthma physiopathology concerns the regulation of mild fibrotic reactions, or the allergy-like response to exogenous inhaled material. In conclusion, our data are in line with recent evidence showing alveolar macrophages play a central role in equine asthma physiopathology, and suggest that further attention should be paid to this cytological aspect of the disease.

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EMERGING DATA ON POSITIVE URINE COLTURE IN CATS WITH NEUROGENIC BLADDER

Annamaria Uva (1), Floriana Gernone (1), Maria Alfonsa Cavalera (1), Grazia Carelli (1), Marco Cordisco (1), Adriana Trotta (1), Rossella Donghia (2), Marialaura Corrente (1), Andrea Zatelli (1)

(1) Department of Veterinary Medicine, University of Bari "Aldo Moro". (2) Unit of Research Methodology and Data Sciences for Population Health, "Salus in Apulia Study" National Institute of Gastroenterology "S. de Bellis" Research Hospital, Bari, Italy.

Corresponding author: A. Uva (Annamaria.uva@uniba.it)

Urinary tract infections (UTIs) are defined as the adherence, multiplication, and persistence of an infectious agent within the urogenital system that causes an associated inflammatory response and clinical sign (1); instead, the presence of bacteria in urine as determined by positive bacterial culture (PUC) from a properly collected urine specimen, in the absence of clinical signs, is defined subclinical bacteriuria (2). On the authors' knowledge, limited data are available for prevalence of UTIs and bacteriuria in cats with urinary incontinence or retention due to neurogenic bladder (NB) associated with chronic thoracolumbar spinal cord injury (SCI). On contrary, in NB dogs and humans the prevalence of bacteriuria is well documented.

The aim of this prospective study, approved by the Ethics Committee of the Department of Veterinary Medicine of Bari (approval number 27/20), was to determine, for the first time, the prevalence of PUC in cats with NB compared to chronic kidney disease (CKD), representing the most common predisposing systemic diseases for bacteriuria in cats, and healthy cats.

Cats of any age, gender, and breeds, were included. For each enrolled animal, signalment data and baseline information [(i.e., clinical history, concomitant treatments relevant to CKD, antibiotics treatment, previous information on laboratory test and imaging, bladder daily management in spinal cord injured cats (manual or with catheterization)] were recorded. Furthermore, a complete physical examination and a complete blood count (CBC), serum biochemistry, electrophoresis of serum proteins, complete urinalysis, urine culture was performed in all included feline population. All urine samples submitted for urine culture were collected by cystocentesis. In cats with NB the neurological examination was recorded too.

Fifty-one cats met the inclusion criteria: 12 cats were affected by NB due to SCI, 22 had CKD and 17 were healthy. The prevalence of PUC was 58,33% and 18,18% in NB and CKD cat populations, respectively.

The incomplete bladder emptying and the decreased resistance in the bladder wall, caused by thoracolumbar SCI, could be considered predisposing elements to PUC in the NB feline population.

The results of this study highlight, for the first time, the very high prevalence of PUC in a feline population affected by NB.

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CONTRAST-ENHANCED ULTRASONOGRAPHY OF FOCAL PANCREATIC LESIONS IN CATS

Silvia Burti (1), Alessandro Zotti (1), Giuseppe Rubini (2), Riccardo Orlandi (3), Paolo Bargellini (3), Barbara Contiero (1), Tommaso Banzato (1)

(1) Università degli Studi di Padova, Dipartimento di Medicina Animale, Produzioni e Salute. (2) Ultravet, Bologna. (3) Tyrus Veterinary Clinic, Terni.

Corresponding author: S. Burti (silvia.burti@phd.unipd.it)

An increasing number of studies has demonstrated the efficacy of contrast-enhanced ultrasound (CEUS) in the characterization of focal pancreatic lesions[1] in human medicine. On the other hand, to date a low number of studies evaluating the CEUS features of normal pancreas[2] and focal pancreatic lesions[3] in cats are available. Therefore, the aim of the study is to describe the CEUS features of focal pancreatic lesions in cats.

A total of 98 cats with focal pancreatic lesions (40 adenocarcinomas, 11 lymphomas, 6 other malignant lesions, 17 nodular hyperplasia, 20 other benign lesions, and 4 cysts), that underwent CEUS and cytopathological examinations between January 2009 and May 2021, were retrospectively collected. Qualitative and quantitative CEUS features of all lesions were described. Differences in the distribution of the qualitative and quantitative CEUS features were calculated. Adenocarcinomas showed both hyper- (20/40) and hypoenhancing (19/40) wash-in, with intralesional microcirculation (27/40), hypoperfused areas were evident in 22 cases, and no wash-out in 27 cases. Lymphomas showed mainly a hyperenhancing wash-in (7/11), with evident intralesional microcirculation (6/11), and hypoperfused areas (6/11), and a hypoenhancing wash-out (6/11). Nodular hyperplasias were all isoenhancing and homogeneous lesions during wash-in phase, with no wash-out. The other benign lesions were mainly hyperenhancing (14/20) and homogeneous (15/20) lesions, with hypoperfused areas in 18 cases, and no wash-out in 17 cases. Cysts showed no contrast enhancement. The other malignant lesions showed mainly a hyperenhancing (5/6) pattern, with evident intralesional microcirculation (4/6) and hypoperfused areas (5/6). Statistically significant differences were evident for all the wash-in and wash-out qualitative CEUS features (except for wash-out homogeneity), and for maximum dimension, and time to peak among quantitative CEUS features.

A machine-learning based decision tree for lesion classification was developed. Based on the decision tree, almost all the adenocarcinoma (34/40), all the nodular hyperplasias, and almost all the other benign lesions (14/20) were correctly classified with a sensitivity of 0.85, 1.00, and 0.70 and a specificity of 0.77, 0.94, and 0.93 respectively. On the other hand, almost all the lymphomas (10/11) were classified as adenocarcinomas. The decision tree showed an overall accuracy of 0.74.

CEUS allowed a good characterization of focal or diffused pancreatic lesions detected at US. Nevertheless, pathological examination remains mandatory to determine the exact histotype of the pancreatic lesions.

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CARDIOVASCULAR CHANGES, LABORATORY FINDINGS AND PAIN SCORES IN CALVES UNDERGOING ULTRASONOGRAPHY-GUIDED BILATERAL RECTUS SHEATH BLOCK FOR UMBILICAL HERNIA REPAIR: A PROSPECTIVE RANDOMIZED CLINICAL TRIAL

Maria Chiara Alterisio (1), Fabiana Micieli (1), Giovanni Della Valle (1), Ludovica Chiavaccini (2), Paolo Ciaramella (1), Jacopo Guccione (1)

(1) University of Napoli Federico II, Veterinary Medicine and Animal Production, Napoli. (2) University of Florida Collage of Veterinary Medicine, Department of Comparative Diagnostic and Population Medicine, Gainesville.
Corresponding author: Maria Chiara Alterisio (mariachiara.alterisio@unina.it)

The frequency wherewith veterinary surgeons still perform herniorrhaphy in the field requires a continuous progression of analgesic techniques to minimize the adverse effects of the procedure and improve the chances of therapeutic success [1]. By evaluating the cardiac dynamics, serum cortisol concentrations, and behavior, the current study aimed to assess ultrasound-guided rectus sheath block (RSB) effects on the health and welfare of calves affected by uncomplicated umbilical hernia undergoing herniorrhaphy under field conditions.

The study methods received institutional approval from the Ethical Animal Care and Use Committee of the University of Naples Federico II (PG/2018/0050013); for all the drugs used the withdrawal times required by the legislation were considered. A group of fourteen calves was randomly assigned to receive either bilateral ultrasound-guided RSB with either 0.3 mL/kg of bupivacaine 0.25% and 0.15 µg/kg of dexmedetomidine (group RSB) or 0.3 mL/kg of 0.9% NaCl (Control group, CG). All animals were monitored by Holter recording to define the effects on the cardiac dynamic and serum cortisol level (SCL) and UNESP-Botucatu pain scale to assess their health and welfare status. Holter monitoring was continuously performed from -120 minutes (min) pre-surgery to +120 min post-surgery dividing the mean results in interval-1 (Int-1) =-120min pre-surgery to the beginning of induction time; Int-2= beginning of induction to end of surgery (EOS-t); Int-3=EOS-t to +120min post-surgery; Int-4=EOS-t to +15min, Int-5=EOS-t to +30min, Int-6=EOS-t to +60min, Int-7=EOS-t to +120min. The SCL was evaluated at -150min pre-surgery (baseline), at induction time, skin incision and EOS-t, as well as at +30min, +45min, +60min, +120min, +360min after surgery. Finally, the pain score was carried out at -150min pre-surgery and at +30min, +45min, +60min, +120min, +240min, +360min post-surgery. No significant difference was observed in heart rate between the two groups. A significant intra-group difference was observed for the RSB group (Int1=99.9±21.0 beat/min vs. Int2=90.7±22.0; mean ±SD, $P<0.01$). SCL was higher in the CG at skin incision (RSB=0.45±0.08 ng/mL ±SD vs. CG=0.82±0.06; mean ±SD, $P<0.01$). Finally, calves receiving RSB exhibited significantly lower median PS ($P<0.05$) at +45min (RSB=1 vs.CG=4), +60min (RSB=1 vs. CG=6), +120min (RSB=0 vs. CG=1) and +240min (RSB=0 vs. CG=1.5).

The current clinical study evaluated the physiological changes associated with using RSB in calves undergoing herniorrhaphy under field conditions for the first time. Although all the techniques selected gave useful information on beneficial effects at the short- and long-term, the overtime combined interpretation of all three made it possible to better define the impact of the procedure. Despite the encouraging results, further studies are needed to confirm the findings before RSB might become a milestone analgesic procedure in bovine medicine.

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SARS-COV-2 SEROSURVEY IN CATS LIVING IN AN ENDEMIC AREA FOR *Leishmania infantum*

Valentina Foglia Manzillo (1), Manuela Gizzarelli (1), Ines Balestrino (1), Nour El Houda Ben Fayala(1), Annalisa Palomba(1), Maria Paola Maurelli (1), Lavinia Ciuca(1), Martina Levante (2), Giovanna Fusco(2), Gaetano Oliva(1)

(1) Department of Veterinary Medicine and Animal Production, University Federico II, Naples, Italy.

(2) Istituto Zooprofilattico Sperimentale del Mezzogiorno, Portici (NA), Italy.

Corresponding author: Valentina Foglia Manzillo (valentina.fogliamanzillo@unina.it)

Since the first episodes of coronavirus disease (COVID-19) several domestic and wild animals have been investigated for their role as reservoir [1]. During the COVID-19 "second wave" pandemic, southern Italy experienced an exponential increase of human cases. The same regions have been historically considered endemic for human and animal leishmaniosis caused by *Leishmania infantum* [2]. Several cases of human co-infection between SARS-CoV-2 and *Leishmania spp*. have been reported all around the world, included in Italy [3]. Cats have been described as a susceptible animal to both infections, also if the pathogenetic role of the SARS-CoV-2 in this species was not definitively demonstrated. The aim of this study was to obtain preliminary data on the presence of SARS-CoV-2 positive cats and their possible co-infection with *L. infantum* in Campania region. One hundred forty cats were included in the study, aged between 6 months and 22 years. Fifty-two cats were owned, the others were stray cats. All the animals were subjected to clinical examination at private Vet Clinics or Public Veterinary Service. Blood samples were collected from all cats and screened for the presence of antibodies against Sars-CoV-2; a sub-sample of 82 cats were investigated also for antibodies against *L. infantum*. Sera samples were quantified by Roche Elecsys® Anti-SARS-CoV-2-S test for spike receptor binding domain (cut off 0.8 U/mL). Antibodies against *Leishmania spp.* were detected by IFAT, using a validated kit for cat provided by CRENAL, Palermo, Italy (cut off 1:40). Of the 140 cats examined for Sars-COV-2-S antibodies, two female stray cats (1.4%; Confidence Interval 95%, CI95%=0.3-5.6), aging two years, resulted positive. One cat showed sneezing and nasal discharge, the second exhibited several clinical signs: gingivitis, stomatitis, upper lip ulcer, dehydration, enlarged popliteal lymph nodes. This cat had an history of calicivirosis and resulted positive at *Leishmania* IFAT (1:40). Of the 82 cats examined for *L. infantum*, 33 (40.2%; CI95%= 29.7-51.7) resulted positive, and among them 13 (39.4%; CI95%= 23.4-57.8) had a titre ≥1:80. Four *Leishmania* infected cats (12.2%; CI95%=4.0-29.1) tested positive to FIV and two (6.0%; CI95%=1.1-21.6) to FeLV. Our results confirm the high prevalence of FeL in the study area and demonstrate that cat may exhibit specific antibodies against Sars-CoV-2. The presence of co-infection seems to be rare but it should be better assessed by the use of molecular specific test.

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METABOLOMIC ANALYSIS OF COLOSTRUM AND MILK IN RAGUSANA JENNIES

Marilena Bazzano (1), Luca Laghi (2), Carmela Scollo (3), Alessandro Fruganti (1), Fabrizio Dini (1), Fulvio Laus (1)

(1) School of Biosciences and Veterinary Medicine, University of Camerino. (2) Department of Agricultural and Food Sciences, University of Bologna (3) Private Practitioner, Catania.

Corresponding author: M. Bazzano (marilena.bazzano@unicam.it)

The growing industry and demand for donkeys is strongly linked to the dairy donkey industry and the production of milk, which is more similar to human milk than other dairy species. This feature makes donkey milk suitable for infants who cannot be breast-fed and people suffering from cow milk protein allergies [1]. Milk metabolomics can also be used in the investigation of physiology of lactation, as the milk metabolites reflect the metabolic activity of the mammary gland [2]. In this study, colostrum (<48h) and milk samples (15th day of lactation) were collected from 20 healthy Ragusana jennies and analyzed by HNMR analysis. Student's T-test was performed for statistical purpose, p values <0.05 were considered statistically significant. A total of 65 metabolites were identified from NMR spectra including sugars, amino acids and derivatives, energy metabolites, fatty acids and associated metabolites, nucleotides and derivatives, and others. A total of 18 metabolites showed different concentrations ($p<0.05$) in colostrum and milk. Namely galactose, galactose-1-phosphate, fumarate, uridine, dimethyl sulphone, creatine phosphate, sn glycerol-3-phosphocholine, o-phosphocholine, o-acetylcarnitine, and ethanol decreased in milk at 15 days of lactation compared to colostrum. Conversely, myoinositol, creatine, acetone, alanine, betaine, valine, glutamate, and caprylate were found at higher concentrations in milk compared to colostrum. Some of the above-mentioned metabolites' trends resemble those seen in human and bovine milk, supporting the hypothesis that also in donkey species both the colostrum and milk metabolomes undergo significant changes over the first two weeks following parturition to support normal newborn development and vital functions.[3,4] The provision of colostrum and its composition and quality is vital to the development and growth of the newborn foal, meanwhile, a deep knowledge of donkey milk quality and composition is of special interest as it represents a main source of food for infants.

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Bartonella henselae MITRAL VALVE ENDOCARDITIS ASSOCIATED TO MULTI-RESISTANT ENTEROCOCCUS FAECALIS BATTERIEMIA IN A CAT

Antonella Colella (2), Grazia Greco (1), Adriana Trotta (1), Marco Cordisco (1), Serena Digiaro (2), Antonella Tinelli (3), Alessandra Recchia (2), Aya Attia Koraney Zarea (3), Paola Paradies (2)

(1) Dipartmento di Medicina Veterinaria, Università degli Studi di Bari "Aldo Moro", Bari, Italia.
(2) Università degli Studi di Bari, Dipartimento dell'emergenza e dei trapianti di organi (D.E.T.O.), Italia.
(3) Department of Microbiology and Immunology, National Research Centre (NRC), Cairo, Egypt.
Corresponding author: P. Paradies (paola.paradies@uniba.com)

A 12-year-old neutered female cat had 1-month history of dysorexia and weight loss. The cat was under treatment with prednisone (1.5 mg/kg PO die) from 4 weeks at time of presentation. On physical examination the cat was hypothermic and dyspnoic with discordant breathing. No heart murmur was audible. Thoracic ultrasound examination demonstrated presence of thoracic effusion. Echocardiography examination revealed an independently iperechoic oscillating lesion attached to mitral valve, suggestive of a vegetative lesion. A sample of blood (2.5 ml) was aseptically collected before starting the antimicrobial treatment, from saphenous vein by using citrate vials. The cat was initially treated with oxygen, furosemide and empirically with ampicillin/sulbactam (20 mg/kg ev TID) plus enrofloxacin (5 mg/kg sc DIE) before results of blood cultures. Twelve hours after hospitalization the cat developed aortic thromboembolism and euthanasia was carried out. The owner consented to perform an autopsy examination. Based on the combined results of *Bartonella* genus-specific qPCR and of cPCR assays targeting the *ssr*A and *pap*31 genes, *Bartonella henselae* DNA was detected in the blood, spleen, liver and pleural effusion samples. From both aerobic and anaerobic blood culturing, a consistent number of smooth colonies was observed. The isolate was identified as *Enterococcus faecalis* by using the API Strep system and 16S rRNA sequencing. The isolate was tested for the antimicrobial susceptibility with Kirby-Bauer method and was found to show a multi-drug resistant pattern. Although a bacteria culture or qRT-PCR has not been carried out on the cat mitral valve proliferation, the postmortem cytological and histological examination revealed the presence of cocci on it, supporting the diagnosis of infectious endocarditis (IE) associated with *B. henselae*. The cat is the primary reservoir for *B. henselae*, the causal agent of the human cat scratch disease [1]. *Bartonella* spp, in particular *B. henselae*, can cause endocarditis and myocarditis in cats, as reported for dogs and human patients [1-3]. In addition, the microbiological evidence of bacteremia due to multi-drug resistant *E. faecalis*, it may have contributed to the worsening of the disease. Enterococci are gram-positive cocci present in the normal intestinal microbial flora of humans and animals [4, 5], but they may cause urinary, intra-abdominal, wound infections bacteremia and IE in different species [4-6]. The resistance of enterococci to adverse environmental conditions and antibiotic resistance have led to the emerging role of these bacteria in nosocomial infections in veterinary medicine as well as in human medicine [4, 5]. Gastrointestinal tract is the suspected port of entry of *E. faecalis* in this cat, accredited by the lymphocytic intestinal inflammation that was found. Although, the urinary tract as origin of bacteremia has not been completely excluded in this case. The prolonged corticosteroid therapy administered in the cat probably played a major role in the development of *E. faecalis* bacteriemia. Despite feline IE is uncommon [3], it should always be considered as differential diagnosis in older cats especially when there are signs of systemic malaise, a history of prolonged corticosteroid treatment or visceral infections.

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INTRACORONARY CYTOPROTECTIVE GENE THERAPY IN A 6-YEAR-OLD ST. BERNARD DOG WITH DILATED CARDIOMYOPATHY: FOLLOW UP POST-TREATMENT

Antonella Colella (1), Lucia Carlucci (2), Serena Digiaro (1), Luca Lacitignola (1), Francesco Staffieri (1), Felix Woitek (3), Beatrice Greco (1), Antonio Crovace (1), Fabio Recchia (2), Paola Paradies (1)

(1) Università degli Studi di Bari, Dipartimento dell'Emergenza e dei Trapianti di Organi (D.E.T.O.). (2) Scuola Universitaria Superiore Sant'Anna Pisa (3) Heart Center Dresden, Technical University of Dresden, Germany. Corresponding author: P. Paradies (paola.paradies@uniba.com)

Dilated cardiomyopathy (DCM) is a myocardial disease of dogs and humans characterized by progressive ventricular dilation and depressed contractility and it is a frequent cause of heart failure (HF). Conventional pharmacological therapy cannot reverse the progression of the disease and, in humans, cardiac transplantation remains the only option during the final stages of HF. Cytoprotective gene therapy with vascular endothelial growth factor-B167 (VEGF-B167) has proved an effective alternative therapy, halting the progression of the disease in experimental pre-clinical studies in dogs [1]. A recent clinical study demonstrated the tolerability and feasibility of intracoronary administration, under fluoroscopic guidance, of VEGF-B167 carried by adeno-associated viral vectors in 10 canine patients naturally affected by DCM [2]. This study reports the clinical and echocardiographic long-term follow up of a 6-year-old St. Bernard dog with DCM and atrial fibrillation included in the above-mentioned study [2] (Ministry Authorization n. 180946122, May 2016). On the day of the procedure (T0) the dog was clinically stable, maintained on standard medical treatment (furosemide, pimobendan, enalapril, digoxin), had normal hematobiochemical parameters and digoxin serum concentration was in the reference range. An echocardiographic and ECG examination was performed at T0. Clinical, laboratory and instrumental (ECG, echocardiography) assessment was performed at 1,3,6,9,12,18,24,36 months post-procedure (T1-T8). Standard echocardiographic parameters were measured and calculated as ejection fraction (EF), end-systolic and end-diastolic volume index (ESVI and EDVI) by Simpson in B mode from left apical four chamber view, EF Teichholz and fractional shortening (FS) in M mode. Sphericity index (SI), E-point to septal separation (EPSS), presence and velocity of mitral and tricuspidal regurgitant jets, left atrium-to-aorta diastolic diameter ratio (LA/Ao) and diastolic pattern from mitral flow were also evaluated. The dog reached T8 in good clinical conditions and was stable in terms of appetite, nutritional status, and vitality. Hematobiochemical and serum digoxin concentration monitoring during all follow-up examinations reported no significant alterations. Comparison of echocardiographic parameters from T0 to the final follow-up examination showed a trend of improved EF: T0 30%, T1 39%, T2 38%, T3 40%, T4 32%, T5 32%, T6 38%, T7 42%, T8 38% (Simpson method). FS did not change (22% at T0; 22% at T8), same as the LA/Ao (2 at T0, 2.1 at T8). EDVI showed an irregular trend, but a mild difference was found from T0 (94.1 ml/m^2) to T8 (110 ml/m^2). Other disease progression parameters varied minimally throughout the study. A tricuspidal regurgitant jet was detected, starting from T4, in addition to the mitral regurgitation already present at T0. From T0 to T8, no relevant change in medical therapy was necessary. The dog survived one more year after the last follow up and the owner was in contact with us to report its clinical status with the support of a practitioner. He died from sudden death 341 days after the last follow-up, i.e. 1436 days after the procedure. Given that the reported survival time of dogs with DCM is approximately 6 months to 1 year from the first diagnosis [3], a 4-year survival in good clinical conditions can be considered a good outcome in our patient. A recent study reported that less than 12% of dogs survived at 500 days from diagnosis [4]. Furthermore, the echocardiographic parameters (including EF) did not significantly worsen during follow-ups, in contrast with the expected derangement in a progressive disease such as DCM. Our results suggest that the administration of VEGF-B167 transgene in this dog suffering from DCM may be effective to slow the progression of the myocardial disease, confirming previous findings in an experimental model [1].

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NEW TOOLS FOR MONITORING HEALTH IN DOGS: VALIDATION OF AN ACUTE PHASE PROTEIN INDEX (API)

Federico Bonsembiante (1, 2), Carlo Guglielmini (1), Martina Baldin (1), Filippo Torrigiani (2), Silvia Bedin (1), Donatella Scavone (3), Maria Elena Gelain (2)

(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. (3) Università degli Studi di Milano, Dipartimento di Medicina Veterinaria e Scienze Animali.

Corresponding author: F. Bonsembiante (federico.bonsembiante@unipd.it)

Acute Phase Proteins (APPs) are part of the innate immune response system, as their concentration or activity changes following any kind of tissue damage [1]. In humans, different studies evaluated the combination of multiple APPs in an index (Acute Phase Index, API) and their results revealed that the simultaneous assessment of various APPs can amplify their ability in monitoring patients' outcome [1, 2]. In veterinary medicine, APIs have been implemented only in cattle, pigs and in a grou p of dogs with Leishmaniosis [3, 4]. However, an API has never been applied to tumour-bearing dogs. The aims of this study were to validate an API and to evaluate its prognostic value in dogs with different neoplastic diseases. Serum samples were prospectively collected from neoplastic dogs, at presentation (treatment-naïve), before chemotherapy administrations and at clinical follow-up. Dogs with other concurrent diseases diagnosed by clinical examination and laboratory tests were excluded. The API was calculated as positive APPs/negative APPs: C-reactive Protein (CRP)*haptoglobin(Hp) / albumin*serum paraoxonase-1 (PON-1). An API without PON-1 was also calculated. Dogs were grouped based tumour type (epithelial, mesenchymal spindle-shaped cells, mesenchymal round-cells and other) and survival time (> or < than 30 or 90 days). Differences in APPs and APIs among the tumour groups were assessed by Kruskall-Wallis test while differences in the survival time were assessed by Mann-Whitney test. Wilcoxon test was used to assess differences in APPs and APIs between the first and the last sample of the same animal. Diagnostic accuracy of APIs in distinguishing survivors from non-survivor dogs at the end of the study was assessed by receiver operating characteristic (ROC) curve. Sixty dogs were enrolled, and multiple samples were collected in 22 of them. Thirteen dogs had epithelial tumours, 18 mesenchymal spindle-shaped cells tumours, 27 mesenchymal round-cells tumours and two other tumours. Dogs with other tumours were excluded from statistical analyses. Forty-nine dogs were alive at 30 days post diagnosis while 39 dogs were alive at 90 days post diagnosis. Epithelial tumours had higher API (median= 0.304; P-value 0.019) and API without PON-1 (median = 0.472; P-value 0.045) compared to mesenchymal ones. The lowest PON-1 activity at first sampling was in mesenchymal round-cells tumors (median= 163.5 U/L; P-value 0.023). In dogs surviving <30 and <90 days, both APIs and CRP concentration were higher compared to that of animals surviving more. A decrease in PON-1 activity was found in all the tumours categories between the first and last sample in dogs with multiple samplings that died before the end of the study (P-value 0.014). API had an AUC of 0.736 (95%CI 0.607-0.842) while API without PON had an AUC of 0.743 (95%CI 0.614-0.847) to predict survival at the cut-off of 0.217. Eighty-four percent of dogs with APIs<0.217 were alive at the end of the study. Our study shows that the APIs could be a promising tool for prognostic purposes in dogs with neoplasia. Moreover, epithelial tumours are associated with a higher degree of inflammation while round cells tumours are associated with a higher oxidative stress, indicated by a decrease of PON-1 activity. Oxidative stress seems to be involved in the progression of the diseases in all the tumours' groups.

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INVESTIGATION ON HORSE' EUTHANASIA: THE VIEWPOINT OF OWNERS

Alessandra Landi (2), Elena Zema (2), Michela Pugliese (1), Vito Biondi (1), Annmaria Passantino (1)

(1) Università degli Studi di Messina, Dipartimento di Scienze Veterinarie. (2) Libero Professionista, Italia.
Corresponding author: M. Pugliese (michela.pugliese@unime.it)

Horse euthanasia represents a practice that should perform with minimum potential stress for the patient and its owner [1]. The current study aimed to evaluate the point of view of a horse's owners regarding euthanasia to improve the management of this procedure by veterinarians in horses. Participants were enrolled through social media and online forum horses to complete a multiple-choice questionnaire regarding the relationship with their horse and the experience of euthanasia. A total of 48 horses'-owners from Sicily (Italy) participated. The 58.3% of participants were female. Horses euthanized have an average age of 13 (SD±6.5). A large part of respondents (68%; n.33) was graduated, faithful (66.7%; n=32) and have only one horse (52.1%; n=25) that considered a companion animal (56.25 %; n=27). 62.5% (n=30) spent time daily with their horse. The majority (72.9%; n=35) of horses euthanized presented a poor prognosis (survival time between 1-30 days). A 72.9% (n=35) accepted the choice of euthanasia but felt anger at the horse's death (54.1%, n=26). A sense of guilt for the horse's death was reported by 35.4% (n=17). The role of the veterinarian in making-decision euthanasia was considered central for 56.25% of respondents (n=27). In 58.3% of cases (n=28) the veterinarian expounded detailly the procedure and 39.5 % (n=19) decided to be present during the procedure. A large part not only deepened emotional bonds with the veterinarian but confronted him about the horse's death. Although potential biases due to recruitment source should be considered, data obtained have shown that the horse euthanasia decision-making is also influenced by a correct explanation of the procedure. In this context, the veterinarian plays a crucial role because he must not only consider the provision of high-quality health care [2] but also evaluate the degree of attachment of the owner towards his horse, considered often as a companion animal, and support the owner during the grieving process. Experts in veterinary communication suggest an owner-centered approach to conducting end-of-life and euthanasia discussions [3, 4]. A good explanation of the euthanasia procedure and a dialogue focused on the patient could reduce the stress on the owner. Also exploring owners' feelings, beliefs, expectancy, or the effect of the animal's disease on the owner and the animal's lives is decisive.

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COLONIZATION WITH METHICILLIN-RESISTANT STAPHYLOCOCCUS PSEUDINTERMEDIUS IN DOGS WITH PRESUMPTIVE DIAGNOSIS OF ATOPIC DERMATITIS: PERSISTENCE AND CLINICAL IMPACT

Michele Berlanda (1), Elena Spagnolo (2), Daniela Pasotto (1), Carlotta Valente (1), Andrea Pivetta (1), Helen Poser (1), Carlo Guglielmini (1), Michela Corrò (2)

(1) Università degli Studi di Padova, Dipartimento di Medicina Animale, Produzioni e Salute. (2) Istituto Zooprofilattico Sperimentale delle Venezie.

Corresponding author: M. Berlanda (michele.berlanda@unipd.it)

Methicillin-resistant *Sthaphylococcus pseudintermedius* (MRSP), is a frequent cause for concern in veterinary dermatology because of difficult-to-treat secondary skin infections in dogs. Information about colonization, persistency of MRSP and implication of MRSP in canine atopic dermatitis is lacking. In addition, the role of environmental contamination and transmission of these bacteria has yet to be fully understood [1]. The present study aimed to monitor over time the presence of MRSP strains in a group of atopic dogs, in cohabiting conspecifics and their living environment. We also investigated whether Canine Atopic Dermatitis and Severity Index (CADESI-04) scores were affected by colonization of MRSP strains.

This prospective study was carried out on dogs visited at the Veterinary Teaching Hospital of the University of Padua with bacteriological analyses performed at the diagnostic laboratory of the Istituto Zooprofilattico Sperimentale delle Venezie as a part of the research project "RC IZSVE 16/18" financed by the Italian Ministry of Health. We reviewed a dataset of 854 skin bacteriological exams, and we identified 16 dogs (11 dogs with a presumptive diagnosis of atopic dermatitis and MRSP, and 5 dogs cohabiting with the former). Considering atopic dogs, we retrospectively searched for the previous bacterial culture results and stored strains. All dogs were then recruited. Clinical history mainly focused on previously recorded antimicrobial treatments. After a complete physical and dermatological examination, CADESI-04 scores were calculated. Samples for bacteriological culture from one armpit, mouth, genitals and body sites of previous infections were collected once a month for six months from each dog. In addition, samples were also collected from the environment where the dogs were living. MRSP colonies were isolated by bacteriological examination and the presence of methicillin resistance gene (mecA) was confirmed by PCR. Bacterial species were verified by MALDI-TOF mass spectrometry. Susceptibility to antimicrobials was determined by the minimum inhibitory concentration (MIC) method. Normal distribution of the data was tested using the Shapiro-Wilk's test. Differences between groups (mecA positive vs mec A negative) were studied using the Mann Whitney test. Value of $P < 0.05$ was considered statistically significant.

Of 229 bacterial cultures, *Staphylococcus* spp. strains were isolated in 146 samples. Seventy-one of 146 cultures (49%) were coagulase-positive. Sixty-four of 71 strains were confirmed as *Staphylococcus pseudintermedius* and 31 of 71 (48%) were mecA positive and multidrug-resistant [2]. Sixteen of 31(48%) were isolated from cohabiting dogs and their living environment. Combining retrospective and prospective data the median MRSP positivity in atopic dogs was 6 months (range 2-20 months). The CADESI-04 scores did not appear to be affected by the colonization of MRSP (p=0.378).

The living environment and the cohabiting dogs could play a significant role in the persistence of MRSP strains, thus representing a public health problem. Further studies may provide a clearer understanding of the clinical significance of these results.

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LUNG ULTRASONOGRAPHY AS A DIAGNOSTIC SCREENING TOOL AND POST-TREATMENT MONITORING WITH TULATHROMYCIN AND KETOPROFEN IN FATTENING BULLS AFFECTED BY BOVINE RESPIRATORY DISEASE (BRD)

Anastasia Lisuzzo (1), Elisa Mazzotta (2), Andrea Beltrame (3), Barbara Contiero (1), Matteo Gianesella (1), Eliana Schiavon (2), Rossella Tessari (1), Massimo Morgante (1), Enrico Fiore (1)

(1) University of Padua, Department of Animal Medicine, Production, and Health. (2) Experimental Zooprophylactic Institute of Venezie (IZSVe). (3) Veterinarian free practitioner.

Corresponding author: A. Lisuzzo (anastasia.lisuzzo@phd.unipd.it)

The clinical presentation of the bovine respiratory disease (BRD) is commonly used for the diagnosis in-field. However, the non-specific manifestation frequently may lead to a misdiagnosis. These contexts represent the critical points in animal's health management and economic losses. The aim of this study was to evaluate the thoracic ultrasonography (TUS) in the assessment of fattening bulls with BRD during the restocking period and the response to tulathromycin and ketoprofen treatment of animals with BRD over 21 days. Animal care and procedure were in accordance with the Guide for the Care and Use of Laboratory Animals and Directive 2010/63/EU for animal experiments. Sixty Limousine fattening bulls were enrolled in this study from a single beef-fattening herds located in Veneto, Italy. The fattening bulls received a clinical examination for body temperature, cough, nasal, and ocular discharges by veterinarian to establish the clinical respiratory score (RS) [1]. The TUS of six lung's areas [caudal (10th–7th intercostal space (ICS)), middle (6th–5th ICS), and cranial (4th–3rd ICS) of both lung sides] was performed to establish the ultrasonography score (US)[2]. The lung lesions such as hepatizations areas and fluid alveolograms were measured for the six lung's areas and to assess the total lung consolidation. The lesion score (LS) was calculated for each six lung areas by converting the lesion findings into a numeric scale. The global lesion score (GL) represents the total of the LS of each investigated lungs area. According to US the control group (Group C; 29 animals; US<3) and the disease group (Group D; 31 animals; US≥3) were established. The TUS assessments were performed at time 0 (T0; day of restocking and treatment for group D) and time 5 (T5; after 21 days) both in group C and group D. Moreover, group D was evaluated via TUS at time 1 (T1; after 1.5 days), time 2 (T2; after 3 days), time 3 (T3; after 7 days), and time 4 (T4; after 14 days). The differences between groups and over time were evaluated by mixed models with the significance set at $p \leq 0.05$. The RS, body temperature, nasal, and ocular discharges, GL, total hepatization, and total fluid alveolograms were greater in group D at T0, whereas only RS was greater at T5. The disagreement between TUS and RS, and the 40% of sick animals in group C based on RS suggest the inaccuracy of RS in the detection of BRD in beef fattening bulls. The most affected areas of the lungs were the cranial and middle lobes with greater hepatizations and fluid alveolograms areas. Furthermore, the difference in total hepatization between groups reduced from 8.05 cm^2 at T0 to 2.50 cm^2 at T5. The reduction in the size of the lesions is reasonably the consequence of recovery processes of the lung tissue, and the reduction of the inflammatory process response due to early antibiotic and anti-inflammatory administration with the resolution of acute pneumonia due to BRD. The treatment was effective in improve the US, and body temperature after 1.5 days, nasal, and ocular discharges after 14 and 3 days, respectively. The total hepatization showed an improvement after 1.5 days, whereas the GL and total fluid alveolograms improved after 3 days, suggesting a better health status of lungs with a resolution of acute lesions. This study confirms that TUS evaluation on the arrival of fattening bulls is a useful diagnostic tool for screening animals and to monitor the effectiveness of the treatment.

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SERUM METABOLOMICS ASSESSMENT OF ETIOLOGICAL PROCESSES PREDISPOSING KETOSIS IN WATER BUFFALO THROUGH THE 1H-NMR SPECTROSCOPY

Anastasia Lisuzzo (1), Enrico Fiore (1), Luca Laghi (2), Kevin Harvatine (3), Elisa Mazzotta (1,4), Maria Chiara Alterisio (5), Paolo Ciaramella (5), Chenglin Zhu (6), Barbara Contiero (1), Vanessa Faillace (1), Jacopo Guccione (5)

(1) University of Padua, Department of Animal Medicine, Production, and Health. (2) University of Bologna, Department of Agro-Food Science and Technology. (3) University of Pennsylvania, Department of Animal Science. (4) Experimental Zooprophylactic Institute of Venezie. (5) University of Napoli, Department of Veterinary Medicine, and Animal Production. (6) Southwest Minzu University, College of Food Science and Technology.

Corresponding author: A. Lisuzzo (anastasia.lisuzzo@phd.unipd.it)

A negative energy balance is still one of the major concerns that may decrease the productivity in buffaloes and predispose to other disorders as ketosis which is characterized by elevated concentrations of β-hydroxybutyrate (BHB). Nevertheless, a specifically BHB threshold for dairy buffaloes was not establish and dairy cows' reference are often used [1]. The aim of this study was to use 1H-NMR to assess the metabolomic profile of Mediterranean buffaloes in early lactation to investigate the metabolic changes associate with different levels of energy deficit. This study received the approval by the Ethical Animal Care and Use Committee of the University of Napoli (protocol number 0099607/2017). Sixty-two Italian Mediterranean Buffaloes (MBs) were selected from a single dairy farm located in Campania, Italy, in early lactation (32±12 days in milk (DIM)). All animals received a clinical examination and BCS evaluation on a 9-point scale. Blood samples were collected by clinical healthy subjects into clot activator tubes to obtain serum. According to serum BHB concentration, MBs were divided into two groups [2]: healthy group (Group H; 37 MBs; BHB<0.70 mmol/L) and group at risk of hyperketonemia (Group K; 25 MBs; BHB≥0.70 mmol/L). The differences between groups were assessed by one-way ANOVA for normally distributed data, and Wilcoxon Test for not normally distributed. The significance threshold was sept at p-value ≤ 0.05. Parameters that presented a p-value between 0.05 and 0.1 were considered as trend to significance. A total of fifty-seven molecules was characterized in MBs serum samples. Six of the quantified metabolites were different between groups: glycerol, taurine, and creatinine reduced in group K, while acetone, acetate and 3-hydroxybutyrate increased in the same group. Additionally, other six metabolites were tended to significance: methanol, proline, and glycine reduced in group K, whereas formate, citrate, glutamate increased in the same group. An increment of body resources mobilization was evidenced by the reduction of glycerol and creatinine. Glycerol may be used for gluconeogenesis or for an increase in milk fatty acids synthesis, a characteristic of ketosis; whereas creatinine derives from muscular metabolism and can be used to supply energy. The altered concentration of acetate, methanol, and formate suggested an alteration of ruminal microbial population and fermentation according to BHB [3]. The reduction of proline, glycine, and taurine may suggest an initial alteration of urea cycle other than a potential state of oxidative stress due to the increased use of glutathione and hypotaurine systems. Furthermore, glutamate and taurine changes may be related to the thyroid hormone synthesis [4]. The reduced concentration of proline and glycine may also suggest a potential alteration of Krebs cycle according to the progressive increment of BHB, even if glutamate and citrate were increased in group K [3]. In conclusion, the increment of BHB below the cut-off of dairy cows revealed different relationships with an early development of ketosis by a metabolomic approach.

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RISK FACTORS FOR ATRIAL FIBRILLATION IN DOGS WITH DILATED CARDIOMYOPATHY

Carlotta Valente (1), Giovanni Romito (2), Chiara Mazzoldi (2), Marco Baron Toaldo (2), Marlos Goncalves Sousa (3), Marcela Wolf (3), Tamyris Beluque (3), Oriol Domenech (4), Valentina Patata (4), Francesco Porciello (5), Paolo Ferrari (5), Domenico Caivano (5), Barbara Contiero (1), Helen Poser (1), Carlo Guglielmini (1)

(1) Università degli Studi di Padova, Dipartimento MAPS. (2) Università degli Studi di Bologna, Dipartimento di Scienze Mediche Veterinarie. (3) Federal University of Paranà, Department of Veterinary Medicine. (4) Istituto Veterinario di Novara. (5) Università degli Studi di Perugia, Dipartimento di Medicina Veterinaria.

Corresponding author: C. Valente (carlotta.valente@unipd.it)

Atrial fibrillation (AF) is the most common supraventricular arrhythmia in dogs and is more frequently observed in large breed dogs, particularly those affected by dilated cardiomyopathy (DCM) [1]. Previous studies identified some risk factors for developing AF in the dog, including increased left atrial (LA) dimension and body weight (BW), but were mainly focused on dogs with myxomatous mitral valve disease [1,2]. The aim of the present study was to identify risk factors for developing AF in dogs with DCM.

In this multicenter, retrospective study, the medical database of five cardiological referral centers were searched for dogs with a diagnosis of DCM phenotype after complete cardiovascular evaluation and cardiac rhythm assessment. Comparison of clinical and echocardiographic variables between dogs developing AF and those not developing AF was carried out using the Student's t-test and the two proportions z test for continuous and categorical variables, respectively. Ability to distinguish between dogs developing AF and those not developing AF was evaluated by receiver operating characteristic curve (ROC) analysis. Univariate and multivariable logistic regression analysis estimated the odds ratio (OR) with 95% confidence interval (CI) of developing AF.

A total of 109 client-owned dogs of different breeds with echocardiographic DCM phenotype were selected, including 26 (23.9%) and 83 (76.1%) dogs with asymptomatic and symptomatic (i.e., DCM associated with congestive heart failure [CHF]) disease. Atrial fibrillation was diagnosed as the prevalent cardiac rhythm in 43 dogs (39.4%), whereas 43 dogs (39.4%) and 23 dogs (21.2%) maintained a sinus rhythm or showed other types of cardiac arrhythmias, respectively. Left atrial diameter had high accuracy (area under the curve = 0.837, 95% CI=0.763-0.912) to predict development of AF at the cut-off of >4.66 cm. Univariate logistic regression showed that developing AF was significantly and positively associated with male sex, BW, presence of CHF, LA and aortic (Ao) diameter and their ratio, fractional shortening, right atrial enlargement (RAE), presence of tricuspid regurgitation, and trans-mitral E wave peak velocity. However, only LA diameter (OR=3.955, 95% CI=2.169-7.214; P<0.001) and presence of RAE (OR=4.81, 95% CI=1.128-8.53; P=0.028) were significant predictors of AF after multivariable stepwise logistic regression analysis.

Atrial fibrillation is a common complication of DCM in the dog and is significantly associated with increased absolute LA dimension, as previously demonstrated in dogs with MMVD [2], but with a higher threshold of LA diameter. Right atrial enlargement is also a risk factor for AF in dogs of different breeds with DCM as previously reported for Doberman pinschers [3].

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IMMUNOMETABOLIC CONDITIONS IN DAIRY COWS DRIED OFF WITH ANTIBIOTIC THERAPY OR TEAT SEALANT ONLY

Claudio Ciaburri, Luca Cattaneo, Andrea Minuti, Fiorenzo Piccioli-Cappelli, Vincenzo Lopreiato, Erminio Trevisi

Università Cattolica del Sacro Cuore, Dipartimento di Scienze Animali, della Nutrizione e degli Alimenti, Piacenza.
Corresponding author: E. Trevisi (erminio.trevisi@unicatt.it)

European policy (Reg. UE 2019/6) provided for a drastic reduction in antibiotics use in dairy farms to contrast the phenomenon of antibiotic resistance. This regulation has banned the use of antibiotics in livestock for preventive purposes since January 2022. Therefore, it is no longer possible to practice the blanket dry-cow therapy, but selective procedures should be applied to treat only cows with overt infections or at risk of developing intramammary infections during the dry period. Several studies have been carried out to identify the optimal procedures to detect cows suitable to be dried off without the antibiotic therapy. Nevertheless, limited data are available about the long-term effects on immunometabolic response caused by different dry-off procedures. The aim of the present study was to investigate the immunometabolic variations in dairy cows with low somatic cell count (SCC) at dry-off treated with an intramammary injection of antibiotic paired with internal teat sealant (AB) or with teat sealant only (TS). Within the project LEO, Livestock Environment Opendata, 16.2 – PSRN 2014-2020 (financed through FEASR), from 86 Holstein cows with SCC lower than 200 (n/µL) and without major intramammary pathogens, blood samples were collected from the jugular vein at -7, 0, 3, 7 days relative to dry-off and at -3, 3, 28 days relative to calving to evaluate a wide hematochemical profile (Aut. Min. 464/2019-PR; Aut. Min. 139/2021-PR). Milk yield was recorded daily, SCC was measured at -14, -7 and -1 days before dry-off and 7, 14, 28 days after the subsequent calving. Data on health status and fertility in the ensuing lactation were also collected. Data at dry-off and calving were analyzed separately with repeated measures mixed models (proc GLIMMIX of SAS), considering dry-off method, time and their interaction as fixed effects, and individual cows as random effect. At dry-off, SCC (163 vs 143 N/µL) and milk yield (20.3 vs 19.3 kg/d) were similar between TS and AB. Regardless of treatment, dry-off causes a decrease in urea and FRAP concentrations. At the same time, metabolites related to mammary involution and immune system activity (ROM and MPO), fat mobilization (NEFA), inflammatory response (ceruloplasmin and haptoglobin), and minerals (Ca and P) increased. However, dry-off method did not cause noticeable changes between groups either at metabolic or inflammatory levels. During the periparturient period, in both groups we observed the well-known variations of indicators of energy metabolism (i.e. decrease of glucose and increase of NEFA and BHB), inflammatory condition (i.e. increase of haptoglobin, ROMt and decrease of zinc), liver fatigue (i.e. increase of bilirubin and decrease of PON and cholesterol), and immune system (i.e. increase of MPO). Nevertheless, all the variations were within the physiological range and limited at 3 days in milk. No differences were observed between groups, as supported by the similar Liver Functionality Index (0.95 vs 0.77, respectively for AB and TS; NS). At 3 d after calving, SCC were slightly higher in TS than AB (146 vs 96 n/µL; NS), and the average milk yield in the first month of lactation resulted slightly lower in TS (38.6 kg/d) compared with AB (40.1 kg/d). Days open and artificial insemination/pregnancy were similar between groups. These results confirmed that drying off dairy cows with internal teat sealant only, if performed in high hygienic conditions, can be applied monitoring the somatic cell content as the only criterion. The threshold of 200 N/µL of SCC is a useful proxy of udder health at dry-off. Drying off healthy cows with the application of internal teat sealant alone can represent a viable solution to reduce the antibiotics use in the dairy sector, as demonstrated by the low SCC after calving and the limited and transient differences between groups in the immunometabolic profile. Therefore, the measurement of SCC immediately before dry off can be used as a routinely procedure to select the proper dry-cow therapy. Nevertheless, other indicators of udder health, such as presence of intramammary pathogens or clinical mastitis history, might be considered in the decision making.

EVALUATION OF ULTRASOUND MEASUREMENT OF SUBCUTANEOUS FAT THICKNESS IN DAIRY JENNIES DURING THE PERIPARTURIENT PERIOD

Irene Nocera (1) , Francesca Bonelli (1), Luca Turini (2), Alessio Madrigali (1), Benedetta Aliboni (1), Micaela Sgorbini (1)

(1) Università di Pisa, Dipartimento di Scienze Veterinarie. (2) Università di Pisa, Dipartimento di Scienze Agrarie, Alimentari e Agro-ambientali.

Corresponding author: I. Nocera (irene.nocera@vet.unipi.it)

The body condition score (BCS) represents a practical but subjective method for assessing body fat reserves, that could lead to inaccurate results (1). Real time ultrasonography (RTU) has been proposed as an accurate method to objectively measure subcutaneous fat (SF) thickness and to routinely predict body fat reserves in cows, horses and donkeys (2). However, there are no study evaluating the use of RTU in pregnant and lactating jennies (1). Thus, the aim of the present study was to describe RTU measures of SF thickness during periparturient period in jennies. The present prospective cohort study evaluated six pluriparous dairy jennies. RTU measurements of the SF thickness were performed in the study population at 15 (T1) and 7 days (T2) before the presumptive delivery, and 2 (T3), 15 (T4) and 30 days (T5) after delivery. Contextually, BCS and a complete physical examination has been performed. RTU was performed using a portable ultrasound machine with a multifrequency linear transducer (5-7.5 MHz). RTU measurements of SF images were obtained in six different truncal sites: site 1 (S1), the probe was place perpendicular to the spine, at the level of wither (7th and 8th thoracic vertebra); site 2 (S2), probe placed perpendicular to the spine, level of the 13th thoracic vertebra; site 3 (S3), probe placed cranial to the tuber sacralis and 4 cm laterally and parallel to the spine; site 4 (S4), probe placed anterior to the tail-head at the level of the 1st to 4th coccygeal vertebrae, parallel to the spine; site 5 (S5), probe placed at the thoracic cage perpendicular to the 6th and 7th ribs, caudal to the point of the elbow; site 6 (S6), probe placed at the thoracic cage perpendicular to the 12th and 13th ribs, midway between the dorsal and ventral mid-lines. In particular, at S5, the SF was measured over the 6th rib (referred on ultrasound image measurements as S5a) and between ribs 6-7 (referred on ultrasound image measurement as S5b); at S6, the SF was measured over the 13th rib (referred on ultrasound im-age measurements as S6a) and between ribs 12-13 (referred on ultrasound image measurements as S6b). Results at each time point were reported as mean±standard deviation and an ANOVA test with Tukey's test for multiple comparison were applied to compare RTU of SF measurements of each site (S1-S6) through time (T1-T5). A multiple linear regression was evaluated between RTU examinations sites and BCS. A total of 180 images were evaluated. No statistically significant differences were found of each site during time. The mean values of RTU measurements from different sites during the whole study period were above those reported by others, except for S5 which were similar to previous studies. BCS and sites through observational time have shown a good and reliable association. Our study could give preliminary indications on fat reserves in different body locations evaluated thanks to RTU, in pregnant and lactating jennies.

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PREVALENCE OF GASTRIC ULCERS IN HORSES INVOLVED IN HISTORICAL HORSE RACES IN CENTRAL ITALY

Sara Busechian (1), Simona Orvieto (2), Fabrizio Rueca (1)

(1) Università degli Studi di Perugia, Dipartimento di Medicina Veterinaria. (2) Libero professionista, Perugia, Italia. Corresponding author: S. Busechian (sarabusechian@gmail.com)

Equine Gastric Ulcer Syndrome (EGUS) is a worldwide disease with different prevalence in different breeds, activities and age of horses: the highest can be found in racehorses, where almost all horses subjected to gastroscopy were found positive. There are currently two specific syndromes recognized, one affecting the squamous mucosa (ESGD) and one the glandular (EGGD). Clinical signs are non-specific and can go from recurrent colic to poor performance, but many horses show no symptoms [1-3]. Historical horse races can be found throughout Italy, and most of the horses involved are Thoroughbred and mix breed, making them quite similar to horseracing. Aim of this study was to evaluate the prevalence of EGUS, ESGD and EGGD in horses used for historical horseracing in central Italy and determine if there was a difference between Thoroughbred and Anglo-Arabians. 38 horses, males, females and geldings, aged between 3 and 14 years, were enrolled in the study. 20/38 horses were Thoroughbred and 18/38 Anglo-Arabians. Gastroscopic examination was carried out according to the literature, the mucosa was cleaned of food material with water passed through the working channel [1, 3]. Presence of lesions on the squamous mucosa was recorded according to the grading system proposed by Sykes et al (grades 0-4/4) [1]. Lesions in the glandular mucosa were only recorded as present or absent due to the current lack of grading system [1, 2]. Horses were considered positive for EGUS when they showed at least 2/4 ESGD and/or were positive for EGGD. Statistical correlation between variables was evaluated with Pearson, with $p<0.05$. 17/38 horses were used for "Palio" in Siena, 15/38 for "Corsa all'Anello" in Narni and 6/38 for "Quintana" in Foligno. EGUS was found in 34/38 (89%) of the horses evaluated, ESGD in 34/38 (89%, grade 0: 1/38, 3%, grade 1: 3/38, 8%, grade 2: 8/38, 21%, grade 3: 12/38, 32%, grade 4 14/38, 37%). EGGD was present as focal hyperemia of the mucosa, without visible macroscopic erosions or ulcers in 21/38 (55%) horses. No statistical correlation was found considering the breeds of the horses or the historical competition they were enrolled in. Presence of EGUS in this cohort of horses is similar to what can be found in animals of similar breeds enrolled in more "conventional" horseracing: EGUS has been reported in almost 100% of racehorses examined in various studies [1-3]. Thoroughbred seem to be particularly predisposed to the development of gastric ulcers also in nonracing settings, LeJeune et al reported high prevalence of EGUS in mares at pasture [4]. The Anglo-Arabians in the group have a high percentage of Thoroughbred descent, making them similar, from a genetic point of view to the Thoroughbreds. Nevertheless, high prevalence of EGUS was found by the authors also in Arabian horses, and belonging to Arabian or Thoroughbred breed seems to be a risk factor for EGUS in Italy [5]. The results of our study and the current literature highlight the need for further studies on the possible risk factors and pathophysiology of EGUS in horses, considering also possible genetic predispositions and heritability.

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EVALUATION OF THE ROLE OF PASSIVE IMMUNITY TO PREVENT THE NEONATAL DIARRHEA IN CALVES INFETED BY Cryptosporidium parvum

Giulia Sala, Antonio Boccardo, Vincenzo Ferrulli, Valerio Bronzo, Davide Pravettoni, Alessia Libera Gazzonis

Università degli Studi di Milano, Dipartimento di Medicina Veterinaria e Scienze Animali.
Corresponding author: G. Sala (giulia.sala@guest.unimi.it)

Neonatal Calf Diarrhea (NCD) is a multifactorial disease that causes economic losses due to treatment costs, mortality, and possible long-term effects [1]. *Cryptosporidium parvum* is one of the most important pathogen for NCD; moreover, its role as a zoonotic agent is not negligible [2]. Prevention of this disease is difficult due to the high resistance of oocysts in the environment, and the treatments of animals are often unsuccessful [2]. Moreover, the role of colostrum immunity in preventing the clinical manifestation of NCD caused by *C. parvum* is unclear [2].

This study evaluated the passive immunity's role in protecting neonatal calves against the NCD caused by *C. parvum*.

Following an outbreak of NCD, an owner of a dairy herd in northern Italy contacted the Clinic for Ruminants of the Veterinary Teaching Hospital (University of Milan) for a consultation on calf management. All calves aged between 1 and 10 days, both male and female Friesian calves, were examined. Animals presenting other pathologies besides the NCD were excluded. As previously described [3], each calf was clinically examined. In addition, a blood sampling was performed for each calf, and immunoglobulin G (IgG) was measured by the radial immunodiffusion technique (RID). Finally, a fecal sample was used for bacteriological and virological examinations and the determination of the presence of *C. parvum* antigens using an immunochromatographic assay.

The possible relationship between *C. parvum* and NCD frequency was analyzed with the Chi-Square test, moreover the role of passive immunity in the prevention of *C. parvum* infection and the association between immune status and mixed infections by *C. parvum* and at least one other pathogen was tested by the way of the U Mann-Whitney test. Statistical significance was considered for $p<0.05$.

Fifty-five calves were enrolled in the, and the prevalence of NCD in the sample was 62.7% (37/55 animals), with a significant association with serum IgG concentration (IgG median of NCD calves: 13.2 g/L; IgG median of healthy calves: 22.8 g/L; p-value=0.005). The *C. parvum* positive calves were 12 (21.8%), and, of these, 10 presented diarrhea: *C. parvum* infection was significantly associated with the clinical manifestation of NDC (p-value=0.047), while it was not statistically associated with the serum concentration of sTP and IgG (IgG median of *C. parvum* positive calves: 14.4 g/L; IgG median of *C. parvum* negative calves: 16.7 g/L; p-value>0.437). The median IgG concentration in calves with *C. parvum* and at least one other pathogen (8.2 g/L) was lower than in calves with only *C. parvum* infection (13.6 g/L; p-value 0.036). No animal included in the study died.

Observed results suggest that passive immunity does not prevent the NCD with *C. parvum* infection. However, an adequate immune status, particularly a high IgG value, seems essential to preventing co-infections. In addition, calves with NCD sustained by more than one pathogen have been found to have a lower serum IgG value.

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ESSENTIAL AND NON-ESSENTIAL AMINO ACIDS IN DOGS AT DIFFERENT STAGES OF CHRONIC KIDNEY DISEASE

Ilaria Lippi (1), Francesca Perondi (1), Alessio Pierini (1), Francesco Bartoli (2), Eleonora Gori (1), Chiara Mariti (1), Veronica Marchetti (1)

(1) Università degli Studi di Pisa, Dipartimento di Scienze Veterinarie. (2) Università degli Studi di Pisa, Dipartimento di Ricerca Traslazionale e delle Nuove Tecnologie in Medicina e Chirurgia.

Corresponding author: I. Lippi (ilaria.lippi@unipi.it)

Abnormalities of serum amino acid profile have been documented in human CKD, and mostly characterized by a reduction in essential amino acids (EAAs), and an increase in non-essential amino acids (NEAAs) [1] . Amino acid disorders have been associated with CKD complications, such as metabolic acidosis, and malnutrition. Serum levels of EAAs and NEAAs in CKD dogs have been scarcely investigated, or limited to particular conditions, such as protein losing nephropathies [2, 3].

The aim of the present study was to evaluate EAAs and NEAAs serum profile in dogs affected by CKD at different IRIS stages, with particular reference to calcium-phosphate abnormalities, metabolic acidosis, and protein-energy wasting syndrome (PEW). Serum EAAs (L-Histidine (HIS), L-Isoleucine (ILE), L-Leucine (LEU), L-Lysine (LYS), Methionine (MET), L-Phenylalanine (PHE), L-Threonine (THR), Tryptophan (TRP), L-Valine (VAL)), L-Arginine (ARG), and serum NEAAs (L-Alanine (ALA), L-Aspartic acid (ASP), L-Cysteine (CYS), L-Glutamic acid (GLU), Glycine (GLY), Proline (PRO), L-Serine (SER), L-Tyrosine (TYR)) were analyzed with an automated high-performance liquid chromatography (HPLC) in a group of dogs with CKD (n=62), and in a group of healthy dogs (n=25). CKD dogs were classified according to IRIS staging, and subclassified according to CaxP in normal (CaxP $\leq$70 mg^2/dL^2), and abnormal (CaxP > 70 mg^2/dL^2), according to the presence (MA) or absence (nMA) of metabolic acidosis, and to the presence (PEW) or absence (nPEW) of PEW[4]. Individual EAAs and NEAAs, and their sum were compared among the different study groups by One-way ANOVA or Kruskal-Wallis, and t-test or Mann-Whitney comparison. Results were considered statistically significant for $p<0.05$.

CKD dogs were distributed in IRIS stage 1 (n=12), IRIS stage 2 (n=16), IRIS stage 3 (n=14), and IRIS stage 4 (n=20), and the majority of them were on a prescription renal diet. Clinically healthy dogs were enrolled among blood doners. CKD dogs showed significantly lower serum levels of HIS (p=0.000), ILE (p=0.000), TRP (p=0.000), ALA (p=0.013), CYS (p=0.000), and SER (p=0.002), and significantly higher levels of PRO (p=0.000), LEU (p=0.001), LYS (p=0.000), VAL (p=0.000), ARG (p=0.002), GLU (p=0.002), and GLY (p=0.010) compared to healthy dogs. Dogs with abnormal CaxP showed significantly higher levels of CYS (p=0.003), and lower levels of TRP (p=0.025) compared to CKD dogs with normal CaxP; dogs with metabolic acidosis showed significantly higher levels of PHE (p=0.035) and LEU (p=0.034) compared to CKD dogs without metabolic acidosis; and dogs with PEW showed significantly lower levels of HIS (0.006), PHE (p=0.049), THR (p=0.007), ILE (p=0.001), LEU (p=0.001), LYS (p=0.014), TRP (p=0.003), VAL (p=0.003), PRO (p=0.011), SER (p=0.031), ALA (p=0.001), GLU (p=0.002), GLY (p=0.045), and TYR (p=0.016), and higher levels of CYS (p=0.010). In PEW dogs, the median distribution of both EAAs (p=0.000) and NEAAs (p=0.001) was significantly lower than nPEW dogs. CKD dogs showed significant abnormalities in both serum EAAs and NEAAs levels. Abnormal aminoacidic pattern was evident since IRIS 1, but no significant association with the progression of CKD was noticed. Although serum HIS, ILE and TRP were consistently lower compared to healthy dogs, our patients did not show the typical reduction in EAAs, usually seen in human CKD. Abnormal serum levels of EAAs and NEAAs may be related to multiple causes, such as increased catabolism rate, worsening of glomerular and tubular functions, and malnutrition. Among the different CKD complications, PEW was associated with significantly lower levels of serum EAAs and NEAAs.

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ERYTHROCYTE SEDIMENTATION RATE IN THE DIAGNOSIS AND TO MONITOR CANINE LEISHMANIASIS USING MINI-PET DIESSE®

Ilaria Lensi (1), Roberto Amerigo Papini (2), George Lubas (1-3)

(1) Clinica Veterinaria Colombo, VetPartners Italia, Lido di Camaiore (LU). (2) Università degli Studi di Pisa, Dipartimento di Scienze Veterinarie. (3) Canine Leishmaniasis Working Group, Seravezza (LU).

Corresponding author: I. Lensi (lensi.ilaria@gmail.com)

Erythrocyte Sedimentation Rate (ESR) is a common "sickness index" frequently used in human medicine to identify inflammation, neoplastic, metabolic, or orthopedic disorders (1). ESR measured with MINI-PET DIESSE® has been proposed as an index of unhealthy conditions in dogs (reference range <10 mm/h) (2). This study investigated ESR and compared it to inflammatory markers commonly used in dogs affected by leishmaniasis. Twenty dogs diagnosed with leishmaniasis have been included (10 mixed-dogs, 10 purebred dogs; 13 males and 7 females; age 2-14 years, median 6 years). Diagnosis of leishmaniasis was carried out with clinical examination and investigations on blood (CBC, serum biochemical profile and protein electrophoresis, ESR, coagulation profile, ELISA or IFAT for *Leishmania*) urine, lymph node or bone marrow for cytology or PCR to detect amastigotes. ESR using MINI-PET DIESSE® is a point-of-care test easily performed in 14 minutes on the same 1 mL K3-EDTA sample for CBC. Dogs diagnosed with comorbidities were excluded. Enrolled dogs were grouped according to Canine Leishmaniasis Working Group (CLWG) at the diagnosis and during the follow-up (3). Ten dogs were treated with a four-week cycle of leishmanicidal protocols: meglumine antimonials and allopurinol in 9 dogs; miltefosine and allopurinol in one dog. These dogs then started maintenance treatment with allopurinol, domperidone, and nutraceuticals PO containing nucleosides and/or zinc. Six of the other 10 dogs were only seen at the diagnosis, while 4 dogs only required maintenance treatment. Applying the Spearman rank correlation test (SRCT, interpretation 0.1-0.3 weak, 0.4-0.6 moderate, and 0.7-0.9 strong), ESR values have been studied in relation to HCT, WBC count, neutrophil-to-lymphocyte ratio, total protein, albumin, A/G, C-reactive protein, iron, ferritin, haptoglobin (HPT), IgG, IgM, and % of gammaglobulin. Patients at the diagnosis were classified (CLWG): 1 dog stage A exposed, 5 stage B infected, 8 stage C sick, and 6 stage D severely sick. Ten dogs which required leishmanicidal treatment showed highly elevated ESR value at the diagnosis (range 12-74 mm/h, median 53.5 mm/h), which remained elevated during the period of follow-up but lower than the initial assessment (range 6-43 mm/h, median 16 mm/h). Ten dogs which had not received the leishmanicidal treatment, with mild disease or only infected, had ESR values within the reference interval or mildly elevated (range 5-13 mm/h, median 12 mm/h). The SRCT for all the samples collected displayed a negative or positive correlation with ESR ($P<0.05$) as follows: strong for HCT (-0.76); moderate with albumin (-0.58) and A/G (-0.41); weak with HPT (+0.36) and iron (-0.31). These preliminary results show a prospective use of ESR in dogs affected by leishmaniasis, together with other commonly used inflammatory markers. ESR values were higher at the diagnosis and lower at the end of the leishmanicidal treatment, while infected or non-ill dogs showed ESR values in the reference interval or mildly increased. Therefore, as ESR is a point-of-care test, it could be easily and promptly added as index of inflammation in the clinical practice. The main limitations of this study were the small sample size and the heterogenicity of the case load, regarding follow-up and stage classification. The study is in the frame of CLWG aims. CLWG is supported by Ecuphar®. DIESSE® supplied the instrumentation.

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ULTRASONOGRAPHIC EVALUATION OF THE UDDER CISTERN AREA IN HEALTHY QUARTERS AND IN QUARTERS AFFECTED BY CLINICAL AND SUBCLINICAL MASTITIS: PRELIMINARY DATA

Chiara Orsetti (1, 2), Micaela Sgorbini (1, 2), Arianna Cervelli (2), Simonetta Citi (2), Francesca Bonelli (1, 2)

(1) Università degli Studi di Pisa, Centro di Ricerche Agro-Ambientali "E. Avanzi", (2) Università degli Studi di Pisa, Dipartimento di Scienze Veterinarie.

Corresponding author: C. Orsetti (chiara.orsetti@phd.unipi.it)

In cows the ultrasound examination is mainly used for the assessment of reproductive conditions but it can be a diagnostic aid to evaluate pathological alterations of the udder [1]. It is a noninvasive technique, easy to perform, and it usually allows to immediate interpretation and diagnosis [2].

The aim of this study was to evaluate the area of udder cistern (UC) in quarter affected by clinical and subclinical mastitis and in healthy quarters. This research was approved by the Institutional Animal Care and Use Committee of the University of Pisa (33479/2016). The study took place at the dairy farm of the University of Pisa (Centro di Ricerche Agro-Ambientali "E. Avanzi"). Twenty-three healthy quarters (HQ), 12 quarters with subclinical mastitis (SCMQ) and 11 quarters with clinical mastitis (CMQ) were included for a total of 46 quarters. Quarters were scanned in both longitudinal (L) and cross section (CS) of the udder cistern. Quarters were defined as affected by clinical mastitis if they showed signs of mastitis in milk and/or quarter and/or systemic signs, while subclinical mastitis was defined as the absence of clinical signs plus a somatic cell count (SCC) > 250.000 cell/ml). As healthy quarters we used the corresponding contralateral udder quarter if it did not show clinical signs of mastitis and a SCC<250,000 cell/ml. Quarters were evaluated in B-mode with a portable instrument (Mindray DPVet, China) as described by literature [3] at the time of the diagnosis of mastitis (T0), 24h later (T1) and 5 days later (T2). The pictures were processed using ImageJ (National Institute of Health, Bethesda, MD, USA). For each ultrasound image, the outline of the UC was traced freehand by following its echogenic margin; the margin appeared clearly visible thanks to the anechoic content. The software automatically processed the cropped image determining the area of the UC expressed in cm^2. Data distribution was assessed with the Kolmogorov-Smirnov test. An one-way ANOVA was performed to evaluate differences through the time. No statistically significant differences were found for HQ-L and HQ-CS. Both SCMQ-CS and CMQ-CS showed statistically significant differences between times, while no differences were found for the L section. In particular, the UC of SCMQ-CS and CMQ-CS was higher at T0 compared to other times. At T0 the UC of SCMQ-CS and CMQ-CS quarters was statistically higher compared to HQ-CS.

These preliminary results showed that the CS section of the UC ultrasound evaluation might be used as an additional tool for the diagnosis of mastitis in field conditions.

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PROPER MANAGEMENT CAN COMPENSATE FOR PARTIAL FPT IN ALPINE GOAT KIDS

Mariana Roccaro (1), Marilena Bolcato (2), Alessandro Tirolo (2), Francesco Dondi (2), Arcangelo Gentile (2), Angelo Peli (1)

(1) Alma Mater Studiorum – Università di Bologna, Dipartimento di Scienze per la Qualità della Vita. (2) Alma Mater Studiorum - Università di Bologna, Dipartimento di Scienze Mediche Veterinarie.
Corresponding author: M. Roccaro (mariana.roccaro2@unibo.it)

The importance of colostral immunity for the health, survival and productive performance of young ruminants is well established [1]. However, differently from calves and lambs, the individual factors that may directly affect passive immune status and its potential long-term effects have not been widely investigated in goats [2, 3]. The aim of this study was to investigate the effect of sex, litter size (singlet vs twin), dam parity (pluriparous vs primiparous) and birth body weight (BW) on serum immunoglobulin (Ig) concentration (of which IgG account for the largest fraction) and to evaluate the effect of passive transfer status on pre-weaning growth performance in Alpine goat kids. The study was approved by the Animal Welfare Body of the University of Bologna (Prot. No. 77988/2022).

Thirty-nine Alpine goat kids (22 males, 17 females) from the same dairy farm were included in the study. All kids were weighed right after birth. They were then allowed to naturally suckle their mothers until weaning (50 days), when they were weighed again. Blood samples were collected 24 hours after birth. Serum Ig concentration was determined by means of electrophoresis. Mean ± SD values for serum Ig concentration, birth BW, day 50 BW, and average daily gain (ADG) from birth to day 50 were calculated. Differences in Ig concentration depending on sex, litter size and dam parity were investigated (Student's t-test). Least squares simple linear regression was used to evaluate the association between birth BW and serum Ig concentration and between serum Ig concentration and pre-weaning growth performance. Statistical analysis was performed using GraphPad Prism (v. 8.2.1).

Serum Ig concentration ranged from 0.20 to 3.30 g/dl (1.37±0.68 g/dl). Birth BW ranged from 2.30 to 4.92 kg (3.81±0.53 kg). BW at weaning ranged from 11.40 to 16.10 kg (13.58±1.37 kg). ADG ranged from 0.16 to 0.26 kg (0.19±0.02 kg). No significant differences in serum Ig concentration between males and females, singlets and twins, pluriparous' and primiparous' kids were found. No association was detected between birth BW and serum Ig concentration, as well as between serum Ig concentration and pre-weaning growth performance in terms of BW at weaning and ADG. Although partial failure of passive transfer (FPT; Ig <0.8 g/dl) [4] was diagnosed in 17.9% of kids (7/39), no effects on morbidity (0%), mortality (0%) and growth performance were observed in our sample.

Whilst the absence of a sex bias for serum Ig concentration has been previously observed [4, 5], our results show that litter size and dam parity do not influence serum Ig concentration, differently from other studies [6, 7]. Moreover, passive transfer status does not affect health and pre-weaning growth performance in Alpine goat kids allowed to remain with their mothers in a nonintensive farming system. This is in contrast with other studies involving lambs and goat kids of other breeds [2, 8]. In conclusion, these findings support the notion that proper management and animal care can compensate for partial FPT in protecting against diseases and enables this goat breed to express its full capacity to raise offspring with optimal health and welfare conditions.

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NONINVASIVE MONITORING OF EQUINE MUSCLE HEMODYNAMICS BY TIME DOMAIN NEAR INFRARED SPECTROSCOPY

Lorenzo Frabasile (1), Michele Lacerenza (1, 2), Mauro Buttafava (2), Davide Contini (1), Alessandro Torricelli (1, 3), Paola Straticò (4), Lucio Petrizzi (4),Emanuela Dalla Costa (5), Michela Minero (5), Vanessa Rabbogliatti (5), Davide Zani (5)

(1) Politecnico di Milano, Dipartimento di Fisica, Milano, Italy. (2) PIONIRS s.r.l., Milano, Italy. (3) Istituto di Fotonica e Nanotecnologie, Consiglio Nazionale delle Ricerche, Milano, Italy. (4) Facoltà di Medicina Veterinaria, Università degli Studi di Teramo, Teramo, Italy. (5) Department of Veterinary Medicine and Animal Sciences (DIVAS), Università degli Studi di Milano, Italy.

Corresponding author: L. Frabasile (lorenzo.frabasile@polimi.it)

Non-invasive and real-time hemodynamic monitoring of equine muscle is crucial to early diagnose the occurrence of myopathies related to prolonged muscle compression, as it often arises during long surgical or diagnostic (e.g., MRI) procedures. In this pilot study, we explored the possibility of detecting equine muscle hemodynamic parameters by means of Time Domain Near-Infrared Spectroscopy (TD-NIRS). The potential advantage of TD NIRS, compared to the state of the art [1, 2] (e.g., hemogasanalysis, continuous wave NIRS), lies in the fact that it allows to monitor the hemodynamic parameters in real-time, during prolonged measurements on hardly accessible investigation sites without interfering with other instruments (e.g. into Faraday cage during MRI) or perturbing the conditions of the animal.

A custom-made portable TD-NIRS tissue oximeter [3] was used to retrieve deoxy- and oxy-genated hemoglobin concentration and tissue oxygen saturation on muscles (Triceps Brachii, Gluteus Superficialis, and Longissimus Dorsi) of 17 live horses, with different hair length and coat color. A custom optical probe was developed, to avoid motion artifacts and to be robust enough to withstand the weight of the horse, and to perform measurements during diagnostic (MRI) and surgical procedures. A group of 9 horses was measured at the veterinary hospital. Measurements were performed before anesthesia (with the horse standing), during anesthesia (with the horse placed on a soft bed), and after anesthesia (with the horse allowed to naturally rise, and measured after 30 min from returning to a standing position). A second group of 8 horses was measured in a horse stable as control measurements with the horse standing (no repetitions were made).

The main result of the study is that the use of TD-NIRS muscle oximetry seems a clinically feasible means to assess tissue oxygenation in most of adult horses, thanks to a sufficiently high signal-to-noise ratio. Moreover, the presence of hair and dark skin did not prevent the possibility of obtaining robust readings. The triceps muscle sites provided the most reliable and repeatable readings. For measurement during anesthesia, no evident changes in physiological parameters emerged. This assumes that the protection systems (e.g. pillows on the bed) were effective and a correct dosage of the drugs avoided anomalies in hemodynamic concentrations.

Work is still in progress to enlarge the sample size and to define possible limitations of the technique. The potential utility of the device is also to be considered under emergency surgery where patient's hemodynamic may be easily compromised.

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RENAL HYPERPARATHYROIDISM IN CATS: PREVALENCE AND SURVIVAL TIMES

Jari Zambarbieri, Emanuele Giacobbe Zampollo, Pierangelo Moretti, Alessia Giordano, Filippo Tagliasacchi, Paola Scarpa

Università degli Studi di Milano, Dipartimento di Medicina Veterinaria e Scienze Animali.
Corresponding author: J. Zambarbieri (jari.zambarbieri@unimi.it)

Chronic Kidney Disease (CKD) is one of the most commonly diagnosed diseases in older cats. Its prevalence increases with age, involving as many as 30% of cats older than 10 years and 50% of cats older than 15 years [1]. Although many aspects of calcium-phosphate metabolism have been explored in the last years [2] and renal hyperparathyroidism (RHPT) is well recognized as a progression factor of CKD [3], its role as a prognostic factor is always under discussion. The aim of this study was to evaluate the prevalence of RHPT in cats affected with spontaneous CKD, staged according to the International Renal Interest Society (IRIS), and its impact on survival times.

Seventy-seven privately owned cats were enrolled for this study. All the animals underwent a complete physical examination, blood works, and urinalysis for diagnostic purposes, during general health checks or internal consultation, requested by the owners (January 2020–December 2021). The animal sample included 64 hyperazotemic cats affected with CKD and 13 clinically disease-free cats (considered as a control group). PTH was measured on leftover sera by an immunoenzymatic method validated for the feline species (ST AIA-PACK® Intact PTH, Tosoh Bioscience, Tessenderlo, Belgium) [4]. Data obtained during the first examinations were analyzed and a telephonic follow-up was obtained by the owner of the nephropathic cats. JMP Pro 16 (SAS Inc. Cary, USA) and MedCalc (MedCalc Software Ltd, Ostend, BE) were used for statistical analysis.

According to the IRIS CKD staging system, cats were distributed as follows: 52 cats (68%) in stage 2, 4 cats (5.0%) in stage 3, and 8 cats (10%) in stage 4. Wilcoxon test revealed a significant difference between PTH values measured in disease-free and CKD cats ($p=0,0145$). Because of the small size of the control group and the consequent absence of a reference interval, the PTH upper value obtained in disease-free cats was used as a possible cutoff between disease-free and CKD groups. RHPT was identified in 30,77% (n=16) of cats in stage 2, in 25% (n=1) in stage 3 and 75% (n=6) in stage 4. Kruskal Wallis test showed a significant difference between sera PTH levels in the different groups, considering stages 3 and 4 were together because of the small number of animals included in these classes ($p=0.0040$). Wilcoxon test as a post hoc showed a significant difference between each group. At the end of the investigation, 45 (70%) cats were alive, 12 (19%) were dead due to CKD and 7 (11.0%) were lost at the follow-up. Wilcoxon test showed that PTH values were significantly different between dead and alive cats ($p<0.0052$).

Survival curves were calculated with Kaplan-Meier analysis. Log-rank test was used to compare curves. PTH values were previously categorized into 4 groups (0-15.2 pg/mL; 15.2-36.5 pg/mL; 36.5-423.25 pg/mL; >423 pg/mL), according to the mean values measured in stages 2, 3 and 4 of CKD. Survival times were significantly different between the groups ($p<0.0001$) and the median survival time was 624, 259, 97, and 3 days, respectively.

Although different survival times could be related to the disease severity associated with RHPT, serum concentration of PTH should be considered a factor affecting survival in cats with CKD.

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FECAL MICROBIOME AND MUCOSAL INFLAMMATION IN HORSES WITH RECURRENT COLIC DUE TO LARGE COLON INFLAMMATORY PATHOLOGIES

Anna Prampolini (1), Matteo Cuccato (2), Massimo Magri (3), Francesca Tiziana Cannizzo (2), Maria Filippa Addis (1), Michela Bullone (2)

(1) Università degli Studi di Milano, Dipartimento di Scienze Veterinarie e Scienze animali. (2) Università degli Studi di Torino, Dipartimento di Scienze Veterinarie. (3) Clinica Veterinaria Spirano.

Corresponding author: M. Bullone (michela.bullone@unito.it)

Colic is a frequently encountered clinical condition in horses, most commonly related with gastrointestinal problems. Recurrent colic occurs when a horse develops colic signs more than twice a year, often associated with uncontrolled parasitism, gastric ulcers, and sand ingestion. Recent data suggest that idiopathic recurrent colic could be associated with underlying chronic inflammatory bowel disease (CIBD) [1]. Equine CIBD is still a poorly defined condition, often diagnosed late during disease progression, mainly due to the lack of standardized diagnostic tools and protocols [2,3]. Rectal biopsy has been proposed as a possible diagnostic tool, but the accuracy of this technique remains unclear [4]. Although studies on the equine microbiome are rising, there is still little knowledge about the physiology of the equine gut microbiota, as well as the pathogenic potential of specific microorganisms. This study investigates changes in the fecal microbiota of adult horses suffering from recurrent colic and their relationship with histological evidence of mucosal inflammation in rectal biopsies. To this aim, 9 horses with recurrent colic and 7 healthy horses sharing the same environment were studied over 7 months (March-September 2021), with the owners' consent. Horses with colic were studied during the period of hospitalization for a colic episode. Control horses were studied within the 7 days following the colic episode of their mate. Stool samples were obtained from all horses enrolled, while rectal biopsies were taken only from pathological cases. Fecal samples underwent macroscopic sediment analysis for the presence of sand and copromicroscopic analysis for parasite burden assessment. As copromicroscopic analysis has low sensibility for small strongyle and tapeworms, further serological analysis were run for excluding parasitism as a possible cause of recurrent colic in these cases. Lastly, 16s sequencing was performed for microbiome assessment in feces. Rectal biopsies revealed eosinophilic, lymphoplasmacytic, or mixed infiltrate in 2, 3 and 2 cases, respectively. Findings about faecal microbiota are coherent with published data which revealed the presence of a 'core' (stable) microbiota, mainly composed by *Phyla Firmicutes* and *Bacteroidetes*. The season (spring vs. summer) was the variable linked with the highest variability in our samples, both from diseased and control animals. This is coherent with previously published data and probably attributable to diet, climatic, and management aspects that need further investigations [5]. After data adjustment, an increase in α-diversity was observed in pathological cases but only during the spring. Also, at the Phylum level, recurrent colic resulted associated with decreased amount of *Elusimicrobia* and with increased *Fibrobacteres* and *Planctomycetes*. Previous studies have suggested their relevance to the physiology of the equine intestinal microbiota, with particularly regard to *Fibrobacteres*. At the class taxonomic level, recurrent colic was associated with a significant reduction in *Clostridia*, whose presence is normally associated with a healthy state [6]. The results gained in this work are a step forward towards understanding the correlations between microbiota and intestinal health in the horse.

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EVALUATION OF THE ERYTHROCYTE MEMBRANE LIPODOME IN YOUNG HEALTHY GERMAN SHEPHERD DOGS

Paolo E. Crisi (1), M. Veronica Giordano (1), Carla Ferreri (2), Chryssostomos Chatgilialoglu (2), Anna Sansone (2), Paraskevi Prasinou (1), Alessandro Gramenzi (1), Andrea Boari (1)

(1) Università degli Studi di Teramo, Facoltà di Medicina Veterinaria. (2) Consiglio Nazionale delle Ricerche, ISOF, Area della Ricerca, Bologna, Italy.

Corresponding author: P.E. Crisi (pecrisi@unite.it)

The analysis of erythrocyte membrane lipidome represents a powerful tool for assessing the quantity and quality of fatty acids (FA) in humans, and recently it was evaluated also in healthy dogs and in dogs affected by different forms of chronic enteropathy (CE) (1, 2). Breed-specific peculiarities have not yet investigated and interbreed differences could be important in the interpretation of the results of erythrocyte membrane lipidome.

The aim of this study was to compare the FA membrane profile of young (<24 months) healthy German Shepherd dogs (GSD; n=9) with 13 age-matched healthy dogs of other breeds.

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 was approved by 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.

Erythrocytes membranes of GSD compared to controls had higher stearic acid (p=0.0003), vaccenic acid (p=0.0008) and DGLA (p=0.005), while lower percentages of palmitoleic (p=0.02) and arachidonic acid (p=0.02) were observed in GSD. Consequently, increased total saturated fatty acids (p=0.003) and reduced PUFA-ω6 (p=0.03), total PUFA (p=0.02), unsaturation index UI (p=0.017) and peroxidation index PI (p=0.02) were calculated. This breed is known for its high trainability and energy levels, which could explain the asset of GSD lipidome to a more resistant lipid configuration by increasing SFA and diminishing arachidonic acid and PUFA. It can be hypothesized that this lipidome profile could sensitize GSD breed toward those health conditions that cause a further increase of SFA, together with the increase of arachidonic acid, UI and PI, such as we have seen in chronic enteropathy (2). As matter of facts, GSD breed is frequently predisposed to contract enteropathy and to be resistant to treatment.

More GSD dog numbers are needed to confirm this hypothesis; however, these data justify the interest for building up a membrane lipidomes data base with dogs of different breeds and conditions.

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DAILY VARIABILITY AND EFFECT OF STORAGE IN URINE SAMPLES FROM MARTINA FRANCA DONKEYS

Alessia Luciani, Francesca De Santis, Renato Ennio Peli, Brunella Anna Giangaspero, Salvatore Parrillo, Andrea Boari, Paolo Emidio Crisi

Università degli Studi di Teramo, Facoltà di Medicina Veterinaria.
Corresponding author: A. Luciani (aluciani@unite.it)

Routine urinalysis is an integral diagnostic test in the clinical evaluation of diseased animals as well as in healthy animals. The performance of human dipsticks was explored in domestics animals included horses (1). The normal values of haematological, biochemical (2) and renal biomarkers (3) in donkeys have been recently published. However no report on diagnostic performance of urinary dipsticks, on daily variability and on the effect of refrigerate storage in urinary parameters in donkeys has been published to date. The aim of the study was to evaluate the daily and monthly variability of urinary parameters, the diagnostic performance of human dipsticks and the effect of refrigeration on urine samples collected from healthy Martina Franca donkeys, considered as endangered breed. Nine adult female healthy donkeys, owned by Faculty, were included based on a clinical examination and complete biochemical profile. Voided urine were collected from each donkey, in the morning and in the afternoon, 2 times a week for four consecutive weeks. The information collected on each urine sample included the urine dipstick (Combur test strip, Roche Diagnostics, Germany) and a miscroscopic evaluation of urinary sediments. To determine urine specific gravity (USG) and pH a manual refractometer and a digital pHmeter (Dostmann pH 80+, DHS, Germany) were used, respectively. On each urine sample protein-to-creatinine (UPC) ratio was evaluated with a clinical chemistry automatic analyzer. To establish effect of storage, an urine sample collected in the morning from each donkey, was stored at 4°C for 24 hours and submitted to the same analysis performed for the other samples. Computer software was used to perform the analysis (Graphpad Prism version 6.01, La Jolla, CA, USA). Normality was checked using the D'Agostino Pearson test. The variations of the urinary parameters were analyzed using 1-way repeated measures ANOVA. A comparison between analytes of samples collected in the two daily time points and after refrigeration was performed using the paired t-test or the Mann-Whitney test, while a comparison between the two methods used to detect USG and pH was performed using Pearson or Spearman correlations. A p value <0.05 was considered significant. The repeated measures 1-way ANOVA did not identify a difference between data from samples collected in the morning and those collected in the afternoon during the entire period. Only USG, detected with a manual refractometer, showed a reduction in urinary samples collected in the morning compared to samples collected in the afternoon ($p=0.0017$). This reduction could be related to the high grass water content in grazing feeding donkeys. Refrigeration brought a significant reduction in protein concentration and pH detected with dipstick, in USG detected with a manual refractometer, and in UPC ($p=0.0105$, $p=0.0028$; $p=0.0139$; $p<0.0001$, respectively). A moderate correlation was detected between pH dipstick and values obtained with a pHmeter only in urine samples collected in the morning ($r=0.6906$, $p<0.0001$) while no correlation was detected between USG tested with a manual refractometer and urinary dipsticks in both daily time points ($r=0.0636$, p=05956 and $r=0.4801$, $p=0.0845$, respectively). Results suggested that human commercial urinary dipsticks were not reliable to correctly estimate USG and pH in urine samples in donkeys as already described in horses (1). Furthermore our results suggested that urine samples should be evaluated after collection to minimize the effects of refrigeration on some urinary parameters.

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PRELIMINARY EVALUATION OF STT-1 IN HOSPITALIZED CATS

Marco Tabbì, Annastella Falcone, Simona Di Pietro, Francesco Macrì, Giuseppe Piccione, Elisabetta Giudice, Claudia Giannetto

Università degli Studi di Messina, Dipartimento di Scienze Veterinarie.
Corresponding author: S. Di Pietro (dipietros@unime.it)

Quantitative evaluation of the precorneal tear film is an essential part of an ophthalmic examination in veterinary medicine, and the Schirmer tear test I (STT-1) is commonly used to measure this value. Daily fluctuation in STT-1 values has been previously described in cats [1]. Circadian rhythm is generated and regulated by the hypotalamic circadian system that serves as a biological clock, being sensitive to light/dark (L/D) schedules [2]. The purpose of this study was to compare the STT-1 values of hospitalized cats (HG exposed to a 24/0 L/D cycle, with lights turned on for all experimental period) with a feline control group (CG exposed to a natural photoperiod of 12/12 L/D cycle), in order to verify if the exposure to a constante light during the hospitalization had an influence on the circadian rhythmicity of STT-1 in feline. The HG consisted of 10 owned-cats hospitalized to perform a sterilization procedure; the CG consisted of 10 staff-owned cats; they were tested in their indoor environment. Prior to the study, complete clinical and ophthalmic examinations were performed in all cats to determine their health status. All animals were free of signs of systemic or ocular diseases and had no history of ocular disorders. Both eyes were tested for every cat in the study (n=40 eyes). The STT-1 was performed at 4 h intervals over 48 h period (starting at 8:00 AM on day 1 and finishing at 8:00 AM on day 3). The first eye measured was chosen at random for each cat at each time point. One examiner performed all the ocular examinations and the STT-1 measurements. A 35x5 mm commercial tear test strip (Schering-Plough Animal Health, Union, New Jersey) was used to record tear production in millimetres wetting per minute. This study was approved by Department's Animal Ethics Council approval (protocol number: 44/2020). Owner consent was obtained. The STT-1 measurements were obtained from the left (OS) and right (OD) eyes in both controls and hospitalized cats. All the results were expressed as mean ± SD. Data were normally distributed ($p<0.05$, Kolmogorov-Smirnov test). Multivariate repeated measure ANOVA was used to compare left and right eye and to determine a statistical significant effect of time and the light/dark schedules on the tear production values for each eye. The data were analysed using the statistical software STATISTICA 7 (StatSoft Inc.). In addition, we applied a trigonometric statistical model on each time series, in order to describe the periodic phenomenon analytically, by characterizing the main rhythmic parameters according to the single cosinor procedure. Four rhythmic parameters were determined: mean level, amplitude, acrophase and robustness. Multivariate repeated measure ANOVA showed a statistical significant effect of time and light/dark schedules on STT-1 values recorded in both eyes. No difference was found between OS and OD tear production. The application of the periodic model and the statistical analysis of cosinor enabled us to define the periodic parameters and their acrophases during the scotophase. Robust daily rhythmicity was exhibited by the STT-1 in both eyes during the entire monitoring period in control cats and only during the day 1 of 24/0 L/D of hospitalized cats. Circadian rhythm was lost in both eyes during the constant light period. Our data confirmed that the circadian pacemaker drives the feline tear production. The loss of rhythmicity in hospitalized cats is linked to the exposure to constant light. Therefore, excluding an ocular response to animals handling that was the same in both groups, it is plausible that the results are related to the change in the photoperiod. These data are a starting point for evaluating the imbalance of ocular physiology observed in hospitalized cats. Further studies on a larger sample size and exposing the animals to various hospitalization procedures are needed to establish whether these alterations are caused by hospitalization procedures or only by the light/dark schedules.

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COMPLETE BLOOD COUNT FINDINGS IN CATS FROM AN AREA ENDEMIC FOR LEISHMANIA INFANTUM AND FELINE IMMUNODEFICIENCY VIRUS INFECTIONS

Giulia Donato (1), Maria Grazia Pennisi (1), Maria Flaminia Persichetti (1), Vito Priolo (1), Germano Castelli (2), Federica Bruno (2), Marisa Masucci (1)

(1) Dipartimento di Scienze Veterinarie, Università di Messina, Messina, Italy. (2) Crenal, IZS-Sicilia, Palermo, Italy. Corresponding author: M. Masucci (marisa.masucci@unime.it)

Feline *Leishmania infantum* (*Li*) infections and clinical cases of leishmaniosis have been described in areas endemic for canine leishmaniosis. In both cases, coinfection with feline immunodeficiency virus (FIV) has been reported but the role of the concurrent retroviral invection is controversial [1, 2]. The aim of this study was to evaluate complete blood count (CBC) changes in cats from regions endemic for both *Li* and FIV infections.

Four hundred and ninety-six cats from Sicily and Calabria regions (Southern Italy) tested for Li [by EDTA blood polymerase chain reaction (PCR) and anti-*Li* antibodies by immunofluorescence antibody test (IFAT), cut off dilution 1:80] and for FIV infections [by enzyme-linked immune assays (ELISA)] were retrospectively evaluated. CBC was performed using the laser hematology analyzer ProCyte Dx (IDEXX laboratories, Westbrook, ME, USA). All low platelet counts as well as any "smart flag" message reported by the analyzer about leucocytes or platelets count (i.e. the analyzer was not able to make the count or accuracy of analyzer count was low) was respectively confirmed or settled by the microscopic examination of blood smears. Blood smears were also examined for morphological alterations of cells and for excluding thrombocytopenic samples where platelets clumps were observed.

Li prevalence (PCR and/or antibody positivity) was 15.5% and FIV prevalence was 10%. Results of CBC with blood smear morphological evaluation were analyzed (Fisher's extact test, $P \leq 0.05$ was considered statistically significant) according to the eight infection patterns observed: Li PCR$^+$ IFAT$^+$ and FIV$^+$ (4 cats), *Li* PCR$^+$ IFAT$^-$ and FIV$^+$ (1 cat), *Li* PCR$^+$ IFAT$^-$ and FIV$^-$ (7 cats), Li PCR$^+$ IFAT$^+$ and FIV$^-$ (4 cats), *Li* PCR$^-$ IFAT$^+$ and FIV$^+$ (11 cats), Li PCR$^-$ IFAT$^-$ and FIV$^+$ (34 cats), Li PCR$^-$ IFAT$^-$ and FIV$^-$ (385 cats), Li PCR$^-$ IFAT$^+$ and FIV$^-$ (50 cats).

Monocytosis was more frequently observed in Li PCR+ IFAT- and FIV- cats (85.7%) compared to the *Li* PCR- cats [Li IFAT- and FIV- (16%, P=0.0001); Li IFAT- and FIV+ (28.1%, P=0.0082); Li IFAT+ and FIV- (22%, P=0.0019); Li IFAT+ and FIV+ (18.2%, P=0.0128)]. Similarly, morphologically activated monocytes were more frequently found in Li PCR+ IFAT- and FIV- (85.7%) cats compared to the Li PCR- and IFAT- [FIV- (2.7%, P= 0.0007); FIV+ (10.3%, P= 0.0489)]. Additionally, they were also more frequent in Li PCR+ IFAT+ and FIV- cats (50%) compared to the Li PCR- IFAT- and FIV- cats (2.7%, P=0.0058). Thrombocytopenia was more prevalent in Li PCR+ IFAT+ FIV+ cats (66.7%) compared to cats Li PCR- IFAT- [FIV- (8%, P=0.0198); FIV+ (3.4%, P=0.0177)] and cats Li PCR- IFAT+ FIV- (4.6%, P=0.0169). Finally, inflammatory leukogram was more frequently detected in Li PCR- IFAT- FIV+ cats (41.4%) compared to both Li PCR- IFAT- FIV- cats (16.5%, P=0.0023) and Li PCR- IFAT+ FIV- cats (16.2%, P=0.0286).

Though other potential concomitant conditions and factors not considered in this study could contribute to CBC changes and some coinfection patterns included few cats, we found that in the CBC of cats from a *Li* and FIV endemic area, a positive *Li* blood PCR is associated with monocytosis and occurrence of activated monocytes, while the inflammatory leukogram is associated with FIV positivity. According to this study, thrombocytopenia is the only CBC abnormality significantly associated with *Li* and FIV coinfection.

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KETAMINE-DEXMEDETOMIDINE COMBINATION FOR THE MANAGEMENT OF STATUS EPILEPTICUS IN A DOG: A CASE REPORT

Cristina De Rose, Roberta Di Prinzio, Andrea Paolini, Giovanni Aste

Università degli Studi di Teramo, Dipartimento di Scienze Cliniche Veterinarie.
Corresponding Author: C. De Rose (cderose@unite.it)

The aim of this case report is to describe the use of a ketamine-dexmedetomidine constant rate infusion (CRI) combination for the management of status epilepticus in a dog. A previous case series reported a successful management of idiopathic epilepsy in 3 dogs with ketamine-dexmedetomidine CRI and a controlled mild hypothermia [1]; this protocol included also a propofol CRI, but since there is a described high incidence of side effects during its administration, we tried to manage this clinical case without it [2]. A male, intact, 7.5-year-old pitbull was presented to the emergency department with ataxia, tremors, hypersalivation and altered mentation. The patient received immediate treatment with intra-rectal (IR) benzodiazepines (diazepam 0,5 mg/kg) as a first line treatment, followed by a peripheral venous catheter placement, IV fluid therapy and overnight monitoring in a stimulus-free environment. Therapy with phenobarbital 3 mg/kg IM TID was then instituted. The biochemical profile showed mildly elevated liver transaminases (AST 172 U/l, ALT 131 U/l), a moderate increase of creatine kinase (2155 U/l) and c-reactive protein (2,40 mg/dl); no alterations have been detected in the haematological profile, as well as in the serological tests to exclude infectious diseases. Twelve hours after admittance, cluster seizures reoccurred with loss of consciousness, tonic-clonic movements, and autonomic symptoms, followed by 3 administrations of IR Diazepam every 5 minutes at the dose of 0,5 mg/kg without effective response. The patient then received a bolus of both ketamine (0.5 mg/kg, IV) and dexmedetomidine (1 μg/kg, IV) followed by a 24-hour CRI of ketamine at a variable rate of 0.2-0.8 mg/kg/h and a 48-hour CRI of dexmedetomidine at a variable rate of 1–2 μg/kg/h. During the 24 hours of ketamine CRI, the dose was progressively reduced by 25% until complete interruption, while the dexmedetomidine CRI was kept for 24 hours longer at the dose of 0.5 μg/kg/h for anxiolysis. No alteration has been found during thoracic radiography and abdominal ultrasound. The patient started to eat autonomously after the ketamine CRI was stopped and fully recovered 48 hours after admittance to the hospital. Treatment was discontinued with no evidence of seizures. The only alteration in physiological parameters noted during the drug infusions was a state of hypothermia, managed with convection heating when body temperature went below 36°C. A mild hypothermia may contribute to decrease intracranial edema formation and prevent or mitigate the excessive intracellular influx of Ca^{2+} and the accumulation of glutamate in the extracellular space [3], thus contributing to prevent the occurrence of a trigger for others epileptic seizures. The patient was discharged clinically stable, with a maintenance dose of phenobarbital at 3mg/kg BID until next neurologic examination. The suspected topographical localization turned out to be encephalic, thus the patient underwent a brain magnetic resonance that didn't show any abnormality. Given the results of all the diagnostic tests, these episodes of convulsive seizures were likely due to an idiopathic epilepsy. In this case, regardless of the underlying cause, with a combination of dexmedetomidine and ketamine, we successfully managed episodes of convulsive seizures, thus leading to the conclusion that this could be a valid alternative to other described treatments for this condition.

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THE EQUINE NASAL RESISTOME: A NEXT GENERATION SEQUENCING APPROACH

Selene Rubiola, Matteo Cuccato, Sara Divari, Francesca Tiziana Cannizzo, Michela Bullone

Università degli Studi di Torino, Dipartimento di Scienze Veterinarie.
Corresponding author: M. Bullone (michela.bullone@unito.it)

Antimicrobial resistance (AMR) is a leading cause of death worldwide, mostcommonly linked with respiratory infections unresponsive to treatment [1]. Pet-associated resistant strains might pose a risk for human health in this perspective and their active monitoring is justified [2]. Veterinarians working with horses have the highest risk of nasal carriage of methicillin resistant staphylococci [3], but less is known concerning other bacterial strains or AMR mechanisms. The term resistome is defined as the complete collective assemblage of antibiotic, antiseptic and heavy metal resistance genes in a microbial ecosystem where both commensal and pathogen bacteria coexist [4]. This study aims at describing the nasal resistome of a heterogeneous population of 93 horses living in North-West Italy. Horses were selected from a convenient sample of 18 barns in the Turin area (4 to 6 horses/barn; ethical approval protocol N. 936, 16/04/2019, DSV-UniTO). An equal number of racehorse, pleasure, and showhorse barns was studied (n=6 per group). Data on horse health and management were obtained from all the animals included. Nasal swabs were obtained from both nostrils of each horse, immediately cooled and stored at -80°C. After thawing, DNA was extracted from each sample using the automated maxwell RSC Buccal Swab DNA Kit (Promega), individually quantified using the quantiFluor dsDNA System (Promega) and then pooled per barn (N=18) before shotgun metagenomic sequencing. Library preparation and sequencing procedures were performed by an external laboratory. Samples were run on a NovaSeq 6000 Illumina platform with a 2x150 bp protocol and to a sequencing depth of 50 M PE reads. Trimmed reads were mapped to the EquCab3.0 equine reference genome with BWA mem and host-filtered reads were aligned to the MEGARes 2.0 database to perform a reads-based resistome characterization; the Resistance Gene Identifier (RGI) was used to rule out genes requiring SNP confirmation. Reads predicted to encode AMR were taxonomically classified using Kraken2. Taxonomic profiling was performed using Kraken2 and Bracken. Reads identified as of equine origin were the vast majority, ranging from 90.15 to 98.92% (median 98.36%). Most commonly observed resistance genes were those conferring resistance against beta lactams, aminoglycosides and tetracycline antimicrobials. Multidrug resistance genes were also commonly observed. This is coherent with the antimicrobial molecules most commonly prescribed in equine practice in our region. Among beta lactams resistance genes, CTX and OXA were the most commonly detected, encoding for different betalactamases. mecA was also detected conferring resistance to beta lactames. Phosphotransferases were the most commonly encountered genes conferring resistance to aminoglycosides in our samples. Genes mediating resistance to tetracycline were those encoding for MFS efflux pumps. Most prevalent bacteria identified as part of the equine nasal microbiota were those belonging to the *Acinetobacter* and *Staphylococcus* species. *Acinetobacter* is a recognized nosocomial pathogen in horses most commonly causing thrombophlebitis and lower airway infections [5]. Staphylococci as well can cause respiratory infections, among others. In conclusion, we report here the first exploratory analysis on the nasal resistome of healthy horses living in our region. Nasal swabs did not guarantee the retrieval of enough material for resistome analysis. Nasal washings may be linked with an increased percentage of microbial *vs* host genome for future studies. Data reported are coherent with current antimicrobial treatments administered in the equine population of our region. Further data are warranted to comment on the clinical and One Health relevance of our observations.

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SICV

CHERRY PICKING IS A COGNITIVE BIAS THAT RESULTS FROM CONSIDERING ONLY EVIDENCE THAT SUPPORTS ONLY THE INITIAL DIAGNOSTIC HYPOTHESIS, IGNORING DATA THAT CONTRADICT IT

C. Spediacci, D. De Zani, S. Biagiotti, K. Gustafsson, M. Di Giancamillo, D. Zani

Università degli Studi di Milano, Dipartimento di Medicina Veterinaria e Scienze Animali.
Corresponding author: C. Spediacci (carlotta.spediacci@unimi.it)

Accurate diagnosis of pain causing lameness can be challenging due to the complexity of interpreting results of diagnostic analgesia and the difficulty in correlating lesions detected in MRI with the clinical signs.

Magnetic resonance imaging (MRI) is considered the gold standard for evaluation of musculoskeletal injuries. However, due to its high specificity several non-significant alterations can be detected (1). Furthermore, in the last decade several studies highlighted the diagnostic limits of perineural anesthesia demonstrating diffusion of local anesthetic and desensitization of anatomical structures proximal to the site of injection (2).

Cherry picking is a cognitive bias that results from considering only evidence that supports the initial diagnostic hypothesis, ignoring data that contradict it.

The purpose of this study is to evaluate the risk of cherry picking in horses with unilateral forelimb lameness responsive to digital palmar nerve block referred for MRI investigation.

A retrospective observational study was performed. Horses with unilateral forelimb lameness positive to DPNB referred to University Veterinary Hospital of Lodi between 2019 and 2021 for MRI examination were included.

Signalment and history were recorded. On the basis of the site of the most significant lesion the sample was divided into two groups: lesions proximal to the middle third of P1 and lesions distal to the middle third of P1.

A total of 67 horses of different breed were included in the study. The sample included 37 females (55%), 16 neutered males (24%) and 14 males (21%), the mean age was 10.3 years with a range of 4-16 years.

In 29.85% of the examined horses the most significant lesion was located proximal to the middle third of P1. In these horses, limiting the MRI protocol to the region of the foot on the basis of positive response to DPNB would have caused a *cherry picking* phenomena.

In conclusion, in order to reduce the risk of diagnostic errors in horses referred for MRI examination with lameness responsive to DPNB, both the foot and the fetlock are recommended to be included in the imaging protocol.

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ULTRASONOGRAPHY OF THE METACARPAL/TARSAL-PHALANGEAL JOINTS IN HEALTHY RACE HORSES: NORMAL APPEARANCE, BREED-RELATED AND AGE-RELATED FEATURES

Irene Nocera, Micaela Sgorbini, Benedetta Aliboni, Emma Bagnoli, Simonetta Citi

Università di Pisa, Dipartimento di Scienze Veterinarie
Corresponding author: I. Nocera (irene.nocera@vet.unipi.it)

In horses, specific ultrasound (US) features and reference values have been reported for joint cartilage appearance ans thickness according to different joints (1). It was shown that cartilage thickness changes according to age, in horses and other species (2). Moreover, since cartilage surface thickness and subchondral bone US appearance markedly change during joint disease in the horse, the knowledge of the normal features is essential during the clinical investigation (3). Thus, the aim of the present research was to evaluate metacarpal/tarsal-phalangeal joints US features in healthy race horses, according to age and breed.

Seventy-one metacarpal/tarsal-phalangeal joints in 28 horses (15/28 Thoroughbred and 13/28 Standardbred) were enrolled in the present study. The horses were sound on orthopedic and radiographic examination and grouped according to age and breed: group A <5 years old vs group B ≥5 years old; Throughbred vs Standardbred. US was performed using a portable ultrasound machine with a multifrequency linear transducer (5-7.5 MHz). Dorsal metacarpal/tarsal-phalangeal joints were scanned, both longitudinal and transversal. The US images were reviewed offline for articular cartilage appearance, thickness and subchondral bone appearance by one experienced observer.

Data were assessed for distribution, median, minimum and maximum for cartilage thickness values were calculated according to both age and breed and differences between groups were evaluated with a Mann-Whithney test. P was set at <0.05.

Cartilage thickness values were statistically lower in group A vs B in Strandardbred, except for the lateral thickness in longitudinal view. No difference was detected in Thoroughbred, according to age. No differences were found comparing breeds. In Standardbred, none of the fetlock joints of group A showed abnormal cartilage and subcondral appearance.

The present study showed a difference in cartilage thickness in healthy Standardbred, according to age, where the cartilage appears thinner and with normal US appearance. On the other hand, no evidence of differences where registered in Thoroughbred within age groups. No differences were detected comparing breeds, probably due to low number of horse enrolled in group, that might have influenced results. Since cartilage might change according to joint growth, horse age and horse training activity (2,3), the present research suggested the use of specific references for US features, that might be pivotal to correctly evaluate the health of fetlock joint.

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YOU CAN LEAVE THE HEAD OUT: A RETROSPECTIVE EVALUATION OF THE UTILITY OF WHOLE BODY CT STAGING IN DOGS WITH MAMMARY TUMORS

Martina Manfredi, Jessica Bassi, Pierantonio Battiato, Mauro Di Giancamillo, Maurizio Longo

Università degli Studi di Milano, Dipartimento di Medicina Veterinaria e Scienze Animali.
Corresponding author: M. Manfredi (martina.manfredi@unimi.it)

Whole-body computed tomography (CT) is generally recommended for staging and surgical planning of canine mammary tumors. However, there are no recent evidence-based guidelines indicating the most suitable staging approach in dogs. Therefore, whole body CT is usually accepted as the best strategy, not considering cost-effectiveness and patient radiation exposure. To raise the attention on ALARA principles application in veterinary imaging, we investigate the utility of including the head in the CT scan of these patients. Whole-body CT exams of dogs referred for mammary tumor staging between 2016 and 2021 were retrospectively evaluated for the presence of tumor related or clinically significative lesions in the region of the head. We included 93 female dogs of different breeds, 33 neutered, median age 10 years old (from 2 to 16 years). Metastatic spread in the region of the head was not detected in any patients. The most clinically significant lesions were an incidental thyroid nodule in three dogs, carotid body paraganglioma in one dog, nasopharyngeal polyp, and pituitary gland enlargement in other two patients. The most observed lesions were teeth infections, otitis externa, and otitis media. Based on the result of the present study, there is no evidence that canine patients might benefit from including the head in the staging process of mammary tumours.

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INJURIES OF THE ARTICULAR SURFACES OF THE FETLOCK JOINT IN HORSES USING LOW-FIELD MRI: 18 CASES

Donatella De Zani, Vanessa Rabbogliatti,Kajsa Gustafsson ,Carlotta Spediacci, Mauro Di Giancamillo, Davide Danilo Zani

Università degli Studi di Milano, Dipartimento di Medicina Veterinaria e Scienze Animali
Corresponding author: D. De Zani (donatella.dezani@unimi.it)

The aim of this retrospective case series was to report the case details and describe the MRI findings and outcome in sport horses with a diagnosis of traumatic injuries of the fetlock joint and to verify the capability of low-field MRI in the classification of these injuries, using a system proposed in human medicine[1]. In the equine practice the terms "short incomplete fracture", "stress fractures", "fissure", transchondral fracture" and "osteochondral fracture" are often used interchangeably. Nevertheless, all these pathological entities have common MRI findings: decreased signal intensity in T1 weighted sequences and increased signal intensity in STIR sequences within the spongiosa and subchondral bone[2,3], loss of definition between the subchondral bone and cartilage layer[4], high signal intensity fissure in the subchondral bone[2,3]and ill-defined subchondral bone loss. In human medicine, a more appropriate classification has been proposed, based on patho-anatomic description and evaluation of the extent of the lesion[1], allowing to reach a definitive diagnosis, to formulate a more accurate prognosis, to choose a correct treatment in order to avoid the progression of an acute lesion into a chronic or catastrophic injury.[4]

Clinical information including signalment, medical history, intended use, onset, duration and lameness grade, response to diagnostic block, radiographic and MRI findings, athletic outcomes of horses included in the study were collected. Based on the magnetic resonance appearance, an incomplete fracture was classified in three categories: subchondral, osteochondral, and chondral fracture.

Eighteen horses with variable lameness localized to the fetlock region were included in the study. All horses had unilateral lameness with sudden onset, localized to the forelimb in 14 cases.

In all horses classification of the incomplete fracture was performed according to the established criteria. Eleven horses had a diagnosis of subchondral fracture and seven of osteochondral fracture, no horse had a fracture classified as chondral. Twelve horses (67%) were sound at the time of re-examination, with a prevalence of horses with a diagnosis of subchondral fracture. Ten horses (56%) returned to compete at previous level.

The result of this study suggest that a more accurate classification of incomplete fracture is possible using a low-field MRI. Adoption of a classification system can be an aid for a more precise diagnosis and prognosis.

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SENTINEL LYMPH NODE BIOPSY IN DOGS WITH SCARS OF PREVIOUS TREATED MAST CELL TUMORS: AN EXPLORATIVE STUDY USING LYMPHOSCINTIGRAPHY

Elisa Maria Gariboldi, Donatella De Zani, Roberta Ferrari, Damiano Stefanello, Davide Danilo Zani, Martina Manfredi, Valeria Grieco, Chiara Giudice, Camilla Recordati, Mario Caniatti, Lavinia Elena Chiti

Università degli Studi di Milano, Dipartimento di Medicina Veterinaria e Scienze Animali
Corresponding author: E.M. Gariboldi (elisamaria.gariboldi@guest.unimi.it)

Sentinel lymph node biopsy (SLNB) has been recently implemented in the surgical treatment of canine mast cell tumors (MCT) [1-3]. To date, access to SLN mapping techniques is limited, and referral centers often treat dogs that have already been received a therapy without SLNB. This study aims to explore the feasibility and utility of SLN mapping and biopsy with lymphoscintigraphy in dogs with scars from previously treated MCT. Dogs with MCT (cutaneous and subcutaneous), previously treated with surgery or intralesionally were prospectively enrolled and underwent SLNB with radiopharmaceutical and blue-dye [4, 5]. Dogs with local recurrence, enlarged regional lymph nodes, stage IV, adjuvant medical treatment were excluded. Recorded variables were: signalment; clinical, pathological, and surgical (marginal vs wide excision; linear vs complex reconstruction) variables of primitive MCT; location and length of the scar; time between MCT excision and SLNB; SLN variables (number for lymphocenters, size, blue dyed, histopathological metastatic status), and status of margins of excised scars (in case of Stage 0).

Twelve dogs with 13 scars were enrolled. Previous treatment consisted of 7 marginal excisions, 5 wide excisions, and 1 Tigilanol Tiglate injection. Scar locations were trunk (53.8%), limb (15,4%), digital site (15.4%), inguinal (7.7%) and head-and-neck (7.7%). Median MCT size was 10 mm (range 5-60 mm); median scar length was 21 mm (range 10-200 mm). The median time between MCT excision and SLNB was 51 days (range 17-99 days). Excised MCT were 9 cutaneous (8 Kiupel-Low grade, and 1 Kiupel-High grade), 3 subcutaneous and histological margins were infiltrated in 7. None had complex reconstruction. Twenty-one SLNs (median diameter 17,5 mm; range 6-30 mm) belonging to 12 lymphocenters were extirpated. One SLN was identified for 5 scars, whilst multiple SLNs were removed in 6 cases. In 2 cases a SLN was not identified: one case had been previously treated with wide margins excision and in the other the lymphosome of interest had been already removed during a mastectomy. Histopathologically, SLNs were: HN2 (5/11) HN1 (3/11) and HN0 (3/11). Blue-dye were not detected in 3 hot SLNs: 2 HN1 and 1 HN0. Histopathologically surgical margins of excised scars were not infiltrated in 6 and infiltrated in 1. Based on these preliminary results, SLN mapping in scars of previous treated MCT should be recommended for correct staging, prognostication and therapy. Future studies should focus on lymphatic drainage alterations in a larger sample population including cases that underwent cutaneous reconstructive plastic surgery.

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VARIABILITY IN FETLOCK JOINT RANGE OF MOTION QUANTIFIED USING WEARABLE INERTIAL MESUREMENTS UNITS IN SOUND AND LAME HORSES

Eleonora Pagliara (1), Laura Antenucci (2), Giacomo Zoppi (1), Mario Giacobini (1), Barbara Riccio (1), Michela Bullone (1), Andrea Bertuglia (1)

(1) Università degli Studi di Torino, Dipartimento di Scienze Veterinarie. (2) Captiks S.r.l., Roma.
Corresponding author: E. Pagliara (eleonora.pagliara@unito.it)

Lameness is a clinical manifestation of a disorder of the locomotor system. Lameness affects horses from all equestrian disciplines and is one of the main reason for veterinary consultation [1]. Its recognition and treatment is an essential part of veterinary competence and monitoring locomotion is a major concern for improvement of the horse welfare [2]. Lameness recognition at naked eye is based upon the detection of asymmetry between bipeds in movement and the deviation from optimal gait but suffer of inherited limitations. Joint angle patterns are indicators of lameness-related gait disturbances in horses and a decreasing fetlock joint range of motion is a sensitive measure of supporting limb lameness [3]. To obtain objective measurements of angular kinematic of the equine joints, a specialized environment and expensive equipment has been necessary in the past. Recently, the use of inertial measurements units (IMU) systems has been growing in the everyday practice because these systems could detect kinematics parameters over different distances and conditions. The aim of the study was to quantify sagittal plane fetlock joint range of motion (sFJRM) using IMU system technology and study its variability in a group of sound animals and affected by natural occurring lameness. Seven sound horses and seven horses showing evident lameness (>3/5 AAEP) were enrolled and analyzed during walk and trot on the same firm surface (approval date 15/12/2020 protocol n 2796/2020). All subjects were instrumented with 8 IMUs (working in pairs for each limb- Movit G1; Captiks S.r.l.,Rome) positioned on the dorsal aspect of the metacarpal/metatarsal bones and pasterns. The coefficient of variation (CV) of sFJRM between the left and right forelimbs and the left and right hindlimbs was calculated on three consecutive strides at two different gaits. In lame horses only the lame limb and its contra-lateral were studied to this aim. A three-way ANOVA ($\alpha = 0.05$) was run to investigate the effect of gait (walk/trot), lameness (sound/lame), and pair of limbs (front-hind) on CVs. In the sound horses, CV between pair of front limbs was 3% at walk and 2.51% at trot. The CV between paired hind limbs was 3.91% at walk and 3.60% at trot. The CV of sFJRM between the lame limb and its contra-lateral was 15.47% at walk and 7.06% at trot. The CV of the sFJRM between paired limbs (right vs left) was significantly affected by lameness ($p<0.0001$), gait ($p=0.0029$), and front vs hindlimbs ($p=0.0233$). Values of sFJRM quantified by the IMU system were in line with previous results of sagittal plane kinematics at walk and trot in sound horses detected with optical motion capture. Larger CVs have been present at walk comparing to trot in lame horses and when the front limb was affected. To the best authors knowledge this is the first study in which IMU sensors were used to quantify and study the variability of sFJRM overground. Further research is warranted to evaluate sFJRM variability in cases of more subtle lameness.

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COMPUTED TOMOGRAPHIC DETERMINATION OF PITUITARY DIMENSIONS IN DOMESTIC SHORT HAIRED CATS: PRELIMINARY RESULTS.

Dario Costanza (1), Adelaide Greco (1), Luigi Auletta (2), Erica Castiello (1), Pierpaolo Coluccia (1), Luigi Navas (3), Leonardo Meomartino (1)

(1) Università degli Studi di Napoli "Federico II", Centro Interdipartimentale di Radiologia Veterinaria. (2) Università degli Studi di Milano, Dipartimento di Medicina Veterinaria e Scienze Animali. (3) Università degli Studi di Napoli "Federico II", Dipartimento di Medicina Veterinaria e Produzioni Animali.

Corresponding author: D.Costanza (dario.costanza@unina.it)

The pituitary gland plays a major regulatory role in the entire endocrine system; neoplastic alterations can produce diseases such as pituitary-dependent hyperadrenocorticism or acromegaly. The detection of subtle changes in the hypophyseal size due to small lesions such as microadenomas has important diagnostic and therapeutic implications. In dogs, the pituitary gland height-to-brain ratio (P/B ratio) was introduced to assess the pituitary gland dimension in subjects of different breeds and sizes [1]. In cats, although few studies established the linear dimensions of the pituitary gland [2, 3], those values were determined on small and inhomogeneous samples, so the established cut-offs may differ between breeds due to anatomical differences in the skull morphotype. The primary objective of this study was to determine the average values for hypophyseal height, length and the P/B ratio in a sample of domestic short-haired (DSH) cats. Secondary objectives were to test sex, age and weight effects on the linear dimensions and P/B ratio. For this aim, post-contrast CT images of DSH cats acquired at the Interdepartmental Center of Veterinary Radiology of Naples with a multidetector CT, using a 0.625–1.25 mm slice thickness and a "standard" convolution kernel, in the period between November 2018 and March 2022 were retrospectively reviewed. Inclusion criteria were the absence of clinical, laboratory and CT alterations related to pituitary gland disease. For each cat included in the final sample, sex, weight (in kg), age (in months), pituitary gland height and length (in mm), and the brain area (in mm^2) were recorded. The CT images were evaluated using a commercial DICOM viewer software through a soft tissue window (WW 500 HU - WL 80 HU). The pituitary height was measured on the axial plane at the level of pituitary fossa where the maximum height was visible; on the same slice, the brain area was measured using an automated segmentation tool. The pituitary length was measured on sagittal plane. The statistical analysis was performed with a general linear model and the correlations between significant effect and the corresponding dependent variables were then further analyzed according to data distribution. Finally, the P/B ratio reference range and the 95% CI of the mean were calculated. Thirty-eight DSH cats (6 males, 7 castrated males, 4 females and 21 neutered females), aged from 6 months to 15 years and weighing between 1.8 and 7.5 kg (mean 4.3±SD 1.4 kg), were included in the sample. The mean ±SD for the hypophyseal height, length and P/B ratio were 3±0.6 mm, 2.9±0.69 mm and 0.39±0.08, respectively. The multivariate analysis showed body weight over the brain area as the only significant effect, but when further analyzed with ANCOVA they did not show any relationship and a fair correlation (rs=0.37, P=0.02). Our preliminary results are different from those already present in the literature and show that the hypophyseal linear dimensions and the PB ratio in the DSH are distributed in a narrow interval. In the authors' opinion, the P/B ratio can be a quick and reliable method for assessing the pituitary dimensions despite the cat's weight, sex and neutering status. The fact that no effect could be detected over the P/B ratio makes it an easy and reliable tool to be used in a clinical scenario but needs to be further explored using different breeds. Based on our results, for the P/B ratio in the DSH cat, an upper limit of 0.53 can be considered.

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THE KIDNEY-TO-AORTA RATIO TO ASSESS RENAL DISEASES IN DOGS

Erica Castiello (1), Leonardo Meomartino (1), Dario Costanza (1), Dario Bruzzese (2), Maria Pia Pasolini (3), Pierpaolo Coluccia (1), Barbara Lamagna (3), Adelaide Greco (1).

(1) Università degli Studi di Napoli Federico II, Centro Interdipartimentale di Radiologia Veterinaria.
(2) Università degli Studi di Napoli Federico II, Dipartimento di Sanità Pubblica.(3) Università degli Studi Federico II, Dipartimento di Medicina Veterinaria e Produzioni Animali.
Corresponding author: E. Castiello (erica.castiello@unina.it).

Ultrasonography (US) is the modality of choice for kidneys imaging in Veterinary Medicine. To assess the renal dimensions, it has been proposed to correlate the kidney length (KL) to the aortic luminal diameter (AoD) [1]. Although this method is easy to perform, it has a wide range of normal cutoff values and, consequently, a low sensitivity and specificity. The wide range of normal cutoff values is most likely related to the different breeds and morphotypes included in the original sample. Indeed, a later study, performed on a single breed, established very narrow reference intervals [2]. Furthermore, this method has not been validated by comparing the KL/AoD ratio in healthy subjects and in those with renal disease. This preliminary study has a double aim: to compare the KL/AoD ratio between healthy dogs and those with clinical diagnosis of kidney disease and to define its sensitivity and specificity. For this purpose, all the clinical records and the ultrasonographic exams performed at the Interdepartmental Center of Veterinary Radiology of Naples between May 2017 and December 2020 were retrospectively reviewed. Dogs were excluded from the initial sample if the images were considered inadequate or if serum creatinine and azotemia levels had not been obtained within 48 hours from the US examination. Breed, sex, weight (in kg), age (in months), length of the right (RKL) and left (LKL) kidney, AoD, levels of serum creatinine and azotemia were recorded on an electronic spreadsheet for each dog included in the final sample. Then, dogs were grouped as healthy or non-healthy based on the serum creatinine and azotemia levels. Finally, the RKL/AoD, LKL/AoD and the ratio for aggregated data (KL/AoD) were obtained. Data distribution and descriptive statistics were obtained for age, gender and weight. Furthermore, 95% confidence intervals and interquartile ranges were calculated for RKL, LKL and AoD in each group and pooled data. The LKL/AoD, RKL/AoD and KL/AoD ratios were compared between the two groups using the Mann-Whitney test. Furthermore, the sensitivity and specificity of the ratios were assessed using the ROC curves. In all analyses, P was set at <0.05. The final sample consisted of 116 dogs (31 females, 33 neutered females, 43 males and 9 castrated males) of 32 different breeds; 95 dogs (81.9%) were classified as healthy and 21 (18.1%) as non-healthy. In the overall population, the median age and body weight (± standard deviation) were 100±41.8 and 17.3±10.6, respectively. The Mann-Whitney test showed a significant difference in the RKL/AoD (P=0.006), LKL/AoD (P=0.014) and in KL/AoD (P=0.009) ratios between the healthy and non-healthy dogs with this last having generally smaller values, although the presence of a moderate overlap between the two groups. The ROC curves showed the best performance in distinguishing diseased from healthy dogs for KL/AoD ratio <6.48. Our preliminary results show that, although there is a partial overlap of the KL/AoD ratio between healthy subjects and those with renal disease, a cutoff value of 6.48 has a good sensitivity for discriminating kidneys with normal dimensions from those with reduced length, confirming the clinical usefulness of the method, and that this limit is significantly higher than the value of 5.5 previously reported. The presence of a partial overlap of some values is probably related to renal disease different from chronic renal failure (CKD), that does not lead to a reduction in renal dimensions. Further studies, considering only subjects with CKD or other single disease will contribute to better define cutoff values.

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POSSIBLE CLINICAL IMPLICATIONS OF ULTRASONOGRAPHIC-DETECTABLE FOCI OF MINERALIZATION IN DOG'S LIVER

Federico Puccini Leoni (1), Caterina Puccinelli (1), Tina Pelligra (1), Veronica Marchetti (1), Eleonora Gori (1), Alessia Diana (2), Nikolina Linta (2), Simonetta Citi (1)

(1) Università di Pisa, Dipartimento di Scienze Veterinarie. (2) Università di Bologna, Dipartimento di Scienze Mediche Veterinarie.

Corresponding author: F. Puccini Leoni (federico.puccini33@gmail.com)

In veterinary medicine the prevalence of intrahepatic bile ducts mineralization is very low in canine population, more frequently observed in small sized old dogs and associated to little or no clinical significance. Hepatic dystrophic mineralizations are often discovered as incidental findings during abdominal ultrasonographic examination (AUE); if they are radiopaque, they can be confirmed by radiographs or computed tomography images. AUE is the most commonly used imaging modality for evaluating small animal patients with suspicion of hepatobiliary disease. The purpose of our study was to assess a possible clinical relevance of ultrasonographic-detectable foci of mineralization in canine liver (FMCL) and a possible relationship between them and other pathological alterations concerning the digestive apparatus. We carried out a retrospective analysis evaluating the database of the canine patients admitted to the Veterinary Teaching Hospital of the University of Pisa and the University Veterinary Hospital of the University of Bologna. All dogs under study underwent an AUE in which FMCL were found and their clinical and anamnestic reports, associated to biochemical profile, were reviewed. A total of 34 patients met the inclusion criteria, 24 females and 10 males. The median age was 11 years and the most represented breed was the crossbreed, followed by the Cavalier King Charles Spaniel breed. Based on FMCL size, we identify two main kind of FMCL: millimetric foci and subcentimetric/centimetric foci. The first ones do not create a distal acoustic shadow and they are often localized in correspondence of the smaller bile ducts or within the hepatic parenchyma. The second ones produce a distal acoustic shadow and they are generally positioned in the lumen of the large bile ducts. These two types of FMCL could result in three main patterns of mineralization: aligned pattern, branched pattern and diffused pattern. Over 90% of the patients showed ultrasonographic alterations regarding the biliary system and over 80% presented ultrasonographic abnormalities of the hepatic parenchyma. Moreover, in 76% of the dogs, ultrasonographic anomalies of the digestive tract were observed, affecting in particular the small intestine. In over half of our patients, we observed increased ALP, ALT and GGT, while hypertriglyceridemia was present in 44% of the cases, hypercholesterolemia in 47%, and a decrease in total protein in 29%. Clinically, 82% of dogs showed signs of gastrointestinal disease, in particular diarrhea and vomiting. We hypothesize, based on biochemical and clinical evidences, that the presence of FMCL could be related to a bile stasis condition and a chronic inflammatory or infectious disease involving the biliary system, the hepatic parenchyma and the gastrointestinal tract. At the same time, colelitis may predispose to a stasis of bile flow and a consequent inflammatory or infectious phenomenon. The presence of FMLC is, in our opinion, an unusual finding that must be taken into consideration as a potential sign of hepatobiliary and/or enteric pathology.

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EVALUATION OF ANALGESIC EFFECTS OF SULFATE MAGNESIUM ADMINISTERED IN COSTANT RATE INFUSION IN DOGS UNDERGOING TIBIAL PLATEAU LIVELLING OSTEOTOMY (TPLO): PRELIMINARY DATA

Caterina Di Bella, Margherita Galosi, Luca Pennasilico, Sara Sassaroli, Angela Palumbo Piccionello

(1) Università degli Studi di Camerino, Scuola di Bioscienze e Medicina Veterinaria.
Corresponding author: M. Galosi (margherita.galosi@unicam.it)

The analgesic role of magnesium sulphate ($MgSO_4$) in veterinary medicine is not yet well defined, although its use and its analgesic potential are widely demonstrated in human medicine. The aim of the study was to evaluate the effects of $MgSO_4$ as an adjuvant to analgesic protocol with ketamine in dogs undergoing TPLO surgery. Our hypothesis is that the use of $MgSO_4$ may play a synergistic role in enhancing intra- and postoperative analgesia. This perspective, randomised, clinical trial included 10 dogs with cruciate ligament rupture to undergo TPLO. The patients were divided in two groups of 5 dogs each: MK ($MgSO_4$ + ketamine) and K (ketamine) groups, respectively. Dogs belonging to MK group received a bolus of $MgSO_4$ (50 mg/kg IV) in 15 minutes followed by a CRI (15 mg/kg/h), as well as a bolus of ketamine (0.5 mg/kg IV) over 2 minutes followed by a CRI (1 mg/kg/h). K group received a bolus and ketamine infusion at the same dosages. All patients were premedicated with methadone (0.3 mg/kg IM) and induced with propofol (5 mg/kg IV). General anesthesia was maintained using isoflurane in pure oxygen. The main haemodynamic and respiratory parameters were recorded for all patients at the following times: 10 minutes before premedication (BASELINE), 10 minutes after the start of general anesthesia (T_0), at the end of the bolus of ketamine (T_1), at the end of the bolus of $MgSO_4$ (T_2), at skin incision (SKIN), at corrective osteotomy (OSTEOTOMY), during the application of the last skin suture (SUTURE) and each 60 minutes until the need of a rescue analgesia. Mean arterial pressure (MAP) was measured by placing an arterial catheter in the metatarsal artery. For both groups the end of the CRI coincided with the SUTURE time. The increase in heart rate (HR) and MAP of 20% compared to T_0 represented the first signal of the occurrence of nociceptive stimuli. In this case the the CRI of ketamine was increased to 2 mg/kg/h in order to induce the analgesic rescue. However, if the parameters did not fall within the predetermined ranges within 10 minutes of their detection or a second peak in HR and MAP was registred, fentanyl (1 mcg/kg IV) would have been administrated and the patient would have been widthdrawn from the study. The post-operative pain (starting from POST60) was assessed using the Glasgow Composite Measure Pain scale (GCPs). Checks were performed every hour until a GCPs score ≥5/20 was reached. At this point, patients received methadone (0.3 mg/kg IM) and the monitoring were stopped. The main electrolytes (Mg^{2+}, Ca^{2+}, Na^{+}, K^{+}) were analysed at different times of the study. In MK group the measure was taken at BASELINE, T2, OSTEOTOMY, SUTURE and POST 60 (for the purpose of recording differences in plasma electrolyte concentration during magnesium CRI), whereas for group K only BASELINE and SUTURE. The Anova one-way test was used to compare all the variables between the two groups and between different time points ($P<0.05$). All patients in the MK group reported, at T_2 time, hypermagnesemia (3.92±0.56 mg/dL) and hypocalcemia (1.03±0.15 mmol/L), as well as no clinical and electrocardiographic changes. However, a decrease of plasma concentration of Mg^{2+} (2.83±0.16 mg/dL) and an increase of Ca^{2+} (1.3±0.01 mmol/L) were detected already at the SUTURE time, before going back to the ranges recorded at POST60 (Mg = 2.67±0.12 mg/dL; Ca^{2+} = 1.33±0.02 mmol/L). There were no statistically significant differences in the request for intraoperative analgesia in the two groups and, in the postoperative period, both groups needed methadone already at POST 60. We should consider the small number of patients as a limiting factor. In conclusion, despite the efficacy of $MgSO_4$ in the management of acute and chronic pain has been widely demonstrated in human medicine [1], the results of this study show that, according to Rioja et al., the use of magnesium sulfate as an adjuvant to ketamine in pain management does not appear to be effective in dogs [2].

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EVALUATION OF THE ADDITION OF CISATRACURIUM IN AN ANESTHETIC PROTOCOL WITH SHORT-ACTING DRUGS IN ASA IV AND V CANINE PATIENTS

C. Interlandi, F. Macrì, F. Spadola, N. Iannelli, G.L. Costa

Università degli Studi di Messina, Dipartimento di Scienze Veterinarie.
Corresponding author: C. Interlandi (cinterlandi@unime.it)

The reduction of drug doses in anesthesia practice results in a valid and safe protocol leading to better management of animal welfare, particularly in patients with high anesthesiological risk (ASA IV and V). Neuromuscular blockers (NMBs) are important adjuncts to general anesthesia [1-3]. Cisatracurium besylate is a nondepolarizing muscle relaxant and could be considered an useful adjuvant to analgesic and anesthetic protocols. The purpose was to investigate whether the use of cisatracurium in association with propofol, remifentanyl and sevofluorane allowed to reduce the dose of drugs making the anesthetic protocol suitable in patients with high anesthesia risk. Ten adult female dogs that met the anesthetic criteria (Group 1), undergoing elective unilateral mastectomy surgery, were evaluated. Subjects received propofol and sevoflurane at variable dosage to induce and maintain anesthesia; analgesia was performed using remifentanyl 0.1 $\mu gkg^{-1}min^{-1}$. After three months, the same subjects (Group 2) underwent contralateral mastectomy and received the same anesthetic protocol with the addition of cis-atracurium 0,2 $mgkg^{-1}$. Postoperative analgesia was provided by intravenously buprenorphine 20 μgkg^{-1} towards the end of surgery, prior to discontinuing the remifentanil infusion. The effects of muscle relaxant on the dose of sevoflurane and propofol were evaluated. During anaesthesia, heart rate (HR), systolic arterial pressure (SAP), end-tidal CO2 (EtCO2), oxygen saturation (SpO2), halogenate concentration in the inspiratory phase (MAC) and rectal temperature were monitored continuously; the measurements were collected before drug administration (basal values, T^0), and at induction time (T^i), at 5 (T^5), 10 (T^{10}), 15 (T^{15}), 20 (T^{20}), 30 (T^{30}), and 35 (T^{35}) minutes after drugs injection. In Group I, HR values (median and range) showed significant differences, compared with T^0 (94; 86/97), at T^i (111; 99/125; p=0.005), T^5 (100; 92/108; p=0.012), T^{15} (74; 65/83; p=0.005), T^{20} (73; 66/80; p=0.005) and T^{25} (70; 64/76; p=0.005). In Group 2, the significant differences of HR values compared with T^0 (94; 89/99) were: T^5 (103; 92/118; p=0.012), T^{10} (103, 85/121; p=0.032), T^{15} (84; 70/98; p=0.028), T^{20} (84; 70/98; p=0.028), T^{25} (78; 68/88; p=0.005); T^{30} (86; 74/99; p=0.018) and T^{35} (84; 69/99; p=0.018). The comparison of HR values between the two groups showed significant differences at T10, T15, T20 and T25 with $p<0.05$. Regarding SAP values, Group 1 showed significant differences in all time points than T^0 (p=0.005). In Group 2, significant differences were found at T^i ((145; 126/164; p=0.008), T^5 (149; 136/162; p=0.012), T^{20} (127; 112/132 p=0.044), T^{25} (119; 108/126; p=0.012), T^{30} (121; 114/127; p=0.012), T^{35} (121; 114/127; p=0.012) in comparison with T^0 (135; 110/158). The comparison of SAP values between the two groups showed significant differences at all data points ($p<0.05$) except at T0. In Group 1, requirement for intraoperative halogenate varied significantly at all time points compared to T^0 with $p<0.001$, while in Group 2 the percentage of halogenate requirement remained constant during the experimental period, showing no significant differences in all data points. In Group 1, the comparison of $EtCO_2$ values showed no significant differences, while in Group 2 a significant difference with $p<0.001$ was found.

The propofol dosage at the induction phase varied significantly between the two groups, with significantly lower dosages in the cisatracurium-treated group (6.9±1.4$mgKg^{-1}$ Group 1 vs 2.45±0.9$mgkg^{-1}$ Group 2, respectively). Estubation time and resumption of station were shorter in Group 2. Subjects treated with cisatracurium showed more stable values of investigated parameters. Its addition determined a clinically relevant reduction in the concentration of propofol and sevofluorane used in dogs, with a good anesthetic and analgesic plan. We recommend the addition of cisatracurium in the anesthetic protocols for canine patients with high anesthesiological risk.

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TRAMADOL VS LIDOCAINE IN SWINE UNDERGOING ABDOMINAL SURGERY

G.L. Costa (1), F. Macrì (1), N.M. Iannelli (1), F. Spadola (1), F. Leonardi (2), C. Interlandi (1)

(1) Department of Veterinary Science, University of Messina, Italy. (2) Department of Veterinary Science, University of Parma, Italy.

Corresponding author: G.L. Costa (glcosta@unime.it.)

The aim of the study was to compare the analgesic efficacy of tramadol to that of lidocaine in local anesthesia in swine undergoing abdominal surgery. The study was approved by the ethics committee of the University of Messina (protocol N 027/2018). The study was performed on 30 large white crossbred swine undergoing abdominal surgery for umbilical hernia repair. The swine received tiletamine/zolazepam 5 mg/kg combined with romifidine 80 µg/kg, administered intramuscularly. The swine were divided into 3 groups which received a local analgesic protocol that provided for the use of 5% tramadol 4 mg/kg and 2% lidocaine 4.5 mg/kg as follows: in the LL group, the lidocaine was infiltrated in the surgical planes (skin/muscles) and administered by intraperitoneal splash; in the LT group, surgical planes were infiltrated with lidocaine and tramadol was administered by intraperitoneal splash; in the TT group, tramadol was infiltrated in the surgical planes and administered by intraperitoneal splash [1]. The following parameters were recorded: heart rate (HR), non-invasive systolic pressure (SAP), and respiratory rate (RR) ;at T5 (five minutes after anesthesia), T10 (skin incision), T15 (incision of the muscular planes), T20 (herniorrhaphy), T25 (suture of the muscular planes), and T30 (skin suture). Intraoperative pain was assessed using a cumulative pain scale (CPS) [2]. A numerical score between 0 and 4 was assigned based on the percentage change of from the reference values (recorded data at T5: HR, SAP, RR) according to the following scheme: 0 = no changes, 1 = > 0% but ≤10%, 2 = >10% but ≤20%, 3 = >20% but ≤30%, and 4 = >30%. The sum of the scores for the three parameters was the intraoperative pain score (CPS score). When CPS score was ≥ 10, the swine received an additional lidocaine bolus as rescue analgesia. The trend of RR, HR and SAP was adequate for the observed anaesthetic and analgesic plans and normal for a patient under anaesthesia. In the LL group, there were significant differences in CPS scores at T15, T25 and T30, compared to that at T10 (p=0.039, p=0.006, p=0.006, respectively). In the LT group, no significant differences in CPS scores were observed. In the TT group there were significant differences in CPS scores at T15, T20, T25 and T30 compared to that at T10 (p=0.006, p=0.013, p=0.006, p=0.013, respectively). Comparison of CPS scores between groups performed for each individual time point showed a significant difference between groups (p=0.000). The TT group showed higher scores compared to those of the LT group at T10 and T15 (p=0.006 and p=0.003); the LT group showed lower scores compared to those of the LL group at T10 and T25 (p=0.034 and p=0.005); the LL group showed lower scores compared to those of the TT group at T10 (p=0.007) and higher scores at T30 (p=0.017). The CPS scores were for all swine < 10 and no subject required rescue analgesia. Tramadol represent an alternative to local anesthetic drugs when local anesthetics are completely or partially inactivated (e.g., in case of tissue inflammation).

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CLINICAL AND RADIOGRAPHIC EVALUATION OF SHORT- AND LONG-TERM OUTCOMES OF DIFFERENT TREATMENTS ADOPTED FOR ELBOW MEDIAL COMPARTMENT DISEASE IN DOGS

Daniele Serrani (1), Sara Sassaroli (2), Francesco Gallorini (3), Alberto Salvaggio (3), Adolfo Maria Tambella (2), Ilaria Biagioli (3), Angela Palumbo Piccionello (2)

(1) Southern Counties Veterinary Specialists, Forest Corner Farm, Hangersley, Ringwood, Hampshire, UK. (2) Università degli Studi di Camerino, Scuola di Bioscienze e Medicina Veterinaria, Matelica, Italy. (3) Clinica Veterinaria San Silvestro, Castiglion Fiorentino, Italy

Corresponding author: S. Sassaroli (sara.sassaroli@unicam.it)

Medial compartment disease is a common disorder in dogs affected by elbow dysplasia [1, 2]. Despite many treatments suggested in the literature, only few studies report comparative outcomes in the short and long term [1, 3-5]. This study reports and compares short- and long-term clinical and radiographic outcomes of dogs treated for medial compartment disease (MCD) by distal dynamic ulnar ostectomy (DUO), bi-oblique dynamic proximal ulnar osteotomy (BODPUO) and conservative management (CM). In this study, medium to large breed dogs, aged between 5 and 12 months, affected by uni/bilateral MCD and treated by DUO, BODPUO or CM from 2016 to 2018, were enrolled and followed up for 24 months. Orthopedic and radiographic examinations were performed at 0 (T0), 2 (T2), 12 (T12) and 24 (T24) months after treatment. Lameness score, elbow arthralgia, elbow range of motion (ROM), osteoarthritis (OA) score and percentage of ulnar subtrochlear sclerosis (%STS) [6] were evaluated at each time point. Dogs were divided into three groups according to the treatment performed: DUO, BODPUO and CM.

Forty-five elbows from 26 dogs, treated with DUO (n=17), BODPUO (n=17) or CM (n=11), were prospectively enrolled in the study. The patients enrolled in the CM group were older and showed more severe radiographic signs of OA compared to those enrolled in the other two groups.

Lameness and elbow arthralgia scores, ROM, OA score and %STS were compared between groups and between different time points within each group. $P<0.05$ was considered statistically significant. Lameness (DUO $p=0.0018$; BODPUO $p<0.0001$) and arthralgia (DUO, BODPUO $p<0.0001$) scores were significantly decreased in patients that underwent surgical treatments and increased in patients managed conservatively (lameness $p<0.0001$; arthralgia $p=0.3068$) at T12 and T24, but not statistically significant difference was detected at short-term evaluation (T2) within each group compared with preoperative values. OA score (DUO, BODPUO, CM $p<0.0001$) and ROM worsened in every study group (DUO, CM $p<0.0001$; BODPUO $p=0.0740$), but %STS decrease in DUO ($p=0.0108$), increase in the CM group ($p=0.0025$) and remaind unchanged in the BODPUO group ($p=0.2740$) at T12 and T24. In particular, in DUO group, %STS significantly decreased at each time point (T2 $p=0.0373$, T12 $p=0.0018$, T24 $p=0.0039$) compared with preoperative values.

This study supports the clinical efficacy of DUO and BODPUO in reducing lameness, arthralgia and progression of %STS. Early diagnosis and surgical attention in patients affected by MCD can improve the short- and long-therm outcome and reduce the progression of secondary changes.

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COMPARATIVE EVALUATION OF EFFECTS AND EFFICACY OF THREE DIFFERENT PREMEDICATION PROTOCOLS IN DWARF RABBITS (Oryctolagus cuniculus) UNDERGOING ORCHIECTOMY

Alessio Angorini, Carlotta Cacchiani, Adolfo Maria Tambella, Alessandro Troisi, Angela Palumbo Piccionello, Caterina Di Bella

Università di Camerino, Scuola di Bioscienze e Medicina Veterinaria.
Corresponding author: A. Angorini (alessio.angorini@unicam.it)

Rabbits have a higher anaesthesiological risk than other small animals, with a percentage of mortality of about 1.39% compared to dogs (0.17%) and cats (0.24%) [1]. The aim of this study is to identify a suitable and effective premedication protocol for elective surgery, reducing anaesthetic complications and perioperative stress in this species. Fifteen male dwarf rabbits, classified as ASA I, were selected for this prospective, clinical study. All patients were randomized into three groups (5 rabbits per group), which were administered three different premedication protocols: MDM (midazolam = 0.2 mg/kg, dexmedetomidine = 25 mcg/kg and methadone = 0.2 mg/kg), DKM (dexmedetomidine = 25 mcg/kg, ketamina = 20 mg/kg and methadone = 0.2 mg/kg) and MKM (midazolam = 0.2 mg/kg, ketamine = 20mg/kg and methadone = 0.2mg/kg). During the preoperative period, heart rate (HR), respiratory rate (RR) and sedation score (0 = normal; 11 = deep sedation) were monitored 5, 10, 15 and 20 minutes after premedication (T5, T10, T15, T20, respectively). Subsequently, the auricular vein was cannulated and the V-GEL mask was positioned in order to administer oxygen and isoflurane. In this preoperative phase, ataxia, incoordination and reactions to manual stimulation were registered. During the intraoperative period, the main cardiovascular and respiratory parameters were monitored 10 minutes before the start of surgery (BASE), during skin incision (SKIN), traction of the funiculus (TESTIS) and suture (SUTURE). Intraoperative nociception was assumed if HR or MAP increased by > 20% from baseline, in which case a bolus of fentanyl (5 µg/kg) was administered. At the end of the surgery, HR, RR and temperature (T°) were monitored 10, 20, 30, 40, 50 and 60 minutes after extubation (Post10, Post20, Post30, Post40, Post50, Post60, respectively). The quality of recovery (QR) was assessed at each postoperative time using a specific score (1 = excellent; 6 = very bad). Cardinal data were compared between the groups and at each time of the study with the ANOVA and the Tukey-Kramer tests; scores data were analysed with the Dunn test ($P<0.05$). In the preoperative period, at T5 (MKM = 72±35.09; DKM = 104.8±60.3; MDM = 115.2±43.9 breath/min), T10 (MKM = 39.2±16.8; DKM = 69.6±52.8; MDM = 96.4±49.6 breath/min), T15 (MKM = 35.2±16.09; DKM = 50±30.1; MDM = 65.6±26.6 breath/min), and T20 (MKM = 35.2±18.4a; DKM = 43.2±21.4; MDM = 64.8±30.2 breath/min), RR was significantly lower in all groups compared to T0 (MKM = 135.2±58.6; DKM = 129.6±14.8; MDM = 151.6±12.3 breath/min). Moreover, in DKM group [9 (8-9)], sedation score (SS) was higher than the other two groups at T10 [MDM=7 (6-8), MKM=6 (6-6)]. However, in DKM group, 4/5 patients showed signs of ataxia and incoordination. During the post-operative phase of the study, the QR, in MDM group [2.5 (1-3)], was already better at Post10 compared to the other two groups [DKM=4 (3-5); MKM=4 (4-4)]. Results of our study showed that the three protocols are valid and safe for routine surgery in rabbits; however, the premedication protocols that include ketamine cause severe ataxia and incoordination, that persist also in the postoperative period [2]. We assume that in short-term surgeries such as orchiectomy (about 20 minutes), ketamine is not the drug of choice as its duration of action is longer than surgical times. In conclusion, considering the sensitivity of the rabbit to the stress and the need to quickly feed and resume large organ functions, protocols including ketamine do not appear to be suitable for rabbit orchiectomy, and, in general, for short-term procedures, compared to protocols that include dugs as benzodiazepines and alpha2-agonists.

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COMPARISON OF TWO VESSEL SEALING DEVICES IN LAPAROSCOPIC OVARIECTOMY IN AFRICAN LIONESSES

Marta Guadalupi (1), Pietro Laricchiuta (2), Marzia Stabile (1), Annalaura Scardia (1), Caterina Vicenti (1), Claudia Piemontese (1), Alberto Crovace (3), Francesco Staffieri (1), Luca Lacitignola (1)

(1) Università degli Studi di Bari, Dipartimento dell'Emergenza e dei trapianti di Organo. (2) Zoosafari di Fasano, Brindisi, Italy. (3) Università degli studi di Sassari, Dipartimento di Medicina Veterinaria.
Corresponding author: M. Guadalupi (marta.guadalupi@uniba.it)

Laparoscopic ovariectomy (OVE) has been described successfully in many big felids and preferred to open surgery because big felids have a very deep and wide abdominal cavity, making it difficult to visualize and ligate the mesovarium, even with a large ventral midline incision (1, 2) in an effort to reduce the risk of suture line dehiscence, self-induced trauma, shorten recovery times, and reduce of post-operative. Many authors, however, found difficulties cutting the cranial tip of uterine horn, particularly in heavy adult or obese lionesses. The primary problems that occurred were multiple coagulation cycles and insufficient hemostasis that needed to be managed and monitored, resulting in a surgical time extension. As a result, the purpose of this study was to evaluate two RFVS devices with different jaw lengths for dissecting ovaries in adult obese African lionesses undergoing laparoscopic OVE. The study was authorized with written informed consent by the Zoo's property and by the Ethical Committee (Approval no. 3/2022).

Six (n=6) lionesses were included in the Atlas group, and four (n=4) in the Caiman group. Laparoscopic Ovariectomy was performed by three portals technique. No significant statistical differences were detected between groups ($p>0.05$) for weight and BCS. No Significant difference was detected between groups for the installation phase ($p>0.05$). The Ovariectomy time was significantly lower ($p<0.05$) for the Caiman group. The dissection at the proper ligament in some cases included the cranial uterine horn, necessitated many applications of the vessel sealing device in the Atlas group. We noticed tissue sliding from instrument's jaws and poor coagulation, necessitating additional coagulation cycles. In the Atlas group, we noticed grade 1 bleeding score in one case, and grade 2 hemorrhage from cranial uterine horn in two cases, which necessitated several coagulations and bleeding monitoring. Nonetheless, the bleeding was successfully managed and did not necessitate conversion.

The mean number of coagulation cycles for resection the cranial tip of the uterine horn mean was 10.83 cycles (±S.D. 11.1; range 2-40) in the Atlas group, and significantly ($p<0.05$) lower for the Caiman group (mean 1 cycle; S.D. 0; range 1-1). The ovary retrieval procedure was simple, with smooth passage from the retractor and no need to widen the portal. The mean size of entire specimens was 42.1 mm length (SD 14.4 mm; range 24.8-61.9 mm;) and 30.7 mm width (SD 12.6; range 14.7-46.1 mm;). The mean total surgery time for the Atlas group was 49.33 min (SD 8.6; range 40-61 min), conversely, in the Caiman group the mean time to complete surgery was significantly lower ($p<0.05$), resulted 33.75 min (± S.D. 11.84 min; range 26-51 min).

Results of our study confirmed significant advantages of employ Caiman 12 vessel sealing devices in comparison with Ligasure Atlas in terms of time need to complete ovariectomy and reduce risks of bleeding from thick uteri. As a results, we recommend the use of Caiman 12 when performing laparoscopic ovariectomies in obese adult lionesses.

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USE OF PULSE OXYMETRY AS A GUIDE OF ALVEOLAR RECRUITMENT IN DOGS UNDERGOING LAPAROSCOPY

Vicenti C., Di Bella C., Stabile M., Lacitignola L., Crovace A., Staffieri F.

Università degli Studi di Bari "Aldo Moro", D. E. T. O., Sezione di cliniche veterinarie e Produzione Animale.
Corresponding author: C. Vincenti (caterina.vicenti@uniba.it)

Blood-gas analysis and respiratory system dynamic compliance (Cdyn) have been widely used and validated to monitor and assess lung recruitment during mechanical ventilation. Hemoglobin oxygen saturation (SpO_2) at room air (SpAT test) has been recently investigated in humans as tool to identify lung recruitment (1).

The aim of this study was to evaluate the SpAT test as monitoring of lung recruitment in dogs undergoing laparoscopy and submitted to a step wise alveolar recruitment maneuver (ARM) to improve lung function. This prospective clinical study was carried out in eight dogs undergoing laparoscopy gastropexis. All dogs were premedicated with methadone intramuscularly (0.3 mg/kg), followed by cannulation of a cephalic vein and induction by intravenous administration of propofol (5 mg/kg). Maintained under general anesthesia with isoflurane and pure oxygen. All patients were mechanically ventilated using volume-controlled mode, setting a baseline tidal volume of 15 ml/kg, an inspired/expired ratio of 1:2, an inspiratory pause of 25% of inspiratory time and the respiratory rate to maintain the end-tidal carbon dioxide between 45 and 55 mmHg. After stabilization of the anesthetic plan, dogs were positioned in dorsal recumbency, and pneumoperitoneum (PP) was induced. Fifteen minutes after PP induction, ARM was performed at FiO_2 0.21. ARM has two steps: the first one, "incremental phase", consists of a gradual increase of positive end-expiratory pressure (PEEP) in successive increments of 5 cmH_2O until an airway plateau pressure of 40 cmH_2O is reached; the second one, "decremental phase", in which PEEP is progressively reduced at intervals of 3 cmH_2O, until return to baseline conditions (2 cmH_2O). Each step of PEEP level was maintained for at least 2 minutes and the values of Cdyn and SpO_2 were recorded at each level. The "decremental phase" was used to the identify the optimal level of PEEP (BEST PEEP) necessary to prevent alveolar collapse (open lung). The open lung condition was defined as the level of PEEP in which was registered the highest level of Cdyn. Arterial partial pressure of oxygen (PaO_2) was also monitored. SpO_2 values recorded above the BEST PEEP level were identified as "recruited", while the values belove as "collapsed". Thereafter, to identify the cut-off level of SpO_2 corresponding to the BEST PEEP a ROC curve analysis was performed.

In all cases, it was possible to identify the BEST PEEP by Cdyn analysis during the decremental phase of the ARM. The mean BEST PEEP value was 5.5 cmH_2O with a range between 2 and 10 cmH_2O. In two subjects it was 2 cmH_2O, in 5 subjects 5 cmH_2O and in 1 subject 10 cmH_2O.

The ROC curve analysis of the SpO_2 threshold value to identify the BEST PEEP corresponding to the open lung condition showed SpO_2>95% as the cut-off value indicating the open lung condition, with a sensitivity of 92.86 % and a specificity of 70.59%, an area under the curve of 0.840 (0.706-0.930) and a $P<0.0001$.

The results of this study proved a good sensitivity and specificity of SpAT to identify lung recruitment. Future prospective studies will provide information regarding the use of this approach in a larger population of dogs.

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TITANIUM MESH, ANGULARIS ORIS FLAP AND OMENTAL TRASPOSITION FOR THE TREATMENT OF MAXILLO FACIAL FIBROSARCOMA IN A DOG

Francesco Collivignarelli, Amanda Bianchi, Andrea Paolini, Massimo Vignoli, Roberto Tamburro

Università degli Studi di Teramo, Dipartimento di Scienze Veterinarie.
Corresponding author: F. Collivignarelli (collivignarelli.francesco@gmail.com)

Oral tumors are relatively common in cats and dogs. Surgical resection is the recommended treatment for all oral tumors. Large defect after resection of maxillofacial tumors need a reconstruction. The object of this report is to describe a case of reconstruction with the use of a titanium mesh, omental pedicule transposition and angularis oris axial pattern flap to close the large residual maxillofacial defect. A 7 years old, male Rottweiler was evaluated for a mass on the caudal aspect of the nose, lost of blood from the upper lip and weight loss. Blood work, urine examination and fine needle aspiration cytology was done. Cytology described sarcoma. CT scan resulted in no metastasis disease. The mass involved the palatine, maxilla and nasal bone. Enbloch surgery excision was done. Reconstruction followed by transposition of a pediculed vascularized omental by a paracostal incisional and subcutaneous tunnel were made to move the elongated omentum to the residual defect. Application of a titanium mesh and angularis oris axial pattern flap was done. The dog was aggressive and in according to the owner came back home in the same day with oral drug administration. There was no major complication. Histopathology diagnosed a fibrosarcoma low grade. Radio therapy followed after twenty days. There was a recurrence after 12 months and no evidence metastatic disease. Serial CT scans were done to monitorate a short and a long term outcome.

Titanium mesh would be an optimal material for oncologic maxillofacial reconstruction.

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COMPARISON OF HIGH FLOW NASAL CANNULA AND HELMET CPAP FOR NON-INVASIVE RESPIRATORY SUPPORT DURING POSTOPERATIVE HYPOXEMIA IN DOGS

Claudia Piemontese, Marzia Stabile, Annalaura Scardia, Claudia Acquafredda, Luca Lacitignola, Caterina Vicenti, Marta Guadalupi, Antonio Crovace, Francesco Staffieri

Università degli Studi di Bari Aldo Moro, Dipartimento dell'Emergenza e dei Trapianti di Organi, Sezione di Cliniche Mediche e Produzioni Animali.

Corresponding author: C. Piemontese (piem.claudia@gmail.com)

Postoperative hypoxemia has been reported to be common during recovery from general anesthesia[1, 2]. Oxygen therapy with a CPAP helmet at 5 cmH_2O of pressure has been described as an effective non-invasive respiratory support technique for treatment of this condition [3]. High Flow Nasal Cannula (HFNC) has been recently introduced in veterinary medicine and provides very high flows of fresh gas with a variable FiO_2 delivered to the nostrils of the patients with specific nasal cannula [4]. In addition, flow is humidified and heated to 37°C, thus enhancing airway clearance and improving patient comfort [5].

In this prospective, randomized clinical trial, the effectiveness of helmet CPAP and HFNC for treatment of postoperative hypoxemia was evaluated and compared. In the study were included dogs matching the following inclusion criteria: ASA status 1-3 (defined by anamnestic, clinical and laboratory assessments), bodyweight >5 kg, surgical procedures not involving thorax and duration of anesthesia less than 3 hours. Patients who did not match the inclusion criteria and cases with poor signal quality of pulse oximeter during measurements were excluded from the study. The percentage of peripheral arterial haemoglobin oxygen saturation (SpO_2) measured in dogs recovering from general anesthesia with a pulse oximeter, at room air, every 15 minutes for one hour (T15, T30, T45, T60), was used as reference to evaluate the effectiveness of helmet CPAP and HFNC. SpO_2 measurements were taken by placing the sensor on one of the following sites: tongue, lip, foreskin, vulva, interdigital space, nipple. If necessary, the sensor was replaced to have a continuous good quality signal, corresponding to a Perfusion index >1%. Dogs that showed an SpO_2 value $\geq$95% five minutes after extubation (T5) did not receive any respiratory support and made up the control group; while those presenting an SpO_2 value <95% were classified as hypoxemic and were randomly treated for one hour with CPAP helmet or HFNC. The study was approved by the Ethical Committee of the Clinical and Zootechnical studies in Animals of the Department of Emergency and Organ Transplantation of the University of Bari, Italy (reference no. 03/2017). From a total of 539 patients enrolled, 493 dogs completed the study. 394 dogs made up the control group (CTR group, FiO_2=0.21), 78 were treated with CPAP helmet (CPAP group, O_2=10L, FiO_2=0.3-0.4, pressure= 5cmH_2O) and 21 with HFNC (HFNC group, O_2= 2-2.5 L/kg/min, FiO_2=0.3-0.4). SpO_2 values of CPAP and HFNC groups at T5 were similar to each other and lower than CTR group. There was an improvement in SpO_2 during both treatments. In CPAP group there was already a significant improvement of saturation at T15, reaching the value of normoxemia at T30. In HFNC group, SpO_2 values at T15, T30 and T45 were lower than in CPAP group and CTR group. In CPAP group, the hypoxemic state lasted 25.26$\pm$2.6 minutes, whereas the HFNC group took longer to reach normoxemia (36.43$\pm$7.95 minutes). At T60 both treatment groups reached a state of normoxemia. These results showed that both CPAP and HFNC are effective in treating postoperative hypoxemia, however CPAP is faster and more effective than HFNC.

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CLINICAL EVALUATION OF TRANSVERSUS ABDOMINIS PLANE AND INTERCOSTAL BLOCK IN DOGS UNDERGOING LAPAROSCOPIC OVARIECTOMY

Andrea Paolini, Amanda Bianchi, Francesco Collivignarelli, Andrea Pratesi, Andrea De Bonis, Ilaria Falerno, Massimo Vignoli, Roberto Tamburro

Università degli Studi di Teramo, Dipartimento di Scienze Veterinarie.
Corresponding author: A. Paolini (apaolini@unite.it)

Transversus abdominis plane (TAP) block allows to desensitize the ventral nervous branches from T10-T11 up to L3 which includes the innervation of the skin, subcutaneous tissue, abdominal muscles, mammary glands and underlying parietal peritoneum [4]. To extend the block cranially, TAP block can be done with a sub-costal approach (up to T9) [3] or mid-abdominal TAP block in combination to intercostal nerve blocks [2]. The aim of the present study is to evaluate the efficacy of TAP and T10-T8 intercostal blocks for nociceptive control of laparoscopic surgery. The study was approved by ethics committee of the university of Teramo Prot. n° 24212. Inclusion criteria were ASA (American Society of Anesthesiologists) status I, age between 12 and 30 months, weighing over than15 kg, no cardiovascular, respiratory, hepatic and/or renal pathologies reported in remote and recent history. Heart rate (HR), respiratory rate (RR), pulse rate (PR), peripheral oxygen saturation (SpO_2), systolic, mean and diastolic invasive blood pressure (IBP) values and esophageal temperature (°C) were recorded during procedures. Bitches were randomly and blindly assigned into two groups. Both groups received the same premedication. TAP group (TAPG) received 0.5 ml kg^{-1} of 2% diluted ropivacaine par hemi-abdomen and 0.05 ml kg^{-1} of 2% diluted ropivacaine from T8 to T10. Control group (FENG) received lactated Ringer's placebo solution at the same volumes. FENG received 5 mcg kg h^{-1} fentanyl in constant rate infusion with bolus of 2 mcg kg^{-1} while TAPG ringer lactate solution. Backhaus positioning, first trocar insertion, CO2 insufflation, second trocar insertion, lifting and cauterization of the first and second ovary were recorded. Bitches were assessed using the Glasgow pain scale short form [1] in the postoperative period. Sternal recumbency and quality of hospitalization were noted. Data was presented as percentages and analyzed with Fischer's exact test. Significance is set at $p \leq 0.05$. Management of perioperative nociception was comparable in both groups but in the TAPG the quality of post-operative hospitalization was better. TAP and intercostal blocks appear to be a valid intra and post-operative analgesia alternative in elective laparoscopic ovariectomy procedures.

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COHORT RETROSPECTIVE STUDY ON DIAGNOSIS AND OUTCOME OF COLIC SYNDROME IN HORSES: PRELIMINARY DATA

Paola Straticò, Gianluca Celani, Giulia Guerri, Adriana Palozzo, Vincenzo Varasano, Lucio Petrizzi

Università degli Studi di Teramo

Corresponding author: G. Guerri (gguerri@unite.it)

Colic syndrome enrolles several disorders mainly involving the gastrointestinal apparatus and is a common cause of morbidity and mortality in horses [1, 2]. Several parameters are considered as predictor factors for the outcome, due to the multifactorial nature of the disorder and variability in horse population [3-6]. Aim of the study was to describe the characteristics, the clinical findings, diagnosis, treatment, and short term survival of horses referred for colic syndrome to the Veterinary Teaching Hospital of Teramo from 2005 to 2022. Collected data included patient signalment (age, gender, body weight, breed), time (morning, afternoon, night) and season of referral, type of treatment (medical, surgical), PCV, Total Protein, euthanasia, survival to discharge. Statistical analysis was carried out with R [7]. Frequency distribution of all discrete variables was provided as well as media, standard deviation (SD) for normally distributed continuous variables, median and interquartile range (IQR) for not-normally distributed continuous variables. One-hundred and fifty-seven horses were included (median age 10 years, IQR 7-13; body weight 550 kg, IQR 407-650). Thoroughbreds, Standardbreds, American and Spanish breeds were represented, as well as Frisian and Italian draft horses. Sixty-two were females (39.5%), 42/157 males (27%), 43/157 geldings (27.5%), 10/157 cases were unidentified (6%). Seventy-three were surgically (46,5%) and 81/157 conservativelly treated (51.5%), 3/157 cases were unidentified (2%). Thirty-nine were euthanized (25%), 5 died spontaneously, 106/157 survived to discharge (67%). In 7 cases the information was lost. When we considered as the outcome variable "survival to discharge", only the explanatory variable"surgery" differed between the two groups. So a subdataset was considered involving only horses that were surgically treated. Horses receiving surgery were not different in age and body weight from those medically treated (t-test, $p>0.05$). Hour and season of referral were statistically different between survived and not-survived horses (Fischer ectact test, $p<0.05$), with more survived horses referred in the afternoon and night, compared to the morning, and less survived horses refererred during winter compared to the other seasons. PCV was statistically lower in discharged horses (38.89% vs 48.17%), and time to standing after surgery shorter (58.61 vs 85.87) (t-test, $p<0.05$). In discharged horses recovery score was better, and not-ischemic lesions were more represented (38 vs 19)(Fischer exact test, $p<0.05$). No difference in discharge rate was observed according to the site of the lesion [large intestine 45/73 (61.64%), small intestine 22/73 (30.14%) or other sites 2/173(2.74%)], or to the administration of lidocaine after surgery (Fischer exact test, $p>0.05$). Overall success rate (survival to discharge) of this observational study was in accordance with previous data, with the most common diagnosis being large colon disorders. Differently, we did not observe a higher rate of survival with this disorder compared to others. Hour and season of referral varied among survived and not-survived horses. A more extensive analysis involving an univariate and multivariate logistic regression model involving physical parameter at referral may be of use to highlight odd ratio for survival and help the clinician in the evaluation of prognosis in case of exploratory laparotomy.

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EFFECTS OF PIEZOELCTRIC CUT IN A LARGE ANIMAL CRANIOTOMY MODEL: HYSTOLOGICAL FINDINGS

Alberto Crovace (1), Gerolamo Masala (1), Nicolò Columbano (1),Stella Romeo (1), Luca Lacitignola (2), Francesco Staffieri (2), Sergio Carnevale (3), Sabino Luzzi (4)

(1) Università degli Studi di Sassari, Dipartimento di Medicina Veterinarie. (2) Università degli Studi di Bari, Dipartimento delle Emergenze e dei Trapianti di Organo. (3) Section of Anatomic Pathology,Cerba Healthcare, Milano. (4) Neurosurgery Unit, Department of Clinical-surgical, Diagnostic and Pediatric sciences, Università degli studi di Pavia, Fondazione IRCCS Policlinco San Matteo.

Corresponding author: A. Crovace (acrovace@uniss.it)

Craniotomy could be a surgery that presents many critical points, like the localization, the protection of brain and also 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 present the hystological findings of piezoelectric cut performed by Mectron Piezosurgery® Touch bone in a sheep craniotomy model. Piezosurgery works using the indirect piezo effect so it allows to execute an osteotomy without damaging softer tissues. The frequencies used are automatically set to 22-30 Hz that are ideal, in case of neurosurgeries, to cut the bone without damaging soft and nervous tissue. An important feature of the instrument is an irrigation system that permits an ideal cooling of the cutting area, crucial to avoid thermic damage [2]. The OT12 tip, that has a saw-like conformation, was selected to perform a 5x3 cm oval cut with a 10 mm depth employing a guide. After the approval of the ethical committee (approval n° 654/2020-PR) 16 sheep were recruited for a study in which a craniotomy was required to evaluate the performances of titanium plates , the cut was performed with the Mectron Piezosurgery® and the bone portion that was removed was put in formalin for histological and SEM evaluations. After the sacrfice of the sheep devided in two groups of 8 subjects at 3 and 6 months from the surgery also samples from brain tissue and dura madre were collected to undergo hystological evaluations. The presence of inflammatory cells and regerneration signs were evaluated in all the samples. In the bone samples was also evaluated the presence of fibroblasts, osteoblasts and thermical damage signs. In the nervous and meningeal tissue was evaluated the presence of gliosis, fibrotic substitusion and damages to the tissues. In all the cases the surgery was smooth without any major complications, in only two cases bleeding occured and was treated using bone wax. The hystological examination performed on the bone gusset removed during surgery showed the absence of thermical damage, the presence of fibrous tissue of new formation, the presence of vascularization and in some cases it was also noticed the presence of woven bone with a good numeber of osteoblasts. The examination performed on brain tissue showed very few inflammatory cells, also only a light gliosis was noticed. The Dura madre samples showed the presence of little infiltration of fibrotic tissue and in only a few cases the presence of fibrotic calcification; a few inflammatory cells were spotted in all the samples. The results of this study confirmed the hypotesis that the piezoelectric cut allows a regeneration of the bone tissue since the precence of cells and vascularization near the cut. The piezoelectric cut allows also to protect the nervous and menigeal tissue from damages linked to the surgery since the presence of inflammatory cells is not severe and also the fibrotic tissue enlighted in some samples can be considered a normal consequence of this kind of surgeries. This finding suggest a quicker recovery without leaving damages linked to the craniotmoy. Thermical damage was absent in all the samples, as underlined by the presence of vital cells, so another important complication can be avoided using this instrument. Concluding, Mectron Piezosurgery Touch can be considered a good alternative to conventional instruments to perform a craniotomy.

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CORRECTIVE OSTEOTOMY TO ADDRESS A SALTER-HARRIS TYPE V DISTAL FEMORAL FRACTURE IN A DOG

Maurizio Isola, Parastoo Memarian

Università degli Studi di Padova, Dipartimento di Medicina Animale, Produzioni e Salute.
Corresponding author: P. Memarian (parastoo.memarian@phd.unipd.it)

Canine angular limb deformities are most often caused by premature arrest of long-bone physis or by fracture malunion. Pure procurvatum of distal femur has been reported sporadically, following malunion Salter-Harris type fractures in young dogs (1-3). Loss of functional stifle extension, quadriceps mechanism impingement, and secondary osteoarthritis are consequences of this deformity which might necessitate corrective surgery (2, 3). A 4-month-old intact male fox terrier dog was presented with persistent weight-bearing lameness of left hindlimb, two months after a vehicle trauma. On orthopedic examination the only abnormal finding includes the loss of functional extension angle of the left stifle joint of 20 degrees. An atypical procurvatum deformity of the left distal femoral metaphysis was diagnosed on radiographs as a cause of a previous Salter-Harris type V fracture of distal femoral physis. Tibial plateau angle of left tibia was increased due to abnormal forces to the proximal tibial physis. Based on CT scans and radiography, the center of rotation of angulation approach (1) was utilized to distinguish the deformity and plan the corrective osteotomy. A cranial closing wedge ostectomy was performed to address distal femoral procurvatum and subsequently stabilized using a locking plate. Proximal tibial epiphysiodesis (4) was performed, under fluoroscopy, to reduce tibial plateau slope. Complete bone healing was achieved 50 days after surgery without any complication. The dog was totally weight-bearing on the limb and reached the normal pain-free range of motion. This case report describes a successful surgical management of a pure procurvatum deformity of the distal femoral metaphysis caused by Salter-Harris type V fracture.

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COMPARISON BETWEEN LOW DOSE INFUSIONS OF DEXMEDETOMIDINE AND FENTANYL ON HEMODYNAMIC AND RESPIRATORY VARIABLES IN DOGS UNDER PROPOFOL ANESTHESIA

Annalaura Scardia, Marzia Stabile, Claudia Acquafredda, Caterina Vicenti, Luca Lacitignola, Annarita Imperante, Marta Guadalupi, Claudia Piemontese, Antonio Crovace, Francesco Staffieri

Università degli Studi di Bari, Dipartimento dell'Emergenza e dei Trapianti di Organi (D.E.T.O.)
Corresponding author: A. Scardia (annalaura.scardia@uniba.it)

In a recent study it has been demonstrated that dexmedetomidine at low dose infusion improves hemodynamic, respiratory mechanics and oxygenation during isoflurane anesthesia in dogs [1]. In the present clinical study, we aim to evaluate the effect of dexmedetomidine compared to fentanyl infusion on hemodynamic, respiratory mechanics and oxygenation in healthy dogs under propofol anesthesia. Our hypothesis is that dogs receiving dexmedetomidine have better hemodynamic and gas exchange conditions compared to those receiving fentanyl.

The study was authorized by Ethics Committee of the Department of Emergency and Organ Transplantation (certificate of approval n. 03/2021) Thirthy female dogs undergoing elective ovariectomy were included in the study. Dogs were premedicated with methadone 0.3 mg/kg IM, induced with propofol 5 mg/kg IV to effect and maintained with propofol at an initial dose of 0.3-0.4 mg/kg/min. the dose of propofol was then adjusted with 20% increments or decrements in order to mantain surgical plane of anesthesia. Ventilation was set in pressure support (PS) mode with 5 cm H_2O of support and FiO_2 100%. Dogs were randomly allocated to one of these three groups: group I (bolus of saline solution followed by dexmedetomidine infusion at 1 µg/kg/h), group BI (dexmedetomidine bolus 1 µg/kg in 5 minutes followed by infusion at 1 µg/kg/h) and group F (fentanyl bolus 3 µg/kg in 5 minutes followed by infusion at 3 µg/kg/h). Heart rate (HR, beats/min), mean blood pressure (MBP, mm Hg), stroke volume index (SVI, ml/kg), minute volume (MV, L/min) and end tidal concentration of CO_2 ($EtCO_2$, mm Hg) were registered at T0, T5 (T post bolus), T15, T30 and T15post-infusion; oxygenation was evaluated at T0, T30 and T15post-infusion with an arterial blood gas analysis. If rescue analgesia was required during the surgery, fentanyl 1-2 µg/kg IV was administered.

Normal distribution of data was assessed using Shapiro Wilk test. Parameters were compared for times and groups using two-way ANOVA. $P<0.05$ was considered statistically significant.

HR decreased while MAP and SVI increased in group BI at T5 compared to other groups. In group I, HR decreased while MAP and SVI increased progressively from T0 to T30. In the same group, MAP significantly decreased at T15post-infusion. In group BI, HR decreased while MAP and SVI increased at T5 compared to T0; HR increased again at T15post-infusion. In the same group, MV decreased and $EtCO_2$ increased at T5 compared to T0. In group F, HR and MAP were lower at T5 compared to T0, and MAP was lower at T15 compared to other groups. Group I received less rescue dose of fentanyl (1.5 µg/kg) compared to group F (2.3 µg/kg).

Low dose dexmedetomidine infusion increased MBP and SVI and decreased HR with or without bolus during propofol anesthesia in dogs. A bolus of dexmedetomidine prior to infusion anticipated the hemodynamic effects of dexmedetomine, while exacerbating respiratory depression. Dexmedetomine improved hemodynamic and had a sparing effect on the rescue dose of fentanyl compared to low dose fentanyl infusion in dogs under propofol anesthesia.

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DESFLURANE REQUIREMENT DURING ANESTHESIA FOR DOG OVARECTOMY AFTER ADMINISTRATION OF MAROPITANT OR METHADONE

Francesca Cubeddu, Gerolamo Masala, Alberto Maria Crovace, Stella Maria Teresa Romeo, Nicolò Columbano, Antonio Scanu, Eraldo Sanna Passino, Giovanni Mario Careddu

Università degli Studi di Sassari, Dipartimento di Medicina Veterinaria.
Corresponding Author: G.M. Careddu (gcareddu@uniss.it)

Balanced anesthesia for ovarectomy in dogs usually includes a strong analgesic agent such as an opioid together with a halogenated anesthetic. Other drugs instead of opioids can be included. Maropitant exerts an analgesic effect through its action on NK-1 receptors. In this study, clinical effects and desflurane requirement during ovarectomy in dogs after administration of maropitant or methadone are compared, with the approval of the ethics committee of the University of Sassari with prot. 1820 of the 20/07/2017. Based on a previous power analysis, sixteen mixed-breed female dogs of 16±7 months of age and weighing 20±5 kg received dexmedetomidine intravenous (IV) 2.0 $\mu g \cdot kg^{-1}$ for premedication and propofol IV 2.0±0.3 $mg \cdot kg^{-1}$ for induction and intubation. The dogs were randomly assigned to two groups of eight dogs each, to receive one of the two agents, i.e. maropitant IV 1.0 $mg \cdot kg^{1}$ (Maropi group) or methadone IV 0.3 $mg \cdot kg^{-1}$ (Metha group) for premedication in a blinded method. Anesthesia was maintained with desflurane in O_2 35±5% with flow of 1,0 $l \cdot min^{-1}$ in spontaneous ventilation. Rescue analgesia was guaranteed by methadone IV 0.2 $mg \cdot kg^{-1}$. End tidal percentage of desflurane (EtDes) was maintained as low as possible based on clinical and cardio-circulatory signs. Values of heart rate (HR), systolic arterial pressure (SAP), diastolic arterial pressure (DAP), mean arterial pressure (MAP) and respiratory rate (RR), EtDes and Italian version of Glasgow Composite Measure Pain Scale-Short Form (ICMPS-SF) score were collected for statistical analysis. Normally distributed data were compared by two tail unpaired t-test. Not normally distributed data were compared by Mann-Whitney test. Statistical significance was considered for $p<0.05$ and statistical comparisons were performed by GraphPad Software. No rescue analgesia has been administered. Mean EtDes was significantly higher in Maropi group than in Metha group at the observed moments of skin incision, ovary tractions, ovary resections, "*fascia*" suture and skin suture ($P<0.05$). At the same moments, depression of HR, SAP, DAP, MAP and RR resulted significantly greater in Metha group than in Maropi group if compared to previous values observed at 10 minutes after equilibration with EtDes 5.5% (basal values) ($P<0.05$). No significant increase in HR, SAP, DAP, MAP and RR values was detected in both groups, at traction and resection of the ovaries compared to basal values ($P>0.05$). ICMPS-SF was found equal in the two groups upon awakening (1/20) and over the following 90 minutes (0/24). Inclusion of maropitant as an analgesic agent in an anesthetic protocol could be advantageous in dogs less responsive to hypotension and bradycardia, i.e. traumatized or young or elderly patients or as a perioperative parenteral option in "opioid-free anesthesia".

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DESFLURANE VS SEVOFLURANE FOR ANESTHESIA INDUCTION, MAINTENANCE AND RECOVERY IN CATS UNDERGOING OVARIECTOMY

Francesca Cubeddu, Nicolò Columbano, Antonio Scanu, Alberto Maria Crovace, Gerolamo Masala, Stella Maria T. Romeo, Eraldo Sanna Passino, Giovanni Mario Careddu

Università degli Studi di Sassari, Dipartimento di Medicina Veterinaria.
Corresponding author: N. Columbano (ncolumbano@uniss.it)

The mask induction technique is very rare in cats. Few studies have evaluated this procedure in cats with sevoflurane no one with desflurane. Aim of this study is to compare the induction, maintenance and recovery from anesthesia with sevoflurane or desflurane in cats under ovariectomy premedicated by alfaxalone, buprenorphine.

Based on a previous power analysis, eighty cats were enrolled in the study. After a intramuscular administration of alfaxalone (2 mg·kg^{-1}), Buprenorphine (10 µg·kg^{-1}) and dexmedetomidine (5 µg·kg^{-1}), the cats scheduled for elective ovariectomy were randomly assigned to one of two groups: Sevo, receiving sevoflurane; Des, receiving Desflurane.

After 15' from premedication, anesthesia was induced with Sevoflurane (group Sevo) or desflurane (group Des) with FGF of 1l·min^{-1} and vaporizer setted at the end of scale (8% Sevo, 18% Des). Time induction and number of attempts were recorded. After intubation FiO2 was set at 0,4 with FGF of 0,5 l·min^{-1}, the % of inahalant anesthetics were adjusted in order to rise and maintain values of 1 MAC (3% for sevo and 10% for des) for 10' (equilibration time).

To keep the ETCO2 between 25 and 35 mmHg each cat was positive-pressure ventilated. Surgery began after the equilibration time. At the end of the surgery and always after 15 minutes, the vaporizer was turned off, the animal allowed to recover from anesthesia and the time period to rise "spontaneous ventilation", to "extubation", "sternal recumbency", "standing" and "walking" were recorded. During the procedure respiratory and hemodynamic variables, were measured and recorded (Heart rate Hr, Blood pressure NBP, Respiratory rate Rr). All continuous data were tested for normal distribution via a Shapiro-Wilk test. Statistical examination of group data was performed using paired/unpaired t tests and Wilcoxon–Mann–Whitney tests for continuous normally and not normally distributed variables, respectively. Group means were compared using analysis of variance (ANOVA) or its non-parametric version (Kruskal–Wallis test) when appropriate. All analyses were two-sided. $P<0.05$ was considered statistically significant. The times "spontaneous ventilation", "extubation" and "sternal recumbency", "standing" and "walking" were highly significant longer in the SEVO group ($p<0.01$). The times "standing" and "walking" were very short and similar. Heart rate (HR) was significantly higher (from $p<0.05$ to $p<0.01$) in the SEVO Group. Mean arterial pressure (MAP) systolic blood pressure (SAP) and diastolic blood pressure (DAP) were significantly higher in the SEVO group (from $p<0.05$ to $p<0.01$). As regards of MAC values these were higher in the SEVO Group ($p<0.01$) during mask induction and immediately before intubation and at the end of the same immediately before extubation. In the DES Group the intubation occurred at 1.47±0.09 in the Sevo Group at 1,91±0.25 multiples of MAC respectively.

The reappearance of the swallowing reflex, which allowed the cat to be extubated, occurred at overlapping values of multiples of MAC (DES group 0.14; SEVO group 0.15).

Under this study conditions, Desflurane allowed a faster induction and faster recovery. On the other hand, sevoflurane at MAC values in cats shows less cardiovascular depressive action and can have its advantage when a slower, quieter awakening is needed.

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CLINICAL EXPERIENCES IN THE BURNS MANAGEMENT IN THE DONKEY

Valentina Secchi, Gerolamo Masala, Nicolò Columbano, Alberto Crovace, Giovanni Mario Careddu, Antonio Scanu, Francesca Cubeddu, Eraldo Sanna Passino

Veterinary Teaching Hospital - Department of Veterinary Medicine of Sassari

Corrispondig Author: E. Sanna Passino (esp@uniss.it)

Fires represent a huge problem in Sardinia, especially during the summer season. They are responsible for huge damage to both local flora and fauna, and constitute a risk for urban centres and human beings. Burn injuries are classified into four categories depending on the depth of wounds. Nevertheless formal guidelines to calculate the total surface area of burns in large animals do not exist.

Two adult donkeys (1 male and 1 female) were admitted to VTH with various degrees of burn injuries. The male presented second-degree deep injuries over the left side of abdomen, thigh, shoulder, chest, scrotum, and various parts of the face (ear, eyelid, muzzle). The female presented third and fourth-degree burns on ventral parts of abdomen, prossimal parts of all limbs and a deep second-degree lesion on the muzzle. Both animals presented detachment of coronal margin of the hoof. At first burn injuries were abundantly washed with clean fresh water to cool the areas and remove the superficial filth. General Therapy during first days of hospitalization: Flunixin meglumine (1 mg/kg/die EV), Dimethyl sulfoxid (DMSO 1 mg/kg EV in 5 L NaCl), buprenorphine (5 µg/kg/die EV), benzylpenicillin procain + diidrostreptomicin (20,000 UI/kg + 11.5 mg/kg IM). Daily local therapy included two steps: disinfection and mechanic debriment to clean and remove necrotic tissues, application of an antibiotic based cream with collagenase. Subsequently, after the all removal of the necrotic tissue, daily therapy consisted in disinfection and application of medical honey gel (Medihoney®, Integra) to allow tissue regeneration. For the male, re-epithelization of the superficial second-degree burns occurred in only two weeks while coat regeneration was again present in a month. Deep second-degree lesions took more than a month to epithelize and after two months coat is still not completely grown. Female burns took a month to start to epithelize, following some phases of purulent exudation and detachment of the white eschars developed. After two months of hospitalization tissue is regenerating and epithelizing but several areas still require time to heal completely and to regrow the coat completely recovered after 10 months. Detachment of coronal margin did not develop, on the contrary it solved positively without severe damages to the hoofs.

Despite the severity of the animals' conditions, the results obtained were promising and successful. A prompt and a daily basis care was fundamental for the good outcome of clinical cases, especially in the first critical days of hospitalization. Mechanic debriment of burns was central for the treatment of lesions. Good results for tissue regeneration were obtained thanks to the use of medical honey used as an alternative product to treat burns. A correct classification of lesions is very important to establish a proper therapy and is fundamental for prognosis and total recovery.

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EVALUATION OF TWO DIFFERENT PULSE OXIMETERS PERFORMANCES IN RELATION TO DIFFERENT POSITIONING IN THE AWAKE RABBIT

Martina Cardinali, Giulia Maria De Benedictis

Università degli Studi di Padova, Dipartimento di Medicina Animale, Produzioni e salute.
Corresponding author: M. Cardinali (martina.cardinali@phd.unipd.it)

Pulse oximetry non-invasively estimates the percent of arterial hemoglobin saturated with oxygen (SpO_2): using a spectrophotometric methodology, it measures the ratio between the red and infrared light absorbance of oxygenated (oxyhemoglobin) and deoxygenated blood (reduced hemoglobin) [1].

It is considered accurate in reflecting arterial oxygen saturation both in humans and animals [2, 3] and its clinical use has been associated with reduced peri-anaesthetic mortality and morbidity in domestic species [4]. Despite its widespread use, there is a lack of information on adequacy of pulse oximetry for rabbit that conversely is a species with one of the highest peri-anesthetic mortality rates of all domestic species [5-7]. Therefore, the aim of this study was to evaluate the performance of two handheld pulse oximetry devices, on two different anatomical locations other than the tongue, in order to find the most reliable and accurate one. The machines tested were: Masimo Rad 5 Pulse Oximeter (Masimo U.S.) equipped with LNCS TC-I ear clip sensor (device 1) and Edan VE-H100B Veterinary Pulse Oximeter (Alcyon, Italy) equipped with a Y clip sensor (device 2). Fifty healthy, adult, privately owned rabbits of 3.2±0.49 kg of body weight were enrolled in the study. After a general clinical examination, each rabbit was auscultated for one minute for three times in order to get the mean clinical heart rate (cHR) which resulted to be 200.46±19.4 beat per minute (BPM).

Thereafter, the probe of each pulse oximeter was placed and a series of 5 measurements of heart rate (mHR) and SpO2 with an interval of 15 seconds was recorded. If the instrument didn't produce any value within a period of three minutes, data were considered missing and the recording as failed. The same procedure was repeated with the same machine on the tail and on the forelimb in random order and the whole process was replicated for the two pulse oximetry devices.

The Pearson correlation coefficients differs between the cHR and the mHR obtained by the two devices (0.613 vs -0.063 for the device 1 and 2, respectively). The squared correlation (R^2) between mHR and the expected values (cHR) was 0.37 for device 1 and 0.004 for device 2, therefore it demonstrates a poor concordance for both the machines with the cHR, although device 1 mHR is closer to the clinical values than device2 mHR.

Besides, device 1 has a higher failure rate than device 2 in both mHR and SpO_2 and in both forelimb and tail. For SpO_2, device 2 provides higher percentages of saturation of haemoglobin with oxygen in both application sites, and has a lower failure rate accounting for 5% only of total measurements.

The main limitation of this study was the unavailability of arterial blood gas analysis and electrocardiography that could have provide a gold standard for the measurements acquired, but it was unfeasible in awake animals.

Pulse oximetry is an equipment easy to use but in awake rabbits its accuracy and reliability needs to be demonstrated. Further studies are warranted to compare the results of different pulse oximeters to the real parameters measured by gold standard instruments.

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EFFECT OF PERI-OPERATIVE VERSUS PERI- AND POST-OPERATIVE ANTIBIOTIC USE AFTER TIBIAL PLATEAU LEVELLING OSTEOTOMY IN DOGS

Roberto Tamburro, Amanda Bianchi, Andrea Paolini, Francesco Collivignarelli, Ilaria Falerno, Andrea De Bonis, Massimo Vignoli.

Università degli Studi di Teramo, Facoltà di Medicina Veterinaria.
Corresponding author: R. Tamburro (rtamburro@unite.it)

The clinical impact of postoperative treatment on infection rates in dogs that underwent tibial plateau levelling osteotomy (TPLO) is still uncertain [1]. The aim of this study was to assess surgical site infection rates (SSI) in dogs having undergone TPLO procedures. The study protocol was approved by the Ethics Committee of the University of Teramo (Prot. 24520 UNTECLE). Dogs were randomly assigned to 2 treatment protocols. The perioperative group (A) was receiving cefazoline (22 mg/kg IV) preoperatively 30-60 minutes before the skin incision repeated every 90–120 minutes intraoperatively and every 4–8 hours for up to 12 hours post-surgically; in the other group (B) antimicrobial therapy was continued during an extended post-operative period with cefazoline 22 mg/kg SID per os for ten days after surgery. For routine surgical site preparation chlorhexidine 4% scrub was used. All TPLO procedures were performed by the same surgeon (RT). Deep intraoperative surgical swab for bacterial examinations was performed at the end of the procedure. Wound care, including three times a day topic disinfection and three times a day ice-pack therapy 5 minutes each, was carried out by the owners for twelve days after surgery. Post-operative re-examinations were performed 14 days, 4 and 8 weeks postoperatively. Any sign of pain, swelling, incisional drainage or dehiscence was recorded, and all cases in which bacterial culture of the surgical site was positive, were considered as having a surgical site infection (SSI) [2]. The surgical site was considered as not infected if no such abnormalities were found in the patient record from the post-surgical follow-up visit and when complete osteotomy healing was observed radiographically. Surgical site infection rate was compared between the two groups using a commercially available statistics software (significance limit of $p<0.05$). Ninety-four dogs (112 TPLOs) met the inclusion criteria. Group A: Forty-three dogs (51 TPLOs); group B: fifty-nine dogs (sixty-one TPLOs). The overall SSI rate was 2/51 (3.9%) in the group A and 2/61 (3.2%) in the group B. No implants were removed. No statistical difference was present between the two groups ($p>0.05$). According to these results, we conclude that perioperative antimicrobial prophylaxis may be sufficient to maintain the overall rate of SSI at a similar low level as in those cases that underwent extended post-operative antibiotic treatment.

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CLINICAL COMPARISON BETWEEN SUBCUTANEOUS AND INTRAMUSCULAR REPEATED INJECTION OF DEXMEDETOMIDINE IN ANAESTHETIZED HORSES: PRELIMINARY INVESTIGATION

Vanessa Rabbogliatti, Martina Amari, Federica Alessandra Brioschi, Federica Di Cesare, Davide Danilo Zani, Donatella De Zani, Giuliano Ravasio

Università degli Studi di Milano, Dipartimento di Medicina Veterinaria e Scienze Animali.
Corresponding author: V. Rabbogliatti (vanessa.rabbogliatti@unimi.it)

Up to 2005, the use of dexmedetomidine (DEX) had not been reported in the equine. Since then, several experimental and clinical studies have been published [1]. The aim of the study is to compare the clinical effects during general anaesthesia and on recovery quality of DEX administered either by intramuscular (IM) or subcutaneous (SC) injection in horses. The study was approved by the Institutional Ethical Committee for Animal Care of the University of Milan (OPBA 17_2020; informed written consent was obtained. Ten horses were included in the study and sedated with acepromazine 0.03 mg kg^{-1} and detomidine 10 µg kg^{-1}. intravenously (IV). Anaesthesia was induced with ketamine 2.5 mg kg^{-1}, diazepam 0.05 mg kg^{-1} (IV) and maintained with a steady state isofluorane (1.3%) in 60 % oxygen. Ringer's lactate and dobutamine were administered IV to maintain MAP >70 mmHg. Mechanical ventilation was immediately initiated with a tidal volume of 15 ml kg^{-1}, peak inspiratory pressure close to 25 cmH_2O, positive end-expiratory pressure of 5 cmH_2O; inspiratory time was set at 2.0 seconds and expiratory time was modified in order to change the respiratory rate and maintain normocapnia. Horses were randomly divided in two groups. One group (DEX SC) received 2 µg kg^{-1} of DEX administered every hour by SC injection, the second group (DEX IM) received 2 µg kg^{-1} of DEX every hour by IM injection. Physiological parameters, arterial blood gases, total dose of dobutamine, ketamine rescue needed, urine production, time required until extubation and time to attain sternal and standing position were noted. The main anaesthesiologist assessed recovery quality graded on a standard scoring 5-point scale [2]. Data distribution was tested with Kolmogorov-Smirnoff test. Student's *t,* Mann-Whitney and ANOVA tests were applied ($p \leq 0.05$). There was no significant difference in body weight (kg: DEX IM 525±66; DEX SC 505±68), age (years: DEX IM 9.8±2.3; SC 10.4±5.1) and ketamine rescue needed (DEX IM 0 horses out of 5; DEX SC 1 horse out of 5). Arterial blood gas parameters were within physiological ranges, with no significant differences between groups. No significant differences in anaesthesia duration (minutes) (DEX IM 133±19; DEX SC 144±15), time until extubation (minutes) (DEX IM 9±3; DEX SC 11±2), time (minutes) to attain sternal (DEX IM 53±20; DEX SC 50±6) and standing position (DEX IM 65±25; DEX SC 56±7) together with recovery score (DEX IM 1.4±0.5; DEX SC 1.6±0.5) were detected. There was significant difference in urinary output (ml kg^{-1} $hour^{-1}$ DEX IM 6.0±2.3; DEX SC 9.1±2.1) and dobutamine administration (µg kg^{-1} $minute^{-1}$ DEX IM 0.33±0.16; DEX SC 0.55±0.140) between groups. To the authors knowledge, this is the first study that investigates the use of DEX administered by subcutaneous or intramuscular repeated injection. Dexmedetomidine administered either by IM or SC injection at indicated dosages, showed similar effect and proved to be useful in balanced isoflurane anaesthesia.

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SIFTVET

BIOACTIVITY OF SKELETONEMA MARINOI AGAINST CHRONIC MYELOID LEUKEMIA:ROLE OF OXIDATIVE STRESS AND APOPTOSIS

Sara Damiano (1), Consiglia Longobardi (2), Gianmarco Ferrara (1), Chiara Lauritano (3), Serena Montagnaro (1), Antonio Rubino (1), Emanuela Andretta (1), Salvatore Florio (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 e Fisica e Medicina Preventiva. (3) Dipartimento di Ecologia Marina Integrativa, Stazione Zoologica Anton Dohrn.

Corresponding author: S. Damiano (sara.damiano@unina.it)

Chronic myeloid leukemia (CML) is a myeloproliferative disease in which a reciprocal translocation between chromosomes 9 and 22 produces the chimeric kinase Bcr-Abl, which is an important therapeutic target that changes the prognosis for CML patients [1]. Unfortunately, currently available tyrosine kinases (TK) inhibitors have a variety of adverse effects, some of which can be attributed to the inhibition of off-target TK [1]. To this end, the discovery of new drugs with low toxicity and more efficacy is an important clinical task. Studies indicate that excessive ROS production induces oxidative stress, disrupts cellular homeostasis and contributes to bone marrow failure and acute myeloid leukemia [1]. In particular, in hematological malignancies, ROS protect the cell from apoptosis and promote cell survival, growth, proliferation, migration and drug resistance [1]. Moreover, markers of oxidative stress, such as malondialdehyde and protein-carbonyls, were found controlled one in the plasma of CML patients compared to controlled one and, in particular, the degree of oxidative stress seems to increase during the accelerated phase of CML [2].

Some marine natural products are actually used to care several of hematopoietic malignancies, such as Cryptotethya crypta and Polatuzumab vedotin [3]. In this work we evaluated the effects of extracts of marine diatom *Skeletonema marinoi* (S.M.) on human cell line K562, that have just shown bioactivity against human melanoma cells [4]. The K562, cultured in a 5% CO2 incubator at 37°C, were treated with vehicle DMSO (0.01%) or S.M. (1 mg/mL). We investigated the effects of the extract on cell viability, measured by MTT test. Probe, 2′,7′-dichlorofluorescin diacetate was utilized to measure the formation of ROS productions. The population of apoptotic cells was analyzed using an AnnexinV-FITC Apoptosis Detection Kit (Strong Biotech, Taipei, Taiwan), according to the manufacturer's instructions and the apoptotic cells were analyzed by flow cytometric analysis. Moreover, the effects of algae extract on the expression of the proapoptotic protein Bax and the antiapoptotic protein Bcl-2 were determined by real time PCR and western blot. Finally, we investigated the link between ROS production and apoptosis through Nicotinamide adenine dinucleotide phosphate (NADPH) oxidases activity, the major sources of ROS induction, by western blot.

Results showed that S.M. extract induced rapid growth inhibitory effects on K562 cells (*P<0.05 vs untreated cells) and the reduction of NADPH oxidase levels in treated K562 cells respect to untreated cells (*P<0.05) showed by ours experiments demonstrated a key role of NADPH oxidase on the ROS production involved in angiogenesis. Finally, the marine extract increased the levels of the proapoptotic protein Bax (*P<0.05 respect to untreated cells) and decrease the antiapoptotic Bcl-2 (*P<0.05), showing that *S. marinoi* induced K562 cell in apoptosis. Taken together, these results describe, for the first time, a possible antitumoral action of S.marinoi against CML and could be used as a potential therapeutic agent in CML patients.

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ROE DEER HAIR AS A POSSIBLE INDICATOR OF ENVIRONMENTAL POLLUTION

Susanna Draghi (1), Federica Riva (1), Giulio Curone (1), Petra Picò Cagnardi (1), Federica Di Cesare (1), Duygu Tarha (2), Bengü Bilgiç (3), Banu Dokuzeylül (3), Alev Meltem Ercan (2), Mehmet Erman Or (3), Francesco Arioli (1)

(1) Università degli Studi di Milano, Dipartimento di Medicina Veterinaria e Scienze Animali. (2) Istanbul University-Cerrahpasa, Cerrahpasa, Biophysics Department. (3) Istanbul University-Cerrahpasa, Internal Medicine Department.

Corresponding author: S. Draghi (susanna.draghi@unimi.it)

Industrialization, intensive farming and breeding practices have led to a significant impact on the natural environment. Several studies have demonstrated that wildlife is strongly affected by a variety of toxic substances which pollute the environment. In this context, wild mammals result as sentinels for environmental studies. This condition of ideal sentinels of pollution is due to several intrinsic characteristics such as their feeding behavior and long lifespan which result in a prolonged exposure to environmental contaminants. The European roe deer (*Capreolus capreolus*) is one of the most abundant ungulate species in Europe and is frequently an indicator of environmental contamination. This animal species is highly adaptable, showing large behavioral plasticity, and occupying a wide range of habitats, including those extensively used for human activities. In the last decades, some ecotoxicological studies were conducted on roe deer mainly focused on metal concentrations in blood and in different types of soft tissues such as liver, kidney, and muscle. Hair has always been underestimated, but represents an interesting matrix because is easily stored, stable, and reflects the accumulation and concentration of metals during previous months and years [1]. Thus, considering all these reasons, the objective of this study is to assess the presence and the quantity of the following elements Al, As, Cd, Cr, Ni, and Pb in wild roe deer to detect the differences between sex, age and geographical areas of life (urban and rural) of animals. To achieve the goals, hair from 39 roe deer was collected during the 2021 hunting season. Animals were hunted in two different areas (urban and rural) in the South area of Oltrepò Pavese (Italy) according to the regional hunting plan. The hair content of different elements was assessed by an inductively coupled plasma-optical emission spectrophotometer (ICP-OES; Thermo iCAP 6000series). The association between hair toxic elements concentrations and age, sex, and geographic area was evaluated by the use of the Unpaired t-test with Welch correction or Mann- Whitney Test, in the case of normally and non-normally distributed data, respectively. Age, sex,and geographic areas were categorized into two different levels. The differences between groups (<3 y.o./ >3 y.o; male/female; urbanized/rural) were considered statistically significant when p-value was <0.05. We found that mean concentrations of Al, Cr, and Pb were significantly higher in the animals hunted in the urbanized area. The mean level of Cr resulted higher in older animals, while in the case of sex the differences between the mean levels of Cd and Cr showed a trend of higher accumulation in females. In agreement with other researchers' results, we found that animals belonging to different environmental compartments contain different accumulation patterns of these toxic elements [2]. Considering classes of age, our findings are in accordance with literature, which reports that the concentrations of several elements vary in an age-related way with higher concentration in adult animals [3]. Finally, as already reported by other authors [3], females show a higher accumulation pattern of these elements. Thus, it is possible to conclude that wild animals, and roe deer in particular, could be a sentinel for environmental pollution and hair can be a good matrix for this type of studies.

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EVALUATION OF ANTIMICROBIAL ACTIVITY OF FOUR ESSENTIAL OILS ALONE AND IN COMBINATION WITH TWO EMULSIFIERS ON BACTERIA OF VETERINARY INTEREST

Alicia Maria Carrillo Heredero, Costanza Spadini, Mattia Iannarelli, Nicolò Mezzasalma, Marica Simoni, Clotilde Silvia Cabassi, Federico Righi, Simone Bertini

Università degli Studi di Parma, Dipartimento di Scienze Medico-Veterinarie.
Corresponding author: A. M. Carrillo Heredero (aliciamaria.carrilloheredero@unipr.it)

Antimicrobial resistance has become a critical problem for infection treatment both in human and veterinary medicine [1]. Finding alternatives is crucial to counteract this phenomenon, as identified by international authorities including EFSA, WHO, and OIE [2]. Essential oils (EOs) are interesting antimicrobial molecules approved by FDA [3, 4], but their inclusion in animal diets requires their adsorption or emulsification on suitable carriers that allow their homogenization in the premixes and feedstuffs. The present study aims to test the antimicrobial activity against four bacterial strains (*Escherichia coli* - EC, *Salmonella* Typhimurium - ST, *Staphylococcus aureus* - SA, and Methicillin-Resistant *Staphylococcus aureus* - MRSA) of four EOs (cinnamon - CIN, lavender-LAV, clove bud - CLO, thyme - THY). The EOs were also tested in combination with two carriers (Tween 20 and Tween 80) at a concentration of 0.5%. The activity was evaluated using a microdilution method. The minimal inhibitory concentration (MIC) against each bacterial strain was tested for each EO, expressed as a percentage. Additionally, the combination between EOs and carriers at increasing concentration was tested by checkerboard assay and the results were expressed as fractional inhibitory concentration (FIC Index) [5]. The MIC values of EOs without emulsifiers ranged from 0.05% (THY against EC) to 3.1% (LAV against MRSA). The most susceptible bacterium to the EOs tested was EC; contrariwise, the most resistant was MRSA. Overall, the MIC of EOs was lower when combined with Tween 20 rather than Tween 80 for all tested bacteria. When EOs were combined with Tween 20 the MIC values ranged from 0.05% (THY against SA and MRSA) to 3.1% (LAV against EC and ST). When EOs were combined with Tween 80 the MIC values ranged from 0.27% (THY against ST) to 12.5% (CIN against SA and MRSA, and CLO against EC, SA, and MRSA). Overall, THY had the most promising antimicrobial activity against the challenged bacteria, whilst CLO had the weaker activity. Considering the higher efficacy of Tween 20 combinations, the checkerboard tests were performed only with this emulsifier. Checkerboard assays showed a prevalence of indifference (43.75%) and additivity (18.75%) among all tested EOs and bacteria. Moreover, synergy was found for THY and LAV in combination with Tween 20 (12.50%). All checkerboards involving the combination of CIN with Tween 20 resulted in antagonistic effects independently from the bacteria challenged (25.00%). In conclusion, the results demonstrate the activity of EOs against the pathogenic bacteria tested. Tween 20 shows potential in enhancing EOs inhibitory activity. Thus, the combinations with Tween 20 could be helpful to lower the doses of antibiotics administered in husbandry, as additive interaction as well as synergy. Considering antagonist interaction between Tween 20 and CIN, further studies should find other possible carriers. In the future, it will be necessary to better understand the possible accumulation and residue issues of EOs components. This project has received funding from the European Union's Horizon 2020 research and innovation programme, under grant agreement No 774340.

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GREEN VETERINARY PHARMACOLOGY (GVP) APPLICATIONS FOR SMALL RUMINANTS AND HONEYBEES WELFARE

Cristian Piras (1), Fabio Castagna (1), Ernesto Palma (1, 2, 3), Enrico Gugliandolo (4), Rosalia Crupi (4), Vincenzo Musolino (1, 2), Antonio Bosco (5), Giuseppe Cringoli (5), Vincenzo Musella (1, Domenico Britti (1)

(1) Department of Health Sciences-University of Catanzaro Magna Græcia, CISVetSUA. (2) Institute of Research for Food Safety & Health (IRC-FSH), Department of Health Sciences-University of Catanzaro Magna Græcia, Catanzaro, Italy. (3) Nutramed S.c.a.r.l. Complesso Ninì Barbieri, Roccelletta di Borgia, Catanzaro, Italy. (4) Department of Chemical, Biological, Pharmaceutical and Environmental Science, University of Messina, Italy. (5) Department of Veterinary Medicine and Animal Productions, University of Naples Federico II, 80137 Napoli, Italy;

Corresponding author: C. Piras (c.piras@unicz.it)

Resistance to drugs for pest control in livestock and honeybees represents a burden for animal production worldwide [1, 2]. For example, the environmental impact of ivermectin and other drugs is also an important aspect to consider for a more conscious use of "mass administration" drugs. In this regard, we believe that more effort should be applied to improve the knowledge about drugs environmental pollution and its consequences.

Green Veterinary Pharmacology aims to reduce the use of synthetic drugs as much as possible by promoting other strategies, such as the integration of feed with bioactive plants/plant products exploiting a similar pharmacological action. Our research group recently demonstrated that (i) aqueous pomegranate (*Punica granatum* L.) and *Isatis tinctoria* extracts are very effective against ewes' Gastrointestinal Nematodes (GINs) [3, 4] and that (ii) *Citrus* spp. and *Origanun* essential oils are extremely powerful in contrasting the parasitic mite *Varroa destructor* [5].

As demonstrated in the first research line (i), *Punica granatum* macerate reduced of 50% the amount of GINs in sheep in "in vivo". The effect persisted up to 21 days after a single shot (50ml "per os") administration. In parallel, the second study highlighted the "in vitro" inhibitory effect of dried I. tinctoria hydroalcoholic extract against sheep's GIN. The inhibitory effect was 84% for the lowest concentration (0.125 mg/ml) of basal leaves extract, only slightly lower than the standard drug thiabendazole (95.6%). Both these studies suggest the application of these plants as alternative natural methods to limit the use of antiparasitic drugs.

The second research line aimed at the detection of natural remedies against one of the major parasitic diseases hampering honeybees farming. Five essential oils (bergamot (*Citrus bergamia*), grapefruit (*Citrus paradisi*), lemon (*Citrus limon*), orange (*Citrus sinensis*), mandarin (*Citrus reticulata*)) and one plant extract (*Origanum heracleoticum* L. (*Lamiaceae*)) were used to assess their "in vitro" efficacy against *Varroa destructor* mite. The essential oils that showed the best effectiveness at 0.5 mg/mL were bergamot, which neutralized (dead + inactivated) 80% ($p \leq 0.001$) of the parasites; grapefruit, which neutralized 70% ($p \leq 0.001$); and lemon, which neutralized 69% of them. *Origanum* extract showed the best efficacy at 2 and 1 mg/mL concentrations, neutralizing (dead + inactivated) 90.9% and 80% of the mites.

Both these examples demonstrate how GVP applications can be valuable alternatives to conventional treatments and that such novel approaches can be used as alternatives to contrast resistance phenomena.

Future directions of GVP pursued by our group are moving towards the study of autochthonous plants to be used against bacterial, fungal and viral infections threating the local animal production system.

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EFFICACY OF THE HISTOLOGICAL MONITORING PLAN: A CASE STUDY

Marzia Pezzolato, Elena Biasibetti, Cristiana Maurella, Francesca Bisso, Marilena Gili, Benedetto Alessandro, Valentina Audino, Elena Bozzetta

Istituto Zooprofilattico Sperimentale del Piemonte, Liguria e Valle d'Aosta -CIBA (CRN Indagini Biologiche Anabolizzanti Animali).

Corresponding author: E. Biasibetti (elena.biasibetti@izsto.it)

Growth promoting agents in food producing animals are strictly forbidden in the European Union (EU) by the Council Directive 96/23/EC [1]. The term 'Illegal treatment' means 'the use of unauthorized substances or the use of substances or products authorized under Community legislation for purposes or under conditions other than those laid down in Community legislation'. The prohibition is due to protect consumers', in fact the assumption of residue of unauthorized drugs poses at serious risk consumers' health. Every year the EU focuses its efforts in fighting the illegal use of substances for growth-promoting purposes, by implementing National Residues Control Plans (NRCPs) to monitor the presence of residues of hormones and veterinary drugs in food and feedstuffs [2]. In spite of the intense official control activity, with over 331,789 targeted samples reported to the European Community by the 27 Member States, the percentage of non-compliant samples in 2020 was 0.27%. Particularly in the group of steroids (A3) and corticosteroids (B2F), only 0.11% and 0.43% bovine samples, respectively, were found to be non-compliant [3]. In 2013, the EFSA, after analyzing data of NRCPs, underlined the inefficiency of the screening strategy and suggested the development and use of biologically-based methods, in order to improve the effectiveness of the official screening steps of Residues Control [4]. To date this recommendation has been followed only by the scientific community through the development of untargeted methods aimed at disclosing illegal treatments [5], while the legislation in force is not yet adequate. To solve this gap National Reference Labs are involved in the revision of the legislation concerning the control of residues of pharmacologically active substances. To tackle the problem, the Italian Ministry of Health introduced, in 2008, the histological monitoring plan as a complementary strategy of control. The objective of this monitoring activity was to verify whether the batches sent to the slaughterhouse exceeded a predefined prevalence threshold level (P=15%) for corticosteroids and sexual steroids. To achieve the objective, histological analyses were performed on a statistically significant number of slaughtered animals by the net of the Experimental Zooprophylactic Institutes official labs. The histopathological approach is able to detect lesions induced by sexual hormones and glucocorticoids in bovine target-organs (sexual accessory glands and thymus) [6]. Sex hormones are known to cause squamous metaplasia in sexual accessory glands [7] while glucocorticoids cause atrophy in thoracic thymus cortex [8]. These changes represent biomarkers that persist after the residues in official matrices have become undetectable by the analytical methods included in NRCPs [9]. Thanks to the monitoring strategy adopted in Italy in 2021, a farm included in the frame of the histological monitoring plan and notified as suspect for sex steroids has been taken care of by the reference center. Indeed, histological analysis performed according to the SOP criteria highlighted severe and diffuse metaplasia in all the animals of the batch and overexpression of PGR receptor in all tissues. Based on reported results the farm was subjected to control by official veterinarians. They collected serum from animals of that farm and its associated farm for NRCP official controls. One serum was non-compliant for progesterone. This work shows how monitoring plans based on an untargeted method can support the fight to drug abuse in farm animals. Considering the rather homogenous picture about veterinary drugs residues in EU, we can expect that the adoption of alternative strategies suggested by EFSA could disclose the real prevalence of illegal treatments and help to address official controls.

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CELL DIFFERENTIATION PROTOCOLS TO ENHANCE THE EXPRESSION OF DRUG METABOLIZING ENZYMES IN BFH12 CELLS: ARE THEY EFFECTIVE?

Silvia Iori, Rosa Lopparelli, Marianna Pauletto, Mauro Dacasto, Mery Giantin

Università degli Studi di Padova, Dipartimento di Biomedicina Comparata ed Alimentazione.
Corresponding author: S. Iori (silvia.iori@phd.unipd.it)

In vitro culture of primary hepatocytes represents the preferred platform for drug development, toxicology and drug-drug interaction studies. Cattle hepatocytes have already been successfully used to investigate xenobiotic metabolism, but they suffer from the same limitations as its human counterpart, specifically a time-dependent reduction of expression and biological activity of drug metabolizing enzymes (DMEs). Additionally, cattle hepatocytes rapidly encounter apoptotic phenomena if the liver is sampled 30 minutes after the sacrifice and the bleeding step of the animal. Since there are no adult bovine hepatoma cell lines, and with the aim of overcoming the disadvantages of bovine primary hepatocytes, a recent immortalized foetal hepatocyte cell line (BFH12) has been established[1]. We recently characterized this cell model; we showed a good responsiveness of BFH12 cells towards an aryl hydrocarbon receptor (AHR) agonist, PCB126[2], and a slight expression of pregnane X receptor (PXR), CYP3A38 and CYP3A48, associated to a low responsiveness to prototypical CYP3A inducers[3]. Therefore, the present study aimed to test different human hepatic differentiation protocols by adding known differentiation inducers to the culture medium, and/or extending the maintenance time, to enhance the mRNA expression of CYP1As and CYP3As and the main nuclear receptors (NRs) involved in their regulation. Specifically, we focused on the mRNA expression of CYP1A1, CYP1A2, CYP3A28, CYP3A38 and CY3A48, as well as their regulators, AHR, PXR, constitutive androstane receptor (CAR), and retinoid X receptor (RXR). BFH12 cells were maintained under 4 different culture conditions: standard medium[4] with 1% glutamine for 4 weeks (A); standard medium (i.e., condition A) plus 1% non-essential amino acids, and 10 µM HEPES for 1 week (B), then supplemented with 1% DMSO, 1µM DEX, 10µM hydrocortisone and insulin for an additional week (C) or 1% DMSO only for further 2 weeks[5] (D). Then, mRNA expression of genes of interest was measured by means of Real-time PCR. Culture condition A enhanced the expression of all four target NRs, even if the statistical significance (P<0.001) was reached for CAR and RXR only (84- and 2-fold induction, respectively). Additionally, CAR was significantly induced (P<0.05) also in condition D, showing a 50-fold increase versus BFH12 cells at T0. As to CYPs, protocol A significantly enhanced (P<0.05) the expression of CYP3A38 and CYP3A48 isoforms (2- and 97-fold induction, respectively), while it sensibly decreased (P<0.01) CYP1A1 and CYP3A28 mRNAs. Conversely, treatment B was effective on CYP1A1 and CYP3A28, leading to a significant induction (P<0.01) of the latter one (1.9-fold). Conditions C and D did not show any improvement on CYP3A38 and CYP3A48 basal level of expression, while significantly decreased (P< .01) both CYP1A1 and CYP3A28 mRNAs. Overall, our findings showed that the most effective differentiation protocols in BFH12 cells were the maintenance of standard culture conditions for one month (i.e., condition A) and the addition of 1% non-essential amino acids and 10 µM HEPES for 1 week (i.e., condition B). Nevertheless, the protocols here used and adapted from human hepatoma cell lines were only partially effective on BFH12 cells, thus supporting the previously observed species-differences between human and cattle in hepatic DMEs regulation, as well as the potential ontogenetic effects (foetus versus adult).

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PRELIMINARY DATA ON ZEARALENONE (ZEA) AND ITS METABOLITES LEVELS IN TISSUES OF WILD BOAR (Sus scrofa) FROM SOUTHERN ITALY

Consiglia Longobardi (1), Sara Damiano (2), Luigi Esposito (2), Nadia Piscopo (2), Antonio Raffaele (3), Valentina Meucci (4), Luigi Intorre (4), Samanta Bacci (4), Roberto Ciarcia (2)

(1) Università degli Studi della Campania "Luigi Vanvitelli", Dipartimento di Salute mentale e Fisica e Medicina preventiva. (2) Università degli Studi di Napoli "Federico II", Dipartimento di Medicina veterinaria e Produzioni animali. (3) Presidente Ambito Territoriale di Caccia Provincia di Avellino. (4) Università di Pisa, Dipartimento di Scienze Veterinarie. Corresponding author: C. Longobardi (consiglia.longobardi@unicampania.it)

Zearalenone (ZEN) is a toxic secondary metabolite synthesized by several *Fusarium* species that grow on crops [1]. ZEN is a mycoestrogen, classified as an endocrine disruptor, having a chemical structure that resembles the endogenous estrogen 17-estradiol and is able to bind estrogen receptors [2]. ZEN and its metabolites, α-zearalenol (α-ZEL) and β-zearalenol (β-ZEL), are considered endocrine disruptors and could cause various toxic effects on animals and humans [3]. ZEN is a mycotoxin with immunotoxic, hepatotoxic and xenogenic effects [3] and it is present in a range of food products including cereals, dried fruits, and spices [2]. Pigs are the most sensitive species to the effects of ZEN [4]. Due to its toxicity, a tolerable daily intake of 0.25 µg/kg body weight and maximum levels in food for human consumption (20–100 µg/kg) have been set by the European Commission by the Regulation EC No 1126/2007. In recent years there has been a significant demographic increase of wild boars (Sus scrofa) in many mountainous and hilly areas of Italy, including the Campania region, mainly due to global climate change. The wild boar can be described as a generalist and omnivorous mammal, capable of varying its diet. Therefore, due to its eating habits, it could be considered a good model of environmental bioindicator towards contaminants, such as mycotoxins. This study has been conducted to evaluate, for the first time, the concentrations of ZEN and its metabolites present in the muscles, liver and kidneys and ovaries of 15 wild boars (40-70 kg), regularly hunted in their habitat by hunters authorized in the scope of the annual hunting plan 2021-2022 in several locations in the province of Avellino. Samples were analyzed with an immunoaffinity clean-up and high-pressure liquid chromatography with fluorescence detection method (LOD = 0.1 µg/kg).

ZEN levels found in the liver and muscles ranged between <LOD-3.25 µg/kg and <LOD-2.22 µg/kg, respectively. α-ZEL levels found in the liver and muscles ranged between <LOD-1.76 µg/kg and <LOD-2.60 µg/kg, respectively. The lowest concentrations were found in kidney with only 1 out 15 contaminated by ZEN and another one contaminated with α-ZEL. β-ZEL was not found in the analyzed samples.

Monitoring the quality of meat is a priority in order to decrease the possibility of toxin carry-over to humans. Traditionally in Italy wild boar meats are used to produce niche products, especially coppa and salami. The present study shows that contamination of meat products by ZEN and its metabolite α-ZEL represents a potential emerging source of this mycotoxin for distinct segments of the Italian population, who are significant consumers of locally-produced wild boar specialties.

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BENTONITE POSSIBLE TOXIC AND PROTECTIVE EFFECTS AGAINST AFLATOXIN B1: AN IN VITRO STUDY IN CACO-2 CELL LINE

Greta Mucignat (1), Irene Bassan (1), Mery Giantin (1), Anisa Bardhi (2), Rosa Lopparelli (1), Andrea Barbarossa (2), Anna Zaghini (2), Enrico Novelli (1), Marianna Pauletto (1), Mauro Dacasto (1)

(1) Università degli Studi di Padova, Dipartimento di Biomedicina Comparata e Alimentazione. (2) Alma Mater Studiorum Università degli Studi di Bologna, Dipartimento di Scienze Mediche Veterinarie.

Corresponding author: G. Mucignat (greta.mucignat@unipd.it)

Feed and food contamination by aflatoxins (AFs) is a serious economic and health concern for both animals and humans [1, 2]. Great efforts are made to minimize risks and losses due to the presence of AFs in feed and food commodities. Bentonite (BEN), is a commonly used sequestering agent adsorbing AFs when added to livestock diets [3]. However, little is known about its possible side effects on animal health and derived food-products. To answer this question, an *in vitro* study evaluating the effects of BEN, alone or in combination with aflatoxin B1 (AFB1), was carried out on the Caco-2 cell line, since the gastrointestinal tract represents the first and foremost interface between AFB1, BEN and the host. To increase the expression of one pivotal enzyme involved in AFB1 bioactivation, i.e. the cytochrome P450 3A4 (CYP3A4), cells were pre-treated with 12-o-tetradecanoylforbol-13-acetate and sodium butyrate [4]. BEN cytotoxicity was assessed by the WST-1 assay, while the effects on cell integrity and monolayer permeability estimated measuring the Transepithelial Electrical Resistance (TEER) and the Lucifer Yellow uptake (LY), respectively. Finally, the uptake of AFB1 in the basolateral compartment and the BEN sequestering capability toward AFB1 and its metabolites, i.e. aflatoxin M1 (AFM1) and aflatoxicol (AFL), were assessed by using set up and validated LC-MS/MS analytical methods.

After 48 hrs of incubation, BEN showed an 50% inhibitory concentration of cell viability (IC_{50}) of 0.08 mg/mL. When tested in combination with a fixed concentration of AFB1 (0.025 mg/mL), BEN showed the maximum protective effect against AFB1 at 0.1 mg/mL ($p \leq 0.01$); by contrast, BEN concentrations over 0.6 mg/mL surprisingly appeared to overcome AFB1 toxicity ($p \leq 0.05$). Additionally, BEN alone showed no effects on the cell integrity and permeability (TEER), while AFB1 alone (0.025 mg/mL) negatively affected the integrity of cell monolayer, as expected. Worth noting, the addition of BEN (0.1 mg/mL) restored the control condition. No statistically significant results were obtained with the LY assay. The adsorbent capacity of BEN against AFB1, AFM1, and AFL was higher for AFL (50%) and roughly 40% and 35% for AFB1 and AFM1, respectively. Finally, the transport rate from the apical to the basolateral compartment was not affected by the addition of BEN.

Present results confirm the protective effect of BEN against AFB1, in the intestinal tract, thanks to its adsorbent capacity. This point was supported also by the preliminary results of transcriptome analysis (RNA-sequencing) in which BEN alone didn't show any effect on gene expression, while proved a consistent involvement in the co-treatment with AFB1. Moreover the aluminosilicate appeared not to interfere with the monolayer physical properties; however, at higher concentrations it showed a certain cytotoxicity, which we hypothesize it is due to potential adsorbent capacity towards medium components like vitamins and minerals.

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PRELIMINARY STUDY ON THE RISK TO HUMAN HEALTH RELATED TO THE PRESENCE OF MERCURY, ARSENIC, AND NICKEL IN PRESERVED ANCHOVIES FISHED IN THE MEDITERRANEAN AND CANTABRIAN SEAS

Federica Di Cesare (1), Sara Panseri (1), Maria Nobile (1), Roberto Villa (1), Irene Rizzoli (2), Stefano Valenti (2), Luca Chiesa (1), Francesco Arioli (1)

(1) Università degli Studi di Milano, Dipartimento di Medicina Veterinaria e Scienze Animali. (2) Delicius Rizzoli S.p.A. Corresponding author: F. Di Cesare (federica.dicesare@unimi.it)

Water pollution by potentially toxic elements (PTEs) is a major concern for the possible effects on human health. Anchovies (*Engraulis encrasicolus*) are the second preserved fish produced in Italy after canned tuna. They are pelagic, zooplankton-eating fishes, inhabiting a very large area from the North Sea to Central Africa, and the whole Mediterranean Sea [1]. For seafood, maximum levels (MLs) for PTEs, i.e. maximum permitted residue concentrations, are defined by Reg. 1881/2006. However, for canned and processed sea products, no MLs are set for PTEs. This study aimed to determine mercury (Hg), arsenic (As), and nickel (Ni) presence in canned anchovies and to characterize the risk for human consumers associated with their consumption. A total of 60 samples of canned anchovies fished in three different FAO sub-areas (Adriatic Sea, Ionian coasts of Tunisia, and Cantabrian Sea in the Bay of Biscay) and marketed in Italy were collected from January 2020 until October 2020. The quantification of Hg, As, and Ni in samples was performed using inductively-coupled plasms-mass spectrometry [2]. The normality of data distribution was evaluated with the Kolmogorov-Smirnov test. The difference in the concentrations of each PTE between FAO sub-areas was assessed by the Kruskal-Wallis test with *post hoc* evaluation adjusted with Dunn's Multiple pairwise comparisons. Considering the PTEs maximum concentrations with a precautionary approach, the risk characterization was performed for the chronic exposure for the three PTEs; moreover, the acute exposure for nickel-sensitive subjects was calculated. Mass spectrometry analyses showed a higher Hg concentration in Adriatic anchovies than in the other two areas (0.270±0.099 µg/kg; $p<0.001$). Regarding As (conservatively considered as the 10% of the total As), Tunisian (0.211±0.103 µg/kg) and Cantabrian (0.209±0.094 µg/kg) anchovies presented significantly higher values than Adriatic ones ($p<0.001$). Finally, anchovies from the Cantabrian Sea showed a significantly higher level of Ni than the other two studied areas (0.060±0.067 µg/kg; $p<0.001$). Considering the maximum levels detected (0.471, 0.413 and 0.332 µg/kg for Hg, inorganic As, and Ni respectively) regardless of the fishing area, for a person of 70 kg was calculated a maximum daily intake of 0.10, 0.09 and 0.07 µg for Hg, inorganic As, and Ni, respectively. These estimated intakes represent less than the 1% of the Health-Based Guidance Levels recommended by EFSA [3-5]. Concerning the acute nickel toxicity in sensitive individuals, using the Margin Of Exposure precautionary approach, it was calculated that a 70 kg person can safely ingest up to a maximum of 23.2 g of the canned anchovies containing the highest concentration detected (0.332 ug/kg). The preliminary results of this study indicate that regardless of the fishing areas, the average Italian consumption of canned anchovies should not pose a risk for consumers.

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FLUMEQUINE CAUSES DEVELOPMENTAL AND REPRODUCTIVE IMPAIRMENTS IN Daphnia magna: AN IN VIVO WHOLE-TRANSCRIPTOMIC APPROACH

Edoardo Pietropoli, Marianna Pauletto, Roberta Tolosi, Silvia Iori, Giovanna Pegoraro, Mery Giantin, Mauro Dacasto, Marco De Liguoro

Università degli Studi di Padova, Dipartimento di Biomedicina Comparata e Alimentazione (BCA).
Corresponding author: E. Pietropoli (edoardo.pietropoli@phd.unipd.it)

Among the environmental pollutants of emerging interest, veterinary medical products are in the spotlight. At present, the effects that the excessive release of drugs, such as antibiotics, can cause on aquatic ecosystems are still unknown. Among antibiotics, fluoroquinolones (FQ), and in particular flumequine (FLU), have been heavily used in aquaculture and fish farming to cope with bacterial infections [1]. Consequently, FLU can be frequently found in river and marine sediments [2]. FQs are detected in the environment at concentrations ranging from ng/L up to µg/L [3], with peaks of around mg/L identified in watercourses near fish farms [4]. In a previous study conducted in our laboratory, 2.0 mg/L FLU has been shown to induce phenotypical and reproductive impairments in *Daphnia magna* [5]. Here we present a second study that aims to understand the molecular mechanisms underneath FLU toxicity. To reach this goal, we assessed chronic toxicity in *D. magna* exposed to 0.2 mg/L and 2.0 mg/L, and carried out a whole-transcriptome analysis by means of RNA-sequencing (RNA-seq). The chronic test was performed in general accordance to the OECD 211 guideline [6], the endpoints being: reproductivity, mortality and growth rate. Total RNA was then isolated from pool of three daphnids. A total of nine RNA-seq libraries, three per experimental group (i.e., FLU 0.2 mg/L, FLU 2.0 mg/L, CTRL), were constructed and sequenced. Looking at the phenotypical end-points, we observed that the population exposed to 2.0 mg/L showed a significant drop in reproduction (-46.50% offspring per mother per day, $p < 0.05$), survival (42.55% mortality, $p < 0.01$), and growth (-8.83% individual growth per day, $p < 0.05$). On the other hand, the group of daphnids exposed to 0.2 mg/L showed no statistical difference in phenotypical characters. These findings were supported by the RNA-seq results. Indeed, daphnid exposure to 0.2 mg/L FLU resulted in less transcriptomic changes compared with daphnids exposed to 2.0 mg/L FLU (43 vs 357 differentially expressed genes, DEGs; fold change greater than 2 and FDR < 5% as default parameters). Interestingly, most of DEGs identified at the lowest FLU concentration were likewise modulated at the highest concentration but with increasing fold changes, thus suggesting a linear transcriptional dose-response. Subsequently, the enrichment analysis revealed that the majority of DEGs reported in the 2.0 mg/L FLU group are associated with development, growth, egg components, detoxification mechanisms and response to oxidative stress pathways. Interestingly, even though the lowest FLU concentration did not induce relevant phenotypical changes, it significantly affected the expression of a certain number of genes (i.e., 43); moreover, many of them are involved in reproduction, as demonstrated by the enriched pathways of egg constituents. In perspective, for a deeper understanding of molecular mechanisms of FLU toxicity, it is crucial to investigate whether environmentally relevant concentrations of FLU are likely to induce transgenerational effects, possibly involving epigenetic variations (e.g. DNA methylation), too.

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USE OF ANTIMICROBIALS IN THE VETERINARY TEACHING HOSPITAL OF THE UNIVERSITY OF PISA: A RETROSPECTIVE STUDY

Samanta Bacci, Luigi Intorre, Valentina Vitale, Francesca Bonelli, Micaela Sgorbini, Valentina Meucci

(1) Università degli Studi di Pisa, Dipartimento di Scienze Veterinarie.
Corresponding author: S. Bacci (samanta.bacci@phd.unipi.it)

Antimicrobials misuse is connected with the emergence of antibiotic resistance (AMR) which represents a serious threat to human and animal health. Antimicrobial stewardship limits the development of AMR and improves patient outcomes by promoting the appropriate use of antimicrobial drugs. Assessment of antimicrobial prescribing practice, a key component of antimicrobial stewardship, in horses is poor reported, despite antimicrobials are commonly prescribed to horses for therapeutic and prophylactic purposes [1]. The aim of this study was to evaluate retrospectively the patterns of antimicrobial use in horses in the veterinary teaching hospital of the University of Pisa between 2019 and 2021 using electronic medical records. Antibiotics were prescribed in 144 (20.2 %) of 714 total visits performed between 2019 and 2021. The most frequently prescribed classes of antibiotics were penicillin (n=50, 34,7%), aminoglycosides (n=48, 33.3%) and sulfonamides (n=24, 16.7%). The most prescribed were gentamicin (25%) mainly employed for the treatment of skin diseases, followed by benzylpenicillin (22.9%) used for prophylactic purposes, and sulfamethoxazole-trimethoprim and ceftiofur (13.2%) prescribed mainly for the treatment of respiratory diseases. Rifampicin was less frequently used (8.3%) in the treatment respiratory diseases. Antimicrobials were prescribed with the support of assay and susceptibility testing only in less than 10% of visits. The present results highlight the need for the development of guidelines for the stewardship of antimicrobials supporting the choice of antimicrobials for empirical use [2]. Because the adoption of a diagnostic approach, including laboratory testing, represents a crucial step that will help in preventing excess use or facilitate the use of specific guidelines for a prudent prescription.

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POLYSTYRENE MICROPLASTIC ALTERS GUT HOMEOSTASIS OF GILTHEAD SEABREAMS EXPERIMENTALLY EXPOSED

Filomena Del Piano (1), Anna Monnolo (1), Doriana Iaccarino (2), Giovanni Piccolo (1), Simone Vozzo (1), Maria Carmela Ferrante (1)

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

Corresponding author: F. Del Piano (filomena.delpiano@unina.it)

During the last twenty years, the massive production and use of plastics have aroused global concern for their effects on living organisms. Indeed, plastics are among the most widely distributed pollutants in the aquatic and terrestrial ecosystems and air (1). Based on their size, little plastic particles are distinguished in microplastics (MPs) (100nm-5mm) and nanoplastics (NPs) (1-100nm) (2). Depending on their shape, MPs are classified in pellets, fibers, fragments, spheroids, and granules. Polystyrene (PS) is one of the most frequently encountered polymer types in biotic matrices (2). After reaching the aquatic ecosystems, MPs and NPs bind to water and sediment phases and undergo trophic transfer along the food chain, through bioaccumulation and biomagnification processes (3). Once ingested, MPs can cause detrimental effects in aquatic organisms ranging from small invertebrates to large vertebrates (4). In this study, we aimed to investigate the effects of a subchronic, oral exposure to PS particles (1-20 µm) in the gilthead seabream (*Sparus aurata*) used as experimental model (Italian Ministry of Health approval n° 1057/2020-PR). In particular, we examined the detrimental impact of PS on intestinal function and integrity and the underlying molecular mechanisms. Gilthead seabreams (81 specimens) were evenly distributed into three groups and fed for 21 days with different diets. The control group received a standard diet, whilst the two exposed groups received a diet with either a low or a high PS concentration, corresponding to a dosage of 25 and 250 mg kg^{-1} b.w./die, respectively. At the end of the experiment, animals were sacrificed, and the intestine was collected for molecular and biochemical analysis. Physiological fish growth and health status were evaluated by Fulton's condition factor, that was significantly reduced ($p<0.05$) at low-dose PS. PS caused a reduced mRNA expression of tight junction proteins (zonula occludens-1, occludin and tricellulin). This effect was nearly always statistically significant (e.g., $p<0.001$ for tricellulin at high-dose PS). PS stimulated local innate immune response through the activation of Toll-like receptors which trigger myeloid differentiation factor 88 signaling pathway required for the induction of cytokines. PS increased gene transcription of pro-inflammatory cytokines (e.g., $p<0.01$ for IL-6 and IL-1β at low- and high-dose PS, respectively) and decreased that of anti-inflammatory ones (i.e., $p<0.01$ for IL-10 at high-dose PS). Moreover, PS-induced increase in nitrosylated proteins ($p<0.05$ at low-dose PS) and malondialdehyde production ($p<0.05$ at high-dose PS) was evidenced. In summary, results indicate that PS determines impairment of the intestinal barrier integrity, gut inflammation, and increased oxidative and nitrosative stress. All these effects impact on gilthead seabreams growth suggesting that PS might compromise their health.

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RESISTANT BACTERIA FROM AQUATIC ENVIRONMENTS, A RETROSPECTIVE STUDY FROM LOGGERHEAD SEA TURTLE *Caretta caretta*

Nicola Pugliese (1), Claudia Zizzadoro (1), Giuseppe Crescenzo (1), Simona Soloperto (2), Olimpia R. Lai (1)

(1) Department of Veterinary Medicine, University of Bari Aldo Moro, Valenzano BA, Italy. (2) Sea Turtles Rescue Center "Luigi Cantoro" Torre Guaceto Protected Area, Carovigno BR, Italy

Corresponding author: O. Lai (olimpia.lai@uniba.it)

In sea turtle rescue medicine, the most frequent occurrences are traumatic injuries (fishing net entrapment, ingestion of hooks and lines, shell fractures, stranding, abnormal buoyancy) or infectious opportunistic pathologies in immunosuppressed animals (e.g., juveniles in cold stunning) (1), and the use of antimicrobial drugs is mandatory. The paucity of data on therapeutic protocols supported by scientific evidence (pharmacokinetics and efficacy trials), together with the repeated reports of antimicrobial resistance in strains isolated from free-ranging sea turtles (2), suggest isolation/sensitivity testing before a therapeutic plan is established as the most correct approach (3). In the present study the results of a four-year investigation about isolation, characterization, and sensitivity testing of isolates from *Caretta caretta* are reported, to explore the diffusion of antimicrobial resistance (AMR) in bacteria form wild species with no history of previous therapies. The animals were presented at the Sea Turtle Rescue Center "Luigi Cantoro" of Torre Guaceto (BR, Italy, Adriatic Sea) for noninfectious occurrences, with lesions at different location (lesions of limbs or carapace with exposition of bone plates, shell fractures, gastrointestinal lesions from hooks/lines). Amies transport swabs were collected from superficial lesions with deep blade scrub, while bioptic samples were collected during surgery for hook/lines removal. Samples were cultured on Columbia Blood agar and McConkey agar. The isolates were identified by Gram staining, oxidase test, API 20E and 20NE systems, then screened for antimicrobial susceptibility by the disk diffusion method (4) with amoxicillin-clavulanic acid (AMC), ampicillin-sulbactam (AMS), cefuroxime (CXM), ceftazidime (CAZ), ciprofloxacin (CIP), norfloxacin (NOR), gentamycin (GEN), and doxycycline (DOX). Samples were cultured also for *Mycoplasma* on Hayflick agar and tested with microbroth dilution for tetracycline, doxycycline, erythromycin, clarithromycin, tylosin, azithromycin, enrofloxacin and marbofloxacin. The prevalent isolates were Gram negative, and 30 of them, belonging to genera *Aeromonas*, *Morganella*, *Pseudomonas*, *Serratia*, and *Vibrio*, are potentially pathogenic. Many isolates resulted resistant to the association of semisynthetic penicillin and beta-lactamase inhibitors (18/32 to AMS and 27/32 to AMC), and to a second-generation cephalosporin (7/32 to CXM), while only two strains of *Vibrio alginolyticus* and *Serratia marcescens* were resistant to the third-generation cephalosporin CAZ. Noteworthy, the same *V. alginolyticus* strain was also resistant to all the tested antibiotics, including GEN and the fluoroquinolone CIP. Four more strains of *Aeromonas, Pseudomonas*, and *Serratia* genera were multidrug resistant (MDR) (semisynthetic penicillins+beta-lactamase inhibitors, second-generation cephalosporin, and doxycycline or fluoroquinolones). A single *Mycoplasma* spp. strain was isolated, proving resistant to tetracyclines and macrolides, with the only susceptibility to fluoroquinolones. The present data confirm the progressive spreading of AMR and MDR bacterial strains in wild animals without previous contacts with antimicrobial compounds, together with the concern about the potential for AMR diffusion linked to mobile genetic elements, frequently reported in many strains of the present isolates (5, 6). At the same time the sea turtles' rehab suffers from the progressive reduction of therapeutic tools and adds zoonotic concerns for operators.

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EFFECT OF SNAIL SECRETION FILTRATE IN A MOUSE MODEL OF CANINE ATOPIC DERMATITIS

Rosalia Crupi (1), Fabio Bruno (1), Laura Messina (1), Patrizia Licata (1), 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 Messin, Italia.

Corresponding author: R. Crupi (rcrupi@unime.it)

Canine Atopic Dermatitis (cAD) is a skin chronic inflammation characterized by lesions that overlap in relation to the stage of the disease. The treatment of cAD is based on the use of moisturizers, cyclosprin, phototherapy, topical corticosteroids and immunomodulators, systemic antihistamines and corticosteroids. The use of non-pharmacological products to treat cAD could be considered a useful and safe alternative to antibiotics and glucocorticoids.

Previous studies, conducted in our laboratories, have shown that snail slime, secreted by the snail *Helix aspersa* Muller, has a strong anti-inflammatory activity, demonstrated in *in vivo* studies. Current knowledge about the mucus produced by *Helix aspersa* Muller indicates that mucus is rich in hyaluronic acid, mucopolysaccharides, polyphenols and bioactive minerals. These substances improve the adhesion of mucus to the skin, acting as a protective barrier, while the polyphenols counteract the damage associated with oxidative stress. Moreover, the mucus is characterized by reparative activity due to emollient, antimicrobial and adhesive properties.

The aim of our study was to evaluate the potential beneficial effects of snail secretion filtrate (SSF) administration in a mouse model of canine atopic dermatitis.

Atopic dermatitis-like lesions were induced by the topical application of Oxazolone (Ox). The mice were sensitized with a single application of 1% Ox. One week later, 0.1% Ox was topically applied every other day for 20 days. Moreover, mice received topical SSF before Ox treatment for 20 days. The desamethasone was used as positive control. The mucus used was obtained after manual stimulation of the snails with a sterile cotton swab tip and then subjected to a series of filtrations to obtain SSF.

Our results demonstrated the protective barrier effects of SSF characterized by a reduction in histological lesions, mastocyte degranulation and marker of inflammatory process (evaluated through ELISA assays for cytokines such as IL-6, IL-1β, TNF-α and Western blot analysis for NF-kB) in the skin following Ox treatment. Our data support the effectiveness of SSF to reduce the skin damage, demonstrating that a product containing SSF could be considered as a potentially interesting approach for the management of cAD.

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MITIGATING EFFECTS OF DIETARY SUPPLEMENTATION WITH *Panax ginseng* ESSENTIAL OIL ON ATRAZINE-INDUCED TOXICITY in NILE TILAPIA (*Oreochromis niloticus)*

Claudia Zizzadoro (1), Alessandro Di Cerbo (2), Nicola Pugliese (1), Olimpia Lai (1), Giuseppe Crescenzo (1), Mahmoud Alagawany (3)

(1) Department of Veterinary Medicine, University of Bari Aldo Moro, Valenzano BA, Italy. (2) School of Bioscience and Veterinary Medicine, University of Camerino, Matelica, Italy. (3) Poultry Department, Faculty of Agriculture, Zagazig University, Egypt.

Corresponding author: C. Zizzadoro (claudia.zizzadoro@uniba.it)

Dietary use of essential oils in aquaculture has been receiving increasing attention as an alternative strategy for improving resistance to biological and non-biological stresses (e.g. pathogens and environmental toxicants), growth performance and overall welfare of farmed fish [1]. In the present study, we tested dietary supplementation with *Panax ginseng* essential oil (GEO) for its efficacy at mitigating the adverse effects that the water-soluble herbicide atrazine (ATZ) is able to induce in Nile tilapia (*Oreochromis niloticus*) as a consequence of contamination of aquatic ecosystems [2]. To this aim, 180 fish were allocated into 6 triplicate experimental groups, where one group (control) was reared in clean water and fed a commercial basal diet, two groups (GEO1 and GEO2) were fed the basal diet supplemented with two different levels of GEO (1.0 and 2.0 mL/kg diet), one group (ATZ) was intoxicated with 1/5 of ATZ 96-h lethal concentration 50 (1.39 mg/L), and the remaining two groups (GEO1+ATZ and GEO2+ATZ) were fed the GEO-supplemented diets and concurrently exposed to 1.39 mg ATZ/L. After a 60-day experimental period, inter-group differences were analysed by one-way ANOVA, followed by Tukey's multiple comparison test. The study was approved by the Institutional Ethics Committee of the Faculty of Veterinary Medicine of Zagazig University (Egypt) and was conducted in accordance with the Local Experimental Animal Care Committee guidelines. Consistently with previous studies using herbal ginseng [3], both levels of dietary GEO supplementation of healthy Nile tilapia exerted significantly positive influence ($P<0.001$) on fish growth and feed utilization parameters, as well as on fish immunity (increased leukocyte counts and IgM levels) and hepatic antioxidant defense systems. Exposure to sub-lethal ATZ concentration was confirmed to significantly reduce ($P<0.001$) fish survivability, growth and feed utilization [4], as well as to induce significant negative changes ($P<0.001$) in intestinal digestive enzyme activity (decreased lipase activity), hematological indices (decreased hemoglobin, packed cell volume, erythrocytes and leukocytes), blood biochemical variables (decreased total proteins, albumin, globulins and IgM; increased total cholesterol, triglycerides and cortisol), hepatic oxidative/antioxidant indices (decreased superoxide dismutase and catalase enzyme activity and mRNA expression levels, decreased glutathione level and increased malondialdehyde content), hepatic reactivity indices (upregulated mRNA expression of the stress- and apoptosis-related genes *Hsp70*, *caspase 3* and *p53)* and hepatic histomorphology (lesions referring to early hepatoma). Of interest, dietary GEO supplementation in GEO1+ATZ and GEO2+ATZ groups significantly attenuated ($P<0.05$) most of the afore-mentioned negative effects of ATZ, though for some parameters still not reaching the control values even at the higher supplementation level. Taken together, the findings of the present study provide further and partly new evidence that dietary GEO supplementation may be useful not only to provide general support to productive performance and health/welfare status of Nile tilapia, but also for mitigating the impact of sub-lethal ATZ toxicity in this fish species.

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EFFECTS ON INTESTINAL PERMEABILITY AND KIDNEY PHYSIOLOGY IN ANTIBIOTICS-TREATED MICE: A MODEL TO STUDY THE GUT-KIDNEY AXIS

Consiglia Longobardi (1, 2), Sara Damiano (3), Antonio Miele (2), Mariavittoria D'Acierno (2), Gianmarco Ferrara (3), Giovambattista Capasso (2), Roberto Ciarcia (3), Anna Iervolino (2, 4)

(1) Università degli Studi della Campania "Luigi Vanvitelli", Dipartimento di Salute mentale e Fisica e Medicina preventiva. (2) Ca.re.Bios "Campus Regi Biologia". (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 Scienze Mediche Traslazionali.

Corresponding author: C. Longobardi (consiglia.longobardi@unicampania.it)

Microbiota and its modulation could influence various physiological functions and the impetuous interest in the field of renal pathologies is becoming an avant-garde research area [1]. However, literature indicates that the gold-standard animal model to study the correlation between the microbiota and its host are germ-free mice, whose generation and maintenance often impractical [2].

This study aims to generate a more advantageous mouse model, in particular an antibiotic-treated one, that exhibits a strong reduction of intestinal microbiota without developing impaired renal function. The potential of this research lies in the chance to carry out future studies on the interaction between the intestinal microbiota and kidney function, the so called gut-kidney axis.

A total of 25 five-six weeks old C57/BL6 male mice were randomly divided into 5 groups of 5 animals/each: Group 1 received a cocktail containing ampicillin, gentamicin, metronidazole, neomycin (1mg/mL each) and vancomycin (0.5 mg/mL) by oral gavage for 10 days; Group 2 received the same cocktail as Group 1 by drinking water (DW); Group 3 was treated with amphotericin-B (2mg/kg) for 3 days, followed by administration for 14 days of vancomycin (100mg/kg), neomycin (200mg/kg), metronidazole (200mg/kg) and amphotericin-B (2mg/kg) mix by oral gavage, and ampicillin (1mg/mL) by DW; Group 4 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 (authorization number 820/2020-PR). DNA extracted from feces (QIAamp Fast DNA Stool kit) of mice undergoing the treatment was used to evaluate the reduction of the intestinal microbiota, by the 16S gene rRNA sequencing. Since the reduction of the microbiota causes an increase in intestinal permeability as well alterations in the morphology of the intestine and lower expression of the tight junction proteins, we evaluated the intestinal permeability, the intestinal morphology and the expression of Zonulin-1 (ZO-1) [3, 4]. To verify the kidney integrity after treatment, renal function was studied by evaluating both the fibrosis at the kidney cortex level with two-phoyon microscope, and some urine and serum parameters, as creatinine, urea and electrolytes. With the exception of Group 1 treatment, with 5% of reduction, the rest exhibited a good percentage of microbiota reduction: Group 2: 93%; Group 3: 70% and Group 4: 58%. ZO-1 showed a reduced expression in all antibiotics-treated groups versus control one ($p<0,0001$): this is symptom of a damaged barrier, confirming that microbiota has been depleted as desired. The fibrosis at the kidney cortex level and urine/serum parameters demonstrate that the Group 4 mice gives the most aboundant percentage of fibrosis while the others show no obvious signs of it.

The study is currently still ongoing and will allow us to have a new mouse model to continue the research in the field of renal diseases and its interaction with the intestinal microbiota, laying the foundations for the evaluation of fecal microbiota transplantation (FMT) for therapeutic purposes in kidney pathologies.

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SIRA

SURGICAL MANAGEMENT OF VAGINAL HYPERPLASIA IN BITCHES BY BÜHNER SUTURE: A CASE SERIES

Roberta Bucci (1), Jasmine Fusi (2), Brunella Anna Giangaspero (1), Serena Florio (1), Maurizio Caputo (1), Domenico Robbe (1), Augusto Carluccio (1)

Università degliStudi di Teramo, Facoltà di Medicina Veterinaria. (1) Università degli Studi di Milano, Dipartimento di Medicina Veterinaria e Scienze Animali (2).

Corresponding author: R. Bucci (rbucci@unite.it)

Vaginal hyperplasia in the bitch is an exaggerated response of the vaginal mucosa to estrogens during the proestral-oestral phase of the cycle, that can protrude through the vulvar lips (1). Although not commonly observed, it represents a clinical condition needing veterinary intervention because the protruding mass could be vulnerable for trauma, ulceration, inflammation and possibly self-mutilation, other than interfere with natural mating. Treatment of vaginal hyperplasia includes pharmacological or surgical approaches. Pharmacological treatments aim to induce ovulation and shorten estrus, counteract the action of estrogens or to hasten the shrinkage of the mass (2). Surgical treatment includes mass amputation or closure of the vulva after mass reposition. The present study refers to the surgical management of vaginal hyperplasia in bitches by Bühner vulvar suture. A total of 14 bitches, 1-7 years old, belonging to several medium and large-sized breeds, refereed to Veterinary Teaching Hospital of the Faculty of Veterinary Medicine, University of Teramo. All bitches showed vaginal hyperplasia and protrusion of the entire circumference of the vaginal mucosa (Type 3), without ischemic or necrotic areas. According to the estrous cycle, all the bitches were in proestral-estral phase. Under general anesthesia, prolapsed mass was cleaned with 50% glucose solution to reduce oedema, and repositioned with a gentle retropulsion; using a Gerlach needle and a sterile vaginal suture tape, Bühner suture was applied as described by Matteuzzi (3) on the vulvar labia maintaining an opening of about one finger in diameter to allow urination. After surgery, all the animals received antibiotic and anti-inflammatory treatment for one week. At the follow-up, none of the bitches showed recurrence during the current cycle, proving the efficacy of the Bühner suture. To prevent the possible recurrence of vaginal hyperplasia at the subsequent estrus, all bitches underwent ovariectomy 2 months later, when Bühner suture was removed. In conclusion, the Bühner suture proved to be useful for the conservative treatment of vaginal hyperplasia in medium or large-sized bitches, as alternative to the pharmacological therapy. However, this approach should be considered only for cases in which the prolapsed mass does not show trauma, ulceration, ischemic or necrotic areas.

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EFFECT OF A SUBCUTANEOUS IMPLANT OF DESLORELIN ACETATE ON SERUM TESTOSTERONE CONCENTRATIONS IN MALE HERMAN (Testudo hermanni SP.) AND GREEK (Testudo graeca SP.) TORTOISES

Maria Carmela Pisu (1), Alice Andolfatto (1), Angelica Ferro (2), Simona Esposito (3), Maria Cristina Veronesi (4), Monica Probo (4).

(1) Centro di Referenza Veterinario, Torino. (2) Libero professionista, Regno Unito. (3) Centro Recupero Animali Selvatici, Cuneo. (4) Università degli Studi di Milano, Dipartimento di Medicina Veterinaria e Scienze Animali.
Corresponding author: M. Probo (monica.probo@unimi.it)

The influence of testosterone (T) on sexual behavior has been long investigated; it has been shown that an elevation of circulating T in males stimulates mate searching effort, territoriality, and aggressiveness, and it promotes the expression of various sexual behaviors and general activity [1]. Male chelonians are regularly presented with an increased sexual behavior due to high T levels [2], resulting in competition and aggression amongst each other, and leading to high stress and traumatic mating conditions in females because of the frequent chasing and sexual acts, as well as claws and bites [1]. Castration can be an option for solving aggression and mating problems in European tortoises if separation of the animals is not possible, but orchiectomy via shell osteotomy, although effective, is time-consuming and associated with prolonged healing times and post-operative complications [3]. Deslorelin acetate is a GnRH agonist formulated in a controlled- release subcutaneous implant, and designed for reversible suppression of T production in dogs. It has been proved to be effective in many animal species, while recently a study [4] demonstrated that neither single nor double deslorelin acetate implant is successful in suppressing gonadal activity and preventing reproduction in adult female pond sliders during a one season follow-up. No data on its effectiveness in male tortoises are available. The aim of this study was to evaluate the effect of a 4.7-mg deslorelin acetate implant on serum T concentrations in male Hermanni (*Testudo hermanni* sp.) and Greek (*Testudo graeca* sp.) tortoises. The study was performed in agreement with the animal welfare committee ethical (OPBA_120_2021). Twenty adult male sliders housed under the same environmental conditions were enrolled according to the following criteria: normal morphology of carapace, absence of previous pathologies, and negativity to parasitosis. Age of the subjects ranged between 10 to 25 years. At the beginning of the study, each subject was weighed, and randomly assigned to a treatment (D=10) or to a control (C=10) group. Starting from May, males of the D group were implanted with a 4.7-mg deslorelin acetate device, whereas males of the C group did not receive any treatment. Blood samples were collected once immediately before implant application and at 20 days, and 2 and 5 months after application. Serum T was measured through a solid-phase, enzyme-labeled, competitive chemiluminescent immunoassay. Testosterone values were analyzed by a two-way ANOVA with interaction. No differences were detected between mean body weight of C (0.60±0.27 kg) and D group (0.67±0.46 kg). Mean serum T concentrations ranged from a minimum of 5.68±4.37 ng/mL to a maximum of 19.11±12.39 ng/mL, and were not significantly different between the two groups in all sampling times; no interaction with time and body weight was observed. The present study suggests that a single treatment with a 4.7-mg deslorelin acetate implant has no effect on short term T production in male Hermanni and Greek tortoises. Further studies are required, as interpretation of these data is hindered by lack of information on physiological pattern of T secretions in this species, and on species specificity in the reptilian GnRH receptors that may limit treatment effectiveness.

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COMPARISON BETWEEN SPLASH BLOCK VERSUS INJECTION OF LIDOCAINE ON THE OVARIAN PEDICLE IN CANINE OVARIECTOMY

Vincenzo Cicirelli, Matteo Burgio, Daniela Mrenoshki, Giovanni M. Lacalandra, Giulio G. Aiudi
Department of Veterinary Medicine, University of Bari "Aldo Moro", Bari, Italy.
Corresponding author: V. Cicirelli (vincenzo.cicirelli@uniba.it)

Ovariectomy is a surgery with medium level of pain and requires an effective analgesic technique. Multimodal analgesia, including drugs administered both systemically and locally, is considered the most effective approach to providing pain relief and has been widely accepted in medicine veterinary medicine to control intraoperative pain (Acquafredda et al., 2021). Splash block is an analgesic technique already described by several authors in domestic animals, which consists of an irrigation of lidocaine in the ovarian pedicle to improve local analgesia during ovariectomy (Cicirelli et al., 2022). Furthermore, Grubb and Loprise (2020) describe direct infiltration of the ovarian pedicle with lidocaine. To our knowledge, the use of lidocaine infiltrated on the canine pedicle during ovariectomy has not yet been evaluated, or compared with other analgesic techniques. Although it is to be expected that additional local anaesthesia confer better analgesia, the aim of this study was to compare the analgesic efficacy of splash block versus the infiltration of lidocaine on the ovarian pedicle in bitches ovariectomy. Forty bitches of various breeds presented for ovariectomy were involved to this study after obtaining informed owner consent. After general examinations, all the dogs had a thoracic radiograph, abdominal ultrasound scan, and routine blood tests. Thus, the bitches were allocated to the very low aesthetic risk class (ASA 1) and randomly assigned in two groups (n=20) to receive topical irrigation (splash block) of 2% lidocaine (C group) on both ovarian pedicle (2 mg/kg each), or an equal volume of lidocaine infiltrated in the same sites (R group). This study was approved by the Ethics Committee for animal testing–CESA of the Department of Veterinary Medicine of the University of Bari Aldo Moro. The same surgical team performed all ovariectomy, in full compliance with the *leges artis*. In C group, prior to manipulation of the ovarian pedicles, 2% lidocaine was dripped on the ovarian pedicle (2 mg/kg each) using a catheter urinary (splash block). In R group, 2% lidocaine was infiltrated on the ovarian pedicle (2 mg/kg each) using a 2.5 mL syringe (23-G). Following lidocaine application, surgical manipulation was stopped for 90 seconds. Before the first incision, the hemodynamic parameters of all animals (preincisional values of heart, respiratory and blood non-invasive pressure values) were recorded to evaluate pain responses to the surgical stimulus. These parameters were registered at six phases of the study: grasping of the ovary (time 1), dissection of the mesosalpinx (time 2), tightening of the first loop ligature (time 3), tightening of the second loop ligature (time 4), transection of the ovarian pedicle (time 5) and release of the ovary (time 6). Repeated-measures ANOVA showed no significant differences in heart rate, respiratory rate and blood pressure values between groups, neither during lidocaine infiltration on the ovarian pedicle. The results of the present study suggest that splash block may provide intraoperative analgesic effects equivalent to injection in the ovarian pedicle in dogs that have undergone ovariectomy. In fact, in both procedure, lidocaine is absorbed quickly from ovarian tissue blocking the ascending afferent input and interfering with ion channels of the nerves of ovaries, allowing a satisfactory intraoperative analgesia.

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PHYSICAL PARAMETERS IN SALERNITANO NORMAL NEWBORN FOALS

Brunella Anna Giangaspero (1), Roberta Bucci (1), Monica Probo (2), Michela D'Angelo (1), Graziano Ippedico (1), Massimo Faustini (2), Domenico Robbe (1), Augusto Carluccio (1)

(1) Università degli Studi di Teramo, Facoltà di Medicina Veterinaria. (2) Università degli Studi di Milano, Dipartimento di Medicina Veterinaria e Scienze Animali.

Corresponding author: B.A. Giangaspero (bagiangaspero@unite.it)

Neonatal survival is the ending purpose of the reproductive process in all species. In the horse, in which gestation is particularly long and only one kid is usuallly delivered each time, neonatal survival is foremost important for the successful of reproduction. To survive, the mammalian newborn should cope with the transition from the intra- to the extra-uterine life, that is assured by a complex process known as neonatal adaptation. The neonatal adaptation includes many physiologic factors, such as maturity, viability and a timed series of behaviors leading to a prompt respiration, stand up, colostrum intake and escretion. In the horse and other equids, the most important physical parameters for a newborn evaluation include the Apgar score, the birthweight, the assessment of the time from birth to stand up and to first suck, and time for meconium expulsion, widely studied in the horse. Among the horse breeds, in Italy some breeds are actually sublitted to programmes for biodiversity conservation. Among them, the Salernitano breed is one of the breeds included in the preservation programmes. The Salernitano is a saddle meso-dolichomorphic horse, reared in the south of Italy, especially in the Campania region, weighing 450-500 kg, with minimum 158 cm in males and 150 cm in females height at the withers at 42 months. The conservation programmes should include all the aspects of reproduction efficency improving, with neonatal evaluation as a main step. This study was aimed to define the main neonatal physical parameters in Salernitano horses in order to detect any possible peculiarities for a better management of the newborns. The study was done on 52 healthy newborn foals born by eutocic spontaneous supervised foaling. Neonatal physical parameters evaluated were: the Apgar score, the birthweight (BW)(kg), gender, time (min) to stand up (TSU), time (min) for first suck (TFS), time (min) for meconium expulsion (TME). Pregnancy length (PL)(d) was also recorded. The results showed that the 52 foals, 31 females and 21 males, were born mature after an average PL of 330±8.49 d (331.7±8.09 in males and 328.9±8.70 in females). Mean Apgar score was 9.4±0.61 (9.3±0.73 in males and 9.5±0.51 in females); BW 45.3±3.59 kg (45.6±4.03 in males and 45.2±3.38 in females); TSU 48.4±24.78 min (47.5±23.69 in males and 49.0±25.86 in females); TFS 85.6±39.66 min (82.5±32.74 in males and 87.7±44.13 in females); TME 109.6±48.40 min (107.6±44.36 in males and 111.0±51.64 in females). The statistical analysis showed a significant correlation between TSU and TFS (Pearson's r =0.744; $p<0.001$), between TSU and TME (Pearson's r =0.687; $p<0.001$), and between TFS and TME (Pearson's r =0.923; $p<0.001$). The foal's BW was significantly correlated to PL (Pearson's r =0.779; $p<0.001$). None of the parameters was influenced by the foal's gender. The results showed that the main neonatal parameters in normal Salernitano foals agree with data reported in literature (Stoneham). Notably, differently to what generally reported for horses (PL>320 d) (Rossdale, 1976), PL was shorter than 320 d in 6 mature, alive and viable foals (303-318 d). This agrees with the reported between individual range of 301-388 for gestations resulting in viable foal (Heck).

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CRYOPRESERVATION MODIFIES TEMPERATURE-RELATED MEMBRANE FLUIDITY IN BOVINE MALE GAMETE

Alessia Gloria (1), Alberto Contri (2)

(1) Università degli Studi di Teramo, Facoltà di Medicina Veterinaria. (2) Università degli Studi di Teramo, Facoltà di Bioscienze e tecnologie agro-alimentari ed ambientali.

Corresponding author: A. Gloria (agloria@unite.it)

Artificial insemination with cryopreserved semen is the most important assisted reproductive technology for dairy farmers around the world to improve the genetic quality of their stock. Cryopreservation has a multifactorial effect on the cell, including stress, osmotic, and peroxidative stresses [1], causing a reduction in cell function and fertilizing ability. Most of these stressors are directed against the plasma membrane, which plays a pivotal role in sperm function and oocyte fertilization ability. Membrane fluidity is largely related to temperature, thus this study aimed to verify the behavior of bovine male gamete plasma membrane [2], in terms of membrane fluidity, at different temperatures both before and after the cryopreservation. To this, diluted samples from 16 bulls were incubated at 25°C, 37°C, and 39°C, before (fresh samples) and after (frozen samples) cryopreservation. Both fresh and frozen samples were analyzed at 10, 30, 60, 120, and 180 min of incubation at each temperature. To assess the effect of temperature and time, the samples were stained with calcein-AM (Molecular Probes Inc., Eugene, OR, USA), for cell viability [3-5], and merocyanine-540 (Molecular Probes Inc., Eugene, OR, USA), as plasma membrane disorder marker [6], and analysed flow cytometrically (CytoFLEX, Beckman Coulter, San Jose, CA, USA). Kinetic attributes of male gametes were also evaluated on fresh and frozen samples using a computer-assisted sperm analyser (CASA, IVOS 12.3, Hamilton-Thorne Bioscience, MA, USA). The data reported in the present study confirmed the detrimental effect of cryopreservation on bovine sperm quality. In the fresh sample, the average percentage of TM was 83%, while in frozen samples a significant reduction was found (TM 52%; $P<0.05$). A similar detrimental effect was found for the other kinetic attributes. The motility evaluated by CASA systems, conventionally used to define the quality of the insemination doses, was found partially adequate to estimate bovine cell function. Thanks to this study, it has been possible to demonstrate that a part of viable and motile cells at the time of thawing tends to destabilize and degenerate after 2-3 hours, since merocyanine positive cells after thawing (26.67±1.83% at 39°C) was significantly higher than the correspondent fresh samples (17.16±1.49%; $P<0.05$) and decreased progressively over time, with a correspondent increase in non-viable destabilized cells. This different behavior of cryopreserved spermatozoa, not detected in fresh samples, appeared temperature-dependent, since in samples incubated at 37°C, and more evident at 39°C, a significant aliquot of sperm population is subject to destabilization, consequently also losing the integrity of the membrane. The same behavior was not detected in cryopreserved spermatozoa incubated at 25°C at any time point. These findings suggest that after cryopreservation part of spermatozoa increased the plasma membrane fluidity at body temperature. This could explain, at least in part, the reduced survival of cryopreserved male gamete in the cow tract.

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ADVANCES IN NEWBORN DOG VIABILITY: DIFFERENCES BETWEEN FRENCH AND ENGLISH BULLDOGS - PRELIMINARY RESULTS

Jasmine Fusi (1), Monica Probo (1), Roberta Bucci (2), Claudia Scabrosetti (1), Maria Cristina Veronesi (1)

(1) Università degli Studi di Milano, Dipartimento di Medicina Veterinaria e Scienze Animali. (2) Università degli Studi di Teramo, Facoltà di Medicina Veterinaria.

Corresponding author: J. Fusi (jasmine.fusi@unimi.it)

Still today, in canine species the perinatal losses reach high values, mainly occurring around the time of birth. For this reason, in the last decade, the Apgar score (AS), adapted to the dog, firstly proposed in 2009 by Veronesi et al [1], was widely used for the evaluation of newborn dog viability, to allow the recognition of normal newborns and the quick detection of those newborns needing special assistance or resuscitation. However, in 2014, Batista et al [2] posed a question about little adjustments of the former scoring system for a more precise newborn viability evaluation in some specific breeds. Whilst Batista and co-authors found that some parameters had to be modified for French Bulldogs (FB) and English Bulldogs (EB), Fusi et al. [3] reported that no changes to the initial scoring system and newborn classification are needed for the newborn viability assessment in Chihuahua puppies. However, questions about peculiarities of bulldogs remain: even if both "bulldogs" and both brachycephalic breeds, FB and EB are different one from the other, especially in body size (FB 8-14 kg, EB 23-25 kg) (ENCI), but up to now no data are available about possible differences in newborn viability between the two breeds. Moreover, according to the present authors clinical experience, differences in newborn viability between FB and EB are suspected. Therefore, this study was aimed to evaluate possible differences in newborn viability between FB and EB puppies. The study was done on 30 (12 males and 18 females) FB and 30 (17 males and 13 females) EB newborn puppies, all born by elective caesarean section from 7 FB (2-5 years old; 1-3 parity) and 5 EB (2-5 years old, 1-3 parity) healthy bitches. At delivery, all puppies were evaluated by AS [1], assessed for physical defects and weighed. Newborns were classified in the three classes of newborn distress: severe distress (AS 0-3), moderate distress (AS 4-6), no distress (AS 7-10) [1]. Survival was checked at 24 hours after birth (S1). Out of all born puppies, 1 FB (3.3%) and 0 EB were born dead. Among born alive, normal puppies, 1/29 FB was dead at S1 (3.4%), while in EB 6/30 (20%) were dead at S1 ($\chi 2$ test: $p<0.05$). A strong difference ($p<0.0001$) in the AS of surviving puppies between FB (7.9±1.15) and EB (6.4±1.13) was found by one-way ANOVA, while no difference was observed for AS in non-surviving puppies: FB (2.0±0.00), EB (1.7±0.52). The results of the present study showed that FB and EB, both brachycephalic and Bulldog breeds, should be considered differently in the evaluation of newborn viability. The reasons for a lower survival at S1 in EB need further investigations, while the lower AS in puppies surviving at 24 hours is very relevant for the neonatologist. In fact, while surviving FB were fully classified in the no distressed newborns, the EB puppies were grouped in between the moderate distressed and no distressed puppies, highlighting that these last newborns need to be closely surveilled, and maybe additional proper assistance is needed to improve their chance of survival.

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RABBIT OVARIECTOMY WITH AIRPLASMA TECHNOLOGY

Giuseppe Bonaffini, Luca Scandone, Chiara Ottino, Elena Colombino, Matteo Serpieri, Ilaria Prandi, Giuseppe Quaranta, Mitzy Mauthe von Degerfeld

Università degli Studi di Torino, Dipartimento di Scienze Veterinarie (DSV), CANC (Centro Animali Non Convenzionali). Corresponding author: G. Bonaffini (giuseppe.bonaffini@unito.it)

Onemytis® is an electro-thermo-clotting-ablation device which employs Airplasma technology. It adopts the gas ionization principle, in which a high voltage electromagnetic field ionises a neutral gas (air). The originated energy (plasma energy) is seen as a "flare" that is only visible, and working, when there is not direct contact between the tissue and the handpiece. The flare is extremely thin (smaller than a cold blade) and develops a medium temperature of 50°C [1, 2]. The aim of this study is to evaluate, from a clinical and surgical point of view, the efficacy of Onemytis® during rabbit ovariectomy, comparing this device with an electrosurgical one (AM 308-N) and with scalpel blade.

The research has been approved by Bio-Ethical Commission of DSV with protocol number 568 on 23/02/2022.

The study was performed on 27 client-owned female rabbits admitted to CANC, a unit of the DSV (University of Turin). Their age ranged between 4 months and 4 years, and they belonged to different breeds. Ovariectomy followed standard procedures and was performed by a surgical veterinary team made up of two surgeons (one of them with expertise in the use of the device) and an anaesthetist. Animals were randomly divided in two groups: in one group Onemytis® was employed for skin incision (Group 1), while in the other it was cut through scalpel blade (Group 2). In each rabbit one ovary was removed through Onemytis®, while the electrosurgical device was employed to excise the other. The intensity of both electrical units was set according to surgeons' previous experience: AM 308-N was regulated at the power usually set for normal use (45kW) while Onemytis® was used at maximum intensity. Both surgeons carried out an equal number of surgeries with both instruments and as lead surgeon (L) and assistant (A). A form to fill out was devised to evaluate the functioning of Onemytis® device. The anaesthetist completed it during surgery while both surgeons filled it in after the operation, without sharing opinions with the colleagues. The surgical wound was monitored through a blind study: a fourth veterinarian, who did not know the devices employed on each animal, checked the wound after 1 (T1) and 8 (T8) days post-surgery and filled out another form. Results were submitted to statistical analysis by Student's t-test.

Veterinarians' evaluations pointed out that, as expected, scalpel blade was more fluent in skin incision than plasma device thanks to its lower resistance (p=0.012) and caused less tissue damage (p=0.002). Onemytis® encountered less resistance in ovarian excision than electrosurgical device (p=0.001) and, even at maximum power, produced less damage in the surrounding tissues (p=0.025). The evaluation of all other parameters (haemorrhage and homogeneity of cutting edges) did not stress any difference in the employment of Onemytis® and cold blade in skin incision or of plasma and electrosurgical devices in ovarian excision (p>0.050). Both L and A, despite their different role during surgery, expressed similar assessments on the use of the tested devices (p>0.050). During surgery, electrocardiographic signal was lost while activating both Onemytis® and AM 308-N and reappeared when their power was turned off. Since cutting with plasma device is faster (due to the less cutting resistance), ECG absence was shorter, allowing a more precise monitoring of the patient. Monitoring of the surgical wound (on T1 and T8) did not highlight any significant difference in the healing process of wounds performed through plasma or scalpel blade. Only tissue warmth was higher on T8 in wounds provoked by Onemytis® device (p=0.010). Results highlight the effectiveness and safety of Onemytis® system. Compared to electrosurgical units, it is faster, more fluent and causes less tissue damage. During surgeries it allows a clean incision, with good bleeding control, and does not alter healing times, even if employed by a surgeon inexperienced in its functioning.

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IMPACT OF THE SEVERITY OF LABOR AND BIRTHPLACE ON HORMONAL CHANGES IN THE SHEEP

Mario Cinone, Antonio Ciro Guaricci, Maria Albrizio

Università degli Studi di Bari "Aldo Moro", Department of Emergency and Organs Transplantation (DETO).
Corresponding author: M. Cinone (mario.cinone@uniba.it)

The onset and evolution of parturition are affected by environmental stressors, number of lambs, and dystocia. The regulation of parturition follows a chronological sequence, whose complete understanding is still rather complex. Progesterone (P_4) is essential for ovine pregnancy maintenance. The placenta is the major source of P_4, but also the fetus plays a critical role in the endocrine mechanisms that regulate the parturition timing. In the last 15 days before term, fetal adrenal gland increases cortisol (F) production which has two remarkable functions: firstly, it stimulates fetal lungs functional maturation and secondly in the placenta it enhances the expression of the steroidogenic enzyme P450c17, thus allowing P_4 conversion to estrogens [1]. The correlation between maternal plasma F levels and the duration of labor as well as the correlation between pain intensity and plasma Beta-endorphin (B-end) concentration have been studied [2]. Still, there are no reports of any correlation between these hormones, the severity of parturition pain and the environment. The aim of this study was therefore to determine whether, in sheep plasma concentrations of F, adrenaline (A), noradrenaline (NA), B-end, estradiol (E_2) and P_4 could be correlated to labor phase and intensity, and to the birthplace (sheepfold versus pasture). Sheep were well suited for handling. Animal care and use, as well as the experimental design of the study, were approved by the local animal ethics committee of the Department (DETO) (protocol n°1091/III/13 del 18/09/2017). The reproductive activity and pregnancy of 20 Comisana sheep (4-8 years old, multiparous) reared with a semi-wild system were monitored. Parturitions were classified in: eutocic, eutocic twin birth, dystocic and dystocic twin birth. Blood samples were taken from day 144 until labor and then during each stage of labor at the following time points: T0 (24 hrs before labor), T1 (prodromic stage), T2 (dilated stage), T3 (expulsive stage) T4 (stage of the fetal membrane expulsion), T5 (24 hrs after labor). Haematic concentrations of F, A, NA, B-end, E_2 and P_4 were evaluated by ELISA kits. Recorded data were then analyzed by SPSS software and statistical significance was set to $P<0.05$. We found that 1) the kind of labor and the place in which it occurred affected significantly F, A, NA, B-end, E_2 concentrations; 2) from T2 until T4 there was a significant increase of A and NA concentrations; 3) at T3 a significant increase of F concentration appeared; 4) B-end was the only compound whose concentration increased from T0 to T5; 5) dystocia induced significantly higher concentrations of all hormones related to stress. The present study shows that during labor a positive correlation between hormonal changes and phases/encountered difficulties exists (labor/F at T3 R=0.628; P=0.003 at T4 R=0.631; P=0.003 at T5 R=0.557; P=0.011: labor/A at T2 R=0.931; P=0.000; at T3 R=0.957; P=0.000: labor/NA at T1 R=0.470; P=0.037 at T2 R=0.982; P=0.000 at T3 R=0.956; P=0.000: labor/B-end at T0 R=0.788 P=0.000; at T1 R=0.562; P=0.10 at T2 R=0.885; P=0.000 at T3 R=0.929; P=0.000 at T4 R=0.720; P=0.000). Moreover, hormonal peaks were reached around the time of expulsion, suggesting that they could have been associated with muscle work, stress and pain.

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EXPRESSION OF CYP27A1, CYP2R1 AND VDR IN EQUINE CRYPTORCHID TESTIS

Maria Albrizio, Salvatore Desantis, Antonio Ciro Guaricci, Luca Lacitignola, Mario Cinone

Università degli Studi di Bari – Aldo Moro, Dipartimento dell'Emergenza e dei Trapianti di Organi (DETO).
Corresponding author: M. Albrizio (maria.albrizio@uniba.it)

A functional vitamin D (VitD) requires a double step bioactivation by six cytochrome P450 (CYP) isoforms. The first step happens in the liver by four D-25-hydroxylase enzymes, among them the most active are CY27A1 and CYP2R1; the activation pathway ends in the kidney by a 1-α-hydroxylase [1]. The biological activity of VitD requires binding to the cytosolic VitD receptor (VDR), which translocate to the nucleus and act as a factor regulating the transcription of more than 200 genes modulating normal and cancer cell growth, differentiation, apoptosis, angiogenesis and metastatic potential [2]. VitD deficiency has been suggested as a risk factor for cancer because it impairs the anti-proliferative properties of vitamin D receptor (VDR) [3], and it adds to cryptorchidism as a cause of testicular cancer [4]. In the horse cryptorchidism is one of the male developmental defect that affects more than 9% of the subjects. Male reproductive tract expresses VDR and the enzymes involved in vitamin D activation through 25-hydroxilation [5]. Therefore, this study examined whether equine testis expresses CYP27A1 and/or CYP2R1 and VDR proteins and whether cryptorchidism may impair their expression thus enhancing the risk of developing testicular cancer. By western blot and immunohistochemistry, CYP27A1, CYP2R1 and VDR proteins were quantified and localized. Results demonstrated that all the three proteins were expressed in equine testis, moreover the expression level of CYP27A1 and VDR were significantly lower ($P<0.01$ and $P<0.05$ respectively) in the retained testis in respect to the contralateral scrotal testis. CYP2R1 protein resulted expressed at the same level both in the undescended and in the scrotal testis. This study showed that also in the horse testes play a role in the vitamin D metabolism.

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DISORDER OF SEXUAL DEVELOPMENT IN A FRIESIAN HORSE

Brunella Anna Giangaspero (1), Paola Straticò (1), Gianluca Celani (1), Giuseppe Marruchella (1), Carlo Tardella (2), Salvatore Parrillo (1), Lucio Petrizzi (1), Augusto Carluccio (1)

(1) Università degli Studi di Teramo, Facoltà di Medicina Veterinaria. (2) Libero Professionista, Narni.
Corresponding author: G. Celani (gcelani@unite.it)

In horses, several presentations of abnormal external genitalia have been described. Disorders of Sex development are associated with an atypical sexual phenotype or cases of intersexuality [1]. Individuals showing atypical sex conditions have been classified according to the gonadal tissue as true hermaphrodites, male pseudohermaphrodites, female pseudohermaphrodites [2]. Genetically they fall into 4 broad categories: XX karyotype, SRY -negative genotype with ovotestis or testis; XY, SRY-negative with gonadal dysgenesis; and XY, SRY -positive with testis or gonadal dysgenesis [2]. The present abstract describes the clinical case of a 3-years-old Friesian horse referred to male-like behavior (nervous temperament, masculine attitude) and ambiguous external genitalia (female phenotype, but anatomical abnormalities of external genitalia). Clinical examination of the external genitalia showed a penis-like structure protruding out of the vulvar region for 8 cm with a cranio-caudad direction. At the inguinal level, mammary development was compatible with the reproductive status of a nulliparous mare, and showed in the intermammary sulcus, a skin lump similar to the penis sheath. Any parenchymatous structures were found with palpation of the external inguinal. Transrettal palpation and ultrasound examination identified aplasia of the body of the uterus and of ovaries. Moreover, the ultrasonographic exam showed segmental hypoplasia of the uterine horns. Laparoscopy through a bilateral flank approach allowed the localization of both gonads and their adnexa into the internal inguinal ring, their dissection, and removal [3]. At the pelvic inlet, a sacculated structure compatible with an aplastic uterus was appreciated. A urethrocistoscopy was performed with a 0.9 mm flexible endoscope. On the floor of the pelvis a masculine urethral opening leads to the urinary bladder through a short urethra. Dorsal to this finding, two sacculated structures were present, a smaller one on the left hand side of the pelvis, a large one on the midline. This structures are similar to the hypoplastic uterine horns.

The endocrine function of the gonads was evaluated using the hCG-stimulation test [4]. Blood testosterone concentrations and histological examination of the tissue removed will confirm the type of tissue. Moreover, the cytogenetic and molecolar analysis will show the karyotype and would allow a genetic classification.

Disorder of sex development in horses has both genetic and phenotypic heterogeneity as occurs in other species [2]. The degree of genetic modification, its site, and the time of onset are resposible of the high phenotipical variability of the intersex [3].

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A RETROSPECTIVE EVALUATION OF RESORPTION RATES AT CANINE PREGNANCY DIAGNOSES

Petra Lascialfari, Matteo Tesi, Cristiana Manetti, Alessandra Rota

Università degli Studi di Pisa, Dipartimento di Scienze Veterinarie.
Corresponding author: P. Lascialfari (petra.lascialfari@phd.unipi.it)

Today most breeders request ultrasonographic pregnancy diagnoses to better manage the bitch during gestation and parturition. These are generally performed around 25 days post-ovulation and at this stage embryo resorption may be observed. According to the veterinary literature, resorptions in canine species affect around 11-13% of conceptuses and occur in 5-15% of pregnancies [1-3]. It was hypothized that resorptions are a physiological event in pregnancies with large litters, due to overcrowding of conceptuses in uterine horns [4, 5]. The aims of this study were to evaluate retrospectively the incidence of embryo resorptions in pregnancies of bitches of different breeds, and to describe the relation with the number of conceptuses present and age and size of the dam. Between January 2020 and March 2022, 87 pregnancy diagnoses were performed by ultrasound examination on 71 different bitches belonging to 19 breeds and aged 13 to 99 months. Bitches belonged to different sizes: Large (L, >35 kg, n=11), Medium (M, 10-34.9 kg, n=19), Small (S, 5-9.9 kg, n=36), Toy (XS, <5 kg, n=21). In the present study, resorption sites were characterized by different ultrasonographic patterns, ranging from a localized horn enlargement with hypoechoic centre and absence or small amount of liquids to a vesicle containing a dead embryo [2-4]. The diagnoses were performed at 21 to 30 days post-ovulation with a Toshiba Aclio 400 ultrasound equipped with linear (5-13 MHz) and microconvex (6.6-8 MHz) probes. Overall pregnancy rate was 90.8% (79/87, negative diagnoses were 1, 2, 4 and 1 in L, M, S and XS bitches, respectively). In 40.5% of pregnancies (32/79) at least one resorption site was visible. The incidence was 50% (5/10), 47.1% (8/17), 37.5% (12/32) and 35% (7/20) in L, M, S and XS bitches, respectively (Chi Square test, $P>0.05$). In 6 of these bitches, no viable embryos were present. Of the 404 structures, 356 were normal conceptuses with a healthy embryo, while 48 (11.9%) were resorptions at different stages of involution. Mean number of structures in the bitches with normal pregnancies or pregnancies with resorptions were 3.0±1.0 and 2.1±0.9 in XS bitches, 5.2±1.8 and 6.1±1.7 in S bitches, 7.1±1.5 and 5.7±1.9 in M bitches, 7.8±2.6 and 4.8±3.6 in L bitches, respectively. Five bitches showed resorptions in both pregnancies evaluated in the study period. Binary logistic regression showed a significant effect of age ($P<0.001$), but not of total number of conceptuses or size of the dam on the presence of resorptions in pregnancy. Age was higher in pregnancies with resorptions than in normal ones (60±20 vs 44±17 months). Even though the results obtained were statistically significant, the regression model could explain only 14% of the variability of our data, showing a low predictivity (R^2 =13.94). The resorption rates in pregnancies and conceptuses observed confirm what previously described, while the incidence among pregnancies was higher; this may be affected by the technical improvement in the ultrasound equipment used in the present study compared to those of last century [1-3]. Although resorptions may physiologically occur in pregnancies with large litters, a relationship between embryo resorptions and litter size could not be identified in our experimental group, while aging increased the resorption rate; this, together with the occurrence of repeated embryonic resorptions in some bitches included in the study, suggest how resorptions may be pathological events. The underlaying mechanisms need to be better identified.

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CLINICAL USE OF CANINE FETAL KIDNEY FORMULA IN DIFFERENT MATERNAL SIZES DURING THE LAST TEN DAYS OF PREGNANCY

Giulia Siena, Francesca di Nardo, Stefano Romagnoli, Barbara Contiero, Magdalena Schrank, Antonio Mollo, Chiara Milani

Department of Animal Medicine, Production and Health, University of Padova, Italy.
Corresponding author: C. Milani (chiara.milani@unipd.it)

Ultrasonographic measurement of canine fetal kidney length is related to gestational age [1, 2]. A specific formula to predict the parturition day was calculated showing differences in its accuracy during pregnancy [1]. Data concerning the use of this formula during the last 10 days of pregnancy are still lacking. The aim of our study was to evaluate the accuracy of the fetal kidney length formula [1] during two-time ranges of days before parturition (dbp): -11/-5 dpb (time I) and -4/0 dpb (time II).

Twenty-five clinically healthy pregnant bitches (Project nr. 69/2018) of 16 different breeds, of 2-9-year-old and 3.5-56.8 kg bodyweight were monitored at least once during the time interval -11/0 dbp, using an 8-5 MHz convex transducer connected to an ultrasound (US) unit (Philips Affiniti 50G, Italy). US was performed in dorsal or lateral recumbency. Two renal sonograms were collected in a longitudinal scan on one kidney for each of the three most caudal fetuses, in both uterine horns. Fetal kidney length (L) was measured and the results of the prediction of parturition day were calculated by using the specific kidney formula [1]. Dbp were counted backward considering day 0 as the day of parturition. The accuracy in the prediction of parturition day was calculated [3]. A statistical analysis was performed using a k-proportion parametric test to identify differences in the accuracy between different maternal sizes (small ≤10 kg, medium 11-25 kg and large ≥26 kg) and sex ratio of pups (percentage of females ≤40%, 41-60%, >60%). The parametric two-proportion z-test was used to identify the differences between litter size classes (≤7 vs >7 pups) and time ranges. Significance was set as $P<0.05$.

A total of 100 measurements of L were obtained from 50 examined fetuses. In two bitches, only two fetuses were examined. Twenty-two bitches were monitored during time I and II. The accuracy of the formula was 28% within ±1 day and 32% ±2 days in the range -11/0 dbp, 31% ±1 day and 35% ±2 days in time I, and 26% ±1 day and 30% ±2 days in time II. A significant difference in the accuracy was not evident between time I and time II ($P=0.931$ within ±1 day and $P=0.925$ within ±2 days). The accuracy was 10% within ±1 and ±2 days in large, 28% ±1 day and 33% ±2 days in medium and 53% ±1 day and 60% ±2 days in small size bitches. A statistically significant difference between small and large size bitches was evident ($P=0.019$, within ±1 day, and $P=0.007$, within ±2 days). The accuracy was 38% ±1 day and 44% ±2 days in small, and 14% ±1 day and 14% ±2 days in large litter size. A threshold value ($P=0.051$) was found between the two litter size classes within ±2 days. The accuracy was not affected by sex ratio of pups.

The results obtained in time II were similar to what reported in previous literature during the same gestational period [1]. In this study, the kidney length formula used during the last ten days of pregnancy may not warrant a good accuracy for the prediction of parturition day. Further studies with a larger sample size, are needed to develop maternal size specific formulas using fetal kidney length, as well as to assess the influence of litter size on the accuracy of the fetal kidney lenght formula.

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EXTRACELLULAR VESICLES BY SEMINAL PLASMA OF BULL OF PROVEN FERTILITY TO IMPROVE FERTILIZING CAPACITY OF LOW-FERTILITY BULLS

Anna Lange-Consiglio (1), Simone Canesi (1), Noemi Monferini (1), Emanuele Capra (2), Marina Cretich (3), Roberto Frigerio (3), Bianca Gasparrini (4), Fausto Cremonesi (1).

(1) Università degli Studi di Milano, Dipartimento di Medicina Veterinaria e Scienze Animali (DIVAS). (2) Istituto di Biologia e Biotecnologia Agraria, Consiglio Nazionale delle Ricerche IBBA CNR, Lodi, Italy. (3) Istituto di Scienze e Tecnologie Chimiche "Giulio Natta", Consiglio Nazionale delle Ricerche SCITEC-CNR, Milan, Italy. (4) Università degli Studi di Napoli Federico II, Dipartimento di Medicina Veterinaria e Produzioni Animali (DMVPA).

Corresponding author: A. Lange-Consiglio (anna.langeconsiglio@unimi.it)

Seminal plasma contains extracellular vesicles (EVs) that vehicle RNA, proteins, and other molecules able to influence the biological function of sperm. Some studies support the role of EVs present in seminal plasma in assisting sperm to reach functional maturity and influence the physiology of female reproductive tract cells to favor reproductive success (1,2). Extracellular vesicles are fundamental regulators of reproductive success, then, it is conceivable that cargo of EVs of seminal plasma from bull of proven fertility could improve the semen quality of low-fertility bulls.

To test this hypothesis, we firstly isolated EVs from seminal plasma of bull of proven fertility and evaluated the EVs incorporation in sperm of low-fertility bulls at different times and after cryopreservation. Then, the effectiveness of EVs in improving the semen quality of low fertility bulls was evaluated after IVF, comparing the *in vitro* outcome of low-fertility semen before and after treatment with EVs.

For the first time, EVs were isolated from the seminal plasma of a bull of proven fertility by ultracentrifugation at 100,000xg for 1h at +4°C and characterized by Nanosight Instruments for size and concentration. The EV concentration was $7.34x10^{11}\pm3.51x10^{10}$ EVs/mL and the EV size was 173.8±59.9 nm. The size data is comparable to that of Ding et al. (3) that isolated EVs from boar semen.

Three low-fertility bulls were used in this study. Suspensions of $5x10^6$ sperm/ml in SPERM-TALP were co-incubated with EVs labelled with PKH-26 for a dose-response curve that was performed to evaluate the incorporation of EVs into spermatozoa by confocal microscope.

Different doses of EVs/ml (50, 100, 150, 200, 300 and $400x10^6$) were tested for different time (1, 2, 3, 4, 5, 6 hours) and the fluorescent signal was detected after only an hour at a concentration of $400x10^6$ EVs/ml in the middle tract with a stay of another 6 hours.

In addition, it has been verified that the incorporation of EVs was maintained even after cryopreservation: spermatozoa with incorporated EVs were diluted with BioXcell freezing medium and cryopreserved for a week. After thawing, EVs were visible in the middle tract.

At last, the spermatozoa of low-fertility bulls, with EVs incorporated, were used for the *in vitro* embryo production. The blastocyst rate *in vitro*, with the use of sperm with EVs incorporated, increased by about twice the embryonic yield obtained with the same sperm in the absence of EVs: bulls having an average embryonic rate of 6.41±1.48%, 10.32±4.34% and 10.92±0.95% improved their yield to 21.21±1.99%, 22.17±6.09% and 19.99±5.78%, respectively ($P<0.05$).

These encouraging results suggest that it might be possible to keep breeding bulls with poor fertility because the incorporation of the EVs in a single hour would allow the cryopreservation of the semen in straws to be used for *in vitro*/*in vivo* insemination.

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SOFIVET

PERINATAL PERIOD, IMMUNITY AND ALLOSTATIC LOAD: WHAT'S THE KEY?

Alessio Cotticelli (1, 2), Francesca Quai (2), Isabella Pividori (2), Antonella Comin (2), Alberto Prandi (2), Tanja Peric (2)

(1) Università degli Studi di Napoli Federico II, Dipartimento di Medicina Veterinaria e Produzioni Animali (2) Università degli Studi di Udine, Dipartimento di Scienze Agroalimentari, Ambientali e Animali.

Corresponding author: I. Pividori (isabella.pividori@uniud.it)

High levels of prenatal stress can have negative consequences for maternal, fetal, and infant health. Cortisol is essential to trigger the process of parturition and to drive the transition to the extrauterine life which consists in radical physiological changes [1]. Aim of the present study was to investigate in calves the interrelationship between the transfer of passive immunity from dam to calf, the allostatic load and zootechnical performances from birth up to pre-weaning period. The study had institutional approval from the Ethical Animal Care and Use Committee of the University of Naples Federico II (code PG/2021/0130478). Colostrum was collected from 12 donor cows at the first milking after calving and given to 12 recipient calves, all of which have been subjected to blood sampling within 3 days after birth. The allostatic load was measured assaying cortisol in hair. The hair of calves was sampled at 15 days of life (T1) and 30 days after (T2). Colostral and plasma IgG concentrations were determined using the bovine IgG ELISA quantitation set of Bethyl Laboratories Inc. (Montgomery, TX). The apparent efficiency of absorption (AEA) was calculated using the equation reported by [2]. Hair cortisol concentrations (HC) were determined as described by [3]. IgG concentrations ranged between 29.30 and 178.90 (mg/ml) in colostrum and between 5.48 and 83.85 (mg/ml) in plasma. AEA showed a minimum value of 5.13 and a maximum of 62.85%. Calves' HC concentrations highlighted significant differences between T1 and T2 (14.49 vs 9.21 pg/mg, respectively, $P<0.01$). In the present study T1 reflected cortisol concentrations of the last third of uterine life and birth, whereas T2 (re-growth hair) recorded the post-natal period. The higher HC found at T1 seem to be linked to the birth process and the immediate perinatal period that brings considerable stress for the newborn calf. Kovács et al. (2021) found a peak of plasma cortisol within 3 hours from delivery. In our study, at T2, HC still does not drop to baseline levels. This could be due to several stressors that occur during the first weeks of life. AEA was positively correlated ($p<0.05$) to daily weight gain (1–75 days of life) and negatively to HC at T2 ($p<0.05$). The absorption of an adequate amount of IgG along with a reduction of the allostatic load seems important for the growth of the calf and an enhance of their performances. Further studies are needed but a proper colostrum management should be considered not only for the welfare of the animals but also for the profitability of the farm.

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PROTECTIVE EFFECT OF BERGAMOT POLYPHENOL-RICH FRACTION ON PORCINE AORTIC ENDOTHELIAL CELL ENERGETIC METABOLISM

Chiara Bernardini (1), Debora La Mantia (1), Cristina Algieri (1), Francesca Oppedisano (2), Ernesto Parma (2), Vincenzo Mollace (2), Giovanni Romeo (3), Salvatore Nesci (1), Monica Forni (1)

(1) Università di Bologna, Dipartimento di Scienze Mediche Vaterinarie. (2) Università della "Magna Graecia" di Catanzaro, Dipartimento di Scienze della Salute. (3) Ospedale Sant'Orsola Malpighi Bologna, Unità di Genetica Medica. Corresponding author: C. Bernardini (chiara.bernardini5@unibo.it)

Porcine Aortic Endothelial Cells (pAECs) provide a useful translational model to study many aspects of cardiovascular function and disease (1, 2). Bergamot (*Citrus bergamia* Risso et Poiteau), a plant endemic to the Calabrian Ionian coast, already utilized in the cosmetic and confectionery industry, is now employed in nutraceutical supplementation thanks to the high concentration of polyphenols that could have beneficial effects on bioenergetic cellular metabolism (3). The aim of the present study was to determine the effect of Bergamot polyphenol-rich fraction (BPF) on pAECs in physiological condition and during toxic stress induced by Doxorubicin (Doxo), a common anthracycline chemotherapeutic drug with a well-known endothelial vascular toxicity (4). (BPF) was obtained from the juice and albedo of bergamot as described previously (5). To investigate the effect of BPF on cell viability pAECs were seeded in 96-well plates ($1x10^4$ cells/well) and the day after were exposed to increasing doses of BPF (1, 5, 10, 25, 50, 100, 150, 200, 250, 300 µg/ml) for 5 h. To evaluate the protective effect of BPF on toxic effect of Doxo treatment, pAECs were pre-treated with BPF at 100 or 200 µg/ml for 5h, then 0.5, 1, 10 µM Doxo were added and incubated for additional 18 hours. The viability was determined using the MTT assay. The energetic cell metabolism was investigated by Seahorse XFp analyzer (Agilent) to simultaneously measure oxygen consumption rate (OCR), an index of cell respiration (pmol/min), and extracellular acidification rate (ECAR), an index of glycolysis (mpH/min). MTT results showed a significant increase of % cell viability was evident at BPF higher doses studied (200, 250 and 300 µg/ml) after 5 h incubation. Furthermore, Doxo exerted an evident cytotoxic effect, producing a significant reduction of pAECs viability in a dose-dependent manner, while BPF significantly restored endothelial viability. The acute effect of BPF on pAECs metabolism showed an ATP rate index greater than 1, which points out a prevailing mitochondrial oxidative metabolism with respect to the glycolytic pathway and this ratio raised to about three times with 100 µg/ml BPF. Consistently, the mitochondrial ATP turnover and the basal and maximal respiration were higher in presence of BPF than in controls. The basal respiration, maximal respiration and the ATP turnover of pAECs were reduced in presence of 1 µM Doxo. pAECs treated with 200 µg/ml BPF can significantly overcome the inhibitor effect of Doxo on bioenergetic parameters of cells. To sum up, BPF might have a beneficial effect on the vascular endothelium by improving the mitochondrial function and avoiding the cardiotoxic action of Doxo.

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STRESS AND INFLAMMATORY RESPONSE OF COWS AND THEIR CALVES DURING PERIPARTUM AND EARLY NEONATAL PERIOD

Francesca Arfuso (1), Vincenzo Lopreiato (1), Andrea Minuti (2), Luigi Liotta (2), Claudia Giannetto (1), Erminio Trevisi (2), Giuseppe Piccione (1)

(1) Università degli studi di Messina, Dipartimento di Scienze Veterinarie. (2) Università Cattolica del Sacro Cuore, Dipartimento di Scienze Animali, Alimentazione e Nutrizione (DiANA).

Corresponding author: F. Arfuso (farfuso@unime.it)

The ability of an animal to produce an appropriate response to a stimulus that trigger a threat to homestosas is essential to survival. Homeostasis is the orchestrated or coordinated control in metabolism of body tissues necessary to support a particular physiological state [1]. On this regard, the activation of Hypothalamic-Pituitary-Adrenal axis (HPA) is one of the most important neuroendocrine responses that occur following a stress condition and it helps the organism to re-establish homeostasis. HPA activity is coordinated by positive or negative feedback loops that increase or decrease, respectively, the production of crucial hormones, including cortisol [2-4]. HPA axis and immune system communicate each other working in concert to maintain animal's homeostasis [3-6]. The framework of this relationship is complex and changes according to physiological condition. On this regard, pregnancy and lactation for the dam, as well as neonatal period for the offspring, are life phases characterized by a combination of multiple stressors which increase susceptibility to diseases and/or negatively impact the animal ability to overcome illness and recover [5]. Peripartum and early life are challenging periods of cattle encompassed by multiple stressors, leading to immune-related disorders. Stress, inflammatory response, and their relationship were investigated in eight Simmental peripartum cows and their calves. From cows, blood was collected at -21 (±4), 0, +1, +7, and +21 days from calving. From calves, blood was collected after birth before colostrum intake (0), and then at 1, 7, and 15 days of age. Cortisol, Interleukin 6 (IL-6), haptoglobin, and white blood cells (WBC) were assessed. Except for lymphocytes count, the effect of time relative to calving was observed for all the investigated parameters ($P<0.001$) in peripartal cows. At calving and 1 d after, cortisol concentration was negatively correlated with levels of IL-6, WBC, and monocytes, whereas levels of IL-6 were positively correlated with WBC, neutrophils, and monocytes count. The age of neonatal calves influenced cortisol, IL-6, haptoglobin, WBC and all leukocyte populations ($P<0.001$) with the exception of neutrophils. A negative correlation between cortisol and IL-6, neutrophils, monocytes and haptoglobin was found at 15 days old. IL-6 resulted positively correlated with haptoglobin at 15 days old and with neutrophils and monocytes at 7 and 15 days old. These data suggest that cortisol plays a crucial role in the immune-modulatory adjustment during the transition period in cows. Furthermore, the cross-talking among HPA axis, innate and adaptive immunity, and liver metabolism of newborn calves seems to be not fully developed in the first 2 weeks of age.

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STUDY ON THE GUT MICROBIOTA MODIFICATION UPON A HIGH-SALT DIET IN SPONTANEOUSLY HYPERTENSIVE RATS

Silvia Bencivenni (1, 3), Patrizia Brigidi (2), Augusta Zannoni (1), Silvia Turroni (3), Federica D'Amico (2, 3), Maria Cotugno (4), Rosita Stanzione (4), Speranza Rubattu (5), Monica Forni (1)

(1) Università di Bologna, Dipartimento di Scienze Mediche Veterinarie. (2) Università di Bologna, Dipartimento di Scienze Mediche e Chirurgiche. (3) Università di Bologna, Dipartimento di Farmacia e Biotecnologie. (4) IRCCS Neuromed, Pozzilli (Is). (5) Sapienza Università di Roma, Scuola di Medicina e Psicologia, Dipartimento di Medicina Clinica e Molecolare.

Corresponding author: S. Bencivenni (silvia.bencivenni2@unibo.it)

Stroke is a leading cause of mortality and disability with a multifactorial pathogenesis including hypertension. Gastrointestinal complications are a common problem following the stroke and the gut has recently gained attention in stroke research [1]. The gut microbiota (GM) is the ensemble of microorganisms that live in the gut, and it is capable of influencing the host health and the functionality of physiological processes [2]. Several studies have focused on the relationship between the GM and acute ischemic stroke, confirming the existence of a bidirectional microbiota–gut–brain axis [1]. The Spontaneously Hypertensive Stroke Prone Rat (SHRSP) represents a suitable model for the study of hypertension-related stroke [3]. SHRSPs spontaneously develop cerebral ischemic episodes within a year of life when fed with a regular diet (RD). Stroke onset is accelerated upon a high-salt Japanese style diet, JD (with an incidence of 100% after 7 weeks of JD) [4, 5]. The aim of this research was to evaluate the effect of JD and RD on the GM composition in SHRPs and in Spontaneously Hypertensive Rats (SHR, as control group). Moreover, the calprotectin level was evaluated, being a marker of gut inflammation and recently used as a marker in the stroke model [6, 7]. A group of 6-week-old SHRSPs (n=8) and a control group of SHRs (n=10) were studied. Each group was further divided into two subgroups. One subgroup received the JD for 4 weeks, whereas the second received RD (Authorization to animal testing n. 1086/2020). Pools of feces were collected from each subgroup from the beginning of the diet (T0) to the end (T28). At T28 the animals were sacrificed and individual blood serum and small and large intestinal content were collected. In order to analyze the GM composition, DNA was extracted from feces (QIAamp DNA stool Mini Kit, QIAGEN) and the V3-V4 regions of the 16S rRNA gene were sequenced through MiSeq Illumina platform [8]. Bioinformatic analysis was performed with R software. Serum and fecal calprotectin level was determined by ELISA kit (Rat Calprotectin CALP, Cusabio). GM variability was represented through α diversity metrics and our results showed an increase of diversity at T28 compared to T0 in JD-fed rats, suggesting that shifting diet had an effect on the GM. The Weighted UniFrac, a β diversity metric, indicated that at T28 the GM profile of JD-fed rats differed from that of RD-fed rats. No statistically significant differences were observed in serum or intestinal content calprotectin level. Further studies on SHRSPs closer to the stroke onset are needed to allow to evaluate the GM dynamics and a potential worsening of intestinal inflammation shortly before the stroke.

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SIMULTANEOUS ESTABLISHMENT OF ENDOTHELIAL, VESSEL WALL MESENCHYMAL AND MAMMARY EPITHELIAL CELL LINES FROM THE SAME GOTTINGEN MINIPIG. REDUCTION & REPLACEMENT FOR TRANSLATIONAL PURPOSES: A CONTRIBUTION FROM THE IMI-CONCEPTION CONSORTIUM

Chiara Bernardini, Domenico Ventrella, Debora La Mantia, Alberto Elmi, Augusta Zannoni, Roberta Salaroli, Camilla Aniballi, Silvia Bencivenni, Maria Laura Bacci, Monica Forni

Università degli Studi di Bologna, Dipartimento di Scienze Mediche Veterinarie.
Corresponding author: C. Bernardini (chiara.bernardini5@unibo.it)

It is now well known that biological models with multiple levels of complexity are necessary for the true translation of biomedical research from basic science to clinical relevance. The last 20 years have seen a transition from an over-reliance on rodent models towards alternative animal models, including the pig species (1). Thanks to its high anatomical, physiological and metabolic similarity to human, the swine has shown to be useful and reliable when used for translational purposes, by bridging the gap between simpler rodent models and humans (2). Standardization of animal models is a prerequisite to achieve efficient Reduction and breed-specific Gottingen minipig assures genetic standards and a high level of microbiological control. In full compliance of both international legislations and the 3Rs principle context to limit the animal for experimentation [3] *in vitro* cell cultures could represent a powerful tool for pre-screening study, also considering the possibility to develop complex 3D models by culturing different cell types that can more faithfully recreate the *in vivo* tissue structure. Moreover, primary cell cultures compared to immortalized cell lines, shows the great advantage of mainly maintaining the *in vivo* functional phenotype and genetic profile. The aim of the present study was to apply some of our previously developed protocols set on commercial swine to Gottingen minipig (mp) with the aim to obtain primary cell cultures of Mammary Epithelial Cells (mpMECs) for the IMI-ConcePTION Project and in the same time primary cell cultures of Endothelial Cells and Mesenchymal Stem Cells from thoracic aorta (mpAECs and mpVW-MSCs respectively). Briefly, mammary lines and thoracic aortic traits were surgically removed and collected from 3 euthanized Gottingen sows (ID1181 Cod 2216A.N.TJ), to generate three replicates primary cell lines for each cell type. For mpMECs isolation, the previously developed protocol (4) was optimized for tissue amount and culture time. For mpAECs and mpVW-MSCs isolation two enzymatic digestion-based protocols (5, 6) were merged mainly to optimize the incubation times for simultaneous isolation of both cell types. The resulting cell lines were tested by immunofluorescence for typical cellular protein markers. mpMECs formed a typical compact monolayer expressing epithelial Cadherin and CK18. mpAECs grew forming a typical cobblestone monolayer and expressed CD31 and vascular endothelial Cadherin. pVW-MSCs showed an elongated, spindle shape, fibroblast-like morphology, and stained positively for, CD105, CD90, CD44. The results obtained demonstrate the possibility to obtain multiple primary cell cultures simultaneously opening up the prospect of developing multicellular 3D models in-subject capable of improving Replacement and Reduction.

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CALCIUM RESPONSES IN SHEEP OOCYTES FERTILIZED BY IVF OR ICSI

Luisa Gioia (1), Luca Palazzese (2), Marta Czernik (2), Pasqualino Loi (2)

(1) Università degli Studi di Teramo, Facoltà di Bioscienze e Tecnologie Agro-Alimentari e Ambientali. (2) Università degli Studi di Teramo, Facoltà di Medicina Veterinaria.

Corresponding author: L. Gioia (lgioia@unite.it)

In mammalian oocytes, at fertilization a rise in the concentration of intracellular free calcium ions (Ca^{2+}) occurs. In particular, repetitive Ca^{2+} transients initiate shortly after sperm-oocyte fusion and stop around the time of the first mitotic cell cycle. Although the mechanism by which the sperm initiates this calcium response has not been fully clarified, it is widely accepted that this series of Ca^{2+} oscillations is sustained by the oocyte's calcium store sensitized by the soluble PLCζ, released from the fertilizing sperm [1], and is necessary for supporting the events of oocyte activation. ICSI, which bypasses fusion of the gametes, has been widely used in several species, with variable efficiency [2]. A great progress has been achieved in humans, making of ICSI the golden standard for embryo production. In this species, it has been also demonstrated that after the injection of spermatozoon, Ca^{2+} oscillations are evoked with a pattern similar to that of IVF embryos that is able to activate the oocytes. In contrast, despite the efforts of several working groups around the world, the success of this technique has been limited in farm animals, especially in ruminants [3]. Particularly in sheep, ICSI outcomes are very limited and despite the resort to chemical activation the development to blastocyst as well as the number of newborns remain critically low compared to IVF [4]. Our previous reports revealed a discrepancy between the activation rate in sheep ICSI, assessed by pronuclear formation (80%), and the first cleavage (35%). Hoechst staining revealed two pronuclei in presumptive zygotes, indicating in the absence of pronuclear apposition and fusion the limiting factor [5]. Given that pronuclear migration and fusion are calcium-dependent processes, we hypothesized that current activation protocol affects the first calcium spikes, but does not induce the later, crucial ones. To date, there are no reports that compare oscillations patterns of ICSI vs IVF in sheep oocytes; therefore, the aim of this study is to analyse the response of Ca^{2+} in IVM oocytes fertilized by ICSI or IVF and microinjected with the Ca-sensitive dye Fluo-4. We have optimized the conditions to analyse the fertilized oocytes over the time to cover the time of pronuclei formation until the first cleavage by using time lapse microscopy and maintaining the oocyte in IVC medium at 38.5°C in 5% CO_2. We demonstrated for the first time that in sheep oocytes fertilized by ICSI Ca^{2+} response is different from that observed after IVF. In fact, the majority of oocytes are unable to produce Ca^{2+} oscillations and, although in some oocytes the initiation of Ca^{2+} response takes place like in IVF oocytes, altogether a defective Ca^{2+} pattern is observed. Once identified the specific defective Ca^{2+} signal occurring after ICSI procedure, it could be interesting to develop ICSI/activation protocols in which the missing Ca^{2+} rises are artificially provided for as long as necessary, in order to better mimicking the temporal Ca^{2+} pattern occurring after IVF and improve the impact on embryo development.

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COMPARATIVE METABOLIC ENERGY PROFILE OF PRIMARY CULTURES OF MAMMARY EPITHELIAL CELLS FROM PIG/MINIPIG ANIMAL MODEL AND HUMAN – A CONTRIBUTION OF THE IMI CONCEPTION CONSORTIUM

Debora La Mantia (1), Chiara Bernardini (1), Salvatore Nesci (1), Cristina Algieri (1), Nina Nauwelaerts (2), Pieter Annaert (2), Maria Laura Bacci (1), Monica Forni (1)

(1) Università di Bologna, Dipartimento di Scienze Mediche Veterinarie. (2) KU Leuven, Department of Pharmaceutical and Pharmacological Sciences.

Corresponding author: D. La Mantia (debora.lamantia2@unibo.it)

The European ConcePTION consortium will develop a non-clinical platform, through the application of in vitro, in vivo and in silico approaches, to predict the transfer of maternal medication into the human milk (1,2). The Göttingen minipig has been selected as the most suitable animal model and offers additional advantages with respect to conventional breeds in terms of reproducibility, thanks to the genetic standardization and similarity to human (3). To compare the possible effects of drugs on the cell metabolism in the different species, the present research aimed to characterize the metabolic phenotype of primary cultures of mammary epithelial cells (MECs) derived from Göttingen minipig, commercial pig breed and human. Primary porcine (p) MECs and minipig (mp) MECs were isolated following the protocol previously published (4). Primary human (h) MECs were purchased from Life Technologies company (Catalog number: A10565, Life Tech, Lot # 2098293). The three primary cell lines, mp-/p- and h-MECs were expanded until the tenth passage of culture and characterized to confirm the epithelial phenotype. The cells showed a cobblestone-like morphology that was maintained throughout all passages during the expansion phase as confirmed by the phenotype characterization of specific epithelial markers.

To investigate the bioenergetic cell metabolism, the three primary cell lines were cultured in 8-well XF miniplates, at 90% confluency, in serum-free human mammary growth medium supplemented with 4 µL/mL Bovine Pituitary Extract (BPE), 10 ng/mL hEGF, 5 µg/mL insulin, 0.5 µg/mL hydrocortisone, at 37°C. Measurements of real-time of oxygen consumption rate (OCR), an index of cell respiration (pmol/min), and extracellular acidification rate (ECAR), an index of glycolysis (mpH/min) were monitored by Seahorse XFp analyzer (Agilent). The three primary cell lines resulted with an ATP rate index greater than 1, with a preference in the use of the oxidative mitochondrial pathway compared to glycolytic one. The results obtained showed that the three primary MECs were characterized by oxidative-type metabolism, with a similar basal respiration and ATP turnover, although with a different ability to respond to stress, by confirming the similarities between the porcine animal model with human.

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SPATIAL MEMORY AND SERUM ALANINO TRANSFERASE PREDICT DIFFERENCES IN SIGNS OF COGNITIVE DECLINE IN A GROUP OF SENIOR CATS

Patrizia Piotti (1a), Holly Memoli (2a), Irit Grader (2), Paola Scarpa (1), Mariangela Albertini (1), Carlo Siracusa (2b), Federica Pirrone (1b)

(1) Università degli Studi di Milano, Dipartimento di Medicina Veterinaria e Scienze Animali. (2) University of Pennsylvania, Department of Clinical Sciences and Advanced Medicine. (a) Shared first authorship. (b) Shared last authorship.

Corresponding author: P. Piotti (patrizia.piotti1@unimi.it)

Age-related degenerative conditions such as feline cognitive decline are on the rise as life expectancy of companion animals increase and veterinary care improves (1). Recently, a novel protocol for companion cats was developed (2), which allowed an objective assessment of medium-short term memory, without the need for prolonged training and multiple testing and could therefore be used outside a laboratory setting. However, it is not known how the performance in the test relates with the clinical presentation of feline cognitive dysfunction. Biochemical parameters associated with cognitive decline have been identified in companion dogs (3), but these have yet to be addressed in cats. Therefore, in this multi-centric study, a group of privately-owned senior cats, without clinical signs of disease, was assessed for their cognitive function and behaviour and clinical presentation. The cats attending the clinic for routine check-up were screened for infective diseases, organ failure and hyperthyroidism. Clinically healthy cats were tested for short-term spatial memory, presenting them over 5 consecutive repetitions with a baited container for which they had to recall the location after a short break (4). They were also assessed for signs of feline cognitive decline dysfunction through a semi-structured interview at the owner, and received a clinical exam and standard blood work (CBC, biochemistry). Preliminary data from 17 cats (Mdn Age = 10 years, range = 7-15; Female = 8) were analysed with an ordinal regression model including a cognitive dysfunction score (frequency x severity of signs) as outcome, and age, the number of correct memory test trials, and the blood parameters WBC, ALT, ALP as fixed factors (AIC = 114.17, Chisq(5) = 14.62, p=0.012). The results indicated that a better performance in the memory test (estimate = 2.84+/-1.16, p=0.014) and higher activity of ALT in the serum (estimate = 0.12+/-0.04, p=0.005) predicted better cognitive function in the group. All other factors, including age, did not have a significant effect on the model (p>0.05). These findings suggest that the memory test and at least one of the considered biochemistry parameters may be good predictors of the decline in the cognitive function of senior cats. It is the first indication of an association between cognitive testing and age-related feline cognitive dysfunction in cats outside a laboratory setting. Further data are collected to confirm these results on a larger scale.

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NUTRITIONAL INDUCED IUGR: EFFECT ON WEIGHT AND LIPID METABOLISM IN SARDA DAIRY LAMBS

Cristian Porcu (1), Francesca D. Sotgiu (1), Valeria Pasciu (1), Maria Dattena (2), Marilia Gallus (2), Andrea Cabiddu (2), Giovanni Molle (2), Fiammetta Berlinguer (1)

(1)Università degli Studi di Sassari, Dipartimento di Medicina Veterinaria. (2) AGRIS Sardegna, Bonassai. Corresponding author: C. Porcu (cporcu@uniss.it)

Intrauterine growth restriction (IUGR) is often defined as the failure of the fetus to reach its genetic growth potential in utero [1]. Maternal nutrition is one of the most important factors influencing foetal programming [2], and often variations of nutrition plans during gestation are used to induce IUGR in experimental conditions. The effect of a induced IUGR also depend on the sex of the fetus and the presence or absence of multiple pregnancies [3]. Moreover, several studies have shown that altered placental function and size are often responsible for IUGR, leading to the birth of low weight lambs [3], and it could occur naturally in ewes carrying multiple fetuses, leading to litter-size-dependent IUGR. As a consequence of IUGR, there is altered programming of adipose tissue and this can be associated with future metabolic diseases [4]. However, in sheep, many studies focused on IUGR-induced changes in the glucose-insulin system [5], whilst metabolic changes on lipidic plasma profile are less studied.

The aim of this experiment was to investigate growth rates, blood glucose levels and lipidic profile during the first 7 months after birth in male and female singleton and twin lambs born from ewes undergoing either normal nutrition or feeding restriction, an established model for IUGR [6]. For this purpose, 57 non-milking ewes were used. Pregnancy occurred after natural breeding following cycle synchronization. From day 24 to 100 of pregnancy, the ewes were fed ryegrass hay and two different iso-protein concentrates fulfilling either 100% of ewes' energy requirements (H - control group; n=24), or only 45% (L - undernourished group; n=23), and up to 60% until lambing. At parturition, H group ewes lambed 16 males (HM – 7 singletons and 9 twins) and 17 females (HM – 7 singletons and 10 twins), whilst L group ewes lambed 17 males (LM – 6 singletons and 11 twins) and 17 females (LF – 5 singletons and 12 twins). After parturition, during suckling, ewes were fed with a diet fulfilling 100% of ewes' energy requirements, as for lambs after weaning. Lambs live weight data and blood samples were collected at birth and monthly for 7 months to monitor the weight, glucose and lipidic profile.

As expected, maternal dietary energy restriction, sex and parity influenced lambs birth weight. Among the groups, L groups were the lightest, in particular twins and females, and the difference was maintained during the following months ($p<0.001$). Throughout the 7 months, both L groups showed lower glycaemic values than controls ($p<0.001$). In the same period, LF showed consistently significant lower levels compared to the other groups: Cholesterol $p<0.001$, Triglycerides $p<0.001$, NEFA $p<0.01$, HDL $p<0.001$, and LDL $p<0.05$. In conclusion, as already described in the literature, IUGR induced by dietary energy restriction led to the birth of lighter lambs [3], but the weight gap within groups remained persistently over time, without showing the catch-up described in other studies [7]. Moreover, the dietary energy restriction during pregnancy was able to influence later offspring metabolism, with more prominent outcome in females. Local Animal Care and Use Committee authorization n 2899 of 17/01/2018. Supported by funds from Regione Autonoma della Sardegna L.R. 7/2007—annualità 2013 (CRP 78167).

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FERTILITY PRESERVATION OF SPIN DRIED RAM SPERMATOZOA FOLLOWING 3 YEARS STORAGE AT ROOM T°

Margherita Moncada (1), Martina Lo Sterzo (1), Luca Palazzese (2), Marta Czernik (1), Pasqualino Loi (1)

(1) Laboratory of Embryology, Faculty of Veterinary Medicine, University of Teramo, Teramo, Abruzzo, Italy. (2) Institute of Genetics and Animal Biotechnology of the Polish Academy of Sciences, Warsaw, Jastrzebiec, Poland.
Corresponding authors: M. Moncada (mmoncada@unite.it), M. Lo Sterzo (mlosterzo@unite.it)

Biobanking allows the conservation of somatic and gametic cells for the preservation of biodiversity and medical applications. Currently, biobanks are maintained in liquid nitrogen (LN). Storage in LN or at -80 ° C requires a great consumption of resources and energy, with a strong economic and environmental impact, thus, alternative methods, such as dry storage, may represent suitable alternatives. Freeze drying has been the first method reported to dehydrate spermatozoa. However, on-going experiments in our laboratory indicate that alternative methods of water removal might be less damaging. One of these methods is spin-dry (SD). SD allows the drying of spermatozoa under vacuum, a gentler procedure comparing with lyophilization.

In this study we set up to investigate the preservation of fertilizing capacity of dry ram spermatozoa following spin dry and room temperature (RT) storage, comparing with the standard procedures of freeze drying. Moreover, a longer storage has been simulated by heating the dry spermatozoa at 65C° for 10 minutes (65C) and 100°C for 10 minutes (100C). Samples dehydrated by SD and stored at RT for 3 years, were subjected to a thermal stress of 65 and 100°C for 10 min. The samples were heated in hot water inside their stainless steel/glass containers, rehydrated with double distilled water and then utilized for intra cytoplasmatic sperm injection (ICSI). In vitro matured sheep oocytes (a total of 280 oocytes – 180 for 65C and 100 for 100C) were fertilized using the ICSI procedures in use in our laboratory, then chemically activated by 5 min incubation in ionomycin followed by 4 hours incubation in cycloheximide. Presumptive zygotes were cultured in humidified atmosphere at 38.5°C, 5% CO2, 7% O2 in IVC-medium (ivf Bioscience, cat. 71005). The highest percentage of cleavage was observed in 65C then 100C (51.38% vs 24%, $p<0.0001$) against 47.16% of the control (ICSI with not treated SD semen on 53 oocytes) while the percentage of blastocysts was 11.6% and 9% respectively against 13.2% of the control. The obtained blastocysts obtained were stored in part in liquid nitrogen for future molecular analysis, and in part were cultured *in vitro* to obtain embryonic cell lines (*outgrowth*) for point-mutation analysis.

These results indicate, for the first time, that spin dried ram spermatozoa, maintain the fertilizing capacity even after 3 years of room temperature storage. Moreover, the preservation of the fertilizing potential even after an intense thermic stress, indicated that long-term room temperature storage of spin dry spermatozoa might be a realistic option.

ESTABLISHMENT OF AN IN VITRO MODEL TO STUDY TROPHOBLAST ADAPTIVE RESPONSE DURING PLACENTA DEVELOPMENT IN SHEEP

Irene Viola (1), Paolo Accornero (1), Silvia Miretti (1), Paola Toschi (1), Mario Baratta (2)

(1) Università degli Studi di Torino, Dipartimento di Scienze Veterinarie. (2) Università degli Studi di Parma, Dipartimento di Scienze Chimiche, Scienze della Vita e della Sostenibilità Ambientale.

Corresponding author: I. Viola (irene.viola@unito.it)

During embryo implantation (Day 18), ovine trophoblast cells (oTCs)[1] invade the endometrium and differentiate to form the syncytiotrophoblast layer, which is the first "bridge-tissue" between foetus and mother. Normal conceptus development is affected by oTCs metabolism whose dysregulation leads to several gestational impairment, such as early pregnancy loss[2], characterized by inadequate nourishment or oxygen supply for developing foetus[3]. However, placental growth adapts to safeguard foetal survival, but how impaired activity of oTCs may be compensated to allow foetal development is still incompletely characterized[4]. In the early stage of placentation, endometrial histotrophic factors support placenta development through the modulation of specific cellular mechanisms, including Akt/mTOR signalling pathway, to balance the effects of stressful environmental conditions[5]. Therefore, the use of oTCs in vitro model may improve our understanding of placenta adaptive response, associated with pathological complication during pregnancy.

The main goal of this project was to set up an in vitro culture system from early sheep placenta in order to study the cellular development in physiological conditions. Preliminary, the cellular model was used to explore how oTCs regulate their adaptive response occurring in suboptimal environment during placenta development. oTCs functionality was assessed under starvation or with FGF2 supplementation and mTOR inhibitor (rapamycin) treatment in order to mimic adverse situations in impaired pregnancy. Firstly, primary oTCs from 21 days old sheep placenta collected at the slaughterhouse were cultured in supplemented medium and characterized by cell morphology, immunofluorescence and PCR with established trophoblast markers. Then, oTCs primary cells and oTr cell line were subjected to different treatment (starvation, 50 ng/ml FGF2, 100 nM rapamycin for 24h) to study their effect on cell functionality, (cell proliferation and migration) and on cell characterization (gene and protein expression profile) through qPCR and western blot analyses. oTCs in vitro model showed mainly mononuclear cells with epithelial cell-like growth and placental morphological properties, such as binucleate cells and multinucleated syncytium-plaques formation expressing peculiar trophoblast markers. FGF2 increased significantly proliferation in both oTCs and oTr ($p < 0.001$). Starvation and FGF2 supplementation stimulated cell migration ($p<0.001$), while rapamycin treatment suppressed it ($p<0.001$) compared to controls. Therefore, the inhibition of mTOR signalling pathway by rapamycin reduced the ability of FGF2 to induce cell migration. Significantly, e-CAD was shown to be up-regulated ($p<0.05$) both in starved and FGF2-treated cells (with or without rapamycin), whereas IFN-τ expression wasn't influenced by 24h treatment.

The results confirm FGF2 induced proliferation and migration activity by phosphorylation of Akt/mTOR in sheep trophoblast cells. Interestingly, the study provides the first evidence that FGF2 up-regulates e-CAD expression in stressful conditions associated with starvation and mTOR-inhibition. As previously shown in vivo experiments, these findings suggest that trophoblast expressed "transitory" epithelial-mesenchymal transition during peri-implantation period.

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HEAVY METALS IN HAIR OF TWO ITALIAN AUTOCHTHONOUS GOAT BREEDS REARED UNDER SEMI-EXTENSIVE SYSTEM

Stella Agradi (1), Gabriele Brecchia (1), Giulio Curone (1), Daniele Vigo (1), Susanna Draghi (1), Duygu Tarhan (2), Alev Meltem Ercan (2), Bengü Bilgiç (3), Banu Dokuzeylül (3), Mehmet Erman Or (3), Laura Menchetti (4)

(1) Università degli Studi di Milano, Facoltà di Medicina Veterinaria, Dipartimento di Medicina Veterinaria e Scienze Animali. (2) Univerisità di Istanbul-Cerrahpasa, Facoltà di Medicina Vetrinaria, Dipartimento di Biofisica. (3) Univerisità di Istanbul-Cerrahpasa, Facoltà di Medicina Veterinaria, Dipartimento di Medicina Interna. (4) Università degli Studi di Bologna, Facoltà di Agraria, Dipartimento di Scienze e Tecnologie Agro-alimentari.

Corresponding author: S. Agradi (stella.agradi@unimi.it)

Livestock autochthonous breeds are considered a pivotal genetic resource for agriculture, rural development, food and nutrition security, mostly when considering the two challenging trends for the future: human population growth and climate change [1]. Lariana and Frisa Valtellinese are double-aptitude autochthonous goat breeds reared in Lombardy on the Alpine arch using the traditional alpine farming system based on the use of meadows and pastures for the production of both fresh forages and hay [2]. Because of their tight role with the territory of origin, local breeds could be used to biomonitor environmental contaminations. Heavy metals are a group of elements that are toxic to human beings, many times also at low doses. The food chain is considered one of the major pathways for human exposure to heavy metals' soil contamination [3]. Herbivorous livestock animals are directly exposed to soil pollution and could be considered good bioindicators for heavy metals contamination. For these reasons, the aim of this study was to compare the heavy metal concentrations in the hair of two autochthonous goat breeds, Lariana and Frisa Valtellinese, reared in two different areas of the Alpine arch. This study is part of a larger research, that will investigate the seasonal variations of heavy metals concentration in goat hair. Twenty-six Lariana and 27 Frisa Valtellinese healthy adult lactating female goats were selected to be included in the study. All the goats were kept under a traditional semi-extensive system in their area of origin on the Alpine arch, Western Lario and Valchiavenna for Lariana and Frisa Valtellinese, respectively. The diet was based on local-produced hay from November to May, and from fresh forages available at free pasture in the remaining part of the year. Hair samples were collected from the left rump region of every goat in April. The collections of hair samples from live animals were performed in the respect of animal welfare according to current legislation. The study was conducted with the approval of the Animal Welfare Organisation of Università degli Studi di Milano (Permission OPBA_4_2021). Chromium (Cr), Copper (Cu), Iron (Fe), Manganese (Mn), Selenium (Se), Zinc (Zn), Arsenic (As), Nickel (Ni), Lead (Pb), and Cadmium (Cd) elements were analyzed by using an inductively coupled plasma-optical emission spectrophotometer (ICP-OES; Thermo iCAP 6000series). Due to the non-normality of the data, non-parametric statistics (i.e. Mann-Whitney tests) were used to compare the concentrations of metals between the two breeds. Lariana goats showed higher concentrations of Mn ($p<0.001$), Pb ($p=0.002$), and Fe ($p<0.001$) than Frisa Valtellinese. Conversely, Cu ($p=0.001$), Cd ($p=0.025$), and Ni ($p<0.001$) were higher in Frisa Valtellinese than in Lariana goats. To date, there are just a few studies investigating the heavy metals concentrations in goat hair [4, 5]. Our results are comparable with these studies, with different variations according to the heavy metal considered, but without major deviations. Future physiological investigations will be needed to establish the specific hair growth rate for these breeds and hair metal-binding properties, considering the tight correlation among these factors and the heavy metals bioaccumulation.

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EFFECT OF TRANSPORT ON STRESS RESPONSE AND CELLULAR OXIDATIVE STRESS MARKERS IN MEAGRE (Argyrosomus regius) JUVENILES

Martina Bortoletti (1), Elisa Fonsatti, Stefano Caberlotto (2), Federico Leva (1), Daniela Bertotto (1), Giuseppe Radaelli (1)

(1) Università degli Studi di Padova, Dipartimento di Biomedicina Comparata e Alimentazione. (2) Valle Cà Zuliani Società Agricola S.r.l.

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

In aquaculture, transportation of live fish is a common practice that includes a suit of factors such as handling, air exposure, constraint and low oxygen levels. All these factors are known stressors to fish. The overall effect of stress is the activation of the brain-sympathetic-chromaffin cells (BSC) and hypothalamic-pituitary-interrenal (HPI) axes with release of catecholamines and corticosteroids. In fish, cortisol is the primary corticosteroid produced by the HPI axis having a key role in stress response. At the cellular level, stress frequently provokes an increase in the synthesis of Heat Shock Protein 70 (HSP70) and a decrease in antioxidant defense capacity. This reduction is due to the unbalance between reactive oxygen species (ROS), which are naturally produced during metabolism, and antioxidants that neutralize ROS. The resulting oxidative stress leads to lipid peroxidation, protein carbonils formation, DNA damage, and eventually to cell death. Lipid peroxides are unstable indicators of oxidative stress that decay to form more complex and reactive compounds such as 4-hydroxy-2-nonenal (HNE), which is a common biomarker of lipid peroxidation. Another important ROS is the superoxide radical, which reacts with nitric oxide giving rise to peroxynitrite, a potent oxidant that may oxidize DNA, lipids and proteins. Among the markers for peroxynitrite production, nitrotyrosine (NT), which derives from the oxidation of the amino acid tyrosine, is a relatively stable one. DNA damage generated by oxidative stress may include single and double strand breaks and the modification of bases, such as the oxidation of deoxyguanosine to form 8-hydroxy-2'-deoxyguanosine (8-OHdG). 8-OHdG is used as a chief biomarker of DNA damage, having a clear mutagenic potential for G to T transversions. The present work aimed at evaluating the stress response of meagre (*Argyrosomus regius*) juveniles exposed to transport stress at four phases (i.e. before transport (control), loading, during transport, end of the transport) using a specific radioimmunoassay (RIA) protocol to assess muscle cortisol level. Moreover, an immunohistochemical approach was carried out to localize the cellular distribution of HSP70, HNE, NT and 8-OHdG in several tissues. Muscle cortisol levels significantly increased after the loading procedure and remained above basal levels until the end of the transport (24h). Therefore, fish did not recover from the loading stress during nor at the end of the transport suggesting the other trasport phases as stressors. On the other hand, immunostaining for HSP70, HNE, NT and 8-OHdG antibodies was detected in several tissues and organs, but without differences among transport stages.

To conclude, the elicited stress response might be primarily associated to fish handling during loading and to the transport itself, highliting the need of adequate operational protocols to preserve fish welfare. However, the unalterated distribution of oxidative stress markers between control and stressed fish might suggest mild levels of stress. The use of muscle instead of blood for the evaluation of cortisol has, once again, proved to be an excellent tool in the assessment of post-mortem stress where blood sampling is impossible.

PROLACTIN AS A POTENTIAL BIOMARKER OF STRESS IN THE DOMESTIC DOG

Jara Gutierrez (1), Angelo Gazzano (1), Asahi Ogi (1), Marco Campera (2), Chiara Mariti (1)

(1) Università di Pisa, Dipartimento di Scienze Veterinarie (Italy). (2) Oxford Brookes University, School of Social Sciences (UK).

Corresponding author: C. Mariti (chiara.mariti@unipi.it)

Prolactin has been reported to be a reliable biomarker of stress in several species, being involved in both acute and chronic stress. This research aimed at investigating the role of prolactin in the stress response of domestic dogs through an extensive analysis on sheltered dogs. Serum prolactin, serum cortisol, and hair cortisol were measured in a large homogeneous sample of dogs, formed by 216 adult Spanish Greyhound dogs (41.2% females; 44.90±21.73 months old) all guested in the same shelter. An assessment of their behaviour at the time of collection and in the box, as well as demographic variables, were considered. Dogs' behaviour in the box was recorded for 10 minutes and then analysed; a stress score was calculated for each dog by summing the number of occurrences of 18 stress-related behaviours (yawning, shaking, paw lifting, tongue out, eliminating, growling, turning head, tail tucked, cowering, trembling, circling, pacing, hiding, panting, salivation, howling, whining, and fence-oriented behaviour). Generalized linear mixed model ($p<0.05$) was used for the following dependent variables: stress score, serum cortisol, hair cortisol, and serum prolactin. The following predictors were included: days of permanence in the shelter, age, sex, neuter status, presence/absence of fear in the collection room, number of dogs in the cage, and number of dogs in the collection room. Stress score was found to be positively related to the levels of hair cortisol, levels of serum cortisol, and presence of fear in the collection room, stress score was instead negatively related to age and serum prolactin. Serum cortisol had a positive relationship with the presence of fear in the collection room and neutered dogs, but it was negatively related to the days of permanence in the shelter. Hair cortisol had a positive relationship with age, stress score, serum prolactin, non-neutered dogs and female sex. Serum prolactin had a positive relationship with hair cortisol, days of permanence in the shelter and male sex, but negative with age and stress score. The different relationship that hair cortisol and serum prolactin show with stress behaviours, although hair cortisol and serum prolactin are positively related one to the other, suggests that the link between prolactin, cortisol, behaviour and chronic stress is complex and needs further investigation. A possible explanation for this complex relationship might be that prolactin, as reported in rats (Torner et al. 2001), could have also in dogs an anxiolytic effect, thus reducing anxiety-related behaviours and having a different impact on stressed animals compared to cortisol. In conclusion, results suggest that prolactin could be used as a biomarker of chronic stress in dogs. The different relationship that hair cortisol and serum prolactin show with stress behaviours might indicate different responses to challenging situations.

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THE SHELTER DOG IN A ONE HEALTH PERSPECTIVE: AN INNOVATIVE KENNEL MODEL IN SOUTHERN ITALY

Claudia Gatta (1), Luigi Sacchettino (1), Michele Visone(2), Angelo Quaranta (3), Luigi Avallone (1), Danila d' Angelo(1) Francesco Napolitano (1-4)

(1) Department of Veterinary Medicine and Animal Production, University of Naples Federico II. (2) Dog Park. Ottaviano. (3) Animal Physiology and Behavior Unit, Department of Veterinary Medicine, University of Bari Aldo Moro. (4) CEINGE - Biotecnologie Avanzate, Napoli.

Corresponding author: C. Gatta (gattaclaudia@gmail.com)

Kennels are nowadays considered one of the main concerns of the human-animal relationship (1), since they're very often considered a sort of dump, rather than a shelter as such, for the animals, namely a place where dogs generally exile, thus representing a burden for society. Therefore, drawing up strategies for a new "kennel conception", as an added value for human society, environment, and dogs (2) as well is mandatory. Accordingly, this shelter dog in southern Italy represents a multifunctional model structure, pointing towards the One Health perspective (3). Animal welfare and environment protection, together with human-dog relationship, represent the "backbone" of such a dog shelter conception (4). In this line, before entering the kennel, dogs should be initially subjected to a careful behavioral assessment and categorization by veterinary behaviorist, to guarantee the most suitable life conditions for the animals, increase the chances of adoption and, interestingly, allow them to be enrolled in educational programs, custom-designed to their aptitudes. In this respect, we enrolled 555 dogs of different ages, who followed a suited-4-month lasting training program between 2018 and 2020, with the aim of increasing the skills to be used in different human social contexts, such as supporting to the inmates, rescuing in the rubble, animal-assisted interventions and, even more, zooanthropology projects in schools. We documented a significant increase of the adoptions for both adult and old dogs, when compared to the age-matched untrained animals, who were housed in the same kennel from 2015 to 2017. Taken together, the present data highlight a crucial role for the training in improving physiological attitudes of the companion dogs, as well as their (maybe) wellbeing, thus harmonizing the human- animal bond. All these activities allow an improvement of the awareness degree, that is at the basis of the human-dog relationship, so reducing the risk aggression against humans and other dogs, along with the development of dysfunctional relationships. The strength of this "novel" shelter conception is represented by the environmental protection schedule, where the objectives, scheduled every three years, are the ongoing improvement and minimization of the environmental impact, which are technically and economically sustainable, actually. It is worth mentioning that the shelter makes use of chemical-physical purification areas and phytovaporation of wastewater, thus reducing environmental pollution of the area. Collectively, this innovative animal care-based strategy for dog kennels can prove successful only if we integrate the dog shelter concept into a wider scenario, including both human and environmental wellness.

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AN INTERDISCIPLINARY APPROACH FOR COMPULSIVE BEHAVIOR IN DOGS: A CASE REPORT

Luigi Sacchettino (1), Claudia Gatta (1), Francesca Ciani (1), Luigi Avallone (1), Francesco Napolitano (1-3), Danila d'Angelo (1)

(1) Department of Veterinary Medicine and Animal Production, University of Naples Federico II, Naples, Italy. (2) Veterinary Behaviorist, Salerno, Italy. (3) CEINGE-Biotecnologie Avanzate, Naples, Italy.

Corresponding author: L. Sacchettino (luigi.sacchettino@unina.it)

Compulsive disorder is a debilitating condition affecting both humans and animals, characterized by intrusive thoughts and recurring out-of-place behaviors (1). Among them, tail chasing might represent one of the most common traits in compulsive dogs (2). Herein, we reported the case of a 7-year-old intact male German Shepherd mixed-breed dog, presenting with tail chasing behavior.

He underwent a first behavioral evaluation 1 year before (at the age of 6), when he injured himself with severe wounds at level of the tail and left thigh. To avoid any specific suffering and increase his physical health, of course, the study was carried out through an interdisciplinary approach, employing a veterinary behaviorist and a rehabilitating dog instructor. Three months after pharmacological treatment with fluoxetine (3) and α-s1 casozepine (4), associated with a behavioral recovery program, the owner reported an improvement of compulsive events in his dog, in terms of intensity and frequency. Interestingly, over the following 3 months, the dog did not experience any new tail chasing episodes. Taken together, the present case study represents a proof-of-concept about the positive impact of employing a cross-sectional strategy, made up of conventional antidepressant (fluoxetine) and bovine-derived α-casozepine, along with a thoughtful behavioral program, thus encouraging further clinical and animal model studies to better characterize, at both behavioral and molecular level, such a psycho-social disorder in dogs.

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ALLOPREGNANOLONE IN MARES' HAIR: BUILDING A PICTURE

Isabella Pividori (1), Letizia Ellero (1), Alessio Cotticelli (1, 2), Antonella Comin (1), Tanja Peric (1), Alberto Prandi (1)

(1) University of Udine, Department of Agricoltural, Food, Environmental and Animal Sciences. (2) University of Naples "Federico II", Department of Veterinary Medicine and Animal Production.

Corresponding author: A. Cotticelli (alessio.cotticelli@unina.it)

Allopregnanolone is an important hormone for the fetal development which determines a maintenance of uterine and fetal quiescence in late gestation [1]. The aim of this study was to investigate hair concentrations of allopregnanolone in healthy mares from 20±4 days pre-partum until ten months post-partum. The study included 5 Italian Trotter mares (*Equus caballus*), aged between 5 and 12 years and bred in a Standardbred. Three of them became pregnant again within 3 months postpartum. Hair samples were collected from the withers region, cutting the hair close to the skin with a hair clipper; this area was chosen because of its cleanliness and easy sampling. The samplings after the first one were performed from parturition every 40 days until 300 days after delivery always from the same area to obtain only re-grown hair. Allopregnanolone concentrations were measured by an ELISA commercially available kit (DetectX®, cat. no. K061-H5, Arbor Assays Inc, Ann Arbor, MI, USA) that has been validated for hair (parallelism y=0.964x+3.028; recovery test rate 88.9±2.3% (mean±SD)). Hair allopregnanolone concentrations increased during the last third of pregnancy reaching a peak of 369.0±44.7 pg/mg (mean±SE) and decreased by 94.62±0.51% (mean±SE) 50-60 days after parturition ($P<0.01$). Its decrease may be related to delivery mechanism and may be involved in readiness for birth [1]. After 240 days of calving, the hair concentration of allopregnanolone rose significantly ($P<0.01$) in pregnant mares compared to non-pregnant which instead maintained basal levels. In the late gestation this assay can provide an opportunity to help completing endocrinological picture of the equine pregnancy and the fetal development. Indeed, since the hair matrix is retrospective, the entire perinatal period and fetal intrauterine development can be indirectly evaluated.

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SOIPA

LEISHMANIOSIS IN PARMA UNIVERSITY HOSPITAL: FIVE YEARS RETROSPECTIVE STUDY

Anna Luciani, Alice Vismarra, Marco Genchi, Gabriele Costantino Melis, Laura Kramer

Università degli Studi di Parma, Dipartimento di Scienze Medico-Veterinarie.
Corresponding author: A. Vismarra (alice.vismarra@unipr.it)

Leishmaniosis is a zoonotic disease caused by the protozoa *Leishmania* spp. worldwide diffused and of particular interest in pets, dogs overall [1].

A retrospective study on the leishmaniosis clinical cases from 2015 to 2020 was performed using the medical records management software of the Veterinary Medicine Hospital of Parma University (Italy).

A total of 46 cases were identified and subjects were divided into two groups. In the first group (21) there were different breeds of dogs and in the second group only boxer (25). The study analyzed data on age, sex, geographical origin, symptomatology, chemical and blood parameters alterations and follow up after 3, 6 and more than 9 months from the beginning of therapy.

Dogs from endemic areas represent almost half of the subjects, but there is a small percentage of individuals who have never moved from the area of Parma, that means that the sand fly is starting to colonize new geographical areas, due to climate change and other factors.

Discordant symptomatology, alteration in laboratory tests parameters and a different follow-up has emerged between the two groups considered. The symptomatology turns out to be more disabling in the group called 'other dogs' than boxers, while laboratory alterations are more significant in the boxer group. In particular, 100% of the subjects were found to be hyperproteinuric, 64% of the subjects had an inversion of the albumin-globulin ratio with a ratio of less than 1, hypoalbuminemia was present in 60% of cases and 36% of the subjects revealed an increase in urea level. Regarding therapy and follow-up, the most responsive group is that of dogs of different breeds, while boxer group showed a recurrence of the disease after 9 months from the start of therapy.

A clear difference in the mode of action of *L. infantum* towards different dogs' breeds was evident, in particular, for boxer dogs. As the main findings described in the literature, there is a suggestion of a susceptibility to visceral leishmaniasis in Boxer dogs. The genetic markers may explain the phenotypic variance in both proinflammatory and anti-inflammatory cytokines and cellular immune responses, including the presentation of the antigen [2]. Many gene segments are involved in the phenotype of canine visceral leishmaniasis and this issue must be investigated more in depth.

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EFFICACY OF TOPICAL ADMINISTRATION OF PRALLETHRIN-PERMETHRIN-PIPERONYL BUTOXIDE COMBINATION FOR THE TREATMENT AND CONTROL OF FLIES AND OTHER NUISANCE INSECTS IN HORSES

Marco Genchi(1), Alice Vismarra(1), Laura Kramer(1), Gaia Valentini(2), Giulia Allievi(2), Lavinia Ciuca(3)

(1) Università degli Studi di Parma, Dipartimento di Scienze Medico Veterinarie. (2) Maneggio Le chianine dei Tognoli. (3) Università di Napoli Federico II, Dipartimento di Medicina Veterinaria e Produzioni Animali.

Corresponding author: M. Genchi (marco.genchi@unipr.it)

Pyrethrins and pyrethroids have been widely used for many years to control insect pests[1-2]. Their effectiveness against arthropods affecting different animal species is well known, but there is a lack of data regarding their use in horses. The aim of the study was to evaluate the repellent activity of a spray formulation based on prallethrin (0.033%) and permethrin (0.10%), synergized with piperonyl butoxide (0.50%), against annoying and harmful insects for horses in field conditions.

The study was conducted in a horse stable in Tuscany region (Italy), in June. Twelve horses infected with a minimum of 15 flies were selected and divided into two groups (control and treated). Insect counts were performed on Day 0 (before product administration), and for three subsequent days, at 1, 10, 20 and 30 minutes and at 1, 2, 3, 4, 5 and 6 hours after the administration. During the counts, some insects were captured and conserved for species identification. The product (Bronco® Equine Fly Spray, Farnam) was applied on all parts of the horses, including mane, head and tail. Insect counts were carried out at different time intervals post-treatment, by three different operators. The percentage of effectiveness for each species of insect was calculated using to the following formula: % efficacy = 100x [(control group – traete group) / control group], arithmetic means.

One minute after the administration of the product, all the horses were negative for the presence of insects. The repellent efficacy for *Hippobosca equina*, tabanid flies and *Simulium* spp. remained higher than 93% for all 4 days pt. Efficacy against *Musca domestica* and *M. autumnalis* was 100% after 1-minute and remained at this level for *M. autumnalis* till 6 hours. The efficacy against *M. domestica* decreased to 89.1% at 10 minutes pt and only reached 53.5% at 6 hours pt.

The treatment is safe and effective in killing and repelling insect pests in horses. Residual activity lasted four consecutive days after treatment.

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EVALUATION OF THE ANTHELMINTIC EFFICACY OF PASTURE PLANT SPECIES TO BIOLOGICAL CONTROL OF SHEEP GASTROINTESTINAL NEMATODES - IN VITRO STUDY

Antonio Bosco (1), Pierpaolo Scarano (2), Maria Paola Maurelli (1), Ciccone Elena (1), Rosaria Sciarrillo (2), Giovanni Quaranta (3), Salvatore Claps (4), Laura Rinaldi (1), Carmine Guarino (2), Giuseppe Cringoli (1)

(1) Università degli Studi di Napoli Federico II, Dipartimento di Medicina Veterinaria e Produzioni Animali. (2) Università del Sannio, Dipartimento di Scienze e Tecnologie. (3) Fondazione per lo Sviluppo Sostenibile del Mediterraneo, MEDES. (4) Centro di Ricerca Zootecnica e Acquacoltura, CRAE.

Corresponding author: A. Bosco (antonio.bosco@unina.it)

Gastrointestinal nematode (GIN) infection endangers livestock health and welfare and is commonly associated with economic losses mostly through subclinical diseases impairing weight gain and milk yields [1]. In recent years, there have been important advances in the biological control of GIN in ruminants. While these measures are still relatively under-utilised in practice, interest will undoubtedly grow due to the emergence of drug resistant parasite populations, the rise in demand for organically farmed products and legislation, which regulates and restricts the use of anthelmintic drugs [2]. The aim of this study was to determine the in vitro anthelmintic activity of aqueous, ethanolic and hydroethanolic extracts from 20 medicinal plants (*Borago officinalis, Malva sylvestris, Matricaria inodora, Mentha suaveolens, Plantago lanceolata, Potentilla reptans, Rosmarinus officinalis, Rumex acetosa, Thymus serpyllum, Thymus vulgaris, Achillea millefolium, Amaranthus hybridus, Caucalis platycarpos, Cichorium intybus, Cirsium arvense, Daucus carota, Dipsacus fullonum, Foeniculum vulgare, Inula viscosa* and *Lepidium campestre*), sampled on pastures of southern Italy. For this purpose, the Egg Hatch Test (EHT) was used to estimate the *in vitro* anthelmintic efficacy [3] of the plant extracts using GIN eggs from sheep naturally infected by *Haemonchus, Teladorsagia* and *Trichostrongylus* genera. Each extract was analyzed in three replicates and tested at decreasing concentrations from 28.0 mg/mL for the aqueous extract, 98.0 mg/mL for the ethanolic extract and 40.0 mg/mL for the hydroalcoholic extract. Thiabendazole and deionized water were used as positive and negative controls, respectively. The results indicated that *Borago officinalis, Malva sylvestris, A. millefolium, A. hybridus* and *C. arvense* extracts caused a high inhibition of egg hatching within 48 hours of exposure, showing efficacy (≥96.4%) at the first two higher concentrations. Further in vivo studies are needed to confirm the obtained results with *in vitro* test, evaluate the therapeutic potential and future applicability in field in order to obtain in sheep farms an "anthelmintic pasture" throughout the year.

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ERYTHROCYTE SEDIMENTATION RATE (ESR) IN CANINE LEISHMANIOSIS DIAGNOSIS: A NEW RESOURCE?

Maria Alfonsa Cavalera (1), Floriana Gernone (1), Annamaria Uva (1), Rossella Donghia (2), Grazia Carelli (1), Roberta Iatta (3), Andrea Zatelli (1)

(1) University of Bari, Department of Veterinary Medicine. (2) Unit of Research Methodology and Data Sciences for Population Health, "Salus in Apulia Study" National Institute of Gastroenterology "S. de Bellis" Research Hospital. (3) University of Bari, Interdisciplinary Department of Medicine.

Corresponding author: M.A. Cavalera (mariaalfonsa.cavalera@uniba.it)

Canine leishmaniosis (CanL) is a sand fly-borne disease caused by *Leishmania infantum* and endemic in the Mediterranean basin (1). The systemic pathogenicity of this intracellular protozoan is strictly dependent on the canine host-parasite interaction. As a part of innate immunity, acute phase response has been reported in CanL, being characterized by variations of acute-phase proteins (APPs) with increases in C-reactive protein (CRP), serum ferritin and haptoglobin (2). Similarly, in humans with visceral leishmaniasis by *Leishmania donovani* and *L. infantum* an inflammatory response is considered an ordinary feature, as indicated not only by the enhancement of APPs but also in the erythrocyte sedimentation rate (ESR) (3). Though ESR has scarcely been investigated in veterinary medicine, it has recently been found to be useful in cases of canine rheumatoid arthritis, osteoarthritis, babesiosis, and ehrlichiosis. Therefore, this study aims to evaluate the ESR in dogs affected by CanL active form and to assess the relationship with the other inflammatory markers (i.e., APPs). From October 2021 to January 2022, kennel and privately owned dogs of any sex, age, weight, and breed referred to the Medical Clinic Unit of the Department of Veterinary Medicine (Valenzano, Italy) were recruited if classified as affected by an active form of CanL (according to 4). For each dog enrolled, a physical examination, a complete blood count (CBC) and biochemical panel, electrophoresis as well as fibrinogen concentration measurement were performed. Dogs were tested for anti-*L. infantum* antibodies by enzyme-linked immunosorbent assay and for anti-*Anaplasma phagocytophilum* and anti-*Ehrlichia canis* antibodies by indirect immunofluorescent antibody test. To evaluate the ESR of the dogs, a point-of-care device (MINIPET, DIESSE, Diagnostica Senese S.p.A., Siena, Italy) was used as previously described. The reference interval of ESR was established as 0-8 mm/h. Moreover, the ESR evaluation has been also performed in clinically healthy dogs (i.e., 'healthy group') based on physical examination, CBC, and biochemical findings. Means and standard deviations of the ESR as well as of the main hematological and biochemical findings were analyzed. Twenty-six dogs affected by a CanL active form and 22 clinically healthy dogs were included in the study. The mean value of ESR in dogs affected by a CanL active form (23.11±16.07 mm/h; 95% CI 16.62 to 29.61) was significantly higher than in the healthy group (9.09±7.97 mm/h; 95% CI 5.56 to 12.62) ($P<0.0001$, Wilcoxon rank sum [Mann-Whitney] test). The ESR level was increased in 100% of leishmaniotic dogs while positive APPs such as CRP, fibrinogen, and serum ferritin were increased in 46%, 50%, and 58% of the animals, respectively. Reduced albumin and increased globulins were detected in 61% and in 69% of dogs, respectively. This study provides for the first time data on ESR in dogs affected by CanL compared with healthy dogs, showing a significantly increased level in *L. infantum*-infected animals. Unlike the positive and negative APPs, ESR was enhanced in all dogs with clinical leishmaniosis. The evaluation of ESR by a point-of-care device proved to be a simple, inexpensive, and ready-to-use bench top tool and ESR can be considered a helpful and timely inflammatory biomarker for the diagnosis of active form of CanL.

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DETECTION OF Leishmania SPP. IN CHRONIC DERMATITIS: RETROSPECTIVE STUDY IN EXPOSED HORSE POPULATIONS

Giulia Rigamonti (1), Alessia Libera Gazzonis (2), Giulia Morganti (1), Ilaria Porcellato (1), Paola Roccabianca (2), Giancarlo Avallone (3), Stefano Gavaudan (4), Chiara Brachelente (1), Fabrizia Veronesi (1)

(1) Università degli Studi di Perugia, Dipartimento di Medicina Veterinaria. (2) Università degli Studi di Milano, Dipartimento di Medicina Veterinaria e Scienze Animali. (3) Università di Bologna, Dipartimento di Scienze Mediche Veterinarie. (4) Istituto Zooprofilattico Sperimentale dell'Umbria e delle Marche.

Corresponding author: G. Rigamonti (giulia.rigamonti@studenti.unipg.it)

Leishmania infantum is a protozoa causing human zoonotic visceral leishmaniasis (ZVL) and visceral-cutaneous canine leishmaniosis (CanL) in the Mediterranean Basin. Further than dogs and cats, *L. infantum* is able to infect a large number of wild and domestic species, including horses, which could participate to the epidemiological scenario of the infection [1]. Since the nineties, clinical cases of Equine Leishmaniosis (EL), typically characterized by cutaneous forms [2,3], have been increasingly diagnosed both in the Old and New World and low to moderate seroprevalence levels have been found in different regions of the Mediterranean area (including Italy).

The aim of the present study was to evaluate the presence of clinical forms of EL in CanL endemic areas of Northern and Central Italy, where the exposition of equine populations was ascertained by recent serological surveys [1]. For this purpose, the repositories of the Veterinary Pathology Services of the Department of Perugia, Bologna and Milan were searched to identify skin biopsies presenting chronic dermatitis compatible with EL. The formalin-fixed and paraffin-embedded (FFPE) skin biopsies of 47 horses were included in the study. The DNA was extracted from the FFPE tissue samples to be subjected to conventional PCRs targeting a 120bp fragment of kinetoplast DNA and a 330bp fragment of the Internal Transcribed Spacer-1 (ITS-1) of ss-rRNA and q-PCR [4,5]. A singular positivity for *L. infantum* was found using both the genetic targets and confirmed also at the q-PCR, with a parasite load of 158,000 copies. The amplicons were sequenced and submitted to BLAST (Basic Local Alignment Search Tool) analysis that revealed a 99-100% homology with *L. infantum* sequences. The ITS-1 sequence was submitted to GenBank under the accession number OM055847.

The positive specimen belonged to a horse from Bologna, showing a nodular lesion on the face, near to the right ear. The histological examination revealed a nodular lymphoplasmacytic and histiocytic infiltrate. The biomolecular positive sample was submitted to an immunohistochemistry (IHC) protocol described by Porcellato et al. [6] that revealed rare macrophages containing numerous positive amastigotes.

The present retrospective study reports for the first time a case of cutaneous lesion by *L. infantum* occurring in an Italian horse. Pathological and healthy skin samples should be investigated on a larger scale to provide information on the potential clinical impact of EL in the practice and to define the role of horses in the epidemiological ZVL and CanL scenario.

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DROPLET DIGITAL PCR: A NEW APPROACH FOR DETECTION OF *Toxoplasma* gondii IN MEAT SAMPLES

Andrea Mancusi[1], *Angela Giordano*[1], *Antonio Bosco*[2], *Santa Girardi*[1], *Yolande T. R. Proroga*[1], *Luigi Morena*[3], *Renato Pinto*[4], *Paolo Sarnelli*[4], *Giuseppe Cringoli*[2,3], *Laura Rinaldi*[2,3], *Federico Capuano*[1], *Maria Paola Maurelli*[2]

(1) Istituto Zooprofilattico Sperimentale del Mezzogiorno, Department of Food Security Coordination. (2) University of Naples Federico II, Department of Veterinary Medicine and Animal Production. (3) Centro di Riferimento regionale per le malattie degli animali domestici (C.Re.San.). (4) Regione Campania, UOD Prevenzione e Sanità Pubblica Veterinaria.

Corresponding author: M.P. Maurelli (mariapaola.maurelli@unina.it)

Toxoplasmosis is a widespread worldwide zoonotic infection caused by the intracellular protozoan *Toxoplasma gondii* [1]. Different direct and indirect techniques have been used to detect *T. gondii* in intermediate hosts and in food products [2]. However, the development of new diagnostic tools more sensitive and specific is always in progress. The droplet digital polymerase chain reaction (ddPCR) is a novel PCR that provides absolute and direct quantification of target DNA, without the need for a standard curve as the qPCR, with a higher sensitivity than other PCR methods [3]. The ddPCR has been used for detection of different parasites in different animal hosts and humans [4, 5]. The aim of this study was to develop and validate a new ddPCR assay for detection and quantification of *T.* gondii DNA in meat samples of intermediate hosts. To optimize the ddPCR, *T. gondii* reference DNA aliquots at five known concentrations: 8000 cg/µl, 800 cg/µl, 80 cg/µl, 8 cg/µl were used. Moreover, results obtained by ddPCR and quantitative PCR (qPCR) were compared using 80 known samples (40 positive and 40 negative), as well as unknown diaphragm tissue samples collected at slaughterhouses.

The ddPCR showed a sensitivity of 97.5% and a specificity of 100%, with a detection limit of 8 genomic copy/µl of *T. gondii*. No positive droplets were detected in the sample used as negative controls. A nearly perfect agreement (κ=0.85) was found between results obtained by ddPCR and qPCR for both positive and negative known samples analysed. On the 171 diaphragm tissue samples from field, 7.6% resulted positive by ddPCR and only 1.2% by qPCR. Therefore, this innovative method could be very useful for the detection of *T. gondii* in meat samples, aiming to prevent human infections.

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EARLY DETECTION OF Besnoitia besnoiti INFECTION IN AN INTENSIVE DAIRY CATTLE FARM IN NORTHERN ITALY: SEROLOGICAL AND MOLECULAR ANALYSIS

Alessia Libera Gazzonis (1), Luca Villa (1), Riccardo Zanchetta (2), Marco Colombo (2), Carolina Allievi (1), Sergio Zanzani (1), Maria Teresa Manfredi (1)

(1) Università degli Studi di Milano, Dipartimento di Medicina Veterinaria e Scienze Animali. (2) Veterinario Libero professionista, Milano.

Corresponding author: A.L. Gazzonis (alessia.gazzonis@unimi.it)

Bovine besnoitiosis (BB), caused by the protozoan *Besnoitia besnoiti* (*Apicomplexa: Sacrocystidae*), is a neglected parasitic disease of cattle considered emerging in Europe. Since no vaccines or chemotherapy drugs are available in Europe, it is necessary to apply control strategies to avoid both the entry of the infection on the farm and the spread of the pathogen among the animals [1]. However, often BB is not included in the differential diagnosis, particularly in the acute phase of the infection when the clinical signs (fever, respiratory problems) are nonspecific. An outbreak of BB in an intensive dairy cattle herd from northern Italy is here reported.

In May 2021 (T0), four cows with clinical signs resembling *B. besnoiti* acute infection and distress as reported by individual animal tracking tags were observed. Detection of specific IgG (ELISA) and IgM (IFAT) and circulating DNA (real time-PCR) [2] were performed. After the diagnosis of the first cases of infection, a control program based on i) the progressive reform of seropositive animals, and ii) the control of vector insects was started. All animals in the farm (both cows and heifers) were screened combining the three tests both in July (T1) and November (T2). Productive data (monthly milk production) and individual records relating to distress (e.g., decreased rumination) as reported by tracking tags were collected during the study period (January-December 2021) and analyzed in relation to *B. besnoiti* seropositivity.

At T0, all suspected cases of BB were confirmed: three cows positive to IgG, and one cow to IgM and real time-PCR. On the whole, 11.1% infected animals (n: IgG=21, IgM=9, PCR=1) were detected at T1; the seroprevalence at T2 showed a slight increase (12.8%), with six new cases of infection (n: IgG=24, IgM=3, PCR=0). During the study period, a total of 30 cows tested positive (at least at one sampling), while heifers always tested negative for *B. besnoiti*. Except from a cow with cutaneous clinical signs and three animals with scleral pearls and/or cysts on teets, after the febrile acute stage of infection no other animal developed detectable clinical signs during the study period. The analysis of the data produced by the individual tags showed a greater number of distress events in infected animals compared to seronegative (40% vs. 21.7%), with a decrease in rumination in 36.6% vs. 13% of cases, respectively. In the context of BB control strategies, 11 infected animals were reformed due to a drop in milk production during or after the study period. However, a statistically significant effect of BB infection on milk production could not be demonstrated.

The combination of different diagnostic tests allowed an early diagnosis of the outbreak, recognizing also animals still in the acute phase of infection. The promptly implementation of a control program effectively limited the spread of the infection within the farm.

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BABESIA CANIS INFECTION IN DERMACENTOR RETICULATUS QUESTING TICKS IN A NATURAL PARK IN ITALY

Luca Villa (1), Sergio Aurelio Zanzani (1), Michele Mortarino (1), Alessia Libera Gazzonis (1), Emanuela Olivieri (2), Maria Teresa Manfredi (1)

(1) Università degli Studi di Milano, Dipartimento di Medicina Veterinaria e Scienze Animali. (2) Università degli Studi di Pavia, Dipartimento di Biologia e Biotecnologie.

Corresponding author: L. Villa (luca.villa@unimi.it)

Dermacentor reticulatus is one of the most important vectors of tick-borne pathogens (TBPs) in Europe causing diseases in animals and humans. Indeed, it is the proved vector of *Babesia canis*, but it can also transmit other pathogens of veterinary importance, such as *B. caballi, Theileria equi*, and *Anaplasma marginale*. The tick could transmit TBPs of public health relevance as *Rickettsia* spp. and some tick-borne encephalitis viruses [1]. *Babesia canis* the protozoan agent of canine babesiosis (CB), a significant hemoparasitic disease of dogs, causing hemolytic anemia, splenomegaly, thrombocytopenia, and fever.

The circulation of *D. reticulatus* and the association of the tick with *Babesia canis* infection was recently reported in northern Italy [2]. Therefore, a longitudinal study was planned mainly aimed to detect the molecular prevalence of Babesia spp. and its seasonal variation in *D. reticulatus* questing ticks collected in a natural park in Italy to define the temporal infection risk for dogs.

The study was carried out in the Groane Regional Park, located in the peri-urban area of Milan and Monza Brianza provinces (Lombardy). Ticks were collected from April 2015 to June 2016 in five permanent transects using both dragging and flagging techniques on the leaf litter and the vegetation. Collected ticks were morphologically identified by taxonomic keys (Arthur, 1962; Pomerantzev, 1950). Overall, 488 adult questing ticks, including 241 female and 247 male exemplars, were processed for DNA extraction. A screening real time PCR on 18S rRNA was performed for the detection of piroplasmid DNA. On positive samples, a conventional PCR for Babesia spp. and subsequent sequencing was carried out.

Out of 488, 58 ticks (24 females and 34 males) were positive for *Babesia/Theileria* DNA, resulting a molecular prevalence of 11.9%. Positive ticks were mostly collected in March (n=30) and April (n=21), when in early spring the peak of tick activity occurred [3]. Positive ticks also occurred in February (2) and May (5). Obtained sequences from 408-bp PCR fragments confirmed a homology of 100% with *B. canis* sequences deposited in GenBank. No tick resulted positive for *Rickettsia* spp. This study evidenced a conspicuous circulation of *Babesia canis* infection in D. reticulatus adult questing ticks and confirms their role in the epidemiology of CB. The risk of acquiring the CB for owned dogs using the peri-urban park is significant in spring months. Considering the efficient transovarial transmission of *Babesia* in the ticks, tick prophylaxis is strictly required and should cover the entire period during which D. reticulatus is active to prevent CB.

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HEALTH AND WELFARE OF HONEYBEES: Origanum vulgare SUBSP. viridulum (MARTIN-DONOS) AND Foeniculum vulgare SUBSP. piperitum (UCRIA) ESSENTIAL OILS FOR SUSTAINABLE CONTROL OF VARROATOSIS

Roberto Bava (1), Fabio Castagna (1, 2), Ernesto Palma (1, 3, 4), Cristina Carresi (1, 3), Vincenzo Musolino (5), Carmine Lupia (1, 6, 7), Maria Rosaria Perri (8), Filomena Conforti (8), Domenico Britti (1, 2), Vincenzo Musella (1, 2)

(1) University of Catanzaro Magna Græcia, Department of Health Sciences. (2) University of Catanzaro Magna, Interdepartmental Center Veterinary Service for Human and Animal Health (CISVet-SUA). (3) University of Catanzaro Magna Græcia, Department of Health Sciences, Institute of Research for Food Safety & Health (IRC-FISH). (4) Nutramed S.c.a.r.l., Complesso Ninì Barbieri, Roccelletta di Borgia (CZ). (5) University of Catanzaro Magna Græcia, Pharmaceutical Biology Laboratory, Institute of Research for Food Safety &Health (IRC-FISH). (6) National Etnobotanical Conservatory, Castelluccio Superiore (PZ). (7) Mediterranean Etnobotanical Conservatory, Sersale (CZ). (8) University of Calabria, Department of Pharmacy, Health and Nutritional Sciences.

Corresponding author: R. Bava (roberto.bava@unicz.it)

Varroatosis, caused by the *Varroa destructor* mite, is currently the main parasitic disease affecting the health of honey bees. The high rate of infestation is responsible for colony collapse disorder, leading to the loss of a honey bee colony within two years or less [1]. Therefore, its negative impact on the health of bees requires the constant use of acaricides which, over the years, has led to widespread phenomena of drug resistance in one or more classes of drugs [2]. The aim of this study was to evaluate the acaricidal efficacy against *V. destructor* of two essential oils of spontaneous native species, typical of the Calabria Region of southern Italy, *Origanum vulgare* subsp. *viridulum* Martin-Donos (oregano) and *Foeniculum vulgare* subsp. *piperitum* Ucria (fennel).

To verify the effect of the essential oils (EOs), a new method has been developed, not previously used to test the fumigation toxicity [3]. The mites were placed in a chamber with a volume of 2 ml (Eppendorf tube) and exposed to the vapors of the oil dilutions. In particular, the EOs were diluted in distilled water (1 mg/mL) and a cotton swab soaked with 40 µL of the dilution was inserted into the recess on the inner surface of the cap, that allows the hermetic closure of the Eppendorf tube. Five adult female mites were transferred to the bottom of the Eppendorf tube using a fine paintbrush. Subsequently, a piece of tulle was immediately inserted into the test tube and interposed between the mites and the cap. Five experimental replicates were performed and a negative control (cotton swab soaked with distilled water) was carried out for each of them. *V. destructor* mites were incubated for different exposure times (15, 30, 45, 90 minutes). At the end of each interval, the alive mites were placed in a Petri dish with a honey bee larva (as nourishment). Finally, the prepared Petri dishes were returned to the incubator and mortality was assessed 24 and 48 hours later. In this way, the onset of mortality due to phenomena of sub-lethal toxicity was verified. After each experiment, the mites were observed under stereomicroscope and classified as inactive if they moved one or more legs after being prodded, instead, if they did not move any of the legs were considered dead. Both dead and inactive mites were considered equally neutralized [3]. Compared to the negative control, the tested EOs returned efficacy results. Particularly, oregano EO has been shown to be more effective than fennel EO. The percentage of dead mites after 48 hours and, in particular, at 90 minutes of exposure to the vapours of oregano and fennel EOs was 88% and 58%, respectively. The activity is presumably due to the synergistic action of the components (linalyl acetate, linalool, linalool propionate, in particular), identified by gas chromatography/mass spectrometry (GC/MS). Therefore, considering that a drug treatment is considered minimally effective if it reduces the phoretic mite population by at least 80%, we can conclude that the oregano EO may be effective for pest control. It is therefore possible to start *in vivo* studies to better define the most appropriate pharmacological formulation for use in the field.

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IN VITRO REPELLENT EFFICACY OF SMOKE WATERS AGAINST LOUSE *Haematopinus tuberculatus*: A PROOF-OF-CONCEPT STUDY

Antonio Bosco (1), Giovanni Jesu (1), Emanuele Bambacaro (1), Mirella Santaniello (1), Maria Paola Maurelli (1), Giuliano Bonanomi (2), Laura Rinaldi (1), Giuseppe Cringoli (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.

Corresponding author: A. Bosco (antonio.bosco@unina.it)

Water buffalo (*Bubalus bubalis*) farming in Italy adopts intensive breeding techniques, whose high density of animals promotes the diffusion of ectoparasites, such as the louse *Haematopinus tuberculatus*, that is widespread and has buffalo as elected host and led to meat and milk production reductions [1]. The current control measures include the exclusive use of chemical ectoparasiticide (i.e., pyrethroids) that, in the long run, can cause the outbreak of resistances in the target organisms. Given the lack of alternative solutions to the chemicals, the aim of this study was to evaluate the in vitro repellent efficacy against louse *H. tuberculatus* of a low-impact by-product: the Smoke Water (SW) [2, 3]. Basing on what has been recorded on other pests the study was performed with an ad-hoc modified set-up test, using lice collected one hour earlier from animals of a commercial buffalo farm located in the Campania region, Southern Italy. The set-up of in vitro test consisted in a glass Petri dish (ø19 cm) subdivided in three separate areas. In the first area was placed a layer of paper soaked with 3 ml of water (starting area), in the second one (obstacle area) was placed a layer of paper soaked in 3 ml of the compound to be tested, and in the last one the paper was previously scrubbed on the host skin and then soaked in 3 ml water, with addition of host fur on it (finishing area). Ten adults (five males + five females) were tested in each plate. Each of the three replications completed until now included the test trial and two controls carried out at the same time. The quantity of 3 ml of SW were used in the SW petri dish obstacle area, while for the obstacle area of the negative and positive control were used 3 ml of water and, basing on its well-known effectiveness on the target, 3 ml of deltamethrin solution (BUTOX® 7,5 Pour-On, MSD Animal Health Srl), respectively. Each of the three replications was carried out at 27°C and 75% Relative Humidity, in the dark. Checks were made after 5, 15, 30, 60, 90, 120, and 180 minutes to evaluate the movement, the living/dead ratio, and the ability to overcome the obstacle area of the lice, in terms of comparative avoidance (%). The results have shown a repellent effect of SW against *H. tuberculatus* adults of both sexes 30 minutes after exposition (85% for males and 75% for females) statistically comparable ($p<0.05$) to the repulsiveness exerted by deltamethrin exposition (65% for males and 75% for females). The deltamethrin though led to the death of all the exposed individuals in that time and SW had not shown any topic effect nor caused direct death on the adults tested. While in the negative control all the lice passed within 15 minutes from the starting area to the final one. Further replications are ongoing in order to evaluate minimum efficacy concentration and quantity of SW to be tested under semi-field and field conditions, thus respecting the economic threshold of convenience for intervention a the 3R principle "reduce, replace and refine", that aims to limit the use of experimental animals in research.

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THE EFFICACY OF A 10% W/V IMIDACLOPRID AND 50% W/V PERMETHRIN SPOT-ON SOLUTION (ADVANTIX®) IN REDUCING THE RISK OF Leishmania infantum INFECTION

Ettore Napoli (1), Gabriella Gaglio (1), Giovanni De Benedetto (1), Domenico Otranto (2), Stefania Latrofa (2), Hannah Ringeisen (3), Timm Dickschat (3), Matthias Pollmeier (1), Emanuele Brianti (1)

(1) Università degli Studi di Messina, Dipartimento di Scienze Veterinarie. (2) Università degli Studi di Bari, Dipartimento di Medicina Veterinaria. (3) Bayer Animal Health GmbH and Elanco Company.

Corresponding author: E. Napoli (enapoli@unime.it)

Visceral leishmaniasis is an important zoonotic vector-borne disease in the Mediterranean area, being a major veterinary problem and increasingly important for human health [1]. Dogs are considered the predominant domestic reservoir, playing the main epidemiological role in the disease. Moreover, the risk of human infection correlates with the degree of infection in dogs. Currently, the most efficient strategy in reducing the risk of *Leishmania* infection in dogs is the use of synthetic pyrethroids [2]. Synthetic pyrethroids, displaying anti-feeding effect against *Leishmania* vectors, are indeed regarded as the most efficient way of controlling transmission, and their use is strongly advocated either in non-infected or infected/diseased dogs. This study evaluated the efficacy of a spot-on solution containing 10% w/v imidacloprid and 50% w/v permethrin (Advantix®) administered every 3 or 4 weeks in reducing the risk of *Leishmania infantum* infection under field conditions in 3 sites in Sicily (Italy) known for high incidence rates (i.e., from 23% to 31%) [2].

Three hundred three dogs were included and randomly divided into 2 groups: G1 (n=163) and G2 (n=140) treated every 3 and 4 weeks, respectively. The study continued throughout 2 consecutive sand fly seasons. Clinical examinations, blood specimen/conjunctival swab collections and ectoparasite burden evaluations were performed on Study Days (SDs) 0, 126, 196, 335, 461, 566, and 640. Blood and swabs collected on the above SDs were analyzed by serology (i.e., ELISA and IFAT for samples positive in ELISA) and PCR.

On SD 0, 46 animals scored ELISA and IFAT positive for *L. infantum* (21 in G1 and 25 in G2) and a total of 28 animals were lost to follow-up during the study. Therefore, on SD 640, 126, and 103 dogs were still present in G1 and G2, respectively. The ectoparasite burden was low in most animals on SD 0 and was well controlled with >99% of animals free of ticks and fleas throughout the seasons. In the first season a total of 9 and 11 new infections with *L. infantum* occurred, corresponding to 93%, and 90% efficacy in the prevention of transmission, in G1 and G2, respectively. These overall efficacies were not statistically significantly different between the two dosing regimens. One new infection occurred in each group during the second season confirming the high efficacy.

Advantix® administered at 3 and 4 weekly intervals is highly effective in reducing the risk of *L. infantum* infection in dogs exposed to severe *Leishmania* transmission challenge.

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SURVEY ON CANINE FILARIOSIS IN SHELTERED DOGS: A NEW FOCUS OF DIROFILARIA IMMITIS IN SICILY

Paola Tropea, Angelo Francesco Basile, Emanuele Brianti, Ettore Napoli

(1) Università degli Studi di Messina, Dipartimento di Scienze Veterinarie. (2) Veterinario Libero Professionista
Corresponding author: E. Napoli (enapoli@unime.it)

Dirofilaria immitis is a widely spread nematode and it is the aetiological agent of Heart Worm Desease (HWD) one of the main canine vector-borne diseases (VBDs) across Europe and Americas [1]. The increased movement of animals from and to endemic areas coupled with climate change favoring the encroachment of vectors into previously free areas has changed the epidemiological patterns of HWD. As matter of facts, in the last decade, *D. immitis* has changed its status in dogs in Sicily from sporadic to increasingly reported [2]. In this survey, we describe the latest focus of HWD observed in a dog shelter located in Catania (Sicily, southern Italy). This finding further supports the spread of the disease in the island and provided some additional data on diagnostic challenges and on the frequency of coinfections with other VBDs, such as leishmaniosis.

In July 2021 all the dogs hosted in a shelter in Catania were sampled, and blood samples analysed with Knott's test for the detection and identification of circulating microfilariae (mfs). Nineteen out of 103 dogs tested positive with a prevalence of 18.4%. The microfilaraemic dogs were also tested for antigenemia and real-time PCR (qPCR). Also, the microfilaraemic dogs underwent cardio-pulmonary echography and were serologically tested for Leishmania infantum infection.

According to anamnestic data, 15 of the infected dogs were housed in the shelter since they were puppies and most of them (14) were found nearby the kennel. Interestingly, for all the infested dogs no history of travel was recorded. The microfilaremia had a mean level of 1032.2 mfs/mL. Out of the 19 microfilaraemic dogs, 13 (68.4%) scored also positive to ELISA rapid assay and all the 19 dogs were molecularly positive for *D. immitis* DNA. Upon echography echoes referable to adults presence were seen in the pulmonary arteries of 4 out 15 tested dogs, and *L. infantum* antibodies were detected in the sera of 16 (84%) *D. immitis* positive dogs.

Findings of this study provide further evidence on the spread and diffusion of *D. immitis* infection in dogs in Sicily. Veterinary practitioners of the area should be aware of this endemicity and specific prevention strategies should be proposed to reduce the risk of transmission in exposed dogs. A multimodal approach is suggested for the diagnosis and coinfections with other VBDs should be always considered and properly diagnosed. Finally, this study highlights that sheltered dogs may represent an important source of maintenance and spread of VBDs. Therefore, proper management and screening of VBDs in this dog population, especially before movement or adoption, is strongly advocated also in light of the zoonotic concerns posed by these infections.

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EMERGING RISK OF Dirofilaria SPP. INFECTION IN SHELTER DOGS IN SOUTHERN ITALY

Lavinia Ciuca, Valeria Caruso, Sergio Illiano, Maria Paola Maurelli, Giuseppe Cringoli, Laura Rinaldi

Università degli Studi di Napoli Federico II, Dipartimento di Medicina Veterinaria e Produzioni Animali.
Corresponding author: L. Ciuca (lavinia.ciuca@unina.it)

Based on the report of two cases of heartworm disease found at post-mortem examination in two roaming dogs from the urban area of Castel Volturno in Campania region of southern Italy (1), the aim of the present study was to better investigate the occurence of *Dirofilaria* spp. in the same area. For this, a local dog shelter from Castel Volturno in southern Italy was selected and screened for the presence of *Dirofilaria* spp. A total of 260 blood samples were collected between June 2020 and July 2021 and examined for identification of microfilariae (mff) using the modified Knott's test (2) and for detection of *Dirofilaria immitis* antigen using the Petcheck Canine Heartworm test (IDEXX). Moreover, all the dogs that showed co-infections with both *D. immitis* and *D. repens* mff were confirmed with molecular analyses (3). Anamnestic data were also collected for the sampled dogs, including age, sex, health status (i.e. activity level, appetite, any health problems, any skin abnormalities). In addition, data regarding the length of stay of the dogs in the shelter at the moment of sampling was also recorded. The dogs were divided into four age classes (class 1: dogs ≤2 years; class 2: dogs >2 ≤ 6 years; class 3: dogs >6 ≤ 10 years; class 4: dogs >10 years old) and three groups of dogs based on the length of stay in the shelter at the moment of sampling (group 1 - new entries: dogs that have been received in the shelter in the last four months; group 2- dogs that were housed in the shelter for more than four months up to 2 years; group 3-dogs that were housed for more than 2 years).

Modified Knott's test reavealed that 188 dogs (72.3%) were positive for circulating mff of *Dirofilaria* spp. Specifically, 113 (60.1%) dogs were positive to *D. immitis* mff and 75 (39.9%) were positive to *D. repens* mff. In addition, 58 (30.8%) dogs presented both *D. immitis* and *D. repens* mff. The antigen test showed 98/260 (37.7%) dogs positive to *D. immitis*. However, 13% of the dogs with *D. immitis* mff were antigen-negative. The PCR test confirmed the co-infections with both pathogens in all 58 dogs. Prevalence was almost twice as high in males (65.2%) compared with females (37.7%). As expected, prevalence was lowest in age class 1 (16.5%) and higher in age classes 2, 3 and 4 (25.5, 27.6% and 30.3% respectively) but these differences were not significant. There was a significant difference regarding the length of stay of the dogs in the shelter, reflecting mainly an increase in prevalence in the group 1 (45.2%, P=0.012) in which all the dogs were new entries in the shelter since four months and their origin was from various locations of the Campania region, including the city center of Naples. The majority of the dogs had no health problems during the examination at the moment of sampling. However, 13 dogs showed some symptoms: skin problems (skin lesions, poor quality of fur, itching) in which five dogs presented small soft nodules in subcutaneous tissues. All the dogs that presented nodules tested positive for *D. repens* in subsequent PCR testing. In addition, no prophylactic treatment against *Dirofilaria* spp. has been performed in the shelter. We conclude that dog shelters from southern Italy constitute hot spots for *Dirofilaria* spp. transmission and we strongly recommend education and veterinary guidance regarding regular testing and systematic treatments.

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75°CONVEGNO SISVET LODI, 15-18 GIUGNO

ABSTRACT POSTER SESSION

AIPVET

SUBCUTANEOUS PANNICULITIS-LIKE T-CELL/NK-CELL LYMPHOMA (SPTCL) IN SIX CATS

Silvia Dell'Aere (1), Paola Roccabianca (1), Clarissa Zamboni (1), Giancarlo Avallone (2), Walter Bertazzolo (3), Luca Crippa (4), Mario Caniatti (1), Verena Affolter (5)

(1) Università degli Studi di Milano, Dipartimento di Medicina Veterinaria e Scienze Animali. (2) Università di Bologna, Dipartimento di Scienze Mediche Veterinarie. (3) Mylav Veterinary Laboratory. (4) Università degli Studi di Milano Bicocca, Dipartimento di Medicina e Chirurgia. (5) VMPIM, UC Davis, Ca, USA.

Corresponding author: S. Dell'Aere (s.dellaere@patovet.eu)

Primary cutaneous lymphoma is rare in cats representing 0.2-3% of all feline lymphomas[1,2]. Panniculitis-like lymphoma (SPTCL) is a primary non-epitheliotropic cutaneous CD3+/CD4-/CD8+ and TCRα/β T-cell tumor that has been described in humans[3,4] and dogs[5].

The aim of this report is to describe the morphological, phenotypical and molecular features of SPTCL in cats. Skin biopsies were obtained from six cats presenting with nodular to plaque like lesions in the abdominal (3/6), inguinal (2/6) and thoracic (1/6) regions. Two cases were initially misdiagnosed as panniculitis and submitted for consult. Samples were formalin-fixed, routinely processed for histology, immunohistochemistry anti-CD18, CD204, Iba-1, CD79a, CD20, CD3, FeLVp27, FeLV gp70 and clonality assessments. Cats with SPTCL were all domestic shorthaired, 4 males and 2 females with a mean age of 11.2 years. Neoplastic cells predominated in the panniculus with septal (6/6) to lobular (1/6) distribution with characteristic rimming of fat lobules in 4/6 cases. Neutrophils (5/6), macrophages (6/6) and variably severe necrosis (6/6) were observed multifocally mimicking panniculitis. Cytomorphology was highly pleomorphic and varied among and in the same cases. Immunohistochemistry and clonality allowed the definition of a T cell origin in 5 cases. Lineage could not be assigned in one case. Less than 20% of neoplastic cells expressed FeLV antigens in 2/6 cats. This is the first description of feline SPTCL. Lesions can be confused with panniculitis and awareness of this entity is relevant to avoid therapeutic delays.

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HISTOPATHOLOGICAL FEATURES FOR LYMPHOVASCULAR INVASION IN 7 CANINE APOCRINE GLAND ANAL SAC ADENOCARCINOMAS

Clarissa Zamboni, Damiano Stefanello, Mario Caniatti, Chiara Giudice, Valeria Grieco, Camilla Recordati, Silvia Dell'Aere, Roberta Ferrari, Cristina Lecchi, Mauro Di Giancamillo, Paola Roccabianca

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

Corresponding author: C. Zamboni (clarissa.zamboni@unimi.it)

Migration of neoplastic cells into the vascular beds is termed Lymphovascular Invasion (LVI). LVI is one of the most relevant criteria of malignant behavior, recurrent and metastatic risk, poor outcome and adjuvant treatment recommendation for tumors in humans such as thyroid carcinoma, melanoma, breast and cervical cancer. Recently, standardization of LVI morphological assessment has been proposed in veterinary medicine (Meuten et al., 2021). The aim of this study was to assess central and perimetral LVI in canine Apocrine Gland Anal Sac Adenocarcinomas (AGASAC) and correlate LVI scores with development of lymph nodes metastases. Peritumoral/intratumoral LVI was scored and the number of true and equivocal LVI foci and presence and extent of nodal involvement were recorded following morphological criteria by Meuten et al.[1]. A total of 7 AGASAC and corresponding regional lymph nodes were examined. Tumor growth was infiltrative in 6/7 cases. LVI sites were as follows: no LVI in 2 cases, <5 sites in 2 cases, 5-10 in 1 case and >10 in 2 cases. Intratumoral LVI was recorded in 2 cases, peritumoral in 7 cases. On a morphological basis equivocal LVI was recorded in 6 cases. Lymph node involvement was observed in 7/7 cases.

In conclusion, LVI in AGASAC was more frequently perimetral and did not correlate with number or extent of lymph nodes metastasis.

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DEVELOPMENT AND SET-UP OF A 3D MODEL OF SWINE RESPIRATORY CELLS FOR THE STUDY OF IN VITRO PATHOGENESIS OF INFLUENZA VIRUSES

Andrea Cacciamali, Michele Tempini, Silvia Dotti

Istituto Zooprofilattico Sperimentale della Lombardia e dell'Emilia-Romagna - Centro di Referenza Nazionale per i Metodi Alternativi, Benessere e Cura degli Animali da Laboratorio.

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

The infectious capacity and spread of influenza A viruses (IAVs) depend on a series of interrelated events that result from complex mechanisms that are still partially known today. Even if they are species-specific viruses, there are increasing reports of the virus passing from one host species to another with the consequent risk of reassortment between different strains and the appearance of new strains with possible pandemic potential. Swine species play a key role in the diffusion of IAVs both for the type of farming and for the enormous variability of the circulating strains deriving from reassortment phenomena between strains of swine, avian and human origin. In vivo experimental models are able to provide relevant elements for the study of IAVs; however, they are subject to numerous ethical and animal welfare implications [1, 2]. It is necessary to standardize alternative models, such as 3D cultures of porcine respiratory tissue, which allows us to evaluate the infectivity and replication capacity of IAVs in systems that maintain the anatomical and functional integrity of the tissues of interest [3]. In this study, we tried to develop a 3D culture system using the New Born Pig Trachea (NPTr) cell line that shows the coexistence of α2,3 and α2,6 receptors, making it suitable for the study of influenza viruses, not only swine, but also human and avian. Cells grow in MEM added with 10% FBS at 37°C and 5% CO_2. In 2D cultures, the morphological aspect, the presence and the type of the cytopathic effect were evaluated when inoculated with swine influenza virus (H3N2 A/swine/1523/98, H1N1 A/swine/1523/98 and H1N2 A/swine/1523/98). Two different matrices are being tested for the 3D model: TissueSpec® Lung ECM Hydrogel (Xylyx, NY, USA) and PeptiGel® (Manchester BIOGEL, UK). For inclusion cell growth, Hydrogel mixes with different stiffness (6 mg/ml, 5 mg/ml and 4 mg/ml), 10% and 20% FBS concentrations and 24 and 48-well plates with a cell concentration of 4.5x10^4 cells/well were tested. No cell growth was observed after 7 days of incubation but was monitored for up to 4 weeks with no results. Other combinations of gel stiffness, FBS concentration and cell number will be tried to achieve optimal growth support. Tests are also underway on cells grown in Hydrogel to develop a protocol for fixing and embedding 3D cultures in paraffin for subsequent immunohistochemical tests. With regard to PeptiGel, different compositional and stiffness formulations have been tested (Alpha 1, Alpha 2, Alpha 2 RGD, Alpha 4, Gamma 2). For growth in inclusion, a suspension of NPTr cells was prepared at a concentration of 1.2x10^6 cells / ml. The matrix enriched with cells was seeded on 12 mm inserts with a transparent PET membrane and 0.4 µm pores (ThinCert®, Greiner bio-one, Austria) suitable for 12-well plates. This facilitates the adhesion of the matrix to the substrate and permits the passage of nutrients from the soil to the gel itself. Cell growth was observed for few weeks and the Alpha 1 and Alpha 4 formulations were found to be the most suitable for the growth of the cells used. These preliminary tests will be useful to identify the most suitable scaffold for the growth of cell lines chosen for virus-cell interaction and viral replication studies. Further developments of the system could concern the culture of other respiratory explants from pigs (nasal, bronchial and pulmonary) allowing the reduction of the number of animals used, in line with the 3Rs principle, improving the in vitro methodology.

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MAIN CAUSES OF DEATH IN INSECTIVOROUS BATS IN TURIN PROVINCE

Elena Colombino, Ilaria Prandi, Giuseppe Quaranta, Mitzy Mauthe von Degerfeld, Maria Teresa Capucchio
Università degli Studi di Torino, Dipartimento di Scienze Veterinarie
Corresponding author: Ilaria Prandi (ilaria.prandi@unito.it)

Several studies describe the presence of biological agents and toxic elements in wild insectivorous bats [1, 2]. However, only few papers analyse the diseases of bats and the causes of their death.

The aim of this study is to examine the main causes of death and macroscopic findings observed during systematic necropsies of wild insectivorous bats. All animals were rescued by the Non-Conventional Animal Centre (CANC), unit of the Department of Veterinary Sciences, University of Turin.

A total of 83 bats belonging to 9 different species of the families *Vespertilionidae* and *Molossidae* (genera *Vespertilio*, *Pipistrellus*, *Hypsugo*, *Eptesicus*, *Myotis*, *Plecotus* and *Tadarida*) underwent anatomopathological examination. Macroscopic lesions were classified and grouped according to type and tissues involved: traumatic lesions (fracture, laceration, hemorrhage), skin or patagium lesions and organs abnormalities.

The main recorded causes of death were: trauma (50.6%), cachexia (15.6%) and predation (8.4%). In 25.3% of bats the cause of death remained unknown. Traumatic lesions were the most common causes of death, in according with literature [2, 3]. Trauma or predation caused bone fracture in 38.8% of cases (which interested mainly humerus, radius/ulna, carpus and phalanxes), while patagium wound occurred in 53.1% of animals. Traumatic hemorrhages involved subcutis, muscles of neck or thoracic/abdominal walls, thoracic and abdominal cavities but also inner organs, such as liver. Regardless of the cause of death, 14.5% of bats displayed gastric dilation and 4.8% presented free nematodes (*Litosomoides* spp.) in thoracic and abdominal cavities.

This preliminary study confirms trauma as the main cause of death in wild bats also in Piedmont region. Histological and microbiological analysis are in progress in order to understand the role of biological agents in the death of these micromammals.

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AN UNUSUAL CASE OF COLLISION TESTICULAR TUMOR IN A DSD SRY-NEGATIVE DOG

Claudia Rifici (1), Letizia Sinagra (1), Mariagiulia Pugliano (2), Viola Zappone (1), Maria Elisa Catalfamo (1), Valeria Grieco (3), Giuseppe Mazzullo (1)

(1) Università degli Studi di Messina, Dipartimento di Scienze Veterinarie. (2) Università degli Studi di Napoli, Dipartimento di Medicina Veterinaria e Produzione Animale. (3) Università degli Studi di Milano, Dipartimento di Medicina Veterinaria e Scienze Animali.

Corresponding author: C. Rifici (crifici@unime.it)

Collision tumors (CT) consist of two independent neoplasms composed of two morphologically distinct neoplastic populations [1, 2]. Disorders of Sexual Development (DSD) are characterized by atypical development of chromosomal, gonadal or anatomical sex, and can occur at any stage of sexual development, leading to various abnormalities of the genital tract [3]. Sex reversal (SR) Syndromes are a type of DSD characterized by a discrepancy between chromosomal sex and gonadal development (testes/ovaries) and the presence or the absence of an SRY gene that is the key regulator of gonadal development [3, 4]. An 8-years-old Jack Russell terrier phenotypically female dog was referred to a gynaecological specialist because of anomalous vaginal discharge and non-pruritic cutaneous bilateral symmetrical alopecia on the flanks. During abdominal palpation, a voluminous mass was detected in the left quadrant area, later confirmed by ultrasound (5x4 cm in size), with the typical appearance of complex mass. Computerized tomography (CT) and explorative laparotomy were proposed but the owner declined. After two months, general health conditions worsened, and a blood transfusion was necessary because of the severe anaemia and mild jaundice. After a further deterioration of clinical status, the owner declined any treatment and decided to proceed with euthanasia and necropsy. In the abdominal cavity, the left gonad was increased in size while the right one was decreased in size as well as the uterus. On the contrary, vagina and vulva appeared thickened. Histologically, the left gonad revealed to be a testis affected by a double neoplastic component (sustentacular tumor and interstitial cell tumor) whereas the right gonad showed coarctated seminiferous tubules. PCR amplification of the genes *SRY* and *AMELX* revealed the absence of a Y chromosome. The relation between DSD and neoplastic processes has often been reported in humans [5] while very few data are available in canine species. It is therefore essential to investigate this aspect of DSD in this species because dog represents an important translational study model as it shares many spontaneous pathologies with humans that are not artificially induced as happens in the mouse model. To the best of our knowledge, this is the first report describing a case of a coexisting testicular collision tumor in a DSD SRY-negative dog.

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SKIN LESIONS AS USEFUL PARAMETER FOR EVALUATION OF DISTURBED HABITAT IN FISH: PRELIMINARY DATA

Teresa Pirollo (1), Maria Teresa Capucchio (2), Maria Perotti (2), Paolo Pastorino (3), Giovanni Loris Alborali (1), Cristian Salogni (1)

(1) Istituto Zooprofilattico Sperimentale della Lombardia e dell'Emilia Romagna. (2) University of Torino, Department of Veterinary Sciences. (3) Istituto Zooprofilattico Sperimentale del Piemonte Liguria e Valle d'Aosta.

Corresponding author: T. Pirollo (teresa.pirollo@izsler.it)

The "disturbed habitat" is a concept indicating a change in the optimal environmental conditions, which can interfere with the normal functioning of all biological systems. In fish, the effects of the disturbances, which may be natural or due to human activity, are closely related to their welfare in the aquatic environment and are very difficult to assess due to the intrinsic variability of species and environment [1]. The farmed fish undergo high levels of stress, and with the increasing development of aquaculture and the worsening of climate change, there is a need to define a practical and holistic approach to assessing fish welfare. Therefore, the aims of this research project are to i) identify the anatomo-histopathological lesions associated with situations of disturbed habitat in fish, and ii) prepare a protocol for the evaluation of fish welfare, to use on-farm. This protocol could be used for the identification of animal-based measures (ABMs) by assessing on the animals, the environmental and farm management effects [2].

Based on the available literature, various situations of disturbed habitat and their effects at the anatomo-pathological level have been evaluated. Fish have been submitted to a diagnostic protocol, which includes anatomo-pathological and histological examinations.

In this preliminary work, the attention was focused on skin and fins lesions. In fact, the epidermis represents a biological barrier between the fish and the aquatic environment, assuming the function of protection against infectious diseases, friction and pollution, and is actively involved in the regulation of ions. Damage to the skin is frequently associated with secondary infections and consequently impacts fish welfare [3]. A total of 170 fish, both farmed and wild, were analysed, after assuming they were living stressful environmental situations. One hundred and forty-six (86%) and 97 (57%) analyzed fish showed lesions of the skin and fins respectively.

The following lesions were observed: change in colour (lighter/darker), hyperaemia, haemorrhages, erosions, ulcers, desquamation, focal lesions due to parasites or fungi, traumas and fraying or shortening of the fins.

The observed findings were evaluated using a semi-quantitative scoring system based on the severity and spread of injuries from absent (score = 0), minimal (score = 1), mild (score = 2) and severe (score =3).

Considering the stressful situations responsible for the observed lesions, animal density producing aggression in the tank (subjects with a high difference in size or during the meal); handling; erosions due to composition materials of the tanks or collisions among fish; and water quality were hypothesized [3–5].

The obtained results confirm the possibility to correlate anatomo-histopathological lesions of skin and fins to stressful environmental conditions. A longer observation period is needed to properly evaluate the efficiency of this protocol.

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ISOLATION OF CIRCULATING EXOSOMES AND EXPRESSION OF EXO-MIR-21 FROM DOGS AFFECTED WITH CUTANEOUS MAST CELL TUMOR

Clarissa Zamboni, Paola Roccabianca, Luiz Gustavo De Matos, Samuele Mauri, Susanna Di Mauro, Fabrizio Ceciliani, Roberta Ferrari, Damiano Stefanello, Lavinia Elena Chiti, Cristina Lecchi

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

Corresponding author: C. Zamboni (clarissa.zamboni@unimi.it)

Exosomes are a class of extracellular vesicles (30-150 nm), delivering molecules including proteins, metabolites, and microRNAs (miRNAs), involved in physiological intercellular crosstalk and disease pathogenesis. Their isolation with reliable quality and substantial concentration is still a major challenge for the feasibility of further analyses. The present study aims are (I) developing an easy and fast protocol for the isolation of exosomes from plasma of mast cell tumor (MCT)-affected dogs; (II) evaluating the ability of exosomes to deliver miR-21 (exo-miR-21), a miRNA overexpressed by MCT[1]. Twelve dogs have been enrolled in the study: 4 healthy and 8 (4 with nodal metastasis and 4 non-metastatic) MCT-affected dogs. Exosomes were isolated using size exclusion chromatography (SEC) (IZON column 35nm) and were characterized by Western blot, Nanoparticle tracking analysis, and transmission electron microscopy. Exo-miR-21 was quantified using digitalPCR.

Exosomes expressed the specific exosomal markers CD9 and TSG101, and their mean concentration and size were 2.68E+10 particles/ml and 99.6 nm, 2.89E+10 particles/ml and 101.7 nm, and 3.21E+10 particles/ml and 124 nm particles/ml in non-metastatic, nodal metastatic, and healthy samples, respectively. The comparative analysis demonstrated that the level of exo-miR-21 ($P<0.05$) was significantly higher in nodal metastatic (110 copies/μL) compared to healthy (19 copies/μL) and non-metastatic samples (21 copies/μL). In conclusion, the present work demonstrated that a pure population of exosomes can be isolated from the plasma of MCT-affected dogs using the SEC approach and that exo-miR-21 is overexpressed in nodal metastatic MCT-affected dogs.

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ARTERIOVENOUS MALFORMATION IN THE ANTEBRACHIAL REGION OF A CAT

Claudio Pigoli (1), Silvia S. Cardinelli (2), Francesco Godizzi (3), Tommaso Furlanello (4), Giovanna Bertolini (4), Lucia R. Gibelli (1), Paola Roccabianca (3)

(1) Istituto Zooprofilattico Sperimentale della Lombardia e dell'Emilia Romagna, Dipartimento Area Territoriale Lombardia. (2) Ambulatorio Veterinario Cardinelli e Mordenti. (3) Università degli Studi di Milano, Dipartimento di Medicina Veterinaria e Scienze Animali. (4) Clinica Veterinaria e Laboratorio d'Analisi San Marco.

Corresponding author: C. Pigoli (claudio.pigoli@izsler.it)

Vascular malformations (VMs) are uncommon congenital anomalies detected early or late according to superficial or deep location growing proportionately with the individual (1, 2). This report aims at describing an arteriovenous malformation (AVM) in the forearm of a cat. A four-month-old domestic shorthaired intact male cat with no history of trauma or venous catheterism presented for right carpus and forearm diffuse soft tissue swelling. On physical examination, the paw was cold with multifocal cutaneous ulcers. A pulsatile lesion was detected in the antebrachial region. Computed Tomography Angiography (CTA) was performed, and finally, the forelimb was amputated and subjected to histopathology. CTA evidenced arteriovenous shunting of the median artery and a tangle of serpiginous venous vessels (nidus) in the soft tissues of middle and distal third of the forearm (3). Bone structures were spared. Histopathology revealed a non-infiltrative vascular lesion in deep dermis and subcutis composed of tortuous thickened or thinned, sometimes misshapen, arteries and veins, dilated vessels of indeterminate type, and a variable number of venules and capillaries. Perivascular fibrosis and occasional internal elastic lamina fragmentation were also visible. No mitotic activity nor cytological atypia were observed. Presentation, CTA and histopathology were diagnostic for AVM. The main clues were the age of the animal, shunting and nidus evidenced by CTA (3-5). AVMs are rarely reported in veterinary medicine (1, 2). In humans, AVMs are considered the most challenging VMs to treat due to their high blood flow (3 ,4). AVM in cats should not be confused with neoplasia or progressive angiomatosis and should be treated accordingly.

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FATAL COINFECTION OF AVIAN POLYOMAVIRUS, PSITTACINE CIRCOVIRUS AND STREPTOCOCCUS GALLOLYTICUS IN A GREY PARROT

Katia Varello (1), Elena Biasibetti (1), Elena Bozzetta (1), PierLuigi Acutis (1), Arianna Meletiadis (1) Davide Mugetti(1), Francesca Rossi (1), Alessando Dondo (1), Simona Zoppi (1)

(1) Istituto Zooprofilattico Sperimentale del Piemonte, Liguria e Valle d'Aosta, Turin, Italy.

Corresponding author: E. Biasibetti (elena.biasibetti@izsto.it)

A 6-months old grey parrot (*Psittacus erithacus*) was presented for necropsy following a sudden death occurred after a brief clinical history of lameness.

Gross findings at necropsy included gelatinous-haemorrhagic oedema of the subcutis, and the muscles extended from the entire left limb to the back and the pelvis. The left limb showed a marked subluxation of the hip joint, knee joint and tibio-tarsal joints. The pericardial serosa showed diffuse thickening and an accumulation of yellowish amorphous inflammatory debris slightly adherent to the basis at the origin of the great vessels associated with haemorrhages and oedema extended to fatty tissue. The liver appeared diffusely marbled by miliary multifocal to coalescing greyish spots, both at the surface and in depth. Samples of all major tissues were collected and fixed in 10% buffered neutral formalin, processed routinely, and stained with hematoxylin and eosin for light microscopic examination. Virus detection on feather, heart, lung, liver, spleen and kidney samples were performed by PCR; also bacteria were tested in lung, liver and bone marrow samples.

A multifocal severe inflammatory infiltration at the origin of the great vessels and subacute multifocal mild bronchitis were histologically observed. Also a multifocal to coalescing hepatocellular necrosis and multifocal granulomatous splenitis were present. In kidney mesangial karyomegaly with intranuclear inclusion bodies were detected. Muscle was characterized by multifocal haemorragia, necrosis and inflammatory infiltrate; moreover, bacteria were identified within the lumen of the vascular spaces indicating a bacteriemia.

Beak and Feather Disease Virus (BFDV) and Avian Polyomavirus (APV) were detected. *Streptococcus gallolyticus* ssp. *gallolyticus* were isolated on tissue cultures.

APV infection in birds is an acute inflammatory disease and can have a mortality rate of up to 100% in fledglings. In Italy APV is rarely observed (0.79%) [1, 2]. BFDV is caused by a DNA virus belonging to *Circoviridae*. Most commonly affecting immature and fledgling birds, where classical symptoms are observed. Other clinical symptoms include beak deformities such as fractures, abnormal elongation and palatine necrosis, lethargy, depression, diarrhoea and immunosuppression, and seldom lead to death [3, 4].

Streptococcus gallolyticus subspecies *gallolyticus* (*Sgg*) belongs to the Group D streptococci, a large group of phenotypically diverse bacteria ranging from those used in food-fermentation, to commensal bacteria of the gut and opportunistic pathogens in both humans and animals [5].

In conclusion, in our opinion, the death of the animal was related at an infection of APV and BFFD that have exacerbate the role of opportunistic bacterium (*Sgg*), accidentally entered in the bloodstream by a trauma.

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SYSTEMIC INFECTION BY ANGIOSTRONGYLUS VASORUM IN A FENNEC (VULPES ZERDA) IN AN ITALIAN ZOOLOGICAL GARDEN

Valentina Galietta (1), Cristiano Cocumelli (1), Caterina Raso (1), Klaus G. Friedrich (2), Pilar Di Cerbo (2), Manuela Iurescia (1), Elena L. Diaconu (1), Patricia Alba (1), Claudio De Liberato(1), Claudia Eleni (1)

(1) Istituto Zooprofilattico Sperimentale del Lazio e della Toscana "M. Aleandri". (2) Fondazione Bioparco, Roma.

Corresponding author: V. Galietta (valentina.galietta-esterno@izslt.it)

Angiostrongylus vasorum (*Nematoda, Metastrongyloidea*) is a parasitic nematode residing in the right side of the heart and pulmonary arteries of many canids [1]. The red fox (*Vulpes vulpes*) is considered its reservoir, but also dog and other canids are natural hosts of *A. vasorum*, where it causes mainly cardiopulmonary disease. Cases of infection have been reported in captive animals, such as red pandas (*Ailurus fulgens*) [2], and the congeneric species *Angiostrongylus dujardini* caused the death of suricates (*Suricata suricatta*) and callitrichid monkeys (*Saguinus oedipus* and *Callimico goeldii*) in an Italian zoo [3]. Here we report a case of an *A. vasorum* systemic infection in a captive fennec (*Vulpes zerda*) in a zoo of Central Italy. The fennec, a 14 month-old female born at Bioparco, used to live in a semi-natural pen with other five conspecific individuals and was brought to the Istituto Zooprofilattico Sperimentale del Lazio e della Toscana "M. Aleandri" (IZSLT) for post-mortem examination. One month before death, it had presented anorexia, weakness, paralysis of hind limbs and at the end respiratory symptoms. A thoracic radiograph showed a diffuse broncho-interstitial lung pattern. It was treated with corticosteroid and antibiotic therapy, but in the end, due to the serious clinical conditions, it was euthanized. Necropsy revealed poor body condition, cardiomegaly and granulomatous pneumonia with adult worms protruding from the lumen of the pulmonary arteries and detected in the right heart ventricle. Kidneys appeared pale with whitish streaks into the cortex and haemorragic petechiae were observed on the gastric mucosa and on the cerebral meninges. Samples of different organs were collected for histopathological examination. Microscopically, lungs showed severe chronic granulomatous pneumonia, with intralesional eggs and nematode larvae. In the lumen of some pulmonary arterioles, thrombotic formations with adult worms were seen. Multifocal lymphoplasmacytic infiltrates and granulomatous foci with nematode larvae were observed in the myocardium of the right ventricle, in brain, in spleen, in intestinal submucosa, in liver and in many renal glomeruli. *A. vasorum* diagnosis was confirmed through both morphological and molecular identification of adult worms recovered at necropsy. No other cases of infection were highlighted between fennec living in the same pen. To our knowledge, this is the first case of *A. vasorum* infection in *V. zerda*. Probably the severe progression of angiostrongylosis in this fennec, with multiorgan involvement, was influenced to age and immune status. Fennecs are active predators and maintain their hunting behaviour in captivity. Hence, it is likely that the animal was exposed to infection by preying on a parasitized gastropod, intermediate hosts of *A. vasorum*, entering zoo enclosures from the surrounding environment. Interestingly, the fennecs' enclosure is just one hundred meters away from the area of Villa Borghese specifically devoted to dogs, therefore characterized by a high dogs fecalization. In conclusion, *A. vasorum* should be considered among the parasites reported to be able to be introduced into zoo enclosures, sometimes leading to captive animals' death. Moreover improved preventive methods and diagnostic tools would be advisable, aimed at preventing their introduction in zoos or at their early detection if introduced.

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PREVALENCE OF ANIMAL ABUSE IN PETS. DATA ANALYSIS OF THE IZSPLVA IN PERIOD 2020-2021

Francesca Rossi, Francesca Cimino, Marilena Gili, Gianluca Ferro, Cristina Marra, Alessandro Dondo, Simona Zoppi

(1) Istituto Zooprofilattico Sperimentale del Piemonte, Liguria e Valle d'Aosta, Turin, Italy.

Corresponding author: F. Rossi (francesca.rossi@izsto.it)

Animal abuse is a social problem, because there is an established link between cruelty to animals and inclination to violence towards humans (1). In Italy animal abuse, including deliberate animal poisoning, is a criminal offense, complying with National Law No 189 of 2004 and Ministerial Order of 18 December 2008.

In order to monitoring this phenomenon, specific forensic expertise of the Istituti Zooprofilattici Sperimentali (IIZZSS) is the focal point to identify and treat properly every single diagnostic case.

The aim of this study was to pay close attention to post-mortem findings suggestive of such crimes against animals in Piedmont, considering anamnestic data, gross lesions and results of diagnostic and toxicological investigations (2, 3).

Six-hundred-sixty-five pets (326 dogs and 339 cats) were examined between January 2020 and December 2021 at the IZS of Piedmont, Liguria and Aosta Valley (IZSPLVA) to define morphological and aetiological diagnosis of death. Each cause of death was classified in 9 categories based on the main pathophysiological processes (PP) normally involved (4). There was a high level of infectious causes (42%; 280/665), followed by degenerative, traumatic , toxic, neoplastic, metabolic, vascular, congenital and inflammatory forms.

From the results obtained, it emerged that 9.9% of total deaths (n=66/665) was attributable to forms of animal abuse and specifically 69.7% (n=46/66) to poisoning, 27.3% (n=18/66) to traumatic lesions, and 3% (n=2/66) to negligence. Anticoagulant rodenticides were the toxic substances most frequently detected, followed by metaldehyde, strychnine, organophosphates and pyrethroids. Traumatic lesions in dogs were attributable to firearm, stabbing weapons, burn and fall from the balcony, while in the cat to compressed air gun. Suffocation by hanging and polytrauma were signalled in both species. 4.3% (n=29/665) of reported cases were defined as suspected abuse but not analytically proved with pathological features suggesting toxic substance intake, or referable to trauma, without any possibility of confirming the malicious intent.

The percentage of suspect cases suggests a possible underestimation of the real number of positive cases and remarks the importance of the improvement of diagnostic protocols to be adopted together with the availability of highly qualified veterinarians.

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FATAL SYSTEMIC TOXOPLASMOSIS IN A CAPTIVE SLENDER-TAILED MEERKATS (SURICATA SURICATTA)

Cristiano Cocumelli (1), Valentina Galietta (1), Caterina Raso (1), Maristella Zambelli (2), Emanuela Bovi (1), Luigi Sorbara (1), Roberta Onorati (1), Carla Gobbi (1), Claudia Eleni (1)

(1) Istituto Zooprofilattico Sperimentale del Lazio e della Toscana "M. Aleandri", Roma. (2) Veterinario libero professionista, GPCert (ExAP).

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

Toxoplasma gondii is a worldwide distributed zoonotic protozoa that can infect many domestic and wild species and *Felidae* are the definitive hosts. The clinical disease can appear with abortion and neonatal mortality in humans and ruminants, while can be fatal in other Families. *Herpestidae* is a family of small nocturnal carnivores and include species hosted as zoo animals or as pets, with slender-tailed meerkats (*Suricata suricatta*) as an example. While the disease is deeply known in many species, in meerkats there are only few references in literature [1-4]. Here is described a fatal disseminated infection in a captive 2 years old male slender-tailed meerkats with acute onset of respiratory distress, anorexia and lethargy, that died in 30 hours. At necropsy a moderate to severe pneumonia with miliary yellowish foci of necrosis and severe hyperemia were evident; blood were detected in the oral cavity. Other lesions were severe enlargement of the mesenteric lymphnodes with evident foci of necrosis at cut, foci of necrosis and diffuse degeneration of the hepatic parenchyma and mild catarrhal enteritis with hemorrhagic mucosal soffusions. Multiple organs were sampled for histological, and bacteriological examination and biomolecular test directed to *T. gondii*. Microscopic observation revealed the presence of 30-50 µm cysts containing uncountable crescent-shaped 7×1.5 µm bradyzoites, particularly in lymphnodes and in less number in lungs and brain; no evident cysts were observed at histology of liver and spleen. Inflammatory lesions and areas of necrosis were evident with different extents in all organs but were particularly severe in organs of the immune system and in liver. Samples submitted for bacterial cultures were negative, while PCR examination confirmed *T. gondii* as the aetiological agents involved in the infection. Clinical, gross and microscopic findings were almost similar to the rare available description of the infections in adult meerkats [2], but differ from other outbreaks in juvenile or adult animals, where neurological signs dominated [1, 4]. This can be explained from the different route of infection or from the animals' age or from the amount of cysts and genotype of *T. gondii* involved. In this report, only one animal died in a group of 4 adults and 3 one month-old merkaats, thus suggesting a focal source of infection (i.e. passive vector or contamination deriving from feral cats). Feral cats are indeed observed around the enclosure and could be putatively the source of the infection of the present animals and in the previuos death suspected to be related to toxoplasmosis reported in the history of the farm. The infections via alimentary route is then unlikely the source of infections, despite it cannot be completely excluded as commercial rodents and birds are part of the meerkats' diet. More studies are necessary to assess the parasite role and its virulence, with a particular attention to the genotypes currently circulating. Clinically, the disease can express with a variety of simptoms complicating the diagnosis. For this reason, and for the high risk of susceptibility for toxoplasmosis, due to an evolutionary recent exposure to *T. gondii*, this agent should always be considered in the differentials in meerkats and other zoo species. Moreover, particular attention is mandatory in the control of contamination from feral cats, from passive vector as rodents, arthropods and birds and from uncontrolled meal (i.e. raw meat) in these particularly vulnerable populations.

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FATAL DISSEMINATED TOXOPLASMOSIS IN CAPTIVE RING-TAILED LEMURS (Lemur catta)

Claudia Eleni (1), Caterina Raso (1), Valentina Galietta (1), Raffaella Parmigiani (1), Klaus G. Friedrich (2), Pilar Di Cerbo (2), Paolo Selleri (3), Fiorentino Stravino (1), Virginia Carfora (1), Cristiano Cocumelli (1)

(1) Istituto Zooprofilattico Sperimentale del Lazio e della Toscana "M. Aleandri", Roma (2) Fondazione Bioparco, Roma (3) Centro Veterinario Specialistico, Roma.

Corresponding author: C. Eleni (claudia.eleni@izslt.it)

Toxoplasma gondii, the causative agent of toxoplasmosis, is an obligate intracellular protozoan parasite that infects a wide range of endothermic species, including humans. Among non-human primates, high susceptibility has been found in New World monkeys and prosimians (lemurs in particular), where the disease often results in an acute and fatal form [1]. Genetic diversity of *T. gondii* strains infecting primates is considered one of the factors involved in determining the different susceptibility [2].

We describe the pathological findings observed in captive ring-tailed lemurs (*Lemur catta*) died for toxoplasmosis. Two lemurs from two different zoological collection of Central Italy, were submitted to the Istituto Zooprofilattico Sperimentale del Lazio e della Toscana for post-mortem examination. Lemur 1, an adult female, was part of a group of four subjects who had all presented the clinical symptoms (malaise, anorexia and dyspnoea for 4-5 days) in the same period and rapidly died in a few days. Lemur 2 was a two-year-old male from a group of 15 subjects, who had showed lethargy, anorexia and progressive wasting for two weeks before death.

Post-mortem examination revealed in both lemurs hepatic degeneration and congestion, severe lung congestion and scattered haemorrhages of small intestine. Lemurs 2 showed also a sero-sanguinous nasal discharge and poor body condition. Specimens of the main organs were collected for histological, bacteriological, virological and molecular tests. Histologically, similar lesions were found, although more severe in lemur 1. Liver presented multifocal necrosis with fibrin deposition and degenerated neutrophils; severe multifocal steatosis was also detected. In lung, congestion and moderate alveolar oedema were observed, together with multifocal fibrinous bronchiolointerstitial pneumonia, particularly in lemur 1. Spleen, heart, mesenteric lymph node and intestine showed multifocal necrosis. In kidney, multifocal moderate membranous glomerulonephritis and lymphoplasmacytic interstitial nephritis were observed. Brain showed a moderate lymphocytic and neutrofilic meningoencephalitis with microhaemorrages; in lemur 2 scattered *T. gondii* cysts were observed. Immunohistochemical labelling for *T. gondii* revealed numerous tachyzoites in several organs, especially in liver necrotic area. Real-Time PCR analysis for *T. gondii* was carried out as previously reported [3] on brain, lung, heart, liver and kidney samples and all tested positive. Bacteriological and virological tests performed on multiple organs were negative.

Pathological findings observed are similar to those already reported in lemurs and New World primates [4, 5]. These results confirm the high susceptibility of the lemurs to *T. gondii* infection and suggest including toxoplasmosis among the important diseases affecting these animals. Furthermore, despite we did not have had the opportunity to examine the other subjects cohabiting with the lemur 1, the presence of the same symptoms and the fatal outcome in all the animals, lead us to hypothesize that toxoplasmosis was the cause of death of the whole group of lemurs. Molecular in-depth studies to evaluate the genetic diversity of the two *T. gondii* positive samples, are still ongoing. Indeed, the results of genetic characterization could be useful to possibly understand the differences in the clinical course of the two cases described.

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TWO CASES OF CAMPYLOBACTERIOSIS IN JAPANESE MACAQUES (MACACA FUSCATA) HOUSED IN AN ITALIAN ZOO

Caterina Raso (1), Cristiano Cocumelli (1), Andrea Caprioli (1), Valentina Galietta (1), Tiziana Palmerini (1), Angelo Giacomi (1), Paola Di Matteo (1), Pilar Di Cerbo (2), Klaus G. Friedrich (2), Claudia Eleni (1)

(1) Istituto Zooprofilattico Sperimentale del Lazio e della Toscana. (2) Fondazione Bioparco, Roma.
Corresponding author: C. Raso (caterina.raso-esterno@izslt.it)

Campylobacter sp. is considered to be the most common bacterial cause of human gastroenteritis in the world and is the most commonly reported foodborne gastrointestinal pathogen in the EU since 2005 [1, 2]. *Campylobacter* sp. are ubiquitous, Gram negative, non-spore-forming, S-shaped or spiral shaped bacteria, with polar flagella conferring motility [3]. The ingestion of contaminated water or food, or direct contact with infected animals are the most important routes of transmission [2]. An acute infection can have serious long-term consequences in humans, including the peripheral neuropathy known as Guillain–Barré syndrome (GBS) and functional bowel diseases [1]. *Campylobacter* species are normally found as commensals of the intestinal tracts of a wide variety of wild and domesticated animals and have been reported as the causative agent of acute and chronic diarrhoea and gastroenteritis in non-human primates (NHP) in the literature [3]. Here we report two cases of campylobacteriosis in Japanese macaques (*Macaca fuscata*) hosted in a zoo in Central Italy, that were submitted to the public health institute Istituto Zooprofilattico Sperimentale del Lazio e della Toscana M. Aleandri for the post-mortem investigation. The macaques were housed in a group of 60 conspecific individuals. The first animal, a 13 years old female, was presented with a history of sudden death in October 2021, without evidence of specific clinical signs. The second animal, a 34 years old male, was euthanized few days later, after showing diarrhoea, weakness, hyporeflexia and paresis of the hind limb in the previous week. Necropsy was performed within 48 hours after death. Both animals were in good body conditions. The anatomopathological investigation of the first macaque revealed moderate serohaemorrhagic effusions in the thorax, abdomen and pericardial sac, hepatomegaly, splenomegaly, moderate gastritis and severe haemorrhagic enteritis. In the second macaque, hepatic degeneration and severe haemorrhagic gastroenteritis were observed. For both animals, histopathological findings in the small intestine consisted of thickening and shortening of the villi, dilated lacteals and infiltration of the lamina propria with lymphocytes, plasma cells and macrophages. Mild to moderate hyperplasia of the epithelium, oedema and lymphoplasmacytic infiltration of the lamina propria and multifocal crypt abscesses were observed in the large intestine. Standard bacteriological, virological and parassitological investigations were performed on fresh samples of liver and intestinal content. *Campylobacter* sp. were isolated from the intestine cultures of both animals and identified as *Campylobacter jejuni* by PCR [4]. No other significant pathogens were isolated. Our findings suggest that *C. jejuni* was the causative agent of disease in the examined animals. Similar anatomopathological and histopathological lesions have been described in infected NHP in the literature [3] and *C. jejuni* has been suspected to play a role in the aetiology of GBS in NHP [5]. Given the clinical history of the second macaque, a peripheral neuropathy consequent to campylobacteriosis could not be excluded, although histopathological findings consistent with GBS were not detected. For its pathogenicity and its potential role as a zoonotic agent, campylobacter infection represents a health concern in institutions housing primates. Further studies will help to better clarify the role of *C. jejuni* in the aetiology of gastrointestinal disease and to unravel the routes of infection and transmission in captive animals, in order to implement the health management of captive NHP colonies.

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THE DIAGNOSIS OF SARS-CoV-2 IN MAMMALS: AN IMMUNOHISTOCHEMICAL APPROACH

Katia Varello (1), Lucia Caterina Florio (1), Elena Berrone (1), Gloria Fornaro (1), Alessandro Benedetto (1), Dashzeveg Bold (2), Jessie Trujillo (2), Jürgen A. Richt (2), Robert Jan Molenaar (3), Cristina Casalone (1), Elena Bozzetta (1)

(1) Istituto Zooprofilattico Sperimentale del Piemonte, Liguria and Valle d'Aosta, Torino, Italy. (2) Department of Diagnostic Medicine/Pathobiology and Center of Excellence for Emerging and Zoonotic Animal Diseases (CEEZAD), College of Veterinary Medicine, Kansas State University, Manhattan, Kansas, United States of America. (3) GD Animal Health, Deventer, The Netherlands.

Corresponding author: E. Bozzetta (e.bozzetta@izsto.it)

The global spread of SARS-CoV-2 in human beings has been accompanied by naturally acquired infections through reverse zoonosis in pets, captive large felids, ferrets and minks [1]. In most cases, animals developed self-limiting, low grade respiratory diseases which did not require further veterinary intervention [2]. However, widespread infection in farmed mink has been reported associated with clinical disease and mortality [3, 4]. Furthermore, virus evolution in farmed mink led to the transmission of mink-adapted SARS-CoV-2 variants to humans. Thus, the surveillance on the emergence of SARS-CoV-2 infection in animals is pivotal to safeguard public health [5]. Aim of the work was to evaluate the feasibility as diagnostic tools of three anti-SARS-CoV-2 antibodies on different animal species and human samples comparing them to molecular diagnostics by Pan-Coronavirus PCR and specific SARS-CoV-2 Real-Time PCR.

Three antibodies, two polyclonal antibodies against the Nucleocapsid and Spike proteins and one monoclonal antibody against the-Spike protein, all developed at the Center of Excellence for Emerging and Zoonotic Animal Diseases at Kansas State University, were applied to paraffinized lung samples from naturally infected humans and experimentally infected hamsters, respectively, and to lung, lymph-node and nose tissues from confirmed naturally SARS-CoV-2-infected minks. Lungs from humans and cats, confirmed negative by molecular analysis, were included as negative controls. The samples were tested in immunohistochemistry with chromogenic and fluorescent detection. Subsequently, paraffinized organs (trachea, lung, spleen lymph-node, intestine) from 1 dog and 3 cats co-habiting with COVID-19 positive patients which died with SARS-CoV-2 suspected infections were also tested by the above antibodies and the molecular methods including: (i) a Pan-Coronavirus RT-PCR test developed to detect a broad range of Coronavirus Genera which includes capillary electrophoresis and Sanger sequencing of positive samples; and (ii) a SARS-CoV-2-sprecific real time RT-PCR assay.

All three SARS-CoV-2 specific antibodies gave positive results on positive control animals and humans and not on negative controls. In mink samples the three immunostaining protocols resulted in positive staining in bronchial and bronchiolar epithelium and in lung parenchyma, while it was negative on the other organs analyzed. The samples from suspected dogs and cats were negative by immunohistochemistry and with the two RT-PCR tests, except for a weak positive signal in the spleen from one cat using the real time RT-PCR test.

In summary, the SARS-CoV-2 specific monoclonal and polyclonal antibodies successfully detected SARS-CoV-2 in infected animal species and humans, thus representing reliable diagnostic tools in support of SARS-CoV-2 monitoring also in the post-pandemic period.

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Brachyspira hyodisenteriae AND *Trichuris suis*: TWO IS WORSE THAN ONE?

Jasmine Hattab, Francesco Mosca, Barbara Paoletti, Pietro Giorgio Tiscar, Giuseppe Marruchella

Università degli Studi di Teramo, Facoltà di Medicina Veterinaria.

Corresponding author: G. Marruchella (gmarruchella@unite.it)

Swine dysentery (SD) is caused by *Brachyspira hyodysenteriae* and still represents a major health issue in the modern pig farming. Clinical signs are most often observed in growing-finishing pigs, which develop loss of body condition and mucohemorrhagic diarrhea. Lesions are typically confined to the large intestine. The affected mucosa is covered by mucus, fibrin, necrotic debris and flecks of blood, while the intestinal lumen is filled by mucohemorrhagic content. Morbidity and mortality rates are strongly influenced by a numer of factors, such as concurrent infections by *Salmonella enterica* Serovar Typhimurium or *Lawsonia intracellularis*. Differential diagnoses include several diseases characterized by bloody faeces or diarrhea, such as gastric ulcer and proliferative enteritis [1].

We describe herein severe outbreaks of hemorrhagic diarrhea, which recently occurred in a small farrow-to-finish pig herd. In summer 2020, several cases of disease were observed in growing-finishing pigs and gilts, few weeks after the introduction of a new breeding stock. About ten pigs spontaneously died and were necropsied for diagnostic purposes. Grossly, lesions mainly affected the spiral colon, being highly suggestive of SD. Biomolecular tests (PCR) were carried out according to previously published protocols [2, 3] and allowed to detect *B. hyodysenteriae* genome, thus confirming the SD diagnosis. Parasitological investigations on stool samples yielded negative results. At this point, biosecurity measures and antimicrobial therapy (lincomycin medicated feed) were implemented to reduce *B. hyodysenteriae* spreading, clinical signs and mortality rate. As a result, no further severe clinical case of SD was observed for 15 months. However, in November 2021 hemorrhagic (even fatal) diarrhea reappeared in some growing pigs, which were housed in two adjacent pens; the administration of lincomycin was only partially effective, thus mimicking the onset of an antimicrobial-resistant strain. At necropsy, the spiral colon appeared enlarged and hyperemic. Surprisingly, a huge number of whipworms (*Trichuris suis*) were seen attached to the cecal and colonic mucosa, which was diffusely thickened and covered by mucus and necrotic debris. In one necropsied pig, PCR proved to be positive for *Brachyspira* spp., but negative for *B. hyodysenteriae* and *B. pilosicoli*. The pathogenic relevance of such *Brachyspira* spp., if any, should be further investigated. Worthy of note, affected pigs had spent a few weeks outdoor, where similar cases of trichuriasis occurred several years before. Accurate cleaning and disinfection of pens, along with anti-parasitic therapy (ivermectin per os), were effective and no further case of hemorrhagic diarrhea has been so far observed.

In our opinion, the events described herein stimulate some useful considerations: 1) planning and respecting strict biosecurity protocols is mandatory in the modern pig herds, even to limit the use of antimicrobials and the occurrence of antimicrobial-resistance; 2) necropsies are always useful to properly drive the diagnostic approach; 3) SD and trichuriasis share many clinical and pathological features, they can occur together, likely worsening each other; 4) although uncommon in conventional "indoor" herds, trichuriasis should be carefully consider in the differential diagnosis of SD.

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ROSTRAL MANDIBULAR OSTEOMA IN A HAFLINGER MARE

Raffaella Maggi (1), Douglas Mudimba (2), Giuseppe Marruchella (3)

(1) Veterinary Practitioner, Rome. (2) University of Namibia, School of Veterinary Medicine; (3) Università degli Studi di Teramo, Facoltà di Medicina Veterinaria.

Corresponding author: G. Marruchella (gmarruchella@unite.it)

Benign bone tumors are poorly documented and illustrated in veterinary medicine. The low prevalence of benign bone neoplasms, along with their favorable prognosis, do not stimulate in depth diagnostic investigations. As a result, morphologic criteria of classification are mostly borrowed from human medicine and arbitrarily applied in domestic animals [1]. Considering that, the present case report aims to highlight the main clinical-pathological features of equine mandibular osteoma.

In December 2021, a 11-year-old, Haflinger breed mare underwent clinical examination because of a lesion affecting the rostral portion of the mandible. Radiographic examination showed a well-demarcated and pedunculated mass, with no evidence of tooth root involvement or osteomyelitis. At this point, the lesion was surgically excised both for therapeutic and diagnostic purposes, promptly fixed in 10% neutral buffered formalin, decalcified in 10% EDTA solution and routinely processed for histopathological investigations (hematoxylin and eosin stain). Microscopically, the lesion mainly consisted of bone trabeculae, covered by a thick layer of connective tissue resembling the periosteum. The appearance of cells and extracellular matrix varied throughout the lesion. In some areas, trabeculae were formed by woven bone, with osteocytes residing in larger and irregularly arranged lacunae, while in other areas they took on the appearance of lamellar bone. A single layer of active osteoblasts usually lined the trabeculae, scattered osteoclasts being occasionally observed at that level. Bone trabeculae bordered bone marrow-like cavities, which were filled by fibrous connective tissue and/or adipose tissue. Focally, the trabeculae were very thick and obliterated the space among them, thus resembling the dense cortical bone. Overall, history, clinical findings, diagnostic imaging and pathological features allowed the diagnosis of mandibular osteoma. Up to date, the clinical outcome is fully satisfactory and no recurrence or complication has been observed.

Equine osteomas usually originate from the cranio-facial complex, in particular the paranasal sinuses and the rostral mandible, although other bones can be occasionally affected [2, 3]. Differential diagnosis includes several neoplastic and neoplastic-like conditions, which can affect the skull: e.g. ossifying fibroma, fibrous dysplasia, trauma-induced bone proliferation. Histopathological diagnosis is often challenging and questionable, as the above disease conditions share many microscopic similarities. Worthy of note, some pathologists consider that osteoma, ossifying fibroma and fibrous dysplasia might represent different stages of the same disease condition, with no clear-cut border existing among those entities [1]. Surgical excision still represents the best therapeutic option for osteomas and the prognosis is fair whenever the neoplasm is fully removed, that being affected by the localization and the size of the mass [1-3].

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IMMUNOHISTOCHEMICAL EXPRESSION OF P62 IN FELINE MAMMARY CARCINOMA AND NON-NEOPLASTIC MAMMARY TISSUE

Gian Enrico Magi (1), Francesca Mariotti (1), Lorenzo Pallotta (1), Alessandro Di Cerbo (1), Franco Venanzi (2)

(1) Università degli Studi di Camerino, Scuola di Bioscienze e Medicina Veterinaria. (2) CureLab Oncology. Inc. Dedham Boston, Massachusetts

Corresponding author: G.E. Magi (gianenrico.magi@unicam.it)

The p62 protein, also called sequestosome 1 (SQSTM1), is an ubiquitin-binding scaffold protein whose function arises at the crossroads between autophagy and apoptosis. In human oncology although several studies have been published the role of p62 in tumorigenesis is not very clear, a key role in autophagy-independent tumorigenesis has beene supposed for p62 (1). The results regarding the expression of p62 in various human cancers are heterogeneo us and in breast cancer p62 is overexpressed in malignant cells (2, 3). Few studies on p62 and cancer have been performed in veterinary medicine. The purpose of this study was to evaluate the immunohistochemical expression of p62 in 12 non-neoplatic mammary glands and in 38 feline mammary carcinomas graded based on the system proposed by Mills et al. (4) (grade I: 5 cases, grade II: 10 cases and grade III: 23 cases), to highlight how this protein is expressed and if there is a correlation with the degree of malignancy of the tumor. Samples were analysed by immunohistochemistry to determine the labelling expression of p62 using a rabbit polyclonal anti-p62 antibody (Sigma-Aldrich) with an avidin–biotin–peroxidase-complex (ABC) technique. Normal mouse pancreas was used as positive control, while for negative control rabbit IgG-isotype control was used instead of primary antibody. Labelling was evaluated by two author semi-quantitatively based on the percentage of immunopositive cells and staining intensity to obtain an immunoreactivity score. A total of 10 HPF were assessed for each sample. Scores between 1 and 2 were considered as low expression of p62, while those from 3 to 4 as intermediate and those greater than 4 as high expression. Score were statistically analyzed with a Mann-Whitney test. The differences were considered statistically significant at $P<0.05$. In this study feline mammary carcinomas had a statistically higher mean expression score for p62 than non-neoplastic mammary glands (8.2 vs 0.3). 28 cases (73.7%) had a high p62 expression score (≥5), three (7.9%) had a score between 3 and 4, while 7 cases (18.4%) had a low score between 1 and 2. Malignant neoplastic cells had cytoplasmic labelling of varying intensity, nuclear positivity was never observed as well as stromal cells positivity was not detected. Among the three histological grades of malignancies there was not statistically significant difference in terms of p62 score expression. All twelve cases of non-neoplastic feline mammary gland tissue were negative for p62: a diffuse negativity was observed in the epithelial cells of the mammary gland, only four cases had some lobules with a slight positivity. Positivity was never observed in the stromal cells. These preliminary observations could suggest a correlation between the expression of p62 and the carcinogenic process, since in carcinomas there was high expression of the protein as opposed to normal tissue. These results are in agreement with the data relating to breast cancer in woman. In fact, two studies demonstrated a higher expression of p62 in breast carcinomas compared to normal breast tissue (2, 3). This preliminary study represents the first approach in the field of feline oncology and the observation of a high p62 immunohistochemical expression in feline mammary carcinomas represents a first basis for considering p62 as a possible oncological target. Notably, our data are in agreement with that reported for woman breast cancer suggesting a possible similarity between human and feline p62.

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PRELIMINARY ASSESSMENT OF SURVIVIN EXPRESSION IN CANINE PERIVASCULAR WALL TUMORS

Francesco Godizzi (1), Damiano Stefanello (1), Patrizia Boracchi (2), Andrea Cappelleri (1, 3), Federico Armando (4), Giancarlo Avallone (5), Roberta Ferrari (1), Lavinia Chiti (1), Paola Roccabianca (1)

(1) University of Milan, Department of Veterinary Medicine and Animal Sciences (DIVAS). (2) University of Milan, Department of Biomedical and Clinical Sciences "Luigi Sacco". (3) Fondazione UniMi, Mouse and Animal Pathology Laboratory (MAPLab). (4) University of Veterinary Medicine, Foundation, Hannover (Germany), Department of Pathology. (5) University of Bologna, Department of Veterinary Medical Sciences (DIMEVET).

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

Survivin is a protein involved in regulation of cell division and apoptosis inhibition with a role in tumor development, progression, angiogenesis, metastasis and chemoresistance (4). Survivin expression correlates with aggressive disease and poor clinical outcomes in several human cancers, including soft tissue sarcomas (STSs) (2). Thus, survivin is a potentially useful prognostic marker and therapeutic target (4). Fragmentary data are available for survivin in canine tumors (3). Canine perivascular wall tumors (cPWTs) display a more favourable behaviour than other STSs (1). However, definition of specific prognostic factors is crucial to identify cPWTs with higher risk for relapse and those cases developing metastases. The aim of the study was to evaluate survivin expression and to explore its role as a potential prognostic factor in cPWTs. Survivin expression was evaluated by immunohistochemistry in 41 surgically excised cPWTs without distant metastases at the time of surgery, in relation to tumor histological grade, mitotic count, Ki67 index and clinical outcome. Nuclear survivin expression was directly correlated with Ki67 index. Higher nuclear survivin expression was observed in grade two and three tumors compared to grade one. Although non-statistically significant, increased nuclear and cytoplasmic survivin expression correlated with increased risk of local recurrence, metastasis, and death, considered altogether as a composite endpoint. The lack of statistical significance can be explained by the low power of statistical tests, due to the small sample size. This research identifies survivin as a potential prognostic factor and therapeutic target in cPWTs. However, further studies on larger set of cases are needed to confirm these results.

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CYTOLOGICAL FEATURES OF EQUINE AND FELINE DERMATOPHYTIC PSEUDOMYCETOMA: ASSESSMENT OF 6 CASES

Francesco Godizzi (1), Clarissa Zamboni (1), Silvia Colombo (2), Paola Roccabianca (1), Gabriele Ghisleni (3), Mario Caniatti (1)

(1) University of Milan, Department of Veterinary Medicine and Animal Sciences (DIVAS). (2) Studio Dermatologico Veterinario, Milan, Italy. (3) Clinical pathology consultant, Morbio Inferiore, Switzerland.

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

Dermatophytic pseudomycetoma (DP) is a rare, atypical, deep dermal to subcutaneous dermatophytosis, mainly caused by *Microsporum canis*, reported in cats (especially Persians) (3), horses (5), dogs (1), ferrets (4), and humans (2). Lesions are single to multiple, often ulcerated subcutaneous nodules occasionally oozing purulent material containing yellow granules. (1-5) Diagnosis is based on histopathology evidencing nodular to diffuse pyogranulomatous dermatitis encircling fungal hyphae surrounded by Splendore-Hoeppli, and on fungal culture. (1-5) Cytological descriptions of DP in domestic animals are scarce with one report in a Persian cat identifying pyogranulomatous inflammation, septate hyphae and arthrospores (6). The aim of this study is to describe the most representative cytological features of 1 case of equine and 1 case of feline DP confirmed by histopathology and fungal culture (*Microsporum canis*) and of 4 feline cases with typical clinical presentation and identical cytology. Fine needle aspirates were stained with May-Grunwald Giemsa and/or Periodic acid–Schiff (PAS). Cytological features included large aggregates of coarsely granular to filamentous dark blue material admixed with PAS-positive hyphae, pyogranulomatous inflammation, multinucleated giant cells and intracytoplasmic phagocytosed dark blue coarsely granular material in macrophages and multinucleated giant cells. Arthrospores were not clearly visible in all specimens, contrasting previous findings (6). Our results suggest that cytological findings, associated with clinical presentation are highly indicative of DP. Cytology with history and clinical presentation may represent a useful tool to diagnose DP thus avoiding biopsy.

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PARAOXONASE 1 (PON-1) ACTIVITY AS A SCREENING TEST IN PIGS AT SLAUGHTER

Donatella Scavone, Alessandra Nesossi, Eugenio Scanziani, Simone Stella Saverio Paltrinieri

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

Corresponding author: D. Scavone (Donatella.scavone@unimi.it)

In livestock, clinical information *in vivo* are often incomplete and measurement of acute phase proteins (APPs) before slaughtering has been proposed as a screening tool to identify animals requiring more accurate inspection of the carcass [1]. Paraoxonase-1 (PON-1) is an antioxidant enzyme and a negative APP, whose serum activity decreases in several diseases mostly characterized by inflammation and/or oxidation [2]. In this study, we assessed whether the measurement of PON-1 at slaughter may predict the presence and severity of lesions. Blood samples were collected during jugulation by percolation from 56 animals (39 sows, 9 piglets, 8 fattening hogs). For each animal, data regarding the age (piglets vs adults) and any information about external lesions or lesions detected during inspection of the carcass (with special attention for pulmonary and hepatic lesions) were recorded. Serum PON-1 activity was measured using a paraoxon-based method. Results recorded in pigs with or without lesions, or with different types and severity of lesions were statistically compared. Overall, 23 animals did not have any lesion, while 33 animals had lesions mostly located in the thoracic cavity (lung nodules with possible purulent or parasitic origin, n=15; chronic pleuritis, n=5; subacute or chronic pericarditis, n=4; subacute or chronic bronchopneumonia, n=2); extra-thoracic lesions were less frequent (milk spot, n=2; umbilical hernia, n=2; pelvic hematoma, n=1; hepatic necrosis, n=1; podal lesions, n=1). PON-1 activity in pigs with (mean±SD: 11.9±2.8 U/mL; median: 11.6 U/mL) or without lesions (12.3±2.2 U/mL, 12.3 U/mL) were not significantly different to each other (P=0.489). However, results from 8 pigs with lesions possibly associated with acute inflammation (10.18±2.67 U/mL; 9.60 U/mL) were significantly lower (P= 0.047), than those of pigs with chronic inflammation (12.52±2.64 U/mL 12.40 U/mL; P=0.019) and without lesions (P=0.027). The Receiver Operating Characteristic (ROC) curve was significantly different from the line of no discrimination (P=0.008) but with a moderate area under the curve (76.1%, 95% confidence interval: 56.6%-95.7%). Moreover, the ROC curve showed that when PON-1 activity is <9.9 U/mL, the specificity is 89.4%, the positive predictive value exceeds 90%, and the positive likelihood ratio is 5.22, although the negative predictivity is low (less than 50%) and the negative likelihood ratio remains close to 1.00. No significant differences were found when animals were classified based on the possible presence or absence of a systemic reaction associated with lesions or based on the site of lesions (intra- vs. extra-thoracic) or the number of pulmonary lesions (<2 vs >2). This study failed to demonstrate a role of PON-1 as a surrogate marker of lesions that may be used to optimize *post-mortem* analysis, possibly because PON-1 values were particularly low also in pigs without lesions. However, pigs with lesions potentially associated with acute and severe inflammation had lower PON-1 activity. Therefore, the detection of low PON-1 activity before slaughtering may suggest a more accurate inspection of the carcass, although the presence of lesions associated with acute inflammation cannot be excluded if PON-1 activity are not low. Further studies are needed to understand why the performances of PON-1 before slaughtering are lower than those of other APPs [3], possibly measuring PON-1 activity with substrates other than paraoxon, since in other species polymorphism of the PON-1 gene, associated with a prevalent lactonase or arylesterase activity has been demonstrated [4].

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TONGUE METASTASIS FROM LUNG CARCINOMA IN A CAT WITH 'FELINE LUNG-DIGIT SYNDROME'

Marcella Massimini, Paolo Emidio Crisi, Leonardo Della Salda, Mariarita Romanucci
Università degli Studi di Teramo, Facoltà di Medicina Veterinaria.
Corresponding author: (mmassimini@unite.it)

Feline lung–digit syndrome (FLDS) refers to a clinical entity in which primary lung tumours present because of metastatic lesions in one or more digits. Nevertheless, metastatic manifestations of primary lung tumours in cats may also variously involve eye, muscle, skin, vertebral bone and distal aorta [1]. On the other hand, to the best of our knowledge, tongue metastasis of feline tumours, including lung neoplasms, has not been reported so far, although lingual metastasis has been rarely described in human patients with lung cancer [2-4]. A spayed female, 15 years old domestic shorthaired cat was presented to the Veterinary Teaching Hospital of Teramo for anorexia and lameness. On physical examination, a whitish, 1.5 cm in diameter, nodule was observed on the third digit of the left forelimb, whereas a whitish, slightly raised, 0.5 cm in diameter, nodular lesion was detected on the dorsal surface of the tongue apex. Radiology of thorax and digits, and fine needle aspirate cytology of the digital lesion, allowed to achieve a definitive diagnosis of FLDS. Given the poor health condition and in agreement with the owners, the cat was euthanized. Necropsy exam revealed the presence of a whitish, about 2 cm mass in the middle right lung lobe, with partial involvement of the adjacent cranial part of the caudal right lung lobe. Multiple, whitish, 0.8-1 cm in diameter, sparse nodules were also observed on the spleen. Histological examination of the pulmonary mass revealed a proliferation of highly pleomorphic neoplastic cells, mainly arranged in a solid pattern, with admixed multifocal aspects of acinar or papillary growth, and characterized by multifocal features of squamous differentiation. Tumours cells showed large, pleomorphic nuclei, with multiple large nucleoli, and a high mitotic count (>20 mitosis/10 hpf – 2.37 mm^2). Extensive metastatic infiltration was observed in tracheo-bronchial lymph nodes. Nodular lesions of the digit, tongue and spleen were also characterized by a neoplastic proliferation of cells with similar features to those observed in the pulmonary mass. Gross and histopathological findings indicated a diagnosis of pulmonary carcinoma with squamous differentiation, associated with metastasis to the digit, tongue and spleen. Since feline primary lung tumours may be clinically silent in themselves, affected cats are usually presented because of metastases to extra-pulmonary sites. Although lingual metastases are rare, the present findings suggest to remind clinicians that metastatic manifestations of primary lung tumours in cats may variably involve not only digits, but also other multiple extra-pulmonary sites, including tongue.

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BOVINE SARCOSPORIDIOSIS: THE COMPARISON OF THREE METHODS OF COUNTING SARCOCYSTS IN MUSCLE SAMPLES OF SLAUGHTERED STEERS

Alessia L. Gazzonis (1), Luca Villa (1), Marta Paroli (2), Daniela Tripolini (3), Massimo Sinelli (2), Annalisa Guida (2), Giulia Sala (1), Maria Teresa Manfredi (1), Pietro Riccaboni (1)

(1) Università degli Studi di Milano, Dipartimento di Medicina Veterinaria e Scienze Animali. (2) Libero Professionista. (3) Distretto Veterinario Basso Lodigiano.

Corresponding author: P. Riccaboni (pietro.riccaboni@unimi.it)

Sarcocystis spp. (*Apicomplexa*: *Sarcocystidae*) is a genus comprising more than 200 species, whose life cycle includes two hosts: an intermediate one, with the development of muscle-based sarcocysts, and a definitive one, with intestinal infection. The bovine is the intermediate host of five species of *Sarcocystis*, two of which, *S. hominis* and *S. heydorni*, are of zoonotic interest having human being as final host [1]. Sarcosporidiosis in cattle is usually asymptomatic and no macroscopical lesions are present in muscle tissue. Therefore, any diagnostic method can be routinely applied at the slaughterhouse to prevent the potential risk for the consumer. With the aim of investigate on the prevalence of sarcosporidiosis and of evaluate three quantitative methods for the count of sarcocysts in histological sections, a survey was planned in cattle slaughtered in Lombardy.

Between January 2020 and March 2021, diaphragm samples were collected from 133 steers at a slaughterhouse in Northern Italy. This study on histological section was performed, on the basis of single sample dimension, applying 3 different methods: Method 1 - the number of sarcocysts per sample, was counted and expressed no considering area dimension; Method 2 - Area sample was manually measured; Method 3 - Area sample was automatically measured via the ImageJ software. Of the values obtained with the three methods, the respective medians were calculated, and compared by the non-parametric Wilcoxon signed-rank test.

The results obtained showed a prevalence of 35.11%. The number of sarcocysts found in the samples was very variable, from 1 to 19 per section. A statistically significant difference was highlitghed between the pure sarcocyst count in a sample (METHOD 1) and the sample area count calculated with both Method 2 and Method 3, as well as between Methods 2 and 3. It is therefore argued that to obtain an objective data regarding the density of infection it is preferable to express the value "number of sarcocysts" not with respect to the sample but with respect to the area of tissue examined.

In light of this data, the advantages and disadvantages of the two methods of measuring the areas were analyzed. Method 3, given the greater standardization, is preferable. Greater standardization of the counting method could represent a valid contribution to the inspection surveillance controls of bovine sarcosporidiosis.

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SEX-DEPENDENT MODULATION OF CAERULEIN-INDUCED ACUTE PANCREATITIS IN C57BL/6 MICE

Luca Bertola (1,2), Giovanna Pepe (3), Arianna Dolce (3), Andrea Cappelleri (1,2), Simone Canesi (1,2), Pierangelo Moretti (1), Alessia Giordano (1), Eugenio Scanziani (1,2), Elisabetta Vegeto (3), Camilla Recordati (1,2)

(1) Università degli Studi di Milano, Dipartimento di Medicina Veterinaria e Scienze Animali. (2) Fondazione Unimi, Mouse and Animal Pathology Laboratory. (3) Università degli Studi di Milano, Dipartimento di Scienze Farmaceutiche.

Corresponding author: Luca Bertola (luca.bertola@unimi.it)

Acute pancreatitis is a sudden life-threatening condition caused by release and activation of acinar enzymes leading to the digestion of the gland, characterized by an increasing prevalence worldwide(3, 6). Although in humans the overall incidence of pancreatitis seems not to differ significantly between sexes, the rate of some forms of pancreatitis as well as pancreatic-related mortality has been found higher in male patients (1). The role of gender in pancreatitis has been investigated using different mice models leading to contrasting results: some studies detecting sex-dependent differences in pancreatitis extent and severity while others being unable to detect such differences (4,5). The aim of this study was to investigate sex-dependent differences in a mouse model of caerulein-induced acute pancreatitis by histology and clinical chemistry. A total of 36 C57BL/6 mice (19 females and 17 males) were treated intraperitoneally with PBS (14 mice) or caerulein (22 mice) (70 µg/kg, 8 times a day for 2 consecutive days) (2) and sacrificed 12 hours, 48 hours and 7 days after last caerulein injection. Blood from each animal was collected from the heart and glucose concentration, and lipase and amylase activity were measured on serum. Pancreas were fixed in 10% neutral buffered formalin and routinely processed for histopathological examination. Pancreatitis was graded histologically for severity by using a semi-quantitative grading system including pancreatitis extension, interstitial edema, interstitial inflammation, and acinar single cell necrosis/apoptosis. Glucose and lipase levels were not affected by pancreatitis in both sexes, while amylase was increased at 12 hours followed by a rapid decrease at 48 hours, without relevant differences between sexes. Histologically, even though no significant differences in severity of pancreatitis were found between males and females, the timing of pancreatitis was different between sexes: in females the peak of pancreatitis was at 12 hours, with a rapid decrease at 48 hours, and an almost complete recovery at day 7, while in males the peak was delayed at 48 hours, with residual acinar single cell necrosis/apoptosis at day 7. The results suggest a sex-dependent modulation of pancreatitis, with females exhibiting a faster onset of pancreatitis but an increased regenerative capacity as compared to males.

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EPITHELIAL-MESENCHYMAL-TRANSITION (EMT): ROLE OF "CADHERIN SWITCH" IN PROMOTING TUMOR METASTASIS IN CANINE MAMMARY GLAND CARCINOMAS

Anna Maria Cantoni (1), Benedetta Passeri (1), Rosanna Di Lecce (1), Chiara Guarnieri (1), Concetta Nigro (1), Luca Ferrari (1), Federico Armando (2), Attilio Corradi (1)

(1) University of Parma, Department of Veterinary Science, Parma, Italy. (2) University of Veterinary Medicine, Foundation, Department of Pathology, Hannover, Germany.

Corresponding author: B. Passeri (b.passeri@unipr.it)

Epithelial-Mesenchymal-Transition (EMT) is a fundamental step during tumor metastasis. EMT process allows epithelial neoplastic cells to modify in order to enhance mobility and invasiveness to favor the metastatic process. One hallmark of the EMT process is the so-called "Cadherin switch" that consists of a gradual replacement of the adhesion molecule E-cadherin by the typical mesenchymal cell protein N-cadherin [1]. Recently, the EMT process has been thoroughly investigated due to its negative prognostic value in carcinomas [2]. The aim of the current study is to investigate the "Cadherin switch" in metastatic and not metastatic canine mammary gland carcinomas, comparing them with benign lesions of the mammary gland in dog.

Thirteen cases of canine mammary gland carcinomas and 5 cases of benign lesions were recruited. 5/13 tumors were metastatic and additional samples from different metastatic localizations were collected during necropsy. All samples were from cross-breed unspayed bitches, aged from 8 to 13.5 years old. Samples were fixed in buffered formalin, pH 7.4, and FFPE sections were processed for routine histology and immunohistochemistry (IHC) for E-Cadherin, N-Cadherin, and Vimentin. Antibody dilutions and incubation times were performed according to the manufacturers' recommendations. The immunolabeling evaluation was carried out using an optical microscope. In all samples, a semi-quantitative score system to detect positive cell percentages and stain intensity was performed as follows. The percentage of positive cells was counted in 10 fields at 40X, for a total of 100 counts. Immunolabeling intensity was assessed using a scale from 0 to 3: absent (0), weak (1), moderate (2), intense (3).

E-Cadherin expression was intense in the membrane and cytoplasm of the physiological, hyperplastic and benign neoplastic epithelial cells, while membrane immunopositivity appeared moderate or intense even in tubulo-papillary carcinomas. E-Cadherin showed a predominantly cytoplasmic localization, weak or moderate, in simple or complex solid carcinomas and squamous carcinomas. The membrane and cytoplasm of the physiological, hyperplastic and benign neoplastic epithelial cells were immunonegative for N-Cadherin, while a weak to moderate cytoplasmic positivity in the epithelia of simple or complex solid primary carcinomas and a weak nuclear expression in comedo-carcinomas and squamous carcinomas were detected. N-Cadherin expression appeared weak or absent in epithelial metastatic cells.

Vimentin expression was absent in mammary gland physiological epithelial cells, hyperplastic cells and in benign tumor lesions. A weak membrane positivity and a moderate cytoplasmic positivity were detected in primary carcinomas (simple and complex solid carcinomas and squamous carcinomas) affecting more than 10% of tumor cells.

The experimental data are in accordance with the "Cadherin switch" reported in veterinary oncology literature. For better defining the immunoprofile of the cells in a dynamic evolution in the "Cadherin switch" process, it could be useful to perform a double staining for E-Cadherin/N-Cadherin and N-Cadherin/Vimentin, using a Confocal Microscope (CLSM), as well as a western blotting analysis for N-Cadherin expression in primary neoplastic epithelial cells of mammary gland and in metastatic epithelial cells.

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UTERINE FINDINGS IN PANTELLERIA JENNIES

Alessandra Sfacteria, Gabriele Marino, Salvatore Monti, Giada Giambrone, Stefania Di Giorgio, Giuseppe Catone, Michele Panzera

Università degli Studi di Messina, Dipartimento di Scienze Veterinarie.

Corresponding author: Alessandra Sfacteria (asfacteria@unime.it)

The donkey of Pantelleria is an autochthonous breed of Sicily counting about 60 animals. Due to the excessive inbreeding rate, matings are strictly monitored and often not authorized. On the other hand, many jennies are quite aged and pregnancy losses reach unacceptable rates. As result, almost all the Pantelleria jennets were barren at the start of the reproductive season of 2022. In this study, 24 barren jennets (almost the entire adult female population) were gynecologically examined in March 2002. The mean age was 15 years (range 9-26 years). The animals had a good temperament but had been only occasionally visited in the past. Sedation of acepromazine (0.03 mg/kg) and detomidine (0.01 mg/kg) was performed to fasten the procedures and avoid distress to the animal. At the ultrasound, 21/24 jennets were cycling, 1 was in anoestrus and 2 were in a transitional period. Four on 22 were found in oestrus, 17 in dioestrus. Double and generally asynchronous corpora lutea were found in 6/17 jennets. Among uterine findings, cysts were found in 3/24, fluid in 1/24 jennets. The asinine cervix was very long, narrow, and sometimes laterally deviated. It was considered stenotic in 2 on 24. However, after a short training, operators were able to access the cervix, using delicate movements of the fingers. The insertion of a bacteriological swab was the first examination. Leaving the external sheath of the swab, cytobrush was inserted secondly to collect a sample for cytology and finally, an alligator Kenney forceps was inserted to collect a biopsy of the uterine body. The uterine culture was positive in 2 cases (*St. epidermidis* and *E. coli*). Cytology allowed a collection of epithelial cells in different secretory phases: a tall epithelium was detected during oestrus and dioestrus, cubic cells characterized the anestrus and transitional period. Inflammatory cells were detected including eosinophils, neutrophils, lymphocytes and macrophages, but a diagnosis of endometritis was not possible following the mare's guidelines. Red blood cells were constantly observed because of the prolonged manipulation of the cervix. Uterine biopsies were evaluated according to the mare's classification [1]. Haematoxylin-eosin and Masson's trichrome were routinely used to evaluate the degree of inflammatory infiltration and fibrosis of the uterine glands. Grade I endometrial alterations (not significant) were observed in 13 cases, grade IIa (mild alterations) in 8 cases and grade IIb (moderate alterations) in 3 cases. The uterine findings reported in barren Pantelleria jennies may reflect the low foaling rate of the population that certainly requires strict management. However, we are not sure that the prognostic factors applied to the mare are exactly transferrable to the donkey. To our knowledge, this is one of the few studies on the uterine environment in donkeys [2, 3]. The approach to this species requires a minimum of training, but many pieces of information can be collected. Especially biopsies were able to reveal chronic endometritis and fibrosis in almost half of the studied population. Although the cervix during oestrus is more accessible, the optimum time for biopsy collection should be dioestrus, to avoid that oedema and an increased population of inflammatory cells may interfere with pathologist diagnosis.

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OUTBREAK OF INTRANUCLEAR COCCIDIOSIS IN YOUNG HERMANN TORTOISES (Testudo hermanni hermanni)

Livio Galosi (1), Rachel E. Marschang (2), Ranieri Verin (3), Lucia Biagini (1), Andrea Piccinini (4), Danilo De Bellis (1), Subeide Mari (1), Valentina Grifantini (1), Giacomo Rossi (1)

(1) Università degli Studi di Camerino, Scuola di Bioscienze e Medicina Veterinaria. (2) Laboklin GmbH & Co. KG, Bad Kissingen, Germania. (3) Università di Padova AGRIPOLIS, Dipartimento di Biomedicina Comparata e Alimentazione. (4) Università di Teramo, Facoltà di Medicina Veterinaria.

Corresponding author: L. Galosi (livio.galosi@unicam.it)

Intranuclear coccidiosis (TINC) is an emerging disease that has been reported in a wide variety of chelonian species, most commonly in radiated tortoises (*Astrochelys radiata*), but detections in Mediterranean tortoises (*Testudo* spp.) are extremely rare, and their pathogenicity in these species has not been well documented [1, 2]. In a mixed collection of Mediterranean tortoises, a 2-year-old spur-thighed tortoise (STT, *Testudo graeca graeca*), purchased from another farm and clinically healthy, was introduced to a fenced enclosure with 74 young Hermann's tortoises (HT, *Testudo hermanni hermanni*) during the summer. The HT were between 1 and 3 years old and born on the farm. The STT was kept in a terrarium during the following winter and an additional 2 specimens were purchase from the same farm, while the HT hibernated naturally in their enclosure. In spring, one of the STT manifested non-specific clinical signs (anorexia, weight loss, lethargy) and yellowish plaques in the oral cavity. After 2 weeks of supportive care, the animal died and a full necropsy was performed. PCR analysis for herpes- and picornavirus gave negative results. At the same time, the breeder noted that several HT had not survived natural hibernation, and 37 surviving HT suddenly died from the spring to the summer, with no clinical signs observed. A complete necropsy was performed on 4 HT, and molecular analyses were carried out on liver and oral swabs of 10 animals. Histopathological analysis indicated the presence of TINC, accompanied by lymphocytic or lymphoplasmacytic inflammation and variable degrees of necrosis. The coccidia, most often detected in the nucleus of infected cells, were most abundant in the kidney and liver, but could be readily found in other tissues such as pancreas, intestine, and spleen. No parasite stages have ever been seen in the cytoplasm of various infected cells types. PCR confirmed the presence of TINC in the colony. This report is the second of TINC in HT and the first detailing a disease outbreak associated with the infection in this species. In order to prevent the spread of this pathogen in breeding colonies of the endangered HT, new animals should be screened for this pathogen and uncontrolled introductions should be avoided.

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AIVI

ANTIMICROBIAL RESISTANT E. COLI IN PORK AND WILD BOAR MEAT: A RISK TO CONSUMERS

Silvia Cavallo (1), Laura Andriani (1), Martina Rega (1), Paolo Bonilauri(2), Mauro Conter (1), Silvia Bonardi (1) Cristina Bacci (1)

(1) Università degli Studi di Parma, Dipartimento di Scienze Medico-Veterinarie. (2) Istituto Zooprofilattico Emilia Romagna e Lombardia- Sede Reggio Emilia.

Corresponding author: Rega M. (martina.rega@unipr.it)

Antimicrobial resistant (AMR) foodborne and zoonotic pathogens can be transmitted from food producing animals to humans through the consumption of meat products thereof. The risk is even higher if the microorganisms are able to produce biofilm [1]. The aim was to evaluate prevalence of these AMR determinants in *E. coli* isolates from pork (n=106) and wild boar meat (n=113) in Emilia-Romagna region (Italy). Given the importance of pig meat, we tested *E. coli* strains isolated from pork for resistance to ciprofloxacin and nalidixic acid (quinolones), amikacin (aminoglycosides) and meropenem (β-lactams). In addition, since the environmental spread of AMR bacteria can be a source of contamination in wildlife [2], *E. coli* isolates from wild boar meat were included in the study. Since several studies reported the co-presence of AMR-associated plasmidic genes, their simultaneous presence was tested [3]. Taking also into account the risk of biofilm production in the food chain, the ability of AMR *E. coli* to synthetize biofilm was evaluated [4]. All the isolates were tested by the Kirby Bauer assay to assess their AMR profile. All *E. coli* strains with a phenotypic resistance profile were tested by PCR for the detection of the correspondent plasmidic resistance genes, namely chromosomal *gyrA* and *parC* mutations, plasmid *qnr* genes (quinolones), *Aac(6′)-Ib*, *Aac(3)-II*, *Ant(3″)-Ia*, *Aph(3′)-Ia* (aminoglycosides), bla_{KPC}, bla_{VIM}, bla_{IMP}, bla_{NDM}, $bla_{OXA\text{-}48\text{-}like}$ (β-lactams) [5-8]. No strains exhibited phenotypic or genotypic resistance to meropenem. In case an isolate was found positive for one plasmid resistance gene, it was tested for all the others to evaluate their simultaneous presence even in absence of phenotypical resistance. Odds Ratio was used to statistically confirm their correlation. Our results showed a higher prevalence of AMR strains in pork than in wild boar meat (50% and 40.7%, respectively). Resistance to quinolones was found in 45.3% and 40.7% of *E. coli* isolates from pork and wild boar meat, respectively. Amikacin resistant strains were 9.4% in pork and 1.8% in wild boar meat. Between the two animal species, the difference in AMR prevalence was statistically significant. The harbouring of resistant genes was higher in porcine *E. coli* than in isolates from wild boars. Statistical analyses showed a positive correlation between the simultaneous presence of quinolone and amikacin resistance genes only in *E. coli* isolates from pork. The ability of AMR *E. coli* to produce biofilm was observed in 86.9% of the wild boar strains and 56.6% of the porcine ones. In conclusion, contamination by AMR microorganisms in pork and wild boar meat could be a threat for the consumer, especially if biofilm-producing strains colonize surphaces and equipments in the food industry.

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CORRELATION BETWEEN PESTICIDE USE IN VITICULTURE AND BEE HEALTH USING A NEW BIOMARKER: A PRELIMINARY STUDY

Paola Mogliotti (1), Annalisa Garrone (1), Cecilia Guasco (1), Eliana Trabunella (2), Patrizia Garbati (3), Paola Ghisellini (3), Cristina Rando (3), Roberto Eggenhoffner (3)

(1) Istituto Zooprofilattico Sperimentale Piemonte, Liguria e Valle d'Aosta, Regional Reference Centre for Bees, Asti, Italy. (2) Istituto Zooprofilattico Sperimentale Piemonte, Liguria e Valle d'Aosta. Epidemiological Observatory, Turin, Italy.

(3) Department of Surgical Sciences and Integrated Diagnostics, University of Genova, Genova, Italy.

Corresponding author: C. Guasco (cecilia.guasco@izsto.it)

Bees are closely linked to human wellbeing by influencing the ecosystem, crop productivity, food security and the reproduction of wild plants (1). In the last decade, a considerable decline in bee colonies has occurred and several studies have established that a variety of factors are involved, including climate change, agricultural practices and pesticide use (2). Piedmont is a highly productive region in terms of beekeeping, moreover it has a strong agricultural vocation; one sector of primary importance is viticulture. In this field, the use of phytosanitary products has increased since the 1990s, with the spread of phytoplasmosis of the vine, known as flavescence dorée and carried by an insect, the *Scaphoideus titanus* (*S. titanus*) (3). Vitellogenin (Vg) plays important roles in protection against oxidative stress, wound healing and insect immunity, with strong activity against bacteria and other pathogens (4). Previous studies have shown that Vg can be used as a biomarker to evaluate bee physiology and health, since its expression can be influenced by different forms of stress, indeed the Vg gene undergoes selection driven by local pathogenic pressures. In terms of the functions played by Vg, the bee is one of the most studied species. Therefore, bees provide a practical and useful model for studying Vg function (5). The study aims to assess the correlation between the use of pesticides in viticulture against *S. titanus* and bee health by detecting Vg as a specific biomarker. Three apiaries close to vineyards were identified (A1, A2, A3); one of them was surrounded by certified organic vineyards (A3). After collecting information on the beekeepers, the apiaries and the surrounding vineyards, three samples were taken: the first one in the period immediately following the treatment of the vine, in August (t0), the second one 15 days later (t15) and the third one 30 days after the treatment against *S. titanus*, between September and October (t30). During all visits, 30 live bees were collected from honeycombs, which were stored at -80°C. The Vg level determination was performed by RT-PCR, using the RNeasy Power Soil Total RNA kit (Qiagen) for total RNA extraction, while DNAase treatment and cDNA synthesis were obtained with the QuantiNova reverse transcription kit (Qiagen). The primers used for the quantification of the Vg expression level were: Forward GCAGAATACATGGACGGTGT and Reverse GAACAGTCTTCGGAAGCTTG (5). The Sybr Green enzyme used in the real-time RT-PCR analysis was provided by the SensiFASTTM Sybr Lo-ROX kit (Bioline). Reactions were conducted using CFX Connect™ real-time PCR detection equipment (BioRad). Relative transcript levels were determined using a standard curve constructed from dilutions of stock cDNA. These data were then compared to the target amount of the housekeeping gene (actin) (6). Results show that the concentration of Vg is significantly higher in October than in August in apiaries surrounded by conventional vineyards (A1 and A2). In the third apiary (A3), however, the October concentration was only slightly higher than in August. Considering the results achieved, it appears that the apiary surrounded by organic vineyards had lower concentrations of Vg than the other two. Consequently, it is reasonable to conclude that treatments against flavescence dorée in organic vineyards have less impact on bee health, both in the short and long term. However, further studies will be needed to confirm this preliminary result.

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IN-HOUSE VALIDATION OF AN ARTIFICIAL DIGESTION PROTOCOL FOR THE DETECTION OF ANISAKIDAE LARVAE IN FISH PRODUCTS ACCORDING TO THE ISO 23063-2:2021

Emanuela Bacchi (1), Gaetano Cammilleri(1), Vito Macaluso(1), Gaspare butera(1), Francesco Giuseppe Galluzzo (1,2), Vincenzo Ferrantelli (1)

(1) Istituto Zooprofilattico Sperimentale della Sicilia, Centro di Referenza Nazionale per le Anisakiasi. (2) Università degli Studi di Modena e Reggio Emilia, Dipartimento di Scienze della Vita.

Corresponding author: E. Bacchi (bacchiemanuela@gmail.com)

Anisakis is a parasitic nematode, of the family *Anisakidae* that infects fish and marine invertebrates, including crustaceans and mollusks. Ingestion of parasitized raw fish with L3 larvae can cause acute gastrointestinal illness; additionally, infection can be accompanied by allergic reactions such as hives, angioedema, and anaphylaxis.

The EFSA (European Food Safety Authority) scientific report (2010) says that, based on current knowledge, for wild-caught fish, no sea fishing area can be considered free of Anisakis larvae.

EFSA, considering the extent of infestation of fishery products from all seas, encourages alternative research methods for Anisakis, including chloropeptic digestion.

The aim of the study is the validation of an artificial digestion based on ISO 23063-2:2021 for the detection of L3 larvae belonging to the family *Anisakidae* .

The method's specificity was calculated with forty samples of farmed sea bream, recognised as free of anisakid parasite infestation according to literature. The samples were preliminarily examined by visual inspection for *Anisakidae* larvae detection, particularly, portions of fish corresponding to lower belly flap, upper belly flap, lower epaxial muscles, upper epaxial muscles, and tail muscles were examined. Then the samples were subjected to a digestive method based on the ISO 23036-2:2021.

A digestion solution containing 2 L of water with 11 ml of HCl≥35% and 10 g of pepsin 1:10.000; 50±5 g of sample to be tested with a digestion time of 40 min at a temperature of 36±1°C were considered the best condition for reliable results. The method's sensitivity was calculated with forty samples of farmed sea bream and sea bass artificially infested with live Anisakis morphotype I larvae identified by light microscopy.

Following digestion, the viability of the larvae was verified by testing motility in acid solution containing acetic acid according to ISO 23036-2:2021.

The analyses performed resulted in a specificity value of 100% and a sensitivity value of 100%, with a Limit of Detection (LOD) of one larva. In addition, undigested cases turn out to be negligible. The results of the in-house validation protocol showed that the chloropeptic digestion method carried out is extremely accurate, therefore, this method can be used as confirmation after screening by visual inspection method.

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SEASONAL TREND OF ANISAKIDAE INFESTATION IN SILVER SCABBARDFISH COLLECTED IN SOUTH MEDITERRANEAN

Emanuela Bacchi (1), Antonio Vella (1), Antonello Cicero (1),Giuseppe Giangrosso (1),Gaetano Cammilleri (1), Maria Drussilla Buscemi (1), Andrea Macaluso (1), Francesco Giuseppe Galluzzo (1, 2), Vincenzo Ferrantelli (1), Stefano Vullo (1)

(1) Istituto Zooprofilattico Sperimentale della Sicilia, Centro di Referenza Nazionale per le Anisakiasi. (2) Università degli Studi di Modena e Reggio Emilia, Dipartimento di Scienze della Vita.

Corresponding author: E. Bacchi (bacchiemanuela@gmail.com)

The nematodes belonging to the *Anisakidae* family are parasites of interest for human health due to their high zoonotic potential. These nematodes if ingested alive with raw fish can cause Anisakiasis, a zoonoses which can compromise the entire gastro-intestinal tract, with several symptoms such as nausea, vomiting and gastroesophageal reflux. A recent EFSA report (2010) urges agencies involved in food safety to get more information about the biology and ecology of these organisms, in order to implement the prevention tools for consumer's protection. Anchovies are one of the most infested fishes by *Anisakidae* larvae. Furthermore fishes belonging to this species are prevalently consumed uncooked (marinades). In this study it was evaluated the seasonal trend of *Anisakidae* infestation in silver scabbardfish (*Lepidopus caudatus*), in order to assess a possible correlation between fish ecology and infestation degree. A total of 60 *L. caudatus* samples were analysed for the *Anisakidae* larvae detection by visual inspection. The sampling was carried out during the whole of 2019, in order to obtain a balanced number of samples for each month. The collected larvae were subjected to morphological (by optical microscopy) and molecular (by RFLP-PCR method) investigation in order to confirm the belonging to the *Anisakidae* family. All the *L. caudatus* samples examined verified the presence of *Anisakidae* larvae, with an overall infestation prevalence of 100% and a mean intensity of 157.67. The infestation prevalence was divided monthly. Given the constant value of prevalence for the silver scabbardfish (100%), we decided to assess the seasonal trend according to the mean intensity values. For the silver scabbardfish a bimodal trend with a peak during March (m.i. = 302.5) and November (m.i. = 250) was found. No statistical differences between sampling seasons were verified (p-value = 0.5064). This significant difference can be attributed to the ecology of the species. As far as we know, this was the first ecological study on the silver scabbardfish population of South Mediterranean Sea. This gives interesting epidemiological data on the prevalence of Anisakis infestation in south Mediterranean fish. The findings seem to confirm the role of Anisakis as ecological tag to deepen the ecology of their hosts as confirmed previously for other species.

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HISTAMINE CONCENTRATION ASSESSMENT IN FISH SAMPLES COMMERCIALISED IN SICILY

Andrea Macaluso (1),Antonio Vella (1), Antonello Cicero (1),Giuseppe Giangrosso (1),Gaetano Cammilleri (1), Emanuela Bacchi (1), Licia Pantano (1), Maria Drussilla Buscemi (1), Vito Macaluso (1), Francesco Giuseppe Galluzzo (1, 2),Vincenzo Ferrantelli (1)

(1) Istituto Zooprofilattico Sperimentale della Sicilia, Centro di Referenza Nazionale per le Anisakiasi. (2) Università degli Studi di Modena e Reggio Emilia, Dipartimento di Scienze della Vita

Corresponding author: G. Cammilleri (gaetano.cammilleri86@gmail.com)

Histamine poisoning is one of the most common form of intoxication caused by seafood consumption. Histamine is a decarboxylation product of histidine, the reaction is catalyzed by histidinedecarboxylase which is found in some bacterial species. This reaction occurs with an unsuitable conditions of fish storage, even in absence of typical signs that would indicate the non-edibility of the product. Mackerel (*Scomber scombrus*), sardine (*Sardina pilchardus*), anchovies (*Engraulis encrasicolus*) and Tuna fish (*Thunnus thynnus*) are the fish species more involved, caused by their rich free-form histidine concentration in meats. Recently in Sicily the consumption of tuna and other fish species has been subject of hundreds cases of food poisoning. The intake of high concentrations of histamine leads to an onset of symptoms called Scombroid Syndrome. A recent report of the European Autority of Food Safety (EFSA) described that the consumption of food containing higher amounts of toxic biogenic amine(s) may cause food intoxication and indicates the need for a better hygiene process and other controls. Member States informed the EFSA that findings of certain levels of toxic biogenic amines (BA) in fermented food could be of concern and reported a recent increase of biogenic amines content in some fermented foods. Presently, only high-performance liquid chromatography (HPLC)-based methods enable simultaneous and high sensitivity quantification of histamine in foods, hence are best suited for monitoring and control purposes. In this study it was evaluated the level of histamine in Sicilian tuna by HPLC DAD method. A number of 627 fish samples were examined in 2018 for the detection of Histamine concentration at the laboratories of Chemistry and Food Technology of the Zooprophylactic Institute of Sicily. All the samples belonging to the species *Scomber scombrus, Sardina pilchardus, Engraulis encrasicolus* and *Thunnus thynnus* and are analyzed with an HPLC DAD method. It was weighed 10±0.1g of samples for the extraction and put in a 50 ml solution with 6 ml of 6% perchloric acid and water. The extract was injected into an Agilent UPLC 1290 for the histamine concentration assessment. Results showed a histamine positive sample rate of 12.6% (79 of 627), according to the limits prescribed on the EC Reg 2073/2005 and subsequent amendments. In the course of the year, the trend of positive samples registered a significant increase during summer period (from May to July), corresponding to the increased activity of fresh tuna fishing. This increase, combined with the high temperatures and bad storage techniques, have led to an increase of histamine positivity cases. Consequently, there has been an increase of scombroid syndrome cases due to consumption of fish. A special case of positivity was observed during December. The samples tested were from a batch of tuna caught in India. This positivity can be attributed to bad techniques of storage and transport of products.

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FOURIER TRANSFORM INFRARED (FTIR) SPECTROSCOPY TO DISTINGUISH AMONG FRESHLY MILKED, KEPT AT REFRIGERATION TEMPERATURE, AND FROZEN MILK, A PRELIMINARY WORK

Carlotta Ceniti (1, 2), Anna Antonella Spina (1, 2), Domenico Britti (1, 2) Valeria Maria Morittu (1, 2)

(1) University Magna Graecia of Catanzaro. (2) Interdepartmental Services Centre of Veterinary for Human and Animal Health, Department of Health Science, Magna Græcia University, Catanzaro, Italy.

Corresponding author: C. Ceniti (ceniti@unicz.it)

Milk has always been considered a fundamental component of the human diet and Italy is one of the leading milk producers in Europe [1]. As regards the timing of heat treatment, our legislation defines "Pasteurized fresh milk" or "high quality pasteurized fresh milk" milk that arrives raw at the packaging plant and which, there, is subjected to thermic processing within 48 hours of milking [2]. Mid Infrared Technology is a rapid tool to quantify milk components such as fat, casein, lactose, and protein working on the interaction between physical matter and electromagnetic radiation in the region between 900 and 5,000 cm-1. Mid-infrared technology was applied to investigate the heat treatment of milk [3], but to the best of our knowledge, nobody investigate storage at low temperatures of fresh milk. In the current study, Fourier Transform Infrared (FTIR) spectroscopy, in combination with principal component analysis (PCA), was applied to spectral averages to distinguish among cow milk freshly milked, stored at 4°C for 7 days, and at -20°C for 7 days. Bulk tank milk samples were collected from 8 different farms located in Calabria, from January to May 2021 and were immediately delivered at 4°C to the Laboratory of the University of Catanzaro. The raw spectra were exported from Milkoscan FT+ software to CSV format. All spectral data were elaborated by the TQ AnalystTM software ver. 8.0 (Thermo Fisher Scientific Inc., Madison, WI), to create a calibration and a validation model based on PCA. As calibration standard, the 18 spectra belonging to fresh, refrigerated, and frozen samples were randomly chosen from 6 farms. The other 6 spectra from the remaining 2 farms were used as independent validation standards. The qualitative composition of milk was also displayed. Only spectral regions containing information related to milk composition were retained for spectral analysis, (3,000-2,800 cm-1; 1,800-1,680 cm-1; 1,600-925 cm-1) regions, as suggested by Bahadi 2021 [4] after processing spectra using a combination of mean center and second derivate with Norris filter. A PCA model was built using 10 Principal components which explained 99.97% of the variance. Our results showed that 4 samples were misclassified. These preliminary findings suggest that FTIR combined with PCA could be a successful strategy to discriminate among milk subjected to different storage treatments. The milk FTIR spectra may provide useful information to verify the times and methods of cold storage of milk, and therefore, the acceptability of milk, in the context of food safety.

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BIOFILM FORMING ABILITY OF DAIRY *BACILLUS* SPP. ISOLATES ON POLYSTYRENE AND STAINLESS STEEL

Angela Maria Catania, Alessandra Dalmasso, Pierluigi Aldo Di Ciccio

Università degli Studi di Torino, Dipartimento di Scienze Veterinarie.

Corresponding author: A.M. Catania (angelamaria.catania@unito.it)

Biofilm formation represents one of the most effective forms of bacterial persistence in surfaces where nutrients are available, such as in the food-processing equipment [1]. The biofilm allows bacteria to better resist harsh environmental conditions [2]. The ability of several bacteria to protect themself from the action of common cleaning agents and disinfectants is very alarming. The production of biofilm in one of the most common strategies adopted by bacteria responsible for food spoilage and foodborne diseases. For this reason, biofilms can be responsible to economic losses and may have a health impact if contamination of pathogenic bacteria occurs.

Bacillus spp. can form biofilm on most surfaces and under almost all the environmental conditions found in dairy industries [3].

In this work, we investigated the biofilm forming ability of *B. cereus* and *B. subtilis*, isolated from dairy products. Firstly, a micro-method screening assay was performed by using two grow media (Brain Heart Infusion, BHI – Tryptone Soy Broth, TSB) at two incubation time (24-48h). Results showed that all tested strains were able to produce biofilm after 24h, with difference statistically significative depending on medium, with best results achieved in BHI. Then, biofilm formation of isolates was assessed in macro-method on polystyrene (PS) and stainless steel (SS). Reference strains (*B. cereus* ATCC 14579 and *B. subtilis* NCTC 3610) were included.

For all tested strains (except ATCC 14579) biofilm formation was higher on PS than SS. Anyway, the determination of viable cell numbers by plate count (CFU) didn't' show a correlation with the total biomass of biofilm.

Bacillus spp. are one well-known biofilm-forming bacteria capable of colonizing dairy industry facilities leading to recurrent contamination of dairy products. Considering the concerns for food safety and food quality associated with *Bacillus* spp. contamination in dairy products, a better knowledge of the *Bacillus* spp. biofilm growth mode is essential. Improving knowledge about biofilm formation on materials, widely employed in dairy processing plants, may represent a starting point to better design intervention strategies to manage this issue.

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METAPROTEOMIC INVESTIGATION OF A TYPICAL GOAT CHEESE: UNRAVELING BACTERIAL COMPETITION IN FOOD SAFETY

Carlotta Ceniti (1), Bruno Tilocca (1*), Viviana Greco (2, 3), Andrea Urbani (2, 3), Valeria Maria Morittu (1), Anna Antonella Spina (1), Paola Roncada(1)*

(1) Università degli Studi Magna Graecia di Catanzaro, Dipartimento di Scienze della Salute. (2) Università Cattolica del Sacro Cuore, Dipartimento di Scienze biotecnologiche di base, cliniche intensivologiche e perioperatorie. (3) Fondazione Policlinico Universitario Agostino Gemelli. *These autors share the first name.

Corresponding author: C. Ceniti (ceniti@unicz.it)

In this work, we focused our attention on detecting bacteria involved in cellulose production in a typical Calabrian goat cheese- Caprino Nicastrese. This artisanal cheese is made with raw milk and no starter cultures were added. Cellulose is a linear polymer of glucose molecules and is the most abundant biopolymer on Earth, and certain bacteria synthesize cellulose as an extracellular polymer for various biological functions [1]. For example, it is well known, that *Gluconacetobacter* species produce ordered cellulose microfibrils and have been used as a valuable model system for several decades to elucidate cellulose biosynthesis and regulation [2]. More recently the discovery of a wide variety of bacteria cellulose producers has opened interesting perspectives on the role of cellulose in bacterial development and in the interaction of the bacterial cell with the environment and the host. Most cellulose-producing bacteria likely produce amorphous aggregates of cellulose as an integral biofilm component [3]. Thus, a detailed understanding of biofilms-like production and how bacteria synthesize and secrete extracellular polysaccharides, especially in food matrices is urgently needed to reveal important information regarding food safety. Heterogeneous microbial polymers prevent spoilage bacteria colonization beside protect from pathogens and or pathobionts occurring throughout the production process and ripening. In this work, we applied metaproteomics analysis of the bacterial compartment of a typical raw goat milk cheese, to obtain information about the whole bacterial composition Cheese samples (Caprino Nicastrese cheese) (30) were collected at three ripening points, 30, 60, and 90 days and two different zones (rind and core). The bacterial fraction was independently isolated from each sample and LC-MS/MS sample preparation was performed according to Marini et al 2020 [4]. Briefly, bacterial protein extracts have been trypsin digested through Filter Aided Sample Preparation (FASP) protocol. Peptides were desalted via C18-Solid Phase Extraction (SPE). Purified peptides were measured in technical triplicates at the high-resolution HPLC-ESI MS/MS Orbitrap Elite. The resulting raw data were processed using the MaxQuant software against the database UniProt Bacteria (ID=2) for the qualitative and label-free quantitative characterization of the cheese-associated microbiota. The metaproteomics investigation suggested different microbiota compositions between the cheese samples: in particular, the analysis revealed twice more proteins ($p<0.01$) related to cellulose production in the rind than in the core of the cheese wheel. Results clearly demonstrated the involvement of bacterial cellulose biosynthesis (cellulose synthase) related to some Gram-negative bacteria, microaerophiles that confirmed the data well documented in literature [5-7]. A better understanding with the metaproteomics investigation of the microbial mechanisms of cellulose biosynthesis and the study of the conditions under which it occurs will also help develop strategies to counteract food-borne bacteria.

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CHANGES IN THE PROTEIN AND LIPID COMPONENTS OF DRY-AGED BEEF FOR UP 120 DAYS

Sarah Currò (1), Federico Fontana (1), Stefania Balzan (1), Luca Fasolato (1), Iuri Martinato (2), Gianluca Nana (2), Barbara Cardazzo (1), Lisa Carraro (1), Enrico Novelli (1)

(1) Università degli Studi di Padova, Dipartimento di Biomedicina Comparata e Alimentazione. (2) Istituto Italiano Assaggiatori Carne.

Corresponding author: S. Currò (sarah.curro@unipd.it)

During the last years the dry aging (DA) process was recovered by some artisanal meat professionals. Unlike wet aging, which is carried out with vacuum-packed primal or subprimal cuts, in DA unpacked cuts are placed in cold room at controlled air temperature and flow and relative humidity for several weeks. DA process is costly in terms of time and labor on the part of the manufacturer but it develops peculiar flavor and tenderness, and meat is a niche product sold in fine restaurant and gourmet butcheries. The duration of aging is variable reaching up to 280 days, generally based on the choices of the operators and chefs [1]. This study aimed to investigate changes in protein and lipid of beef meat during DA. Two sirloins (m. *L. dorsi;* Limousin and Polish crossbreed) were DA at 1.5-3.5°C and 80% moisture for 120 days by a butchery laboratory. At 30, 60, 90, 120 d, proximate composition, fatty acids profile, titratable acidity (TA; [2]), proteolysis index [3], conjugated dienes (K232; [4]) and total volatile basic nitrogen (TVBN; [5]) were measured. From 30 to 120 d protein (21.8÷25.3%) and ash content (0.67÷1.08%) increased meanwhile fat (13.4÷6.9%) decreased compared to a less variable water content (64.1÷66.9%). The variation of fat and protein were related and this trend could depend both on differences in the composition of the m. *L. dorsi* and on the lipolytic action. The aging determined a progressive increase in proteolysis index (10.0÷15.2%), TVBN (13.9÷24.4 mg N/100 g) and conjugated dienes (11.1÷16.5), meanwhile TA decreased (11.2÷4.2% oleic acid). Data also showed a progressive decrease in saturated (40.3÷37.5% total fatty acids identified) and monounsaturated fats (46.9÷43.8%), while polyunsaturated fats increased (4.5÷9.4%). Similar results were observed by [6]. The modification of meat protein complexes during aging was due to the activity of endogenous proteases, this determined both the softening and the reduction of water binding [6], and to microorganism (molds and yeast) growing on the crust that increased the concentration of flavor precursor. The increase in oxidation products was due to the activity of endogenous and exogenous lipolytic enzymes [7], forming products that were precursors for the generation of thermally induced aromatic volatile compounds. Further studies will concern the evaluation of microbial grow on the crust and the impact of lipid oxidation and microbiological flora on food safety.

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CHARACTERIZATION OF SALMONELLA EMERGING SEROTYPES IN NORTHWESTERN ITALY: A RETROSPECTIVE STUDY

Monica Pitti, Clara Tramuta, Andrea Vannuccuni, Daniela Adriano, Daniela Manila Bianchi, Lucia Decastelli

(1) Istituto Zooprofilattico Sperimentale del Piemonte Liguria e Valle d'Aosta, SC Sicurezza e Qualità degli Alimenti

Corresponding author: A. Vannuccini (andrea.vannnuccini@izsto.it)

CeRTiS is the Piedmontese Regional Reference Centre for *Salmonella* Typing: it is based in Istituto Zooprofilattico Sperimentale of Piemonte Liguria and Valle d'Aosta, in Turin. It is in charge of serological and biomolecular typing of *Salmonella* strains isolated from environments, animals, foods and human in the area of Piedmont Region. It collaborates with competent authorities during epidemiological investigations in the frame of suspected foodborne or zoonotic outbreaks. The serotyping analysis is a fundamental investigation to correctly define the more than 2000 different serotypes of *Salmonella* spp.; moreover, since some serotypes are characteristic of certain animal species, serotyping represents a valid tool for epidemiological investigations. Biomolecular investigations, and in particular the Pulsed-field Gel Electrophoresis (PFGE), allow to correlate from an epidemiological point of view, strain isolated from different sources. *Salmonella* infection, as reported by EFSA (1), is second food-borne pathogen in humans in the European Union. *Salmonella* enterica serotypes more involved in zoonoses and FBO are represented by S. Enteritidis, S. Typhimurium, monophasic variant S. Typhimurium. Recent data observed by CeRTiS showed that monophasic variant of S. Typhimurium (48%), S. Enteritidis (18%), S. Typhimurium (8%), S. Derby (5%), S. Napoli (3%) and S. Infantis (2%) were the most frequent serovars of 450 *Salmonella* isolates of human origin starting from 2016. The aim of the present work is to investigate, through PFGE, the genetic relatedness of emerging *Salmonella* serotypes circulating in North-West of Italy isolated from human and the creation of a Regional database to facilitate epidemiological correlation. One hundred and twelve strains, isolated from 2016 to 2018 in hospital laboratories, were characterized by PFGE, following the PulseNet protocol, after identification as *Salmonella* according to standard microbiological techniques and serotyping was performed according to ISO 6579-3 (2) and the Kaufmann-White scheme using O and H antisera (Statens Serum Institut®). PFGE was performed on a CHEF Mapper system for the separation of large fragments generated with the restriction enzyme XbaI to generate on agarose gel comparable genetic patterns. The agarose gel was stained with GelRed® and photographed under ultraviolet transillumination using a Gel Doc System. The patterns obtained were compared using Bionumerics software (version 7.6) using Dice coefficient with 2% band tolerance and 2% optimization and the data obtained for each serotype were compared according to the geographical origin and the year in which they were isolated. All the isolates highlight an appreciable restricted digestion patterns ranging and a fairly heterogeneous distribution of pulsotypes has emerged in the different provinces. Cluster analysis indicated high genetic similarity (≥ 83%) among strains of S. Derby (n. 30; 88%), S. Infantis (n. 36; 95%) and S. Napoli (n. 38; 95%) circulating in north-western Italy. The present work show the genomic similarities shared by the emerging *Salmonella* strains in Northwest Italy and allowed to create a database to detect outbreaks in an early stage. Therefore, the results confirmed that PFGE is a powerful and discriminatory tool to investigate the genetic relationships among strains in order to monitoring and control Salmonellosis outbreak spread. PFGE still represents one of the most suitable approaches to characterize strains, in particular for the laboratories for which NGS techniques are not available.

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SCREENING OF QUINOLONE RESIDUES FROM SLAUGHTERED BOVINE URINES

Gianluigi Ferri (1), Carlotta Lauteri (1), Luca Maria Pennisi (1), Giacomo Scorzetti (2) Domenico Pellei (3), Alberto Vergara (1)

(1) Università degli Studi di Teramo, Settore di Ispezione degli alimenti. (2) Magazzini Gabrielli S.p.A., Ascoli Piceno. (3) Global Concept srl., San Benedetto del Tronto AP.

Corresponding author: G. Ferri (gferri@unite.it)

Due to the global increasing request for food products of animal origin as primary protein resource, productive pressures have induced livestock and animal husbandries to implement intensive farming systems characterized by high animal densities. It has encouraged uncontrolled drugs use especially in developing countries (Van Boeckel et al, 2015). Consequently, residual drugs are often identified from food matrices (i.e., xenobiotic substance persistence in slaughtered animals) and has also induced to a selection of resistant bacterial strains to the most used antibiotic molecules (Ramatla et al., 2017).

The present study aimed to evaluate quinolone residues in urine specimens collected at the slaughterhouse. This antibiotic family is commonly used in bovine bacterial disease (Tessa et al., 2016).

The survey involved 32 female bovines (mixed-breed) aged from 18 to 24 months from 2 farms located in North-Est of Italy, Veneto region, during 2021.

Urine samples were collected from each animal in 2 slaughterhouses. During transportation and storage to the laboratory, all aliquots were individually packed and kept at refrigerated temperature (+4°C).

The evaluation of quinolone residual presence was performed by using the liquid chromatography with mass spectrograph (Mieriex NutriSciences Rasena, Treviso, Italy).

The screened antibiotics were: nalidixic acid, oxolinic acid, carbadox, cinoxacin, ciprofloxacin, danofloxacin, enoxacin, enrofloxacin, enrofloxacin, ciprofloxacin, flumechin, lomefloxacin, marbofloxacin, norfloxacin, ofphloxacin, norfloxacin, ofphloxacin.

The results demonstrated total absence of residual antibiotics.

The 73% of antibiotic global use has been employed in the meat supply chain, and forecasts, regarding consumption of these drugs, predict a growth of 11.5% by 2030 (Treiber et al., 2021).

Quinolone family is identified as a "critical important antibiotics" by the World Health Organization.

The thermal treatments can reduce the risk ingestion of sulfonamides, tetracyclines and fluoroquinolones, but do not guarantee their total dissolution and degradation.

Quinolones and beta-lactamics are characterized by high stability to heat processings and, for this reason, represent huge risk to human health. There is no study about detection of quinolone residues in cooked meat, but their residues can persist in milk after pastorization treatments and reach the dairy industry and the final consumer.

Metabolic and catabolic toxic effects need more knowledge and detailed studies. Due to their possible persistance and presence in cooked meat, antibiotic residues should be inserted in maximum daily intake, if limits were established by Legislations.

Another important concern is antimicrobial resistance phenomenon that hasn't physic or geographic limits. EFSA Report in 2019 showed that 31.705 samples were not conform on 1191 analyzed; 0,41% of these came from beef food chain (0,04% in Italy) (EFSA, 2021). Hence, our results are in line with national data.

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AMV

BRDU: A MARKER OF DNA SYNTHESIS

Nadia Gionchiglia, Adalberto Merighi, Laura Lossi

Università degli Studi di Torino, Dipartimento di Scienze Veterinarie.

Corresponding author: N. Gionchiglia (nadia.gionchiglia@unito.it)

The existence of two major neurogenic niches in the adult brain has been established in numerous studies. However, the number of dividing cells, their nature and their significance in the postnatal brain is still debated: some studies have found that only few new neurons are added to the adult dentate gyrus every day, while others have counted hundreds of them. Many studies, both in rodents and humans, were carried out with the use of the nucleotide analogue 5-bromo-2′-deoxyuridine (BrdU) as a marker for proliferating cells.

We hypothesized that BrdU incorporation in aging brain cells is mostly correlated to mechanisms of DNA repair, rather than true events of cell proliferation. We separated six CD1 mice (18-24 months) into three groups and used for labeling experiments with BrdU (authorizations n. 65/2016 PR of March 2016 to the University of Turin and n. 130/2012-B of January 2012 to Enea, Rome). All animals were injected with BrdU at time 0, while only the two experimental groups underwent X-ray induced DNA damage after 90 minutes from injection. First group was then sacrificed 5 minutes after radiation (Group I), while the second group was sacrificed after 30 minutes (Group II). After paraffin embedding, brain sections were single- or double-stained with antibodies against BrdU, the phosphorylated form of histone H2AX (γH2AX) (which is required for efficient repair of DNA double strand breaks), p53-binding protein 1 (53BP1) (which is recruited during the DNA damage response), and phosphorylated histone H3 (pHH3) (which labels proliferating cells).

BrdU+ cells were seen especially in the ventricle walls, hippocampus and cerebral cortex. Moreover, we saw an increase in the number of positive cells in both experimental groups, and especially in Group II, compared to controls. We then performed double stainings of BrdU and γH2AX, 53BP1 and pHH3. In all three experiments, we observed colocalization of the markers, and a strong significant difference in the number of double positive cells between treated animals and the control group, and especially in Group II. The results show an increase in the number of cells co-expressing BrdU with any of the other markers in animals that survived longer times after radiation.

This demonstrates that at least a fraction of the cells labelled with BrdU in old animals (>18 months) undergoes DNA damage and repair. So, we can say that DNA repair activity is not only present during development, but also in specific areas of the old mouse brain and that BrdU is indeed a marker of DNA synthesis rather than proliferation.

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ONLINE BIOGRAPHICAL DIRECTORY OF ITALIAN VETERINARIANS

Associazione Italiana di Storia della Medicina Veterinaria e della Mascalcia
c/o Museo di Scienze Veterinarie – Università di Torino
Corresponding author: segreteria.aismevemnito.it

The main goal of this report is to build a biographical collection of Italian veterinarians and, most importantly, farriers, to be posted on the website of the Italian Association of History of Veterinary Medicine and Farriery (A.I.S.Me.Ve.M.) Through this collection of biographical profiles, trusting that many Colleagues will be willing to give their contribution to it, we intend to create an archive of information about how many, veterinarians or not, have contributed to the development of veterinary medicine by giving prestige and emphasis to the profession, not only in the field of research and professional practice, but also in the cultural and socio-political one.

We hope that this list will become a point of reference for those who need to deepen their knowledge of the veterinary world, be it for work, teaching or simple curiosity. The idea is not new: it is inspired by a similar international initiative promoted in 1993 in Cordoba during the 29th Conference of the World Association for the History of Veterinary Medicine.

The idea was proposed by Guus Mathijsen and Ivan Katic, their slogan was *better one person too much than one person missing*. The writing of the biographical profiles follows the structure of the Dictionary of American Medical Biography.

Hitherto, thirty-one biographies have been posted on the Association's website, accompanied by photos or portraits. It is a very small number if compared to the total number of *zoojatri*, once, and veterinarians, later, graduated since the foundation of the first School of veterinary medicine in Italy, in 1769, until today.

The thirty-one biographies collected, all relating to a period of time ranging from the early 19th century to the end of the 20th century, concern sixteen university teachers (whom it is easy finding information about), and fifteen practitioners who worked in different fields of the veterinary medicine.

Among the biographies there are also those of the four Colleagues who died in war, who were awarded the Gold Medal of Military Valour.

On the website it is also possible to find all the useful information about how to write the biographical profiles.

All the details at the following link: https://storiamedicinaveterinaria.com/biografie/

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Fare clic o toccare qui per immettere il testo.

EXPRESSION OF NESFATIN-1 IN THE GASTROINTESTINAL TRACT OF THE BOTTLENOSE DOLPHIN Tursiops truncatus

Elena De Felice (1), Claudia Gatta (2), Daniela Giaquinto (2), Federica Fioretto (1), Paola Scocco (1), 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, Dipartimento di Medicina Veterinaria e Produzioni Animali.

Corresponding author: P. Scocco (paola.scocco@unicam.it)

Nesfatin-1 (Nesf-1) was first identified as potent anorexigenic peptide [1]. Successive studies addressed to further characterization of Nesf-1 actions have highlighted a pleiotropic role at central nervous system and in peripheral organs [2]. In several species of vertebrates, from fish to mammals, Nesf-1 appears abundantly expressed in the digestive tract [3, 4]. Here we questioned whether also in marine mammals Nesf-1 has a peripheral expression and maybe implicated in the peripheral regulation of food intake. By relying on the availability of tissue samples available in the Mediterranean Marine Mammal Tissue Bank (MMMTB), we conducted immunohistochemistry and western blot analyses on the digestive tract of the bottlenose dolphin *(Tursiops truncatus*). *T. truncatus* shows a diverticulated composite stomach, consisting of three chambers, while the intestine does not exhibit any macroscopic subdivision into small and large intestine. Our data document that Nesf-1 is widely distributed mainly in the epithelial cells of the gastric chambers with higher distribution in the second chamber compared to the first and third, whereas immunopositive nerve fibers and neurons, scattered or/and clustered in ganglion structures, displayed a similar distribution along all the gastrointestinal examined tracts. Western blot analyses confirm protein expression in all samples. The pattern observed propts us to further emphasize the evolutionary conserved expression of Nesf-1 in marine and terrestrial mammals.

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NEW OLD TECHNIQUES: COMBINED APPROACHES FOR SUCCINATE DEHYDROGENASE ASSAY OF SKELETAL MUSCLE IN RABBIT, PIG AND COW

Fabrizio De Luca (1), Alessia Di Giancamillo (2), Lucia Aidos (2), Margherita Pallaoro (1), Giampaolo Bosi (1) Valentina Herrera (2), Silvia C. Modina (1)

(1) Università degli Studi di Milano, Dipartimento di Medicina Veterinaria e Scienze Animali. (2) Università degli Studi di Milano, Dipartimento di Scienze Biomediche per la Salute.

Corresponding author: F. De Luca (fabrizio.deluca1@unimi.it)

Succinate dehydrogenase (SDH) is a mitochondrial heterodimeric enzyme complex located in the inner mitochondrial membrane that has a key role in both oxidative phosphorylations, regulating the electron transport, and the Krebs cycle, through the oxidation of succinate to fumarate [1]. SDH enzymes play an essential role in the oxidation of proteins, carbohydrates and lipids and the estimation of their enzymatic activity goes back to approximately 70 years ago, when Kun for the first time used tetrazolium compound for their quantification [2]. However, the use of frozen sections for the estimation of the SDH has been demonstrated only a few years later by Seligman and Ruthenberg, using blue tetrazolium in frozen tissue from the brain, liver, kidney and heart, until 1953, when the presence of high enzymatic activity was confirmed even in mitochondria of the skeletal muscle [3, 4]. Nowadays, SDH histochemical staining is widely used in the assessment of muscle biopsies and represents a useful strategy for the identification of skeletal muscle fiber types, allowing discrimination between slow and fast fibers. However, this assay has several disadvantages. This histochemical method does not allow to identify intra- (e.g. nuclear and cytoplasmic compartments) or extracellular (e.g. endomysium and perimysium) structures: To facilitate the identification/discrimination of the cellular boundaries of the marked fibers, we carried out an histochemical evaluation of SDH enzymatic activity, in frozen tissue from pig, rabbit, and bovine samples of commercial meat, together with different histochemical stains. In particular, it was decided to combine the classical SDH reaction with different nuclear counterstains, using haematoxylin and methyl green, as well as histological stains (i.e. eosin and trichrome). The combination between SDH and nuclear counterstaining has thus allowed a faster and more specific identification of the different cellular population. On the other hand, eosin stain better highlighted the evaluation of SDH-negative cells. Finally, the use of trichrome has allowed us to enhance several intra- and extracellular components (e.g. nuclear chromatin and collagen fibers), allowing us to relate the expression level of the SDH enzyme with possible alteration of other tissue components. In conclusion, the use of a histochemical technique proven by decades of study is always a certainty, and the combination of this staining with other historical methodologies could represent a good strategy for the evaluation of muscle structure, as well as for the assessment of muscle biopsies to reveal underlying pathology.

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IMPACT OF SEMEN CRYOPRESERVATION ON SKELETAL AND MUSCULAR DEVELOPMENT OF MARBLE TROUT (SALMO MARMORATUS) LARVAE FROM THREE RIVER BASINS

Lucia Aidos (1), Silvia Modina (2), Fabrizio De Luca (2), Giorgio Mirra (1), Valeria Bornaghi (3), Lorenzo Proietti (3), Katia Parati (3), Alessia Di Giancamillo (1)

(1) Università degli Studi di Milano, Dipartimento di Scienze Biomediche per la Salute. (2) Università degli Studi di Milano, Dipartimento di Medicina Veterinaria e Scienze Animali. (3) Istituto Sperimentale Italiano "Lazzaro Spallanzani".

Corresponding author: L. Aidos (lucia.aidos@unimi.it)

The marble trout (*Salmo marmoratus*) is a freshwater species from the family of the salmonids. Due to several human activities and to the hybridization with brown trout, marble trout was included in the European Union Habitat Directive and in the 2013 IUCN Red List, classifying it as "Critically Endangered" (1). In this context, the existence of a sperm cryobank assumes a great importance on this species preservation and natural stocks enhancement. The aim of this study was to assess the viability of the marble trout larvae from three river basins, Adige, Piave and Brenta, obtained with fresh (CTR) and cryopreserved semen (CRYO, from the Spallanzani cryobank). It is important to refer that, within each river basin, the genetic line of this species is independent. Therefore, differences between rivers are not relevant for the purpose of this study.

Sperm samples were collected and cryopreserved according to the protocol developed ad hoc for marble trout. Fertilization took place and hatching occurred at 44 dpf (days post-fertilization). Larvae were evaluated in terms of weight and total length, muscle development (histometric analyses for fibers density, FD and cross-sectional area, CSA) as well as the occurrence of skeletal abnormalities, using a double staining whole-mount technique with Alcian Blue and Alizarin for cartilage and bone tissues respectively. The fish handling procedures and sampling methods used in the trial followed the guidelines of the E.U. directive 2010/63/EU on the protection of animals used for scientific purposes.

For each river basin, no significant differences were found between treatments regarding larvae total length. Moreover, no differences were observed in Adige in weight between treatments. Interestingly, in Piave, larvae from the CTR group were heavier than the CRYO ones; on the opposite, in the river Brenta, the heavier larvae were the ones obtained with CRYO. For each river basin no differences were found regarding FD and CSA, except for Brenta, where larvae from the CRYO group showed the highest CSA. These differences may be due to a different balance between hyperplasia/hypertrophy as well as to exogenous factors related to the different basins and fish genotypes.

Regarding skeletal development, whole-mount double staining showed no abnormalities in larvae from all the river basins and from all treatments.

Overall, taking into account these results, it would seem safe to state that cryopreserved semen produces equally good quality larvae as the ones obtained with traditional, fresh semen, for all the three river basins. Acknowledging no negative effects on the use upon the descendants, semen cryopreservation from cryobank can be an additional strategy to protect endangered species.

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NORMAL SKIN IN CULTURE CAN REPRODUCE ALLERGIC AND SEPTIC FEATURES

Giulia Lazzarini (1), Chiara di Franco (1), Angela Briganti (1), Francesca Abramo (1), Denise Biagini (2), Andrea Pirone (1), Fabio Di Francesco (2), Vincenzo Miragliotta (1)

(1) Università degli Studi di Pisa, Dipartimento di Scienze Veterinarie. (2) Università degli Studi di Pisa, Dipartimento di Chimica e Chimica Industriale.

Corresponding author: V. Miragliotta (vincenzo.miragliotta@unipi.it)

Atopic dermatitis is a common chronic inflammatory skin disease with high prevalence in both humans and dogs, whose pathophysiological background is still under debate [1, 2]. Sepsis is currently defined as life-threatening organ dysfunction caused by a dysregulated host response to infection [3]. The aim of this study was to investigate whether allergic/septic conditions can be established ex vivo in a canine skin organ culture model [4].

Skin biopsies obtained during surgery unrelated to the purposes of the study were cultured and treated in triplicate: a) a first study was performed by treating biopsies with either compound 48/80 alone (10 or 100 µg/mL) or in the presence of palmitoylglucosamine (30 µM) for either 24 or 48h; mast cell degranulation, microvessel size and histamine content in the culture medium were determined; b) a second set of biopsies was cultured and treated in triplicate with LPS (10µg/ml); the concentration of 60 oxylipins was measured in the culture medium by UHPLC-MS/MS analysis and microvascular size was determined by histology.

The first study showed a dose-dependent increase in mast cell degranulation, histamine content and microvascular dilation subsequent to treatment with compound 48/80 that was reverted by PGA. In particular, either 24 and 48 hours after administration, compound 48/80 induced statistically significant degranulation of mast cells at both concentrations compared to vehicle. PGA reduced this effect with differences between the two time-points: PGA significantly controlled the degranulation induced by the higher concentration of 48/80 at the first time-point, while it significantly reduced the degranulation induced by both concentrations of 48/80 at the second time-point. Histamine content in the culture medium significantly increased with both 48/80 concentrations but not when PGA was present concomitantly. Microvessels size was increased with 48/80 but not when PGA was present concomitantly.

The second study showed a time-dependent (2h, 24h and 48h) increase of the level of 15-F2t-IsoP, 15-E2t-IsoP, TXB2, PGE2, and lipoxin B4 induced by LPS treatment.

Here we show that mast cells degranulation and subsequent histamine content in the culture medium and microvascular size can be specifically modulated by administering compound 48/80 and palmitoylglucosamine (PGA), and that isoprostanes and related oxylipins (proposed as plasma biomarkers of oxidant stress and development of organ failure in severe sepsis) increase in the culture medium after administration of LPS [5]. This canine skin organ culture model can be used to study treatments for canine atopic dermatitis or other skin allergic conditions as well as for the investigation of changes related to sepsis and subsequent therapies. In a 3R perspective, other measurable endpoints might be implemented that may provide relevant information on pathogenic mechanisms.

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ANIV

RELIABILITY STUDY OF AN IN-CLINICS TEST FOR THE DETECTION OF THE SPECIFIC ANTIBODY TITER FOR DISTEMPER, PARVOVIRUS INFECTION, AND INFECTIOUS HEPATITIS IN THE DOG Fare clic o toccare qui per immettere il testo.

Paola Dall'Ara, Yvonne Sessolo

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

Corresponding author: P. Dall'Ara (paola.dallara@unimi.it)

Current vaccination guidelines published by many veterinary associations worldwide (WSAVA, AAHA, BSAVA, etc.) and experts recommend administering core lived vaccinations (MLVs) to all dogs and cats on a three-year basis, although this type of vaccines can confer even longer-lasting protection, as demonstrated by numerous studies; on the other hand, there are many factors that can negatively influence the success of vaccination [1-4]. For these reasons, veterinary clinical medicine is trying to abandon the practice of a "blind" vaccination, which can be useless if not risky, preferring vaccinations and boosters only if necessary. Knowing the dog's antibody titers specific for core vaccinations is therefore essential, and many tests are available today to evaluate post-core vaccination antibody titers. The purpose of this study was to analyze the SensPERT CADP Ab Test Kit (simplified CADP), a new in-clinics test (developed by the Korean company VetAll and supplied in Italy exclusively by AlphaVet), to determine the dog protection against parvovirus infection (CPV), distemper (CDV) , and infectious canine hepatitis (CAV).

A total of 56 tests/intertests on blood samples of 16 dogs have been performed to evaluate: (a) the practicality in daily use; (b) the repeatability of the results by testing different temperatures (18.5°C, 24°C and 30°C), different combinations of instruments (kit supplied devices and lab pipettors) to collect and to pour the diluent, different sample types (whole blood, fresh plasma, fresh serum, frozen plasma, frozen serum), and different times of reading (5, 10, 15, and 20 minutes); (c) the test reliability comparing the results with those of another in-clinics test of ascertained validity (ImmunoComb VacciCheck Canine, simplified VacciCheck, developed by the Israeli company Biogal and supplied in Italy exclusively by Agrolabo). CADP shows an excellent practicality and a very good repeatability in all tests, and it is very easy to perform. All the instruments supplied by the kit showed a good accuracy even in collecting very small sample volumes (1 mL), and readings were stable within 20 minutes. Moreover, CADP can be used with many types of samples (fresh whole blood with or without anticoagulant, fresh or frozen plasma, and fresh or frozen serum), but it can't be used with hemolytic samples. CADP and VacciCheck results showed a substantial agreement (calculated by Cohen's kappa) for CPV and CAV, but not always for CDV, for which the agreement was low using the kits' thresholds (62.5%), and substantial considering a 1:16 threshold for both (89.2%). CADP is then a practical, reliable, and straightforward test, but is a little less accurate than VacciCheck, that allows more precise titration thanks to its colorimetric scale. Therefore, the recommendation is to use CADP to evaluate the protection of adult dogs in anticipation of a possible booster vaccine and in all other cases in which it is not necessary to obtain a precise antibody titration (for example old or kennel dogs). For puppies VacciCheck remains preferable: puppies are indeed more susceptible to diseases (particularly to parvovirus infection [1, 5]), and therefore a more precise antibody titration is recommended, especially during the "window of susceptibility" when the interference of maternally-derived antibodies (MDA) doesn't allow a successful vaccination [1, 2, 5].

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PRESENCE OF CAMPYLOBACTER IN CANINE FECAL SAMPLES AND RELATION WITH SOME PREDISPOSING FACTORS

Francesca Molinari (1), Gabriele Meroni (2), Valerio Massimo Sora (2), Giulia Laterza (2), Piera Anna Martino (2)

(1) Practitioner, Genova Pegli. (2) Università degli Studi di Milano, Dipartimento di Scienze Biomediche, Chirurgiche e Odontoiatriche - Sezione One Health.

Corresponding author: P.A. Martino (piera.martino@unimi.it)

The intestinal microbiota both in humans and in animals includes more than 400 different microbial species adapted to their niches. The "positive" flora is also composed of potentially pathogenic bacteria (e.g., *Clostridium*, *Campylobacter*) that can live in the host in a condition of equilibrium with other microorganisms or can cause clinical diseases in presence of impaired conditions (i.e., dysbiosis). In dogs and cats *Campylobacter* species (in particular *C. upsaliensis*, *C. helveticus* and *C. jejuni*) are frequently isolated from faecal samples but the majority of the animals are subclinically infected acting as reservoirs and shedding the microorganism in the environment. However, animals with concurrent disease or stressed can develop clinical signs (mild to moderate enteritis) [1, 2]. Moreover, asymptomatic carriers can be a potential source of *Campylobacter* infections in humans (e.g., zoonotic risk for owners) [2].

The aim of this work was to evaluate the prevalence of *Campylobacter* from faecal samples and rectal swabs, collected by 150 dogs and to point out a relation between the presence of *Campylobacter* and the possible predisposing factors. The dogs located in Liguria and Lombardia regions showed heterogeneous characteristics (e.g., gender, age, breed, stool appearance, type of diet, previous gastrointestinal diseases, etc.). The samples were streaked onto Charcoal Cefoperazone Deoxycholate Agar (CCDA) (Oxoid ThermoFisher, Italia) under microaerophilic conditions at 37 °C for 48 hours [3]. The bacterial identification was made according to the literature [3, 4].

The results showed a prevalence of 42.4% (64/150) for *Campylobacter* according to literature. Moreover, the sensitivity of cultural procedures seems to increase using faeces instead of rectal swabs. High positivity has been observed in subjects with stools of normal consistency (43.5%) confirming the role of *Campylobacter* as an intestinal commensal bacterium (canine healthy carriers), but also in dogs fed with raw meat based diet (100%) or with a homemade diet (52%) and in dogs with enteritis or Inflammatory Bowel Disease (IBD) (100%). In our results older animals harbored the microorganism more than the adult and the younger ones (63.3% *versus* 39% *versus* 34.5%); in particular, the lower isolation of *Campylobacter* in young dogs was in contrast to literature but it will be necessary to increase the number of samples to make the data more robust.

The presence of *Campylobacter* in dogs without clinical signs draws the attention to their role as carriers/reservoirs in transmitting the microorganism to humans.

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ANTIVIRAL ACTIVITY OF THE FUNGAL METABOLITE 3-O-METHYLFUNICONE IN CANINE CORONAVIRUS INFECTION

Claudia Cerracchio (1), Maria Michela Salvatore (2, 3), Valentina Iovane (4) Francesca Paola Nocera (1), Luisa De Martino (1), Rosario Nicoletti (4, 5), Anna Andolfi (2, 6) Filomena Fiorito (1, 6)

(1) University of Naples Federico II, Department of Veterinary Medicine and Animal Production. (2) University of Naples Federico II, Department of Chemical Sciences. (3) National Research Council, Institute for Sustainable Plant Protection. (4) University of Naples Federico II, Department of Agricultural Sciences. (5) Council for Agricultur Research and Economics, Research Centre for Olive, Fruit and Citrus Crops. (6) University of Naples Federico II, BAT Center-Interuniversity Center for Studies on Bioinspired Agro-Environmental Technology.

Corresponding authors: F. Fiorito (filomena.fiorito@unina.it); A. Andolfi (anna.andolfi@unina.it)

Fungi produce a large number and variety of secondary metabolites (SMs), many of which have been exploited for application such as antibiotics, fungicides, plant growth regulators, and phytohormones. In relation to their great adaptability to the most varied habitats and lifestyles, fungi in the genus *Talaromyces* (Eurotiales: *Trichocomaceae*) show high biosynthetic versatility. In addition, they represent a source of many bioactive products of pharmaceutical interest, including potential antiviral drugs (1, 2). Indeed, screening performed on benzo-γ-pyrone 3-*O*-methylfunicone (OMF), a secondary metabolite produced by *Talaromyces pinophilus*, revealed that it reduces infectivity of hepatitis C virus (3), as well as of bovine herpesvirus 1 (4).

The emergence of SARS-CoV-II has been sparking wide interest in coronaviruses, whose mutations can result in deadly diseases in new hosts. To evaluate the antiviral efficacy of OMF, we performed *in vitro* tests against CCoV-II, a classical canine coronavirus, generally responsible for self-limiting enteric infections, characterized by high morbidity and low mortality in dogs (5, 6). Drugs such as indomethacin has been shown to have potent antiviral properties against CCoV (7 ,8).

Herein, during infection with the reference CCoV strain S/378 in a canine fibrosarcoma (A-72) cell line, *in vitro* bio-screen, immunofluorescence staining, cytomorphological and virus yield analyses were performed (4).

Following infection, the non-toxic concentration of 0.5 μM OMF noticeably decreased signs of cell death. In addition, OMF considerably decreased the cytopathic effect and intensely downregulated the expression of the nucleocapsid protein (NP), a structural protein of CoVs responsible for binding the viral RNA genome, packing viral genome RNA into ribonucleoproteins, and compressing it into a compact virion core. In addition, NP is generally more stable than CoV spike protein, which has a higher mutation rate (9).

Overall, our preliminary results suggest that OMF, a nontoxic concentration, shows potential activity against CCoV infection. Up to now very few antiviral compounds to fight CCoV infection have been described (7, 8). Further, in the screening of potentially antivirals our *in vitro* animal model of CoVs avoids the manipulation of extremely dangerous human CoVs (SARS-CoVs, MERS-CoV).

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A RETROSPECTIVE SEROSURVEY OF THREE PORCINE CORONAVIRUSES AMONG WILD BOAR POPULATION IN CAMPANIA REGION

Gianmarco Ferrara (1), Consiglia Longobardi (2), Antonella Rossi (1), Sara Damiano (1), Roberto Ciarcia (1), Giuseppe Iovane (1), Ugo Pagnini (1), Serena Montagnaro (1)

(1) Università degli Studi di Napoli Federico II, Dipartimento di Medicina Veterinaria e produzioni animali. (2) Università della Campania "Luigi Vanvitelli", Dipartimento di Salute Mentale e Fisica e Medicina Preventiva.

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

The growing interest in porcine coronaviruses (CoV) is due to both their negative impact on the swine industry and their propensity to mutate and overcome host barriers. A total of six viruses belonging to this family can infect pigs and mainly cause respiratory and intestinal diseases. Among them, Porcine Epidemic Diarrhea Virus (PEDV), Transmissible Gastroenteritis Virus (TGEV) and Porcine Respiratory Coronavirus (PRCV) are widely distributed and frequently reported in Europe [1]. Since information on CoV in wild boar is limited, especially in Italy, a serosurvey was conducted to assess the epidemiological situation in the Campania region and to clarify the role of wild boar as a reservoir for enteric and respiratory porcine CoVs. During the 2016-2017 hunting season, serum samples were collected from 434 feral pigs (from 119 hunting areas in four provinces) and analyzed for antibodies to PEDV, TGEV, and PRCV using specific and commercial ELISA assays. The most common pathogen in our study was PEDV with a percent positivity of 3.83% (CI 95%: 2.05-5.6; odds ratio: 5.85 vs. TGEV and PRCV), while very low seroprevalence was detected for TGEV and PRCV (0.67%; CI 95%: 0-1.44 in both cases). The combined prevalence was 4.73% (CI 95%: 2.76-6.7). Only two wild boars were co-infected, the first with PEDV and TGEV, the second with TGEV and PRCV. Surveys conducted in other European wild boar populations revealed low prevalence values; for example, seroprevalences of 0.7% for PRCV and 0.4% for TGEV were found in Croatia. In Germany, a large-scale survey of 1,221 blood samples found 7.87% PRCV-positive and 1.59% TGEV-positive feral pigs. In Slovenia, a PRCV seroprevalence of 3% was described, although a very high seroprevalence in domestic pigs (65%) was reported. In a study conducted in the Czech Republic, only 1% of the wild boars tested had antibodies to TGEV. In Poland, PEDV seroprevalence was found to be 3.2%, while the virus was not detected by the RT -qPCR assay [2]. These data suggest that CoV infections are not widespread in European wild boar. Considering the very low seroprevalence observed in our study, TGEV and PCRV were not included in the statistical analysis of risk factors. The PEDV prevalence of infected animals was positively correlated with age (0 - 12 months old) (7.2%; CI 95%: 2.89-11.49), while univariate analyzes showed no correlation between PEDV seropositivity, sex (P=0.8473) and location (p=0.66), although a higher prevalence was observed in female wild boar (4.4%; CI 95%: 1.21-7.55) and in Avellino province (4.8%; CI 95%: 0.21-9.31). The lack of correlation between positivity and sex is an unexpected result, since close contact between sows and piglets (which are the main vectors of the virus) should increase the risk of exposure for females. Conversely, we observed a statistical correlation between age and PEDV seropositivity. Indeed, the highest seroprevalence (7.2%) was observed in young animals (<12 months), with a decreasing trend of seroprevalence in the three age groups tested (young animals = 7.2%, subadult animals = 3.7%, adult animals = 0%). The absence of positive adult animals could be explained by the short duration of circulating detectable antibodies, which is typical for CoV [3]. The low prevalence detected in our study suggests that these viruses are not widespread in the wild boar population in the Campania region and do not represent a concrete risk for CoV transmission to domestic pigs.

Considering the regular epidemics reported in the swine industry, we can assume that these infections have obvious effects only when predisposing factors typical of intensive farming (such as stress, overcrowding, etc.) induce higher susceptibility and ensure wide dissemination.

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TEN-YEARS OF INTEGRATED APPROACH IN SURVEILLANCE OF FOOD-BORNE SALMONELLA IN PIEDMONT REGION, 2011-2020

Aitor Garcia-Vozmediano (1), Monica Pitti (1), Clara Tramuta (1), Giovenale Moirano (2), Giuseppe Ru (1), Cristiana Maurella (1)

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

Corresponding author: A. Garcia-Vozmediano (aitor.garciavozmediano@izsto.it)

Foodborne zoonotic diseases and multi-drug resistance are of global concern for public health. Nontyphoid serotypes of *Salmonella enterica* subsp. enterica are the second most reported cause of food-borne outbreaks in Europe [1]. Disease incidence in humans have generally declined in recent years thanks to improvements in veterinary health associated to both *Salmonella* occurrence and rational use of antibiotics [2]. Due to the existing interrelations between animals, humans and environment a multidisciplinary One Health approach in *Salmonella* surveillance might ensure a high food safety level and reduce the risk of pathogen transmission to humans. We aim at investigating the geographical and temporal distributions of *Salmonella* infection in humans, farmed animals and wildlife in Piedmont region over the 2011-2020 period, identifying serotypes involved and evaluating their antimicrobial-resistance profiles. All isolates were identified and serotyped according to ISO/TR 6579; then antimicrobial resistance, against 18 molecules in human and environmental isolates and 12 molecules in animals' isolates, was evaluated by the Kirby-Bauer method according to Clinical and Laboratory Standard Institute guidelines. To achieve a good representativeness of the geographic distribution of the infections in farmed and wild animals, poultry and cattle livestock and red fox, roe deer and wild boar species were considered. We successfully characterized 4,440 isolates from symptomatic human patients requiring clinical care in different inpatient facilities across Piedmont region. Our results showed clear differences in pathogen infections by age and gender strata, being male children (<9 years-old) the most affected class. The two livestock populations showed similar levels of *Salmonella* prevalence: however, we detected differences according to farming methods and productive orientations, with organic poultry (P=22.2; 95% CI=14.1–32.2), layer hens' production (P=14.8; 95% CI= 11.3–18.9) and dairy cattle (P=21.1; 95% CI=14.5–29.9), showing the largest pathogen occurrence. In wildlife species, *Salmonella* infections were restricted to only few geographic areas, and prevalence was particularly relevant in red fox (P=7.8%; 95% CI=4.7–12.1), compared with wild boar (P=4.4%; 95% CI=1.6–9.3) and roe deer (P=2.5%; 95% CI=1.0–5.0). Phenotypic analyses uncovered a great variety of *Salmonella* serotypes, especially in humans (n=151) and poultry (n=34). *Salmonella* Typhimurium monophasic variant 1,4,[5],12:i:- was the most prevalent serotype involved in human infections, *S.* Typhimurium prevailed in cattle and wildlife and *S.* Enteritidis in poultry. Among these serotypes, the highest number of multidrug resistant strains were observed for *S.* Typhimurium monophasic variant 1,4,[5],12:i:- of human origin (up to 11 antibiotics); in particular, more than 70% of these strains were resistant to some antibiotics used in clinical practice (tetracycline, ampicillin and streptomycin). High levels of antimicrobial resistance also occurred in isolates from poultry (up to 9 antibiotics), cattle (up to 12 antibiotics) and wild species (up to 8 antibiotics).

Our study provides a broad picture of the epidemiology of nontyphoid salmonellosis in Piedmont region during the last decade. We underline the importance of adopting a multidisciplinary approach to develop effective strategies to comprehensively respond to salmonellosis and antibiotic resistance globally.

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ANTIMICROBIAL RESISTANCES OF *SALMONELLA* STRAINS ISOLATED FROM HUMAN, WILD BOAR, AND ENVIRONMENTAL SAMPLES FROM 2018 TO 2020 IN THE NORTHWEST OF ITALY

Valeria Listort , Aitor Garcia-Vozmediano, Monica Pitti, Cristiana Maurella, Daniela Adriano, Carlo Ercolini, Monica Dellepiane, Lisa Guardone, Elisabetta Razzuoli

Istituto Zooprofilattico Sperimentale del Piemonte, Liguria e Valle d'Aosta
Corresponding author: V. Listorti (valeria.listorti@izsto.it)

Salmonellosis is the second most common zoonosis reported in Europe in 2020 [1], and antimicrobial resistance (AMR) in foodborne pathogens such as *Salmonella* is a major concern for public health safety [2]. The interface between the environment, wild animals and humans contributes to the emergence of antimicrobial resistant bacteria [3]. Integrated surveillance of AMR is one of the top priorities of the European Union [4]. From a one-health perspective, this study aimed to investigate the serotypes and antimicrobial resistance profiles of *Salmonella* spp. strains circulating in humans, environment and wild boars in Liguria region from 2018 to 2020. A total of 517 *Salmonella* spp. strains were analysed. In details: Sixty strains were isolated from liver of wild boars hunted in Liguria, according to ISO 6579:2002/COR. 1, 2004, 193 environmental strains have been previously isolated and 264 human strains were conferred by different hospitals in Liguria, isolated from patients showing clinical signs referred to salmonellosis. Serotype identification was carried out in accordance to ISO/TR 6579-3, 2014. Antimicrobial resistance was evaluated in 415 typed isolates using the Kirby-Bauer method according to Clinical and Laboratory Standard Institute guidelines. Human and environmental isolates were tested against to 18 molecules and isolates from wild boar against 12 molecules. *S.* Typhimurium monophasic variant 1.4.[5].12:i:-, *S.* Veneziana and *S.* Newport were the most prevalent serotypes identified in humans, environmental samples and wild boar, respectively. The degree of antimicrobial resistance varied according to different sources, with 80.4% of *Salmonella* isolates from humans, 50.0% of those obtained from environment and 7.7% from wild boars displaying resistance for at least one molecule. We uncovered highest levels of resistance against to the combinations of sulfadiazine + sulfamerazine + sulfamethazine, in human (93.3%; 95% CI=87.0–97.0) and environmental strains (74.2%; 95% CI=62.0–84.2) and trimethoprim + sulfamethoxazole (human strains: 66.7%; 95% CI=43.0–85.4), (environmental strains: 58.3%; 95% CI= 36.6–77.9), while only one resistance pattern was observed against to trimethoprim + sulfamethoxazole in wild boar isolates. Multiple resistant patterns (≥ 2 molecules) were observed in both environmental and human isolates, which concurrently displayed resistant behaviour to up to 5 and 8 antibiotics, respectively. Notwithstanding, multiple resistant patterns often occurred among human serotypes (58.9%; 95% CI=51.5–66.1) compared with environmental isolates (16.4%; 95% CI=8.8–27.0). Fortunately, apart a high resistance observed against azithromycin in environmental strains, the resistances against high critically important molecules observed were generally low. Considering the public health safety, this is an important observation, being these molecules essential for specific treatments in humans.

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STAPHYLOCOCCUS PSEUDINTERMEDIUS FROM ANIMAL CLINICAL SPECIMENS: CHARACTERIZATION AND STORAGE IN A NATIONAL BACTERIAL COLLECTION (TUCC)

Patrizia Nebbia, Patrizia Robino, Alessandro Bellato, Maria Cristina Stella, Daniela Scalas

Università degli Studi di Torino, Dipartimento di Scienze Veterinarie.

Corresponding author: P. Nebbia (patrizia. nebbia@unito.it)

The adoption of a One Health perspective for improving knowledge and understanding of antimicrobial resistance (AMR) in global public health has determined the need for a common approach to human and non-human sources for collection, storage and use of pathogens. To accomplish this goal, since 2016 the University of Torino has been developing a biobank named Turin University Culture Collections (TUCC, http://www.tucc.unito.it/), which includes more than 700 microbial resources (i.e. bacteria, yeasts, filamentous fungi) collected by four different departments. As part of this project, the Department of Veterinary Sciences has gathered 100 veterinary pathogenic strains, belonging to various species all relevant for animal health.

The aim of this study was to improve the TUCC repository by focusing our attention on methicillin-resistant *Staphylococcus pseudintermedius* (MRSP), an increasing public health concern in dogs and cats mainly due to clonal spread. MRSP itself is not a problem for healthy humans, but it could affect hospitalized patients, thus rising future concerns if this bacterium is transmitted from colonized or diseased animals to pet owners or veterinarian staff. A total of 40 *S. pseudintermedius* isolates (35 from dogs and 5 from cats) were obtained from clinical samples of the Veterinary Teaching Hospital of the University of Torino between 2019 and 2021. *S. pseudintermedius* strains were isolated by standard culture methods, identified by MALDI-TOF MS and by PCR (*nuc* gene). Finally,16S-rRNA sequencing was performed as this technique is required for inserting strains into the collection. Antimicrobial susceptibility testing (AST) towards a panel of 21 antibiotics belonging to 18 different classes, including the Highest-priority Critically Important Antibiotics (HCIA,), was performed by disk-diffusion method and E-test MIC assay. Results were analysed according to EUCAST clinical breakpoints.

All collected strains were found to be resistant at least to one antimicrobial drug. Based on class resistance, 75.0 % of strains (90% C.I.: 63.8–86.2%) were defined as multi-drug resistant, whereas no extensively-drug resistant strains were found. All strains were selected and included within the TUCC biobank, with at least two different long-term storage methods (-150°C and lyophilization), according to standard protocols.

Biobanking of highly characterized bacterial strains might represent a key strategy for advancing basic research against emerging AMR pathogens either in veterinary or human fields, as it might ensure high-quality biological resources and associated data for clinical and translational research, with special emphasis on phenotypic and antimicrobial profiles.

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ANTIBIOTIC RESISTANCE PROFILES IN COAGULASE-NEGATIVE STAPHYLOCOCCI ISOLATED FROM EQUINE FARMS

Patrizia Nebbia, Alessandro Bellato, Maria Cristina Stella, Erica Milani, Michela Bullone, Alberto Tarducci, Patrizia Robino

Università degli Studi di Torino, Dipartimento di Scienze Veterinarie.

Corresponding author: P. Nebbia (p.nebbia@unito.it)

Coagulase-negative staphylococci (CoNS) are a widespread group of bacteria that colonize the skin and mucous membranes of humans and animals. However, these species could also cause critical infections in animals such as mastitis, urinary tract infections, osteomyelitis and dermatitis (1). Moreover, CoNS easily acquire resistance, not only to b-lactams but also to other antimicrobial classes. For those reasons, these bacteria are useful indicators of the degree of antimicrobial resistance (2).

The aim of this study was to (I) assess the prevalence of methicillin-resistant CoNS strains (MR-CoNS) in the upper respiratory tract of healthy horses and their caregivers from local farms (Piedmont) and (II) to evaluate the antibiotic resistance against molecules used both in veterinary and human clinical practice. MR-CoNS were initially isolated from nasal swabs of 158 subjects (121 horses and 37 men) by culturing them on a selective medium. Then, the strains were identified by MALDI TOFF-MS and tested towards twenty-one epidemiologically relevant molecules, which belong to eighteen different classes of antimicrobials. Strains resistant to three or more classes were defined as multi-drug resistant (MDR) (3).

Forty-four strains were isolated from 29 horses and 9 caregivers, with a 24.0% (90%Confidence Interval [CI]: 17.6–30.3%) prevalence in horses and 24.3% (CI: 12.8–35.9%) in the caregivers. They belonged to 10 different CoNS species; the most prevalent was *S. sciuri*, followed by *S. fleuretti*, and *S. equorum*. In these strains, we observed high rates of resistance towards antibiotics that are usually administered in veterinary medicine (e.g., 34% gentamicin,25% tetracycline). What is worse is that they were also endowed with resistance to antimicrobial classes for exclusive human use such as glycopeptides (18% of resistant strains to teicoplanin, 14% to telavancin, 2% to vancomycin), ansamycins (12% to rifampicin), oxazolidinones (2% to linezolid), lipopeptides (2% daptomycin), and streptogramins (2% toquinupristin-dalfopristin). In addition, a high prevalence (64%, CI: 57.7–70.3%) of MDRs was observed in both caregivers and horses, suggesting the possibility of horizontal transmission of CoNS strains between animals and humans in close contact.

Since selective pressure from antibiotics is the main cause of the emergence of MDR bacteria, it is essential to reiterate the importance of more prudent use of antimicrobials in the veterinary field, in order to limit the phenomenon of antibiotic resistance. Nonetheless, the presence of strains resistant to exclusively-human antibiotics suggests that a one-health approach is badly needed.

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ANTIMICROBIAL RESISTANT ACINETOBACTER SPECIES IN WILD BIRDS

Anna-Rita Attili (1), Martina Linardi (1), Livio Galosi (1), Francesca Paola Nocera (2), Patrizia Robino (3), Patrizia Nebbia (3), Filomena Fiorito (2), Lucia Biagini (1), Giacomo Rossi (1), Vincenzo Cuteri (1), Luisa De Martino (2)

(1) Università di Camerino, Scuola di Bioscienze e Medicina Veterinaria. (2) Università di Napoli "Federico II", Dipartimento di Medicina Veterinaria e Produzioni animali. (3) Università di Torino, Dipartimento di Scienze veterinarie.

Corresponding author: A.-R. Attili (annarita.attili@unicam.it)

Acinetobacter species belonging to the *Acinetobacter calcoaceticus-Acinetobacter baumannii* (ACB) and not-ACB complex have become of particular concern in veterinary and human medicine because of their antimicrobial resistance phenotypes and their involvement in case of coinfection with the pandemic SARS-CoV-2 [1, 2]. As wildlife is still overlooked in the epidemiology of medical important antibiotic-resistant bacteria, two free-ranging populations of Scarlet macaw (*Ara macao cyanoptera*) in Guatemala (n=10) and Belize (n=15) and a rescued group of Yellow-headed amazon (*Amazona oratrix belizensis*) in Belize (n=15) were sampled to investigate the epidemiological role in AMR *Acinetobacter* species spreading.

Faecal samples (n=16), cloacal (n=34) and choanal swabs (n=14) were collected and cultured for *Acinetobacter* spp. isolation. Colonies were identified at level species (bioscore >2.300) by matrix assisted laser desorption ionization-time of flight mass spectrometry (MALDI-TOF MS: SOP Direct Transfer Procedure Revision.4; Bruker Daltonics, Germany). Susceptibility to a panel of 17 human and veterinary antibiotics, belonging to 11 different categories (aminoglycosides, penicillins, antipseudomonal penicillins+β-lactamase inhibitors, 1st 2nd 3rd and 4th generation cephalosporins, antipseudomonal fluoroquinolones, antipseudomonal carbapenems, monobactams, tetracyclines, macrolides, folate pathway inhibitors, polimixins), was assessed by Kirby-Bauer and MIC (E-test) methods, according to the EUCAST guidelines [3]. Data were analyzed using Chi Squared (STATA 13.0). In wild birds, 30 different bacterial species (n=218) were isolated, 67.4% belonging to Gram positive (n=147) and 32.6% to Gram negative (n=71). The family *Moraxellaceae* (4.2%, n=71) was represented by *Acinetobacter* spp. strains, cultured from choanae (P=0.0001). Members of ACB-complex (*A. dijkshoorniae*) accounted for 33.3% of isolates and not ACB-complex strains (*A. haemolyticus*) for 66.7%. While not ACB-complex strains resulted sensitive to all antibacterial categories tested, the AMR strains were significantly represented in ACB-complex (P<0.0001). In particular, according to Magiorakos et al. [4], *A. dijkshoorniae* resulted multidrug-resistant (MDR), showing a 100% of resistance to penicillins, penicillins+β-lactamase inhibitors, 1st, 2nd, 3rd generation but not to 4th generation cephalosporins, carbapenems, monobactams, tetracyclines, and macrolides.

These findings suggest that Scarlet macaw and Yellow-headed amazon populations can harbour *Acinetobacter* spp. members and not member of ACB-complex. Wild birds could act as carriers of MDR *Acinetobacter* strains, spreaders in the environment, and vectors of AMR as secondary sources of AMR for humans and other animals, representing sentinels mirroring the presence of AMR in human and animal influenced-environment.

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MOLECULAR CHARACTERIZATION OF SELECTED CANINE VIRUSES IN DOGS TRADED FOR CONSUMPTION, NIGERIA

Linda A. Ndiana (1, 2), Gianvito Lanave (1), Costantina Desario (1), Aya A.K. Zarea (1), Francesco Mira (3), Nicola Decaro (1)

(1) Department of Veterinary Medicine, University of Bari, Valenzano, Bari, Italy. (2) Departement of Veterinary Microbiology, Michael Okpara University of Agriculture, Umudike, Nigeria. (3) Istituto Zooprofilattico Sperimentale della, Palermo, Italy.

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

Canine parvovirus (CPV), canine distemper virus (CDV), canine adenovirus types 1 and 2 (CAdV-1/2) and canine circovirus (CanineCV) are well-known pathogens, which pose a threat to domestic dogs worldwide (1, 2, 4). In some states in Nigeria, dog meat is a widely consumed delicacy. Dogs of indigenous breeds and with no clinical history are transported from the north to other regions of the country for human consumption. To date, while circulation of CPV has been previously reported, the circulation of CDV, CAdVs and CanineCV has not yet been described in the country.

The aim of this study was to investigate the presence of CPV, CDV, CAdVs, and CanineCV in slaughtered dogs from Nigeria to elucidate the role of trade dogs in the epidemiology of these canine viruses.

A total of 100 blood samples were collected from dogs with no clinical history at slaughter slabs in Uyo, Akwa-Ibom State, Nigeria. DNA and RNA extracts were screened by real-time PCR (qPCR) assays with primers and probes specific for CPV, CAdVs, CanineCV DNA, and CDV RNA respectively. Samples testing positive for CPV were characterized by minor groove binder (MGB) probe-based qPCR assays. Full VP2, partial rep, and partial N genes were amplified from CPV, CanineCV and CDV-positive samples, respectively, and subsequently sequenced. Ethical approval was obtained from the ethics committee of the College of Veterinary Medicine, Michael Okpara University of Agriculture, Umudike, with number MOUAU/CVM/REC/202118. The sampling points were mapped using ARCGIS® software.

Out of 100 collected dog samples, 83 tested positive for CPV (83/100, 83%), of which 27 (32%) were characterized as CPV-2c and 5 (6%) as CPV-2a. Three dogs (4%) were co-infected with CPV-2a and 2c, whereas 48 (58%) samples were not typed. No CPV-2b was detected in this study. In addition, 14% (14/100) dogs were positive for CanineCV, 17% (17/100) for CDV and no sample for CAdV1/2.

Full VP2 capsid gene sequences were generated for 21 CPV-2c and for one CPV-2a positive samples. The VP2 protein of all CPV-2c strains displayed residues 5Gly, 267Tyr, 297Ala, 342Ile, and 370Arg.

By phylogenetic analysis of the VP2 capsid gene, 22 Nigerian CPV strains segregated into the CPV-2c clade together with European, Asian and African strains detected in dogs. Strain NIG/2021/265.21-76 (Accession nr ON063555) segregated into the CPV-2a clade together with other Asian, American and Indian strains retrieved from different dog and coyote host species.

Circovirus (CV) sequences from dogs in this study displayed a 92.0-98.5% nucleotide (nt) identity to CanineCVs detected in domestic dogs. CDV from two dogs were characterized as European viruses.

This report provides the first molecular evidence for circulation of CanineCV and CDV in Nigeria and corroborates a previous study that detected CPV-2c of Asian origin at a high frequency (3). The high infection rate could be explained with the lack of prophylaxis in trade dogs which may serve as vessels for virus amplification and dissemination across the country. Further surveys prior to and after arrival of dogs at the slaughtering points are required to clarify the real virus burden in these animals.

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PARATUBERCULOSIS IN SHEEP AND GOATS' FARMS: SEROLOGICAL INVESTIGATION AND PROPOSAL OF RISK ASSESSMENT CHECKLIST

Elisabetta Mondo (1), Raffaele Scarpellini (1), Federica Giacometti (1), Federica Savini (1), Angelo Peli (2), Mariana Roccaro (2), Federico Tomasello (1), Silvia Piva (1)

(1) Alma Mater Studiorum - Università di Bologna, Dipartimento di Scienze Mediche Veterinarie;
(2) Alma Mater Studiorum - Università di Bologna, Dipartimento di Scienze per la Qualità della Vita

Corresponding author: E. Mondo (elisabetta.mondo2@unibo.it)

Paratuberculosis is a chronic and contagious disease caused by *Mycobacterium avium* subsp. *paratuberculosis* (MAP), which affects mainly domestic and wild ruminants (1). MAP has a global spread and causes serious economic losses to the livestock industries and the only measures to control it are based on biosecurity practices and direct prevention that give good long-term results (2). The study aimed to determine the seroprevalence rate of MAP among sheep and goats on 33 farms in the Emilia Romagna region and to produce a proposal of a checklist relating to the risk of introduction and transmission of MAP in small ruminant herds based on the one available for bovine (3, 4). The study was conducted on 498 sera samples (392 sheep and 106 goats) collected during the Transmissible Infectious Diseases internship activities of the Degree Course in Veterinary Medicine (University of Bologna), from 2016 to 2020. The overall seroprevalence rate of MAP among sheep and goats was 4.5% (23 out of 498 samples), with a significantly higher percentage in goats (14%) than in sheep (2%). This result is in line with the range of seroprevalence of MAP in Italy that is between 1.6% and 24.9%, a wide range given by the differences in herd management (5,6). As regards animal age and farm's size, the highest percentage of positives (30%) was observed in animals aged from 2 to 4 years and in herds with more than 170 animals.

As regards the checklist, the bovine' s one was applied in 30 farms and its application in small ruminant herds needed to be adapted. The changes were related in particular to the animal species (goat and/or sheep), the number of the production group, the delivery and grazing management.

In conclusion, the results of this study allow us to increase the knowledge of MAP in small ruminants, since they are currently scarce and to propose a possible checklist to apply in small ruminant herds. Further studies, increasing the number of samples and herds with a standardized sampling will be necessary to evaluate the current spread of paratuberculosis in small ruminants and the effectiveness of the checklist to produce a program control for the risk assessment and the implementation of management strategies to prevent the disease.

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CHARACTERIZATION OF SULFONAMIDE RESISTANCE GENES IN GRAM NEGATIVE STRAINS ISOLATED FROM FARM AND DOMESTIC ANIMALS IN ITALY

Angela Maria Catania (1), Maria Cristina Stella (1), Matteo Pirotta (1), Valentina Meloni (1), Francesca Cimino (2), Carlo Castellina (3), Elena Grego (1)

(1) Università degli Studi di Torino, Dipartimento di Scienze Veterinarie. (2) Istituto Zooprofilattico Sperimentale del Piemonte, Liguria e Valle d'Aosta, Torino. (3) Aglietto Natura s.r.l.

Corresponding author: A.M. Catania (angelamaria.catania@unito.it)

The use of antibiotics in animals' farms contributes to the enhancement and spread of antibiotic resistance bacteria [1]. In veterinary medicine, sulfonamides belong to first-line antibiotics for treatment of animal infections, according to EMA (European Medicines Agency). Bacteria in the gut microbiota of animals constitute a large reservoir for antibiotic resistance genes (ARGs), and mobile genetic elements associated to antibiotic resistance, can be propagate ARGs between environment, animals, and humans, with consequent health risk [2].

Resistance to sulfonamides is mediated by four plasmid-borne genes (*sul1*, *sul2*, *sul3*, and *sul4*).

In this study the presence and the frequency of *sul* genes was investigated in 48 *Escherichia coli* strains, isolated from five species: cattle, swine, poultry, dogs, and cats, previously characterized as resistant to sulfamethoxazole and trimethoprim, based on phenotypical evaluation.

PCR reactions were optimized to assess the presence of *sul* genes. A total of 45/48 samples (94%) showed positivity for at least one *sul* gene, whereas 3/48 (6%) resulted negative.

Seventy-six *sul* genes were identified: fifteen in cattle (6 *sul1*, 7 *sul2*, 1 *sul3* and 1 *sul4*), thirteen in swine (3 *sul1*, 8 *sul2* and 2 *sul3*), fifteen in poultry (8 *sul1*, 5 *sul2*, 1 *sul3* and 1 sul4), seventeen in cat (4 *sul1*, 7 *sul2*, and 6 *sul4*), and sixteen in dog samples (7 *sul1*, 8 *sul2*, and 1 *sul4*).

The most frequent gene identified was *sul2*, with an incidence of 78%, followed by *sul1* (62%), *sul4* (20%) and *sul3* (9%). Moreover, in more than half samples, the presence of two or three sulfonamides resistance genes was assessed.

Positive PCR results were confirmed by Sanger sequencing and phylogenetic analysis was performed. Results highlighted how sequences related to the same gene, although isolated in different animal species, resulted highly conserved.

It was interesting note the presence of *sul* genes in animals of zootechnical interest and, for the first time, also in pets.

The present study contributes to underline the importance to study pets resistome, moreover

the presence of *sul4* gene not only in environmental and human, but also in animals resistome (including cats and dogs), highlighted the importance of a "one health" approach recognizing the interdependence of animal, human and environmental.

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MONITORING OF VIRAL AGENTS IN UNTREATED AND TREATED WASTEWATERS IN NORTHERN ITALY, PIEDMONT

Angelo Romano (1), Cristina Pignata (2), Ilaria Silvia Rossella Gorrasi (3), Claudio Caruso (4), Daniela Manila Bianchi (1), Lucia Decastelli (1)

(1) Istituto Zooprofilattico Sperimentale PLV, SC Sicurezza e Qualità degli Alimenti. (2) Università degli Studi di Torino Dipartimento di Scienze della Sanità Pubblica e Pediatriche. (3) Dipartimento di Prevenzione – Azienda Sanitaria Locale CN1, SC Igiene degli Alimenti e Nutrizione. (4) Azienda Sanitaria Locale CN1, U.O.T. Racconigi.
Corresponding author: A. Romano (angelo.romano@izsto.it)

Infectious diseases caused by viruses represent a significant public health problem. Enteric viruses have been recognized as the causative agents of many sporadic cases and outbreaks of gastroenteritis and other illness originating from contaminated water. In recent decades, there have been several epidemics caused by pathogenic viruses, for example, the SARS-CoV-1 epidemic that occurred in 2003, the H1N1 flu pandemic in 2009-2010 and the current SARS-CoV-2 pandemic [1]. Commonly studied enteric viruses belong to the following families: *Picornaviridae, Adenoviridae, Caliciviridae* and *Reoviridae* and the most important from a public health point of view are rotaviruses (RV), adenoviruses (AdV), noroviruses (NoV), enteroviruses (EV) and hepatitis A (HAV) and E (HEV) viruses [2]. Human enteric viruses are excreted in large quantities in the faeces of infected individuals (symptomatic and asymptomatic) and its spread in the environment can also derive from livestock sewage from farms which are not collected to purification plants.

The aim of the present work was the evaluation of the occurrence of viral pathogens (EV, AdV, NoV GI and GII, HAV, HEV and RV) in raw and treated wastewaters from Wastewater Treatment Plants (WTP) and livestock sewage. Sixteen raw and 16 treated samples were collected from two WTP in the Piedmont area, northern Italy, between August 2020 and April 2021. Twelve samples of livestock sewage were collected from 3 livestock between December 2020 and July 2021. Wastewater samples were processed following, for concentration, the standard WHO procedure for Poliovirus surveillance with modifications [3] and then nucleic acids were extracted from 5 ml of concentrated water samples using the eGeneUP® semi-automated extraction system (bioMérieux) with magnetic silica NucliSENS (bioMérieux). Livestock sewages were directly processed with AllPrep® PowerViral® DNA/RNA Kit (QIAGEN) for total nucleic acid extraction. Adenovirus, EV, NoV GI/GII, HAV, HEV and RV were analyzed by molecular kit available at CEERAMTOOLS® in all samples.

All wastewater samples (100 %) tested positive for at least one viral pathogen. RV and NoV GII were the most detected pathogens, 93.75% and 62.50% of the analyzed samples respectively. NoV GI, EV and HEV showed positivity of 37.50%, while AdV was found in 43.75% of the samples. In both WTP, a decrease in positivity was observed after treatment in all samples, with the exception of RV, however, a decrease in viral load was observed. Concerning the livestock sewages, 91.6 % of the tested samples were positive for at least one viral pathogen and RV was the most detected virus, found in 83.33 % of the samples and EV, HAV and HEV were reported in 25.00 % of the analyzed samples.

Even if molecular methods are capable of detecting only pathogen genomes and do not provide information on infectivity, the approach could be used as monitoring tool. In conclusion, despite the frequent occurrence and diversity of enteric viruses found in raw sewage, a considerable reduction in the presence of viral genomes was detected after treatment.

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THE CASE OF WEST CAUCASIAN BAT LYSSAVIRUS IN AREZZO (ITALY): A SCIENTIFIC STORYTELLING FOR THE HEALTH SYSTEM

Ginevra Palmerini (1), Maria Luisa Marenzoni (2), Raoul Ciappelloni (3)

(1) Medico Veterinario Libero Professionista, (2) Università degli Studi di Perugia, Dipartimento di Medicina Veterinaria, (3) Istituto Zooprofilattico Sperimentale dell'Umbria e delle Marche "Togo Rosati".

Corresponding author: R. Ciappelloni (raoul@ciappelloni.it)

This work deals with the case of West Caucasian Bat *Lyssavirus*, transmitted for the first time from a bat to a cat in the territory of Arezzo City (Italy) and isolated from the Experimental Zooprophylactic Institute of Venice (IZSVe) in June 2020. A journalistic interview with the Veterinarian who visited the cat for the first time, is used as an educational resource, in order to convey its medical and scientific contents to Veterinarians and health operators. The story, from the analysis of the first sign of disease, the evolution of the events that made the suspicion of Rabies and finally the isolation of the *Lyssavirus* in June 2020, is narrated in detail. Through a specific Web interface, developed in the "Micro Epidemic One Health" - MEOH Project by Experimental Zooprophylactic Institute UM and the Department of Veterinary Medicine of Perugia University - Italy, a broad diffusion of this story is promoted. Our experience shows that the engaging storytelling and the MEOH reading environment interface, ensures high visibility and communicative value to the *Lyssavirus* story for different types of stakeholders in the scientific and social communities. Since this interview with the Veterinarian is a "true personal story", it loses the dryness of the technical report and pleasantly guides the reader through the biomedical information. Moreover, it actively involves and interests the reader, also for its simple structure and content rich in personal considerations or emotional implications related to the zoonotic potential of the virus. That's why this Vet story solicits healthcare Professionals participation in the commentary of the storytelling, through the set of informatic tools available in the MEOH interactive platform. This "publishing System" can foster the diffusion of sound medical information, critical to learning and innovation, in the public health sector. In conclusion, this work points out that if we really intend to preserve the territory from severe zoonoses, we must collect their stories from health Practitioners working in the territory and convey them, through advanced publishing Systems, to the scientific community and civil society.

ARNA

EFFECTS OF OLIVE CAKE INCLUSION IN THE DIET OF MODICANA DAIRY COWS ON MILK QUALITY

Annalisa Amato (1), Luigi Liotta (1), Carmelo Cavallo (1), Vincenzo Chiofalo (1), Esterina Fazio (1), Arianna Bionda (1, 2) Vincenzo Lopreiato (1)

(1) Università degli Studi di Messina, Dipartimento di Scienze Veterinarie. (2) Università degli Studi di Milano, Dipartimento di Scienze Agrarie e Ambientali, Produzione, Territorio, Agroenergia.
Corresponding author: Amato A. (annalisa.amato@unime.it)

Olive cake (OC) is a typical by-product of the Mediterranean area, which disposal has a significant environmental impact, but due to its richness in polyphenols and in Unsaturated Fatty Acids (UFA), when pitted, it is used in the diet of monogastric [1] and ruminants [2, 3]. For this reason, to contribute to the sustainable recovery of this by-product, in Sicily, a disciplinary was created and approved by EU, called QS Sicilia (Safety Quality Sicily) which allows the inclusion of olive cake in feed for dairy cows up to 10%. The aim of this study was to evaluate the effects of OC integration on diet of a local dairy cattle breed on milk quality. A total of 120 multiparous Modicana breed, were sampled and divided in two homogeneous groups, according to BCS (2.75±0.15) and lactation period (30±5d), called CTR and BIOTR. The control group (CTR) was fed a conventional diet, whereas BIOTR group was fed with an 8% inclusion of dried pitted olive cake. Diets were isonitrogenous (20% of CP/kg DM) and isoenergetic (1.05 UFL/kg DM) between groups and the experiment lasted 120 days. Bulk milk samples were collected, and milk analysis (Fat, Protein, Casein, Lactose, Solids, Urea, Citric Acid, Fatty acids profile) was monthly assessed using MilkoScanTM 7RM (FOSS Analytical A/S, Hilleroed, Denmark) through MIR spectrometry, according to Tullo [4]. Data were subjected to SAS software for statistical analysis. No significant difference was observed on milk gross composition, while a different fatty acid profile was recorded. In particular, BIOTR group showed a significant increase in UFA (BIOTR 1.29 g/100 mL vs CTR 1.06 g/100 mL; $P<0.05$) and PUFA (BIOTR 0.13 g/100 mL vs CTR 0.08 g/100 mL; $P<0.05$) content of milk and a higher PUFA/UFA ratio (CTR 0.07 vs. BIOTR 0.10; $P=0.08$). Whereas the CTR group showed a higher ratio for both MUFA/UFA (CTR 0.94 vs. BIOTR 0.89; $P<0.05$) and MUFA/PUFA (CTR 12.77 vs. BIOTR 8.93; $P=0.06$). There were no significant effects of diet on milk concentration of SFA and MUFA, and SFA/UFA ratio. These results, also in agreement with our previous studies [2], showed that as well as for widespread breed, in local breeds such as Modicana, the administration of OC had positive effects on milk fatty acids profile; as well known, unsaturated fatty acids, are positively related with human health [5], thereby, their increase in milk brings added value to dairy products [6]. Furthermore, the use of OC in Modicana diet could represent an opportunity to reduce cost associated with animal nutrition; that is important in a context in which the cost of raw materials is increasing worldwide.

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CHEMICAL CHARACTERISTICS AND DAIRY YIELD OF SICILIAN CANESTRATO CHEESE PRODUCED WITH KIWIFRUIT EXTRACT

Luigi Liotta (1), Fabrizio Nicosia (2), Federica Litrenta (3), Vincenzo Lopreiato (1), Carmelo Cavallo (1), Rosita La Cava (4), Marco Scalisi (1), Cinzia Caggia (2), Cinzia Randazzo (2)

(1) Università degli Studi di Messina, Dipartimento di Scienze Veterinarie. (2) Università degli Studi Catania, Dipartimento di Agricoltura, Alimentazione e Ambiente. (3) Università degli Studi di Messina, Dipartimento di Scienze Chimiche, Biologiche, Farmaceutiche, ed Ambientali. (4) Caseificio La Cava srl, Catania.

Corresponding author: L. Liotta (lliotta@unime.it)

The worldwide increase in cheese production, combined with the reduction in supply and religious reasons (Islam and Judaism), have led to the search for alternative enzymes for coagulation of milk, as appropriate substitutes of animal rennet [1]. Enzymes extracted from plants, proteases like ficin from *Ficus* sp. latex, papain from *Carica papaya* and cardosins from *Cynara* sp. have become a subject of growing interest in dairy technology [1]. Among plant coagulants, kiwifruit (*Actinidia deliciosa*) contains high amounts of actinidin (EC. 3.4.22.14), a cysteine protease, which showed a high potential for its use as a milk-clotting agent in cheese making process [2, 3]. The aim of the present study was to evaluate the effect of kiwifruit enzyme on the quality of Sicilian Canestrato cheese. In addition, the quality parameters were compared to those of Canestrato cheese made with commercial lamb rennet (Caglio pasta provo 5, Caglificio Clerici, Italy). In detail, pasteurized milk was clotted by kiwifruit (*Actinidia deliciosa*) extract (3% v/v) following the traditional flowchart for the Canestrato cheese production. The curd was broken into small granules with a thorn, and then pressed, to facilitate the purging of the whey, then brined and stored at 14°C and 70-80% humidity at least for 15 days. The pasteurized milk and the resulting cheese were subjected to chemical determinations. In particular, a total of 12 semi-matured cheese, 6 for each experimental thesis (7 days; 0.5kg each, by mixed milk: 50% sheep/50% cow) were used and analyzed in triplicate. Moreover, total polyphenols content of experimental and commercial Canestrato cheese samples was determined spectrophotometrically using Folin-Ciocalteu's reagent. Results showed that no significant differences were observed for total lipids (20.54% vs. 19.97% for CONTROL and CHEESHAL, respectively; P=0.15), total proteins (24.44% vs. 24.80% for CONTROL and CHEESHAL, respectively; P=0.32), whereas significant differences were recorded for the polyphenols content (mg/kg of cheese: 88.02 for CONTROL vs. 141.07 for CHEESHAL; P<0.05) according to Serra et al. [4]. Cheese made with kiwifruit extract showed a significant lower (P<0.05) cheese yield (13%) than cheese from lamb rennet (16%). Preliminary results of this study point out the opportunity of using kiwifruit extract as a promising alternative to commercial rennet in milk coagulation, but improvements in cheesemaking technology are needed to increase milk yield. The results, however, suggest that kiwifruit extract could be a potential plant source of coagulant to produce Sicilian Canestrato cheese with improved nutritional characteristics.

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E-NOSE AND E-TONGUE FOR SENSORY QUALITY EVALUATION OF CANESTRATO CHEESE PRODUCED WITH KIWIFRUIT EXTRACT

Francesca Accetta (1), Luigi Liotta (1), Annalisa Amato (1), Rosita La Cava (2), Doriana Aliquò (1), Gianluigi Agolino (3), Cinzia Randazzo (3), Ambra Rita Di Rosa (1)

(1) Università degli Studi di Messina, Dipartimento di Scienze Veterinarie. (2) Caseificio La Cava srl, Catania. (3) Università degli Studi Catania, Dipartimento di Agricoltura, Alimentazione e Ambiente.

Corresponding author: A. Amato (annalisa.amato@unime.it)

The development of texture, aroma, and flavor of most cheese types is directly affected by casein proteolysis, which is one of the major biochemical changes occurring during cheesemaking and ripening [1]. The most widely used proteases in cheesemaking is the animal rennet, which is extracted from the abomasum of the newborn ruminants. Recently, plant derived enzymes are proposed as valid substitute for the milk clotting and among them the kiwifruit extract appears very promising [2]. Up to now, little data are available on the effects of kiwifruit extract on the organoleptic characteristics of the cheese. The aim of this work was to assess odor and taste properties of cheese made with kiwifruit extract as milk coagulant, using a sensor-based instruments platform. The study was conducted on two set of 6 samples each of fresh cheeses (15 days of seasoning), obtained from a blend of pasteurized cow and ewe's milk, which was coagulated using kiwi extract (H cheese). Cheese samples made with commercial lamb rennet was used as control (C). The cheese samples were analyzed using an E-nose (FOX 4000, Alpha M.O.S., Toulouse, France) with 18 MOS sensors and a potentiometric E-tongue (αAstree, Alpha M.O.S., Toulouse, France) with 7 chemical sensors. For each sample, 10 replicates were analyzed using both instruments.

Exploratory data analysis was performed with principal component analysis (PCA) and expressed based upon the discrimination index (DI). The E-tongue PCA plot shows that the C group was well discriminated from H cheese with a discrimination index of 92%. The first two components (PC1 and PC2), represent 99.70% of the total variance between the sample measurement. The sensors named AHS and SCS showed a greater intensity of response towards the H cheese. The two sensors respond to acid and bitter taste respectively [3] highlighting a strong difference between the two groups of cheeses, in accordance with other studies [4]. The E-nose PCA plot shows a discrimination index of 91%. Moreover, PC1 and PC2, explain a variance of 99.94%, showing also a clear separation between the two groups considered. Specifically, the LY-type sensors, sensitive to short-chain volatile fatty acids and aldehydes, moving towards the C group, while the H group is defined by the P-type sensors, sensitive to methane, propane and other aliphatic non-polar molecules and the T-type sensors, sensitive to polar alcoholic and chlorinated compounds [5]. The artificial senses showed the effect of bitter compounds in the final products underlying the limitations of plant coagulants in cheese production; these results, although preliminary, can be very useful for developing cheese making strategies aimed at reducing the bitterness in the final products. Certainly, at the end of the trial, a panel test combined with E-nose and E-tongue will be carried out to quantify the effect on the consumer.

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EXPLORING THE FECAL MICROBIOTA OF RAGUSANO DONKEY

Viviana Floridia (1), Grazia Galeano (1), Letterio Giuffrè (2), Alessandro Zumbo (1), Orazio Romeo (2), Enrico D'Alessandro (1)

(1) Università degli Studi di Messina, Dipartimento di Scienze Veterinarie. (2) Università degli Studi di Messina, Dipartimento di ChioBioFarAm.

Corresponding author: V. Floridia (viviana.floridia@studenti.unime.it)

The "Ragusano" is a donkey breed reared on the Mediterranean island of Sicily (Italy), where for a long time it has played an important cultural role both as a working animal, for carrying goods, and for its high-quality meat and milk which are still consumed by people today [1,2]. Recently, thanks to the development of the high-throughput DNAs sequencing techniques, most of the scientific researches focused their attention in understanding the taxonomic diversity of microbal communities populating animal gastrointestinal tracts, as the gut microbiota is known to play a crucial role in modulating host health [3-6]. The intestinal tract of animal belonging to the *Equidae* family is colonized by a diverse community of microorganisms such as bacteria, archea, fungi, parasites, protozoa, and viruses. Unfortunately, no data are currently available about microbial diversity of ragusano donkey gut microbiota. For this reason, the aim of this study was that to characterize, for the first time, the fecal microbiota of this autochthonous donkey, in order to explore the taxonomic and functional profiles of gut microbes potentially associated with this animal. Genomic DNA was extracted from each fecal sample taken directly from the rectal ampoule of 18 female of Ragusano donkeys, officially registered in "Herd Book". The V3-V4 region of the bacterial 16S rDNA gene and the ITS1 fungal region using paired-end strategy (2 x 300 bp) on an Illumina Miseq platform. Bioinformatics analysis included raw reads processing "Amplicon Sequence Variants" (ASVs) identification and classification, functional prediction and statistical analysis. In this metagenomic study, a total of 23 phyla, 190 families and 350 bacterial genera were identified using an identity threshold of 74%, 85% and 95% respectively; of these, only 11 phyla, 69 families and 125 genera showed a relative abundance greater than 0.1%. The most abundant phylum was *Bacteroidetes* (~44%) followed by *Firmicutes* (~40.8%), *Spirochaetes* (~8.9%), *Fibrobacteres* (~3.2%) and *Proteobacteria* (~2%). However, this trend was not always confirmed as in some fecal samples (6/18 samples), the most abundant phylum was that of *Firmicutes*, with an abundance rate ranging from ~42% to ~51%. Regarding fungal diversity, our analysis identified a total of 11 phyla, 125 families and 165 fungal genera of which 9 phyla, 59 families and 70 genera showed a relative abundance rate higher than 0.01%. At the phylum level, *Basidiomycota* was the most abundant fungal group (~36.6%) followed by *Neocallimastigomycota* (~24%), *Ascomycota* (~11.3%), and *Mucoromycota* (~3.7). Overall, the most abundant phylum/microbial families (both bacteria and fungi) found in this study reflect the remarkable ability of donkeys to better digest fibers. The complex function of Ragusano donkey' s microbiota is further supported by prediction of thousands of enzymatic classes involved in a number of biochemical and molecular pathways. In fact, in this study over 2000 enzymatic classes and 404 metabolic pathways were identified. Overall, the results of this study provide a first overview of the microbial communities colonizing the intestinal tract of the Ragusano donkey. Studies like this, offer a cutting-edge tool for the development of optimal animal husbandry and management solutions, as well as useful information for the welfare and enhancement of the Ragusano donkey breed. The obtained results are encouraging and may serve as a starting point for future microbiome investigations in this endangered autochthonous Sicilian donkey breed.

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A MULTI-SCALE PERCEPTION TO PERFORM THE SUSTAINABILITY OF BUFFALO LIVESTOCK

Angelo Fierro (1), Annachiara Forte (1), Gabriele Di Vuolo (2), Giovanna Cappelli (2), Esterina De Carlo (2), Luis Fernando Zuin (3), Mario Giampietro (4), Domenico Vecchio (2)

(1) Laboratorio di Urbanistica e Pianificazione Territoriale (LUPT) University of Naples Federico II, Naples, Italy. (2) Unit Animal Science and Welfare, Istituto Zooprofilattico Sperimentale del Mezzogiorno – CReNBuf, Portici, Italy. (3) Departamento de Engenharia de Biossistemas da Faculdade de Zootecnia e Engenharia de Alimentos da Universidade de São Paulo (FZEA-USP), São Paulo Brasil. (4) Institut de Ciència i Tecnologia Ambientals, Universitat Autònoma de Barcelona Barcelona, Spain; ICREA, Catalan Institution of Research and Advanced Studies, Barcelona, Spain.

Corresponding authors: A. Fierro (fierro@unina.it), D. Vecchio (domenico.vecchio@izsmportici.it)

The sustainability of animal husbandry represents a topic, often with explicit accusatory arguments, on which a substantial part of the narrative is focusing. The following contribution intends to present a relational model of representation of animal husbandry which partially rehabilitates the sector but which in any case highlights the many metabolic criticalities of the sector. The approach used is that of MuSIASEM (MultiScale Integrated Accounting of Societal and Ecosystem Metabolism), a relational multicriteria approach that allows to evaluate different metabolic features of the system. Through a structural and managerial characterization of the system (including animal welfare strategies) and of the related matter, energy and monetary flows, it is possible to represent the system in a more comprehensive way. The accounting method allows to evaluate the metabolic performance of the system under observation by means of 4 level: (i) feasibility, shows the constraints imposed by the ecosphere (both resources availability and the ability to receive wastes); (ii) viability, characterizes techno-economic strategies adopted by the system; (iii) desirability, is about normative and institutional behavior of the several social actors involved; (iv) externalization, describes the opening of the system and therefore its dependence on external resources, with the related environmental and social externalized impacts. Specific analytical examples will be presented for the buffalo livestock sector analyzed for the Campania Region, Southern Italy.

Results highlight a sector with a high degree of externalization, which highlights a tension with the expected strategies of the farm to fork. We will also discuss the criticalities of some solutions considered sustainable in the main stream of the narrative.

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THE EFFECT OF WHEY SUPPLEMENTATION ON FAECAL MICROBIOME COMPOSITION IN PIGS

Anna Maria Sutera (1), Viviana Floridia (1), Valentina Riggio (2), Giuseppe Tardiolo (1), Alessandro Zumbo (1), Enrico D'Alessandro (1)

(1) Università degli Studi di Messina, Dipartimento di Scienze Veterinarie. (2) University of Edinburgh, The Roslin Institute and R(D)SVS.

Corresponding author: A.M. Sutera (asutera@unime.it)

The gut microbiota plays a pivotal role in different physiological processes. The composition of the microbial community can be influenced by several factors, including nutrition [1-3]. Liquid whey (LW) represents a highly nutritious byproduct of the dairy industry. Its administration can affect body weight gain, feed efficiency, protein and fat digestibility, as well as mineral absorption and retention [4]. This study explored the effects of LW integration on faecal microbiota in 20 crossbred pigs (Landrace x Large White). The animals were homogeneous for body weight, age, and breeding management, and were divided in two groups (i.e., 10 individuals each), with the control group (CTRLg) fed with a pellet complete feed and the treatement group (LWg) receiving the same diet supplemented with LW, *ad libitum*. The trial lasted for a total of 60 days, from post-weaning (30 days old) until growing (90 days old), including an adaptation period of 15 days. Stool samples were collected at 30 (T0), 60 (T1), and 90 (T2) days of age. The environmental parameters, as well as the physiological condition of the animals, were daily monitored. The V3-V4 hypervariable regions of the bacterial 16S rRNA gene were sequenced in two MiSeq (Illumina) runs with 2 × 300-base paired-end reads. Raw sequences were processed using a pipeline combining QIIME2 and DADA2. High-quality reads were clustered into high-resolution Operational Taxonomic Units (OTUs). Using taxonomic assignment, OTU tables were collapsed from phylum to genus level. At the phylum level, *Firmicutes* and *Bacteroidetes* were the most abundant phyla at all times regardless of treatment. At genus level, the most prevalent genus was *Prevotella* regardless of the diet, whereas *Lactobacillus* showed differences between the two groups, being the predominant genus ($p = 0.002$) in LWg. Chao1 and ACE indices and Shannon, Simpson, and Fisher's alpha were used to calculate within sample microbial richness and diversity, respectively. Beta diversity was estimated by computing Bay-Curtis distances into Principal Coordinates Analysis (PCoA). Significant differences in alpha or beta diversity were assessed by a Kruskal–Wallis and Wilcoxon test, with significant differences observed between CTRLg and LWg ($p<0.001$) and at T2 ($p<0.002$). Alpha and Beta diversity analyses indicate that LW addition shifted the gut microbial community structure. In conclusion this study has shown that LW supplementation to the diet can have an effect on the bacterial community in the pigs' gut and should be further investigated.

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EFFECTS OF SUPPLEMENTARY FEEDING USING A DAIRY BY-PRODUCT ON FAECAL MICROBIOTA COMPOSITION IN NERO SICILIANO PIG

Anna Maria Sutera, Viviana Floridia, Laura De Maria, Giuseppe Tardiolo, Alessandro Zumbo, Enrico D'Alessandro

Università degli Studi di Messina, Dipartimento di Scienze Veterinarie.

Corresponding author: A.M. Sutera (asutera@unime.it)

Pigs' gut harbours thousands of different bacteria, whose composition and relative proportions vary depending on the breed, age, nutritional and environmental factors. In particular, genetic background is strongly associated with the host's gut microbial taxa and characteristics [1]. The Nero Siciliano is an autochthonous breed of a domestic black pig reared in Sicily (Italy) [2]. Recently, the faecal microbiome of the Nero Siciliano pig was explored [3] by using whole-metagenome shotgun sequencing approach, to increase the knowledge regarding the taxonomic and functional profiles of microbes associated with this rare and endangered-maintained pig breed. The present study evaluated the effects of supplementing the diet with liquid whey (W), a highly nutritious dairy by-product on faecal microbiota in 12 Nero Siciliano pigs. Six individuals were assigned to a control group (Cg) fed with a pelleted complete feed and the remaining six to a treated group (Wg) that received the same diet supplemented with W, *ad libitum*. The animals were homogeneous for body weight, breeding condition and age. The trial lasted 60 days (including ten days of adaptation to the diet). Faecal samples were collected from the rectal ampoule of each animal at different time points (T0, T1 and T2). The interval between T1 and T2 collection was of twenty-five days. The procedure of samples collection, storage, DNA extraction and sequencing, bioinformatic and statistical analysis followed a defined pipeline. The extracted DNA was prepared for the sequencing of the V3 and V4 regions of the 16 rRNA gene with a MiSeq (illumina, San Diego, CA, USA) in a 2×300 paired-end mode. Raw sequences were processed using the bioinformatics program QIIME 2 and Phyloseq packages of R. The alpha-diversity was similar between the groups and a tendency to a higher evenness was observed in the Wg. The classes *Clostridia*, *Bacteroidia*, *Bacilli* and *Spirochaetia* accounted for more than 90% of the community (~40, ~39, ~8, and ~7%, respectively), regardless of the diet. Concerning beta-diversity, the gut microbiome clearly changed between treatments and time-points (p-value=0.04). Despite the small sample size, these preliminary results show that W supplementation can have an effect on gut microbiome of the the Nero Siciliano pig. Further studies are therefore required, using bigger sample sizes.

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NIR SPECTROSCOPY AND MULTIVARIATE DATA ANALYSIS TO DETECT UNDECLARED MECHANICALLY SEPARATED MEAT (MSM) IN SAUSAGES

Francesco Pennisi, Martina Vona, Giovanna Esposito, Marzia Pezzolato, Elena Bozzetta

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

Corresponding author: F. Pennisi (francesco.pennisi@izsto.it)

In recent years, meat authenticity awareness has increased related to fraudulent activities spreading through the meat chain production. The European Food Safety Authority has expressed concern about mechanically separated meat (MSM) used in meat products [1]. MSM, as defined in Reg. (EC) No 853/2004, is obtained by mechanically removing meat from flesh-bearing bones after boning or from poultry carcasses [2]. Thus, the normal structure of the muscle fibre is mostly lost or modified in such a way that it is not comparable with regular meat, and that's why they are considered as secondary products, cheaper to produce and less valuable from a nutritional and qualitative point of view. In this context it's easy to understand why they can be used in a fraudulent way, undeclaring or minimizing their presence in products. Furthermore, there is a public health issue associated to the increasing of microbial activities caused by muscular fibre degradation and nutrients release.

Although the European Union obliges producers to indicate the presence of MSM on the label [3], there are no rapid, reliable and validated methods capable to detect it in food directly in field, in order to reveal frauds whenever MSM is not expressly indicated on the label. In fact, MSM is mostly identified in meat products by applying analytical methods, mainly based on calcium ion detection. Meanwhile, this project aims at developing a fast and non-destructive approach like NIR spectroscopy coupled with chemometric models, which represents an easy-to-use and high throughput method that could be directly applied on site productions.

In the present study, MSM was searched in frankfurter sausages made of meat of different species by acquiring NIR spectra with the portable laptop-controlled spectroscope MicroNIR 1700 PRO ES (VIAVI Solutions, San Jose, CA, USA). A dataset of reference samples made by 60 frankfurters containing MSM and other 60 clearly declared without MSM among ingredients, was collected and used as calibration set for the development of a prediction model. A discriminant model (PLSDA – Partial Least Square Discriminant Analysis) was built on calibration set using the software The Unscrambler X (VIAVI Lite Package, version 10.4.1, 2016 CAMO Software).

Firstly, an exploration analysis using Principal Component Analysis (PCA) was carried out on NIR spectra acquired on the outer side and on the inner side of samples. Since variables that mainly influenced the separation of samples were in the spectroscopic range between 1130 nm and 1350 nm, a PLSDA model to discriminate the two categories (MSM and NoMSM) of products was performed considering the reduced range. The model recognized MSM samples with a sensitivity of 100% in cross-validation and even the total accuracy was 100%. The predictive model was tested by acquiring spectra from commercial products bought in the local superstores. 64 sausages of different meat species containing MSM (32) and without MSM (32) were analysed by the PLSDA predictive model. The model sensitivity was 74,60%: poultry products (43) were mainly correctly classified, meanwhile MSM pork products (20+1 poultry product) represented the percentage of MSM not recognized.

The good classification results of the approach combining NIR spectroscopy and simple chemometric classification methods, especially for poultry samples, suggest great applicability directly in the marketplace by the consumers at the moment of purchase, as well as by the reselling companies when dealing with suppliers, or by authorities whenever making in site official controls.

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RESIDUAL FEED INTAKE AND ITS ASSOCIATION WITH BLOOD INFLAMMOMETABOLIC PROFILE IN PREWEANING SIMMENTAL CALVES

Abdulrahman Alharthi (1), Giulia Ferronato (2), Andrea Minuti (3), Ahmed Elolimy (4), Fiorenzo Piccioli-Cappelli (3) Erminio Trevisi (3), Annalisa Amato (5), Luigi Liotta (5), Vincenzo Lopreiato (5)

(1) Department of Animal Production, College of Food and Agriculture Sciences, King Saud University. (2) Dipartimento di Ingegneria Civile Ambiente Territorio Architettura e Matematica (DICATAM), Università degli Studi di Brescia. (3) Dipartimento di Scienze Animali, della Nutrizione e degli Alimenti, Università Cattolica di Piacenza. (4) National Research Centre, Department of Animal Production, Giza. (5) Dipartimento di Scienze Veterinarie, Università di Messina.

Corresponding author: A. Alharthi (abalharthi@ksu.edu.sa)

Identifying biological regulators of feed-efficiency in young dairy cattle from a physiological standpoint would maximize profit margins [1]. The RFI is defined as the difference between measured and predicted feed intake [calculated using a linear regression of actual intake on metabolic body weight ($BW^{0.75}$) and average daily gain (ADG)]. The most-efficient animals (M-Eff) have actual intakes smaller than predicted resulting in negative RFI coefficients, whereas the opposite is true for least-efficient (L-Eff) animals [2]. Hence, the aim of this study was to investigate the immunometabolic profile of calves categorized as most-efficient (M-Eff) or least-efficient (L-Eff) using RFI divergence during the preweaning period. Twenty Simmental calves were monitored through 60 d of age. Calves received 3 L of colostrum from their respective dams. From 2 to 53 d of age, calves were fed a milk replacer twice daily, whereas from 54 to 60 d (weaning) calves received only one meal. Calves had ad libitum access to concentrate and intakes were recorded daily. The measurements of BW and blood samples were performed at 0, 1, 7, 14, 21, 28, 35, 45, 54, and 60 d of age. The RFI coefficient was -0.11 and 0.10 kg of DMI/d for M-Eff and L-Eff calves, respectively. Despite colostrum IgG content was not different between groups, M-Eff calves had numerically greater plasma IgG content compared with L-Eff calves at 24h after colostrum intake (22.24±2.75 vs. 16.91±2.30, respectively; P=0.15). Throughout the entire pre-weaning period no differences were found for BW, with a weaning BW of 88.37±12.73 for L-Eff and 88.96±9.41 kg for M-Eff calves at 60 d (P>0.05). Overall, average daily gain did not differ between groups (0.68±0.35 for L-Eff and 0.69±28 kg/d for M-Eff calves; P>0.05). However, the concentrate DMI was greater for L-Eff calves compared with M-Eff calves (at weaning: 1.51±0.73 for L-Eff and 1.11±0.44 kg/d for M-Eff calves; P<0.05). Overall, M-Eff calves had a greater Gain-to-feed ratio compared with L-Eff calves (0.71±0.03 vs. 0.61±0.04, respectively; P=0.02). Plasma ceruloplasmin, myeloperoxidase and reactive oxygen species were greater in L-Eff than M-Eff calves (P<0.05). L-Eff calves had greater plasma haptoglobin at 21 and 60 d than M-Eff calves (P<0.05). M-Eff calves showed greater globulin (likely due to the greater plasma IgG content, even though only numerically) and Zn, greater GGT at 1 d, greater FRAP at 7 d, and lower BHB at 28 d of age compared with L-Eff (P< 0.05). Retinol and urea were greater in L-Eff than M-Eff calves (P<0.05) due to higher intake with concentrate. The divergence in RFI during early life is associated with the immunometabolic response. A low-grade of oxidative stress and a lower production of biomarkers associated with the systemic inflammation (ceruloplasmin and haptoglobin) in M-Eff calves is associated with a better efficiency of nutrients utilization. This idea is supported by the similar performance obtained in M-Eff calves that consumed less concentrate than L-Eff calves, where the latter likely had a greater energy expenditure to maintain the higher activation of the immune system mirrored by inflammation and oxidative stress biomarkers.

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ADVANCED ON THE USE OF MILK THISTLE IN ANIMAL SCIENCE

Alessandro Guerrini, Doriana E.A. Tedesco

Università degli Studi di Milano, Dipartimento di Scienze e Politiche Ambientali.

Corresponding author: D. Tedesco (doriana.tedesco@unimi.it

The MT, *Silybum marianum* L. (Gaertn.), is a medicinal plant grown for the bioactive compounds present in the seed, silymarin, that has well-documented antioxidant and hepatoprotective properties used in humans, for almost two millennia [1]. The pharmacological activity of milk thistle is well documented by existing data that confirm the safety and tolerability of its herbal preparations in various settings related to hepatic disorders [2]. Other than in human treatment, milk thistle and its derivative products are successfully used to improve animal health and productivity. This study aimed to update information on trials done with MT and derivative products on different species and categories of animals, selecting about 80 different studies. MT can be administered predominantly as silymarin extracts (from seeds) or milk thistle expeller/cake, the product remaining after the removal of oil/fat, still containing bioactive compounds. From the literature, in poultry, the administration of MT seed cake or silymarin, ameliorates the feed conversion ratio, feed intake, daily weight gain, final body weight, and gut health. In laying hens, increases the villus height and villus to crypt ratio, egg mass and reduces serum cholesterol, triglyceride and MDA concentrations in blood. In swine, enhances the growth performance, nutrient digestibility, and meat quality of finishing pigs. In silymarin treated sows from late pregnancy to weaning, evidences an increase in litter weight. In dairy ruminants, silymarin administration around parturition improves the milk yield without influencing quality parameters and reduces body condition loss. In fish, confirmes its usefulness with control of hepatic enzymes activity, better performance associated with improving intestine morphology and immune system activity. In sport horses, milk thistle cake shows several positive effects and a faster return of cortisol to the resting values before exercise occurred. Furthermore, in some species such as ducks, rabbits and fish, silymarin denotes hepatoprotective and antioxidant effects from toxic agents. In dogs and cats with liver damage, silymarin reduces the hepatic enzyme activity and the adverse effects of some drugs (antimicrobial, antiarrhythmics or chemoterapics). A few report no effects, but none report problems connected to MT administration. In certain studies, the composition and dose of MT administered are not always clearly reported. However, overall the research shows positive effects from the administration of MT and its derivatives and a recent increase in studies regarding the use of MT in animal species. In conclusion, the studies confirmed the potential of MT administration to reduce liver damage, and improve the oxidative status and performance in animals.

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THE EFFECT OF LIFT CRATES ON PIGLET SURVIVAL RATE AND SOW STRESSLEVEL DURING FARROWING

Eleonora Buoio (1), Annamaria Costa (1), Cecilia Salvagnini( ), Silvia Michela Mazzola (1)

(1) Department of Veterinary Medicine and Animal Sciences, Università degli Studi di Milano. (2) Department of Biotechnology, University of Verona.

Corresponding author: A. Costa (annamaria.costa@unimi.it)

In intensive farms, the mortality of newborn piglets during farrowing is a critical aspect that represents a significant cause of economic loss in pig production, and the deaths, representing 5–25% of newborn piglets, depend on several factors, and greatly on crushing [1]. The term "crushing" indicates that death of the newborn is due to trauma caused by the sow, during her movements; laying from standing, in particular way.

The first days of farrowing are the most critical for piglets: in the first 72 h, the mother can crush some newborns due to the significant size difference and for her rolling movements [2].

The design of the farrowing pen and of the farrowing crate is fundamental of piglet survival before weaning time [3].

The aim of the present study was to determine the effect of the lift farrowing crates on piglet mortality by crushing, and on sow welfare, using hair cortisol to assess their chronic stress during farrowing, in traditional and lift farrowing crates. Cortisol concentration as long-term stress indicator in animals was pioneered by Koren et al. in 2002 [4], it represents a reliable tool to obtain information on long-term HPA axis activity in a non-invasive way.

The lift farrowing crates (Nooyen Balance frame, Nooyen pig flooring, Deurne, The Netherlands) is an innovative farrowing crate equipped with a self-propelled floor that uses sensors to detect where the animal is standing or lying down. The floor of the sow is raised by an air-hydraulic system 28 cm higher than the creep area, when she stands, and gently lowered when she returns to lay, to limit the probability to crush her newborn piglets.

The experimental study was conducted in a pig farm located in Northern Italy, using eighty-four sows (Landrace X Large White X Duroc) in a one-year experiment organized in in three monitoring sessions. The number of piglets crushed by the mother within 72 h, and at weaning (28th day) was assessed by the farm veterinarian.

In each session, 14 sows were housed in a room with conventional crates (CC), and 14 sows were lodged in a room equipped with lift crates (LC).

The sows were randomly distributed in CC and LC rooms. No primiparous sows were considered in the study to avoid sows unexperienced with the dynamics of lift crates.

Hair cortisol concentration (HCC) was measured, by shaving hair of the sows in the transition area between neck and shoulder, upon entry and exit from farrowing to evaluate stress level variation. Also feet diseases and backfat thickness were evaluated to assess sows' potential diseases induced by lift crates and potential metabolic problems.

The results show that the number of crushed piglets, per sow, during the 72 h was higher in the CC rooms (0.44 vs 0.15; $P<0.05$) and up to weaning (0.50 vs. 0.37; $p<0.05$) than in the the LC rooms. Mean values of HCC variation in sows during farrowing were significantly different in the two housing systems and higher for the LC sows (0.53 pg/mg vs. 0.22 pg/mg; $P<0.05$).

No significant differences were detected for backfat variation and feet disease scoring between LC and CC sows. In conclusion, LC sows evidenced an increase in hair cortisol values during farrowing, probably caused by a higher stress status induced by the dynamics of the lift crate, along with the benefit of the higher survival rate of piglets before weaning.

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SHEEP NEUTROPHILS-DERIVED EXOSOMES: MIRNAS AND PROTEOMIC CHARACTERIZATION

Maria Giovanna Ciliberti, Antonella della Malva, Martina di Corcia, Antonella Santillo, Rosaria Marino, Agostino Sevi, Marzia Albenzio, Mariangela Caroprese

Università degli Studi di Foggia, Dipartimento Dipartimento di Scienze Agrarie, Alimenti, Risorse Naturali e Ingegneria, DAFNE.

Corresponding author: M.G. Ciliberti (maria.ciliberti@unifg.it)

Neutrophils are considered the primary mobile phagocytes recruited for the suppression and clearance of pathogens to an inflammatory site. Therefore, it is considered crucial to maintain their optimal functions to guarantee animals' health performance [1]. Recently, there is growing interest in the post-transcriptional role of miRNAs contained in exosomes (EV), considered as promising biomarkers of an inflammatory state, in both human [2] and animal studies [3]. The aim of this study was the isolation of EV from sheep peripheral neutrophils and the characterization of esosomial miRNAs and proteomic profile. Dairy ewes from Comisana sheep breed were recruited and neutrophils were isolated from peripheral blood according to Ciliberti et al. [4]. A final concentration of $0.5x10^6$ cells were cultured for 24h at 37 °C, in presence with fetal bovine serum exosomes-free culture media. Supernatants were collected and EV isolated using a specific reagent (REA) for obtaining intact EV from culture media. Briefly, supernatants were subjected by a first centrifugation at 2000 g for 30 minutes, followed by an incubation of recovered supernatant with REA at 4°C, overnight. The last centrifugation step was at 10.000 g for 1 h at 4°C. The number of EVs isolated was determined using a CD81 ELISA Kit, obtaining on average $1.9x10^{10}$ EV isolated and resuspended in PBS. On EVs collected was performed the miRNAs characterization using smallRNA-Seq data collected from 5 Homo sapiens samples obtained with Illumina NextSeq and sequenced in single-end mode. Moreover, the proteomic analysis of lysed EVs, using RIPA buffer, was performed by HPLC-MS/MS. A number of 101 miRNAs were identified among which the main expressed were the miR-16-5p, miR-423, let-7a-5p, miR-21-5p, let-7f-5p, miR150-5p, let-7g-5p, miR92a-3p, let-7b-5p, miR24-3p, miR-142-5p, miR-423-3p, miR-26a-5p, miR-191-5p, miR23a-3p, miR-26b-5p, miR-22-3p, miR-342-3p, miR-30e-5p, miR-29a-3p, miR-10a-5p, miR-486-5p, miR-486-5p, miR-184, and miR-122-5p. The proteomic cataloguing of isolated EVs revealed a set of 64 proteins with the presence of many proteins associated to controls of the classical pathway of complement activation among which the Complement C3, acting as chemoattractant for neutrophils, the C4b-binding protein alpha chain, the Complement factor H, and the Complement factor B. Moreover, the presence of alpha-1-anti-trypsin, an inhibitor of serine protease, implicated in the neutrophils degranulation pathway was found. In conclusion, miRNAs and proteomic characterization of neutrophils-derived EV could be useful for further selection of new biomarkers to study deeply animals' innate immune responses.

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EXPERIMENTAL USE OF THE GAMMA-INTERFERON TEST IN THE DIAGNOSIS OF BRUCELLOSIS ON BUFFALO FARMS: PRELIMINARY FIELD STUDY

Lorena Schiavo (1), Alessandra Martucciello (1), Piera Mazzone (2), Celestina Mascolo (3), Anna Donniacuo (1), Michele Napoletano (1), Esterina De Carlo (1), Giorgio Galiero (1)

(1) National Reference Centre for Hygiene and Technology of Breeding and Buffalo Production, Istituto Zooprofilattico Sperimentale del Mezzogiorno, Salerno. (2) Istituto Zooprofilattico Sperimentale dell'Umbria e delle Marche "Togo Rosati", Perugia. (3) Azienda Sanitaria Locale Caserta, Caserta.

Corresponding author: L. Schiavo (lorena.schiavo@izsmportici.it)

Brucellosis, a worldwide zoonosis with a strong economic and public health impact (1), continues to be a critical issue, especially in some areas of Campania Region in Italy where the close proximity between farms, the high number of animals reared, and the limited presence of proper biosecurity procedures allow the rapid spread of the microorganism among farms making the eradication process particularly difficult. In buffalo species BRC is due to *B. abortus* and *B. melitensis* and the diagnosis is generally based on indirect serological assays that mainly detect antibodies against smooth lipopolysaccharide (S-LPS). However, false positivity could occur due to cross-reactivity with other species of bacteria with S-LPS similar to those of *Brucella spp.* For this reason, the use of at least two serially applied tests is recommended to optimize specificity, and it is generally assumed that a combination of the Rose Bengal Test (RBT) and Complement Fixation Test (CFT) is the most suitable serial scheme (1). To increase the sensitivity of BRC diagnosis, it is recommended to use at least two serological tests in parallel, or further increase sensitivity with parallel testing by both serology and brucellin skin test (BST) that detects the cell-mediated reaction (1). Another valuable tool for the diagnosis of brucellosis in buffaloes could be the γ-IFN test that, similar to BST, is based on cell-mediated immunity. Actually, little is known about the use of γ-IFN test in the diagnosis of brucellosis infections in buffalo, while this test had been recently used for the diagnosis of bovine tuberculosis in this species (2). Basing on encouraging results of previous research conducted in Campania Region in 2003 e 2017 (3, 4), the present study aims to evaluate the use of γ-IFN test in the buffalo species for diagnostic purposes. Serum and heparinised blood samples of buffaloes, no. 261 from 11 BRC-free herds and no. 170 from 15 brucellosis-infected herds confirmed by bacteriological test were analyzed. RBT and CFT were carried out according to the official procedures (1). Blood samples (1 mL) for the γ-IFN test were stimulated with Brucellergene OCB (Zoetis, France) (40 U per well) (3) and phosphate-buffered saline (PBS) as negative control. The γ-IFN production was assayed with the BOVIGAM TB kit (Thermo Fisher Scientific) according to the manufacturer's instruction. All 261 buffalos from BRC-free herds were confirmed negative by the three in vivo tests. Among 170 buffaloes from BRC-outbreak herds, 92.9% (158/170) resulted RBT positive, while 83.5% (142/170) resulted positive by CFT and 91.1% (155/170) were positive to γ-IFN test. Out of these 155 γ-IFN test positive buffaloes, 146 were also positive to RBT and 135 were also positive to CFT, suggesting that γ-IFN test may have revealed an early stage of BRC infection in some of the animals, thus increasing the sensitivity of the diagnostic protocol. However, this hypothesis will have to be confirmed with the post-mortem investigations currently ongoing. According to our data, the γ-IFN test could be used rather than the BST that, like the intradermal tuberculin test, is still a subjective and more time-spending test. It could be used in addition to the tests currently provided for BRC diagnosis, in particular to increase Sensitivity (use in parallel) and Specificity (confirmation test).

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ASSESSMENT OF THE STRESS RESPONSE IN YOUNG BULLS OF THE AUTOCHTHONOUS VALDOSTANA BREED

Giulia Pagliasso (1), Vitale Nicoletta (1), Vevey Mario (2), Dellepiane Lucrezia (1), Dondo Alessandro (1), Razzuoli Elisabetta (1), Bergagna Stefania (1)

(1) Istituto Zooprofilattico Sperimentale del Piemonte, Liguria e Valle d'Aosta. (2) Associazione Nazionale Allevatori Bovini razza Valdostana (A.N.A.Bo.Ra.Va.).

Corresponding author: L. Dellepiane (lucrezia.dellepiane@izsto.it)

Transport, weaning, new environments, mixing with other animals, represent stressful events for animals (1). These critical factors have negative consequences on both animal health and welfare. One of the concerns regarding cattle management is that any resultant stress may be immunosuppressive and render the animals more susceptible to disease (2). For example, transport stress is considered to be one of the triggers for bovine respiratory disease, also known as shipping fever (3).

The present study aimed to evaluate various blood chemistry and innate immunity parameters in order to describe the degree of adaptation of young bulls delivered to the genetic centre of the Italian Valdostana Breeders Association (A.N.A.Bo.Ra.Va.).

A total of 45 bulls, specifically 28 Aosta Red Pied and 17 Aosta Black Pied, coming from 40 different farms located in the Valle d'Aosta territory, were included in the study. The animals were 60.7 ± 8.5 days old and their weight was 84.2±12.1 kg. Sampling was carried out in five times: in the farm of provenance before transport (T0), upon arrival at the Control Station (T1), 7 days from arrival (T2), 30 days from arrival (T3) and 4 months from arrival (T4). Blood samples were taken from the jugular vein of bulls using vacutainer tubes with K3EDTA anticoagulant and without anticoagulant. Whole blood samples were processed for blood count, while serum samples were employed for clinical chemistry, for protein electrophoresis and for the evaluation of innate immunity parameters.

The analysis of the blood count didn't show significant changes in the parameters evaluated. Hematocrit (HCT) was not increased after transport and haemoglobin (HB) value was always higher than the minimum limit set by law, equal to 7.25 g/dl (Dlgs. 126/2011, Annex I, point 11). Between T0 and T1 there was an increase in neutrophils and a simultaneous reduction in lymphocytes, which can be figured as a stress leukogram, but this deviation is not statistically significant. Liver, kidney, muscle function and the energy and electrolyte profile were normal and without any significant changes. Electrophoresis showed an increase in gamma globulins at T3 and T4. Albumin and total proteins remained steady before and after transport. Among the acute phase proteins, Haptoglobin (HP) did not show significant alterations over time; as for Serum Amyloid A (SAA), the peak was observed before the start of weaning, at T3. Concerning the pro-inflammatory interelukins evaluated, Interleukin-6 (IL-6) showed a peak at the end of weaning, at T4. Finally, as regards Tumor Necrosis Factor alfa (TNF-α), the peak was observed before the start of weaning, at T3 and continued until the end of weaning, at T4. The results of analysis reveal a good general condition of bulls before and after transport, as well as after mixing with other animals and adapting to a new environment. In particular, hematocrit, total protein and albumin values did not increase after the trip, indicating that the animals did not show signs of dehydration. The increase of gamma globulins and pro-inflammatory cytokines could be related to an activation of the immune system linked to the vaccination of the animals, which occurs between T2 and T3 or to weaning which occurs between T3 and T4.

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FIRST DETECTION OF EQUUS CABALLUS PAPILLOMAVIRUS TYPE-9 (ECPV9) IN ASYMPTOMATIC ITALIAN HORSES

Livia De Paolis (1), Chiara Grazia De Ciucis (1), Katia Cappelli (2), Samanta Mecocci (2), Tiziana Nervo (3), Lisa Guardone (1), Maria Ines Crescio (4), Floriana Fruscione (1), Gian Guido Donato (3), Cristiana Maurella (4), Paola Modesto (4), Alessandro Ghelardi (5), Elisabetta Razzuoli (1)

(1) Istituto Zooprofilattico Sperimentale del Piemonte, Liguria e Valle d'Aosta, Centro Di Referenza Nazionale oer l'Oncologia Veterinaria e Comparata (CEROVEC). (2) Università degli Studi di Perugia, Dipartimento di Medicina Veterinaria. (3) Università degli Studi di Torino, Dipartimento di Scienze Veterinarie. (4) Istituto Zooprofilattico del Piemonte, Liguria e Valle d'Aosta, Sede Centrale di Torino. (5) Azienda Usl Toscana Nord-Ovest, UOC Ostetricia e Ginecologia, Ospedale Apuane.

Corresponding author: L. De Paolis (livia.depaolis@izsto.it)

Papillomaviruses (PVs) are a group of highly host adapted, small, non-enveloped DNA viruses with a specific tropism for cutaneous and mucosal keratinocyte. PVs infections may be related to anogenital lesions and cancer development, in humans and several other animal species [1, 2]. To date, 13 different PVs have been found in Equidae [3]. Among these, the newly described *Equus caballus* Papillomavirus Type-9 (EcPV9) was so far only reported in the semen of a stallion with penile lesions in Australia [3]. The aim of this study is to report for the first time the presence of EcPV9 in asymptomatic Italian horses. From July 2020 to January 2022, penile and vulvar swabs were sampled with sterile cytobrushes from 209 horses (195 females, 11 stallions and 3 geldings) with no apparent sign of neoplastic disease and no PVs associated lesions. The sampling was conducted during clinical examination at the Didactic Veterinary University Hospital (OVUD) of Perugia and at the Veterinary University Hospital (OVU) of Turin. DNA extracted using QIAamp DNA Mini Kit (Qiagen) was submitted to real-time PCR targeting the *L1* region, using primers and probes specifically developed. As first, an amplification targeting a EcPV9-L1 region of ~125 bp was performed; for samples resulted positive to this first gene amplification, a second ~500 bp EcPV9-*L1* sequence amplification and sequencing was carried out. Moreover, the presence of EcPV2 was assessed in horses resulted positive for EcPV9, following the procedure previously described [4]. EcPV9-*L1* DNA was found in 11 out of 195 horses (5.3%), all female and mainly English Thoroughbred (OR 26.4, IC95% 3.2-214.7) compared to the other breeds. This may be due to the fact that these animals were all subjected to natural mating. The positive animals lived in 5 different regions, and 7 out of 11 positive horses were also positive for EcPV2. The obtained results contribute to the description of the exposure prevalence or infection to EcPV in the horse populations in Italy, considering the scarce knowledge on this group of viruses. Further investigation of the EcPV9 clinical impact on horse health is clearly merited [3], also considering its great sequence similarity with EcPV2 [3], a major aetiologic agent of equine squamous cell carcinoma (SCC) [4]. The impact of EcPV coinfections is still scarcely investigated. Our data suggested that EcPV9 infection circulates in an asymptomatic manner in Italy. In this light it is necessary to further investigate its genoprevalence, in order to evaluate its impact on fertility in breeding horses and its possible role in the pathogenesis of equine genital SCC.

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COW COLOSTRUM-DERIVED EXTRACELLULAR VESICLES CAN IMPROVE IMMUNORESPONSE INDUCED BY ETEC INFECTIONS

Floriana Fruscione (1), Roberto Zoccola (2), Livia De Paolis (1), Chiara Grazia De Ciucis (1), Zoppi Simona (2) Katia Cappelli (3), Samantha Mecocci (3), Daniele Pietrucci (4, 5), Giovanni Chillemi (5), Luisa Pascucci (3), Mariella Goria (2), Elisabetta Razzuoli (1)

(1) Istituto Zooprofilattico Sperimentale del Piemonte, Liguria e Valle d'Aosta, Centro Di Referenza Nazionale oer l'Oncologia Veterinaria e Comparata (CEROVEC). (2) Istituto Zooprofilattico del Piemonte, Liguria e Valle d'Aosta, Sede Centrale di Torino. (3) Università degli Studi di Perugia, Dipartimento di Medicina Veterinaria. (4) CNR, Institute of Biomembranes, Bioenergetics and Molecular Biotechnologies. (5) Università della Tuscia, Department for Innovation in Biological, Agro-food and Forest systems (DIBAF).

Corresponding author: F. Fruscione (floriana.fruscione@izsto.it)

Extracellular vesicles (EVs) are membranous spherical structures secreted by multiple cell types under physiological and pathological conditions. By acting as signalling mediators between distant cells, EVs can modulate immunity and inflammation [1]. Milk contains high amounts of EVs and colostrum is particularly enriched in vesicles enclosing proteins able to regulate the immune response [2]. Calf neonatal diarrhea caused by enterotoxigenic *E. coli* (ETEC), represents a pathology of zootechnical interest where chemotherapy often has poor results. In this context, this study aims to investigate the beneficial effects of cow colostrum-derived EVs (colos-EVs) in an *in vitro* model of ETEC infection using porcine intestinal epithelial cells (IPEC-J2) and two ETEC strains. Colostrum was collected from Piemontese cows within 24 h of calving (the first 3 milkings) and colos-EVs were isolated and characterized through TEM following the methods used in a previous study [3]. IPEC-J2 were seeded and treated with 10^8 colos-EVs into 12-well culture plates. Untreated wells were used as control. After 48 h of incubation IPEC-J2 were infected with ETEC strains prepared as follows: bacteria were grown overnight in brain heart infusion broth (BHI) medium at 37°C; then inoculated into fresh BHI and incubated for 1–2 h at 37°C to obtain mid-log phase cultures; finally, a pool for each strain was pelleted, re-suspended at 10^8 CFU/ml and used for IPEC-J2 infection. After 2 h of bacteria incubation, medium was removed, and cells monolayers were washed. Two experiments were then performed: 1) cells were lysed and serial dilutions were seeded on tryptone bile x-glucuronide agar (TBX) plates to evaluate the adherent bacteria; 2) cells were again incubated in their medium for 3h, then RNA was extracted from monolayers to evaluate cell gene expression and supernatants used to measure the IL-8 release. Each experiment was performed in triplicate. The colos-EVs administration significantly (P=0.045) reduced the ability of ETEC strains to adhere to cell surface. In particular, we detected 5.7±0.2 $\log_{10}$ bacteria/500,000 cells in untreated wells and 5.4±0.2 $\log_{10}$ bacteria/500,000 cells in wells treated with colos-EVs. An increase of *IL8* gene expression and cytokine production was detected, together with a significant modulation of parameters involved in innate immunity. Our data shows that colos-EVs can modulate *in vitro* host-pathogen interaction confirming EVs role in modulating immune response in *E. coli*-induced diseases. These results might be interesting to investigate differences in colostral EV cargo between ETEC-vaccinated and ETEC-non vaccinated subjects.

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CHARACTERIZATION OF PROBIOTIC LACTOCOCCUS LACTIS ENGINEERED FOR THE EXPRESSION OF ANTIGENS AGAINST PORCINE ESCHERICHIA COLI

Serena Reggi, Antonella Baldi, Matteo Dell'Anno, Luciana Rossi

Università degli Studi di Milano, Dipartimento di Medicina Veterinaria e Scienze Animali (DIVAS).

Corresponding author: S. Reggi (serena.reggi@unimi.it)

Lactic acid bacteria (LAB) are the most prevalent microbiota utilized as probiotics and owing to their significant immunomodulatory effects, they have garnered attention as promising candidates for developing the edible vaccines [1]. *Lactococcus lactis* is a Gram-positive LAB used in food fermentation, at the same time is easily genetically accessible therefore a variety of new applications have been developed [2].

In the present study, we describe a regulated gene expression for the production of antigens of major porcine *E. coli* pathotypes in *Lactococcus lactis*. In particular, F4 adhesive fimbriae (FaeG), F18 adhesive fimbriae (FedF), and the B subunit of the verocytotoxin-e (Vte2-B) were considered antigens for the development of a model of a probiotic-based vaccine against Enterotoxigenic *Escherichia coli* (ETEC) and verocytotoxic *Escherichia coli* (VTEC) strains [3]. With this proposal a double approach was adopted: the vectors were designed for FedF, FaeG, and Vte2-B expression to both the cytoplasm and anchored the cell wall. For intracellular expression, FaeG, FedF, and Vte2-B genes were amplified and cloned in the NICE vector pNZ8148 under the control of the nisin-inducible *nisA* promoter [4]. The competent cells of *L.lactis* NZ9000 were transformed by electroporation with the three recombinant plasmids. After induction with nisin, the western blotting demonstrated that FaeG, FedF, and Vte2-B were accumulated in the intracellular soluble protein fraction. In a second way, FaeG, FedF, and Vte2-B genes C-terminal fused with a cell wall anchor CWA (amplified from *S. pyogenes*) were cloned in the NICE vector pNZ8123 under the control of the *nisA* promoter and USP45 signal peptide in order to secretion the proteins [4]. The western blotting confirmed the antigenic protein expression in the cell wall fraction. The double way of expression (intracellular and cell wall) was estimated to maximize the immunogenic activity of FedF, FaeG, and Vte2-B by increasing the survival rate along the intestinal tract. Finally, in the simulated *in vitro* digestion assay, engineered *Lactococcus lactis* strains showed a high tolerance confirming their potential to exert probiotic activity and as valuable delivery system of immunogens through the intestinal tract [5].

This research is under patent control (Patent Filling 102021000006506).

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SICLIMVET

A RETROSPECTIVE EVALUATION OF NEUTROPHIL-LYMPHOCYTE RATIO, MONOCYTE-LYMPHOCYTE RATIO AND PLATELET-LYMPHOCYTE RATIO IN CATS WITH OBSTRUCTIVE UROPATHY

Federica Cagnasso, Barbara Bruno, Renato Zanatta, Claudio Bellino, Silvia Roncone, Franca Borella, Paola Gianella, Antonio Borrelli

Università degli Studi di Torino, Dipartimento di Scienze Veterinarie.

Corresponding author: F. Cagnasso (federica.cagnasso@unito.it)

The veterinary literature has reflected growing interest in the neutrophil-lymphocyte ratio (NLR) and platelet-lymphocyte ratio (PLR) as diagnostic and prognostic markers for both neoplastic and inflammatory conditions.[1-4] However, no information exists on their use in cats with obstructive uropathy (OU). In the human counterpart, NLR, monocyte-lymphocyte ratio (MLR) and PLR have been studied in many cancers and infections, with higher ratios indicating poorer overall disease and prognosis.[5, 6] The aims of this retrospective study were to describe a population of cats with OU referred to a Veterinary Teaching Hospital (VTH), to compare NLR, MLR and PLR between healthy and OU cats and to evaluate correlations between NLR, MLR and PLR and selected clinicopathological variables in OU cats. Data from healthy owned-cats presented at the same VTH for their annual check-up and vaccination or pre-anesthetic evaluation before neutering served as a retrospective control group. At presentation, the following information from cats with OU was gleaned from the medical records: signalment, body weight, body temperature, complete blood count, serum concentration of creatinine (CREA), blood urea nitrogen (BUN), and potassium (K), concurrent urinary tract infection (UTI). In addition, length of hospitalization (LO) was recorded. Only cats that underwent abdominal imaging (abdominal ultrasound, radiographs), urine analysis and culture (cystocentesis), and indwelling catheterization with urine output monitoring, intravenous fluids, and pain medication were considered for inclusion. For statistical purposes subgroups A (idiopathic urethral obstruction, urethral plugs) and B (urethrolits) were created. Data distribution was assessed by Shapiro Wilk normality test. Wilcoxon rank sum test, Spearman correlation test and contingency tables analysis were performed where appropriate. P value was set at 0.05. Seventy-one healthy cats and 151 OU cats were included. Median age of OU cats was 5 years (1-11), median weight was 5 kg (2.6-12). CREA, BUN and K were increased to a variable extent in 82, 84 and 28 OU cats, respectively. Temperature was increased and decreased in 8 and 32 OU cats, respectively; 47 cats had concurrent UTI. Median LO was 4 days (1-21). Seventy-three and 32 cats were assigned to groups A and B, respectively. Median NLR, MLR and PLR in OU cats were 5.3 (0.3-139), 0.1 (0-3.7) and 129.3 (2.4-2160), respectively. OU cats showed significantly higher NLR and MLR compared to healthy cats ($P<0.001$). Subgroup B showed significantly higher PLR compared to subgroup A (P 0.02). A positive correlation ($P<0.01$) was found for BUN with NLR ($R_S=0.23$) and MLR ($R_S=0.25$), and for CREA with NLR ($R_S=0.24$) and MLR ($R_S=0.31$). When considering subgroup A, CREA correlated positively ($P<0.05$) with MLR ($R_S=0.27$) and NLR (0.28), while BUN ($P<0.05$) with NLR ($R_S=0.25$). In subgroup B, CREA ($P=0.02$) correlated positively whit NLR ($R_S=0.45$). The results suggested that NLR, MLR and PLR are elevated in cats with OU and that could be used as inflammatory markers of OU. Further studies are expected.

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ASSOCIATION BETWEEN DYNAMIC UPPER AIRWAY OBSTRUCTION AND FITNESS PARAMETERS IN STANDARDBRED RACEHORSES DURING TREADMILL EXERCISE

Chiara Maria Lo Feudo, Luca Stucchi, Giovanni Stancari, Bianca Conturba, Enrica Zucca, Francesco Ferrucci

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

Corresponding author: C.M. Lo Feudo (chiara.lofeudo@unimi.it)

Dynamic upper airway obstruction (DUAO) is a common cause of poor performance in racehorses. During supramaximal exercise, the respiratory system is considered as a limiting factor for performance, and the occurrence of DUAO is believed to impact negatively on athletic capacity by impairing ventilation [1]. However, contrasting results have been reported by different studies compairing blood gas exchanges and lactate concentrations between healthy horses and DUAO-affected horses during exercise [2, 3]. The inconsistency of these findings may be attributable to the fact that different forms of DUAO exist and may affect fitness differently based on the severity of airflow limitation. The present retrospective study aims to evaluate the relationship beween the severity of DUAO detected during high-speed treadmill endoscopy (HSTE) and selected fitness parameters measured by an incremental treadmill test in Standardbred racehorses. One hundred ninety-one Standardbreds referred for poor performance to the University of Milan between 2002 and 2021 were included in the study. All horses underwent a complete diagnostic protocol for performance evaluation, in order to exclude the presence of other concomitant diseases which could affect performance. After a resting evaluation, horses underwent an incremental treadmill test, during which the following fitness parameters were measured: speed at a heart rate of 200 bpm (V200), speed and heart rate at a blood lactate of 4 mmol/L (VLa4, HRLa4), peak lactate concentration, minimum pH and maximum hematocrit reached during the test. After one day active rest, horses underwent HSTE, and the absence or presence of specific types of DUAO was recorded, including dorsal displacement of the soft palate (DDSP), nasopharyngeal collapse (NPC), medial deviation of aryepiglottic folds (MDAF), dynamic laryngeal collapse (DLC), epiglottic entrapment (EE), and epiglottic retroversion (ER). Based on the impact on airflow limitation, DUAOs were considered as severe, when mostly/totally obstructing the laryngeal lumen (DDSP, NPC, DLC, ER), or mild, when only partially reducing the laryngeal lumen (MDAF, EE), and horses were divided into 4 groups: no-DUAO, mild-DUAO, severe-DUAO and multiple-DUAOs. Fitness parameters were compared between groups using Kruskal-Wallis and Dunn's multiple comparisons tests or ordinary one-way ANOVA and Hold-SIdak multiple comparison test. Statistical sifgnificance was set at $p<0.05$. Among the study population, 108 horses were included in the no-DUAO group, 9 in the mild-DUAO, 52 in the severe-DUAO, and 22 in the multiple-DUAOs. The V200 was significantly lower in the severe-DUAO ($p=0.0412$) and the multiple-DUAOs ($p=0.0181$) groups compared to the no-DUAO group; similarly, the VLa4 was higher in the no-DUAO group compared to the severe-DUAO ($p=0.0468$) and the multiple-DUAOs ($p=0.0158$) groups. Moreover, horses in the multiple-DUAOs group reached a higher peak lactate compared to horses in the no-DUAO group ($p=0.0134$). The other fitness parameters did not differ significantly between groups. These results suggest that aerobic capacity is impaired by DUAO based on the entity of airflow limitation: the more severe a DUAO is, the more it interferes with ventilation and gas exchanges: therefore, hypoxemia may occur at lower speeds and the switch to anerobic metabolism may take place earlier with lactate accumulation. Another hypothesis is that early lactate accumulation may induce muscle fatigue, also involving the upper respiratory tract musculature, which may lose the ability to contrast collapsing forces during strenuous exercise, and therefore prevent the development of DUAO.

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FECAL PROTEOMICS IN PARASITISED DOGS – A PILOT STUDY IN TRICHURIS VULPIS NATURALLY INFECTED ANIMALS

Silvia Vincenzetti (1), Giacomo Rossi (1), Fabrizia Veronesi (2), Manuela Diaferia (2), Alessandra Gavazza (1), Andrea Marchegiani (1), Beniamino Tesei (1), Gianni Sagratini (3), Massimo Ricciutelli (3), Luca Scortichini (4), Stefania Pucciarelli (1), Matteo Cerquetella (1)

(1) University of Camerino, School of Biosciences and Veterinary Medicine. (2) University of Perugia, Department of Veterinary Medicine. (3) University of Camerino, School of Pharmacy. (4) DVM, Maiolati Spontini (AN), Italy.

Corresponding author: M. Cerquetella (matteo.cerquetella@unicam.it)

The present pilot study aimed at deepening the knowledge on fecal proteomics in dogs; it has been chosen to study the fecal proteome from dogs affected by helminthoses, using *T. vulpis* as a model of infection, since it is localized and harms even severely large bowel, thus expecting damages more evident in fecal samplings. The feces from 15 animals, tested positive only to *T. vulpis* by flotation in $NaNO_3$ solution (density 1.350) and having a Mc Master fecal egg count (FEC) higher than 100 eggs for gram (epg) [1], were stored at -20°C for two-dimensional electrophoresis (2DE) analysis. All fecal samples were from dogs submitted to routine coprological screening (i.e. floatation, Baermann, coproantigen) for endoparasites at the Parasitic Laboratory of the Veterinary Teaching Hospital of Perugia and did not receive any anthelmintic treatment in the last two months. Four out of the 15 parasitized dogs showed diarrhea, and only three hematochezia; any other clinical signs in addition to those due to the parasitosis were recorded. The experimental design of the present proteome analysis is based on the complete sample pooling strategy [2]. Preparation of fecal samples for 2DE, and liquid chromatography-tandem mass spectrometry (LC-MS/MS) analysis were performed as described previously [3-5]. Among the spots expressed in our samples, only those that showed a normalized quantity greater than $20x10^3$ were selected for subsequent analysis by LC-MS/MS. Seven proteins/enzymes (plus 3 isoforms for myosin), or their fragments, were identified in feces: Cu-Zn superoxide dismutase, titin isoform X1, immunoglobulin lambda-1 light chain isoform X8, alkaline phosphatase, myosin, enolase, and albumin. Of interest, considering the parasitosis, is the finding of albumin, myosin, and titin (involved in muscle development and contraction) which presence is possibly the direct expression of the damage caused by the sub-epithelial localization of the parasite as well as of the normal mucosal turnover (for albumin) [4,6-8]. Also, the enzyme Cu-Zn superoxide dismutase is noteworthy, as it acts as enzymatic antioxidants during oxidative stress [9], considering that an increased level of oxidative stress has been reported in human chronic intestinal helminthic parasitosis e.g., roundworms and whipworms infections [10-11]. The main limitation of the present pilot study is the absence of the comparison with a healthy control group, which was however out of our aim. Fecal proteomics is a technique that is showing interesting perspectives in the study of gastrointestinal diseases in the dog. The present study provides indications that this technique has the potential to identify the presence of a GI damage, suggesting its study also in other digestive disorders, for possible diagnostic/prognostic/monitoring markers discovery.

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PRO-INFLAMMATORY AND IMMUNOLOGICAL PROFILE IN DOGS WITH MYXOMATOUS MITRAL VALVE DISEASE: PRELIMINARY RESULTS

Francesco Pizzo (1), Diego Piantedosi (1), Anna Teresa Palatucci (2), Flavia Carriero (2), Nadia Musco (1), Pietro Lombardi (1), Giuseppe Molinaro (1), Valentina Rubino (3), Giuseppe Terrazzano (2), Laura Cortese (1)

(1) Department of Veterinary Medicine and Animal Productions, University of Naples Federico II, Naples, Italy. (2) Department of Science, University of Basilicata, Potenza, Italy. (3) Department of Translational Medical Sciences, University of Naples Federico II, Naples, Italy.

Corresponding author: D. Piantedosi (dapiante@unina.it)

In humans, heart failure is associated with induction of pro-inflammatory cytokines such as TNF-α, IL-1 and IL-6, that may contribute to the pathogenesis of cardiac remodeling, and systolic and diastolic dysfunction [1]. In addition, during CHF, the SRAA is activated. AG II and aldosterone are responsible for ventricular and renal remodeling over time, through a pro-inflammatory and fibrosis-promoting action. Furthermore, a reduction in CD4+ CD25high FoxP3+regulatory T cells (Treg) was observed in aldosterone-treated mice, compared with placebo-treated controls [2]; on the contrary, a modulation of the SRAA by ACE inhibitor therapy induces an increase of Treg cells and a decrease Th1/Th2 cytokine ratios and inflammatory cytokine production in human patients with CHF [3]. MMVD is the most common acquired cardiac disease in small and medium-sized dogs , and is responsible for CHF [4]. The involvement of the immune system and a pro-inflammatory cytokine response in mitral canine patients with CHF has not yet been extensively investigated. The aim of this study was to verify the existence of a pro-inflammatory condition and a dysfunction of the immune system in dogs with MMVD in different stages of severity through the blood assessment of immunological profile, leptin, and pro-inflammatory cytokines. Thirty-six dogs with MMVD, from a client-owned referral population of the Veterinary Teaching Hospital, were classified according to the ACVIM clinical classification. The immunological evaluation of the dogs enrolled in the study was performed by analyzing CD4+, CD8+ and Treg lymphocytes populations. For this purpose immunofluorescence techniques combined with flow cytometry were used. Canine cytokines (TNF-α, IL-1β and IL-6) and leptin blood levels were assessed by ELISA. According to the stage of the ACVIM classification, the echocardiographic examination showed a higher end-diastolic volume index, EDVI ($P<0.05$) and end-systolic volume index, ESVI ($P<0.01$), and left atrium/aorta diameter ratio, LA/AO ($P<0.01$) in C-D group compared to B1-B2 group.

The immunological profile of dogs with MMVD was compared with the immunological profile of healthy age-matched control dogs and also with the immunological profile of healthy young control dogs. MMVD dogs belonging to C-D group showed a higher percentage ($p<0.02$) of CD4+ T lymphocytes, and a lower percentage ($p<0.05$) of CD8+ T lymphocytes, when compared to age-matched control dogs. Moreover, the Treg percentage was increased in B1-B2 ($p<0.0005$) and C-D group ($p<0.0005$) when compared to age-matched control dogs. In particular, the Treg percentage in C-D group was equivalent to that observed in young control dogs. In addition TNF-α resulted significantly higher in MMVD groups compared to young control dogs ($P<0.01$) while, IL-1β and IL-6 that resulted significantly higher ($P<0.01$) as the disease progressed compared to both control dogs groups. Treg increase in C-D MMVD dogs could be induced to contrast such pro-inflammatory cytokines. This study opens new perspectives in the understanding of the relationship between the homeostatic mechanism underlying the reduction of inflammatory parameters and the immune-regulation in dogs with MMVD according to the stage of the disease.

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CAN THE LIVER FUNCTIONALITY INDEX (LFI) IDENTIFY THE LESS RESILIENT COWS?

Luca Cattaneo, Claudio Ciaburri, Andrea Minuti, Fiorenzo Piccioli-Cappelli, Riccardo Negrini, Erminio Trevisi

Università Cattolica del Sacro Cuore, Dipartimento di Scienze Animali, della Nutrizione e degli Alimenti, Piacenza.

Corresponding author: E. Trevisi (erminio.trevisi@unicatt.it)

LFI is calculated with the contribution of 3 blood indices, Albumin, Total Cholesterol and Total Bilirubin, whose plasma concentrations are measured at 3 and 28 days of lactation and it was proposed to provide a summary of cow adaptation to the transition period [1]. The aim of this study was to evaluate whether LFI is related to inflammometabolic responses of months before its assessment and, thus, if it is able to identify the less resilient cows in the herd. In this study, 121 Holstein cows were monitored from the week prior to dry-off to the first month of lactation. Cows enrolled were clinically healthy in the 2 months preceding the dry-off and nulliparous heifers were not included. Within the project LEO, Livestock Environment Opendata, 16.2 – PSRN 2014-2020 financed through Fondo Europeo Agricolo per lo Sviluppo Rurale (FEASR) to calculate the LFI and evaluate the metabolic and inflammatory status, blood samples were taken from the jugular vein at -7, 0, 3, 7 days from dry-off and at -3, 3, 28 days from calving. Milk yield and SCC were measured at -14, -7 and -1 days before dry-off and at 7, 14, 28 days after calving (Aut. Min. 464/2019-PR; Aut. Min. 139/2021-PR). Data on fertility, production, and health status in the two lactations considered were also collected. Cows were classified according to their postpartum health status: healthy (H; n=83) and diseased (D: at least one disease detected among retained placenta, metritis, ketosis, mastitis, displaced abomasum, and milk fever in the first 30 days of lactation; n=38). Data at dry-off and calving were analyzed separately with repeated measures mixed models (proc GLIMMIX of SAS), considering health status, time and their interaction as fixed effects, and individual cows as random effect. LFI clearly defined the two groups as it resulted significantly higher in H than in D group (0.85 vs -0.55; $P<0.01$). Groups had similar characteristics in the previous lactation, except for the lower milk yield at dry-off (16.5 vs 20.5 kg, $P<0.01$) and higher SCC at dry-off (2.19 vs 2.01 log n/µL; $P<0.05$). Moreover, these differences were confirmed during the first month of the next lactation, as SCC were higher in D vs H cows (2.01 vs 1.77 log n/µL; $P<0.05$), and milk yield lower (36.2 vs 40.0 kg, $P<0.01$). It is noteworthy that cows that will develop a postpartum disease (D group) had already blood alterations in some metabolic and liver function biomarkers before the dry-off. In fact, before dry-off, compared with H, D cows had lower level of cholesterol (considered as a negative acute phase index: 4.38 vs 4.82 mmol/L, $P<0.01$) and higher level of creatinine (an indicator of the ability of kidney to remove it from the bloodstream: 98.7 vs 93.7 µmol/L, $P<0.01$). Surprisingly, after calving, when diseases occurred, the differences at blood level between groups were moderate, with minor alterations in liver activity (higher bilirubin in D vs H: 4.46 vs 3.11 µmol/L, $P<0.01$; higher GOT at 3 d: 119 vs 104 U/L, $P<0.05$), kidney function (higher creatinine in D vs H: 95.2 vs 90.7 µmol/L, $P<0.01$) and oxidative stress (higher ROM at 28 d in D vs H: 15.7 vs 14.5 mg H_2O_2/100mL; $P<0.05$). The latter could be related with the several diseases considered, which might have confounded the results. Nevertheless, it also highlighted how, regardless of health disorder, LFI pointed a worse condition in diseased cows (lower and negative values). Conversely, higher LFI was related with better health status and an overall improved ability to cope with the transition period challenges. However, LFI could be influenced by previous conditions or have a genetic basis. Moreover, it can be calculated only after the most critical phase, making necessary to find further predictive proxies of inflammometabolic status.

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SERUM AMINO ACIDS IN CANINE CHRONIC HEPATITIS: RESULTS IN 16 DOGS

Verena Habermaass, Eleonora Gori, Francesco Bartoli, Alessio Pierini, Chiara Mariti, Ilaria Lippi, Veronica Marchetti

(1) Università degli Studi di Pisa, Dipartimento di Scienze Veterinarie.

Corresponding author: V. Habermaass (v.habermaass@phd.unipi.it)

In humans, chronic liver disease may cause alterations in amino acids (AAs) metabolism with serum branched-chain AAs (BCAAs) decreasing, and aromatic AAs (AAAs) increasing (1). It results in decreased Fischer ratio (BCAAs/AAAs) and BCAAs/Tyrosine ratio, which are reported to be useful to assess human cirrhosis prognosis (2,3). In veterinary medicine, few studies have been performed to evaluate whether serum AAs may vary in chronic hepatopathic patients, and it seems that BCAAs/AAAs tends to reduce only in case of congenital portosystemic shunts (4).

The aim of this study was to evaluate serum AAs pattern in dogs with chronic hepatitis (CH) compared to a healthy control group.

Leftover serum samples of 16 client-owned dogs with histological diagnosis of CH (group A) and 25 healthy dogs (group B) were included in this case-control study. Dogs with AAs supplementation in recent clinical history were excluded. Glycine (GLY), alanine (ALA), valine (VAL), leucine (LEU), isoleucine (ILE), proline (PRO), serine (SER), threonine (THR), cysteine (CYS), methionine (MET), phenylalanine (PHE), tyrosine (TYR), tryptophan (TRP), aspartate (ASP), glutamate (GLU), histidine (HIS), lysine (LYS) and arginine (ARG) were measured with High Performance Liquid Chromatoghaphy (HPLC). Total Serum protein were recorded. Normally distributed data are expressed as mean±SD, whereas non-normally distributed data as median and range. Unpaired t-test or Mann-Whitney U-test were used to investigate differences between groups based on normality distribution.

Quite a number of serum AAs resulted significantly higher in CH dogs than healthy dogs as follows: SER 58.5 nmol/ml ±23.6 vs 41.3 nmol/ml ±14.7; GLU median 19.2 nmol/ml (4.9-102.6) vs 8.8 nmol/ml (4.4-20.6); GLY 70.6 nmol/ml ±21.8 vs 48 nmol/ml ±16.47; HIS 246.8 nmol/ml ±82.7 vs 198 nmol/ml ±58.83.8; PRO median 55.9 nmol/ml (22.96-76.46) vs 6.3 nmol/ml (3.9-67.46); TYR median 20.5 nmol/ml (11.42-67.72) vs 11.3 nmol/ml (6.3-54); VAL median 34.9 nmol/ml (17.8-63.3) vs 4.8 nmol/ml (3-14.2); MET median 8.6 nmol/ml (3.4-36.6) vs 4.8 nmol/ml (0.3-58.5); LYS median 55.9 nmol/ml (29.1-96.4) vs 7.3 nmol/ml (3.8-64.1); LEU median 26.7 nmol/ml (8.6-51.7) vs 10.7 nmol/ml (7.8-34.4); PHE median 25.3 nmol/ml (10.4-63.1) vs 10.9 nmol/ml (4.5-24.6). Contrarily, CYS, ILE (5.8 nmol/ml ±1.3 vs 38.3 nmol/ml ±15.3; 9.3 nmol/ml ±3.6 vs 26.1 nmol/ml ±8.3, respectively) and TRP (median 19.8 nmol/ml (9.9-33.8) vs 48.2 nmol/ml (19.7-108.3), P value<0.0001), were lower in group A. BCAAs/AAAs did not significantly differ between the two groups. No difference in serum protein between CH and healthy dogs was found (6.2 g/dL±1.1 vs 6.4 g/dL±0.8, respectively).

Several serum AAs concentrations are significantly different between dogs presenting CH and healthy dogs. Many metabolic mechanisms could be involved, as already known for human liver disease. According to human medicine, AAAs seems to increase during CH disease, instead of decreased isoleucine. BCAAs/AAAs ratio not differ significantly from healthy controls. Even if proteinemia did not significantly differ, we observed changes in the proportions of serum AAs between healthy and CH dogs, reflecting qualitative AA imbalances.

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ELECTROCARDIOGRAPHIC AND ECHOCARDIOGRAPHIC FINDINGS IN HYSTRIX AFRICAEAUSTRALIS: PRELIMINARY DATA

Carlotta Valente (1), Helen Poser (1), Luca Bellini (1), Francesca Zanusso (1), Carlo Guglielmini (1), Laura Voltan (2), Giulia Maria De Benedictis (1)

(1) Università degli Studi di Padova, Dipartimento di Medicina Animale, Produzioni e Salute. (2) Parco Faunistico Valcorba.

Corresponding author: C. Valente (carlotta.valente@unipd.it)

The Cape porcupines (*Hystrix africaeaustralis*) are rodents native from central and southern Africa. Information regarding the prevalence, clinical signs and diagnosis of different diseases affecting these animals are lacking, probably because of their peculiar anatomical and behavioral characteristics. Post mortem examination can provide additional information about pathological processes observed in this species [1, 2], however cardiovascular system has never been studied, even if peripheral circulation has been described [3, 4]. The aim of this study was to describe some electrocardiographic (ECG) and echocardiographic parameters of anesthetized Cape porcupines.

Six *Hystrix africaeaustralis* from a zoological park undergoing orchiectomy were sedated using a combination of dexmedetomidine 0.008 mg/kg (Dexdomitor 0.5 mg/ml), butorphanol 0.3 mg/kg (Dolorex 10 mg/ml), ketamine 5 mg/kg (Nimatek 100 mg/ml) and midazolam 0.2 mg/kg (Midazolam 5 mg/ml) injected intramuscularly; then, they were intubated and maintained with isoflurane in oxygen.

To integrate the clinical health care program of the park, electrocardiographic and echocardiographic examinations were performed on six and three anesthetized porcupines, respectively. Animals were positioned on right lateral recumbency for 3-minute ECG recording. Heart rate, duration and amplitude of P wave and QRS complex, mean electrical axis of QRS and duration of each interval were analyzed on a six-lead ECG. Echocardiographic examination was performed by a single trained operator (HP), from the right and left parasternal windows using standard views. Parameters of cardiac structure and function were recorded. Normality of ECG data was assessed through Kolmogorov-Smirnov's test and data were reported as median [min-max].

Sinus rhythm was detected in all animals and median ECG parameters were the following: heart rate 92 bpm [80-101 bpm], P wave duration 43 ms [40-54 ms], P wave amplitude 0.1 mV [0.1-0.7 mV], QRS complex duration 50 ms [41-55 ms], QRS complex amplitude 0.4 mV [0.3-1.1 mV], PQ interval 136 ms [112-151 ms], QT interval 266 ms [238-297 ms] and mean electrical axis 64.5° [20.7°- 82°]. Echocardiography was easily performed in this species and it allowed to obtain good quality images. Cardiac chambers and walls as well as atrioventricular and semilunar valves had anatomical and functional features similar to those of other domestic mammals. Moreover, persistent left cranial vena cava was found in all animals.

This study is the first to describe ECG parameters in the anesthetized *Hystrix africaeaustralis*. Persistent left cranial vena cava seems to be a consistent anatomical presentation in Cape porcupines, although additional studies with larger number of animals are necessary to establish reference ranges for abnormalities recognition in this species.

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COMPLETE BLOOD COUNT PATTERNS IN CANINE CHRONIC HEPATITIS

Eleonora Gori (1), Verena Habermaass (1), Ilaria Lippi (1), George Lubas (2), Alessio Pierini (1), Francesca Abramo (1), Veronica Marchetti (1)

(1) Università di Pisa, Dipartimento di Scienze Veterinarie. (2) Clinica Veterinaria Colombo.

Corresponding author: E. Gori (eleonora.gori@vet.unipi.it)

Erythrocyte abnormalities are frequently described in canine vascular hepatopathy [1]. However, little is known in canine chronic hepatitis (CH). The aim of this study was to evaluate complete blood count characteristics and possible erythrocyte abnormalities in dogs with CH and their association with necroinflammatory and fibrosis scores.

The review of the Veterinary Teaching Hospital database was conducted searching for dogs with CH diagnosed from 2011 to 2022. All liver biopsies were reviewed by a single pathologist to confirm the CH diagnosis [2]. Following current WSAVA guidelines, necroinflammatory activity (A score) was graded with a 6-point scale from absent (A=0) to very marked (A=5), and fibrosis (F score) was staged with a 5-point scale from absent (F=0) to very marked (F=4) [2]. Dogs were divided into A score groups: A0-1 (absent to slight) and A>1 (mild to very marked), and into F score groups F<2 (absent to mild) and F≥2 (moderate to very marked). Data from erythrocyte (RBC, hematocrit [HCT], hemoglobin, mean corpuscular volume [MCV], RBC distribution width [RDW], reticulocyte count), leukocyte (WBC, neutrophils count, presence of left shift and monocytosis/activated monocytes) and platelet values (platelet count [PLT] and mean PLT volume [MPV]) were recorded for each dog. In addition, occurrence of RBC morphological abnormalities (e.g., schistocytes, acanthocytes, target cells, echinocytes, dacrocytes,...), anisocytosis, polychromasia, anemia (HCT<37%), microcytosis (MCV<61 fL), and leukocytosis (>16,000/μL) were also collected. Relationships between continuous and categorical variables and A and F score groups were investigated.

Fifty-four dogs were included. Seventeen dogs belonged to A0-1 group, whereas 37 to A>1 group; 23 dogs were in F<2 group and 31 in F≥2. Twenty-eight dogs (52%) had anemia and 19 dogs (35%) had microcytosis. Based on microscopic evaluation, 15 dogs (28%) showed poikilocytosis, ranging from slight (+; 4 dogs) to marked (++ and +++, 9 and 2 dogs respectively). The most frequent RBC abnormalities were: schistocytes, acanthocytes, echinocytes (7 dogs; 46% of dogs with poikilocytosis) and dacrocytes and echinocytes (4 and 3 dogs, respectively). Twenty-eight dogs (52%) had anisocytosis (+, 17 dogs; ++, 11 dogs) and 18 (33%) showed polychromasia. No association or differences in any of the RBC parameters between A and F score groups were found. Leukocytosis was detected in 12 dogs (22%). Five and 12 dogs had left-shift and monocytosis/activated monocytes, respectively. None of the WBC findings were associated neither with A score, nor with F score. PLT count and MPV was not significantly different in A and F score groups. Despite the lack of association with histological inflammatory and fibrosis, anemia, microcytosis and poikilocytosis have been fairly frequently observed in dogs with CH. Oxidative stress, lipidic alteration and/or microangiopathic pathways may be involved. Signs of systemic inflammation (leukocytosis) are not associated with necroinflammatory activity grade of CH (A score).

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PREVALENCE AND FEATURES OF INCIDENTAL FINDINGS IN VETERINARY COMPUTED TOMOGRAPHY: A SINGLE-CENTER FIVE-YEARS' EXPERIENCE

Tiziana Caspanello (1), Diego Iannelli (2), Nicola Maria Iannelli (1), Federica De Paoli (2), Massimo De Majo (1)

(1) Università degli Studi di Messina, Dipartimento di Scienze Veterinarie. (2) Clinica Veterinaria Camagna (RC) – VetPartners.

Corresponding author: T. Caspanello (tizianacaspanello@gmail.com)

Computed Tomography (CT) is an advanced imaging technique, whose use may lead to incidental findings or "incidentalomas", asymptomatic lesions found by coincidence during diagnostic tests aimed at other suspects. This retrospective analysis of incidentalomas, found by CT scans in a veterinary clinic over a five-year period, aimed at providing a contribution about prevalence, location and types of incidental findings and their correlation with species, sex and age of the patients. The reports submitted by Diplomates of European College of Veterinary Diagnostic Imaging of CT scans, performed between 2015 and 2020 with a *Revolution ACT* 16-slice scanner (*GE Medical Systems*, Italy), were examined and compared to the history of the patients. All the CT findings not mentioned in the history nor matching the indication of the CT exam were included. Follow-up data of the patients included were also collected. 426 CT scans were performed and 96 different incidentalomas (i.e. 22.54%) were found in 58 dogs (29 males, 29 females) and 6 cats (4 males, 2 females). The 15% of patients undergoing a CT scan had at least one incidentaloma and the 31.25% of them had more than one. The dog/cat ratio was 9.66 in patients with incidentalomas and 10.81 in patients undergoing CT. The most common incidentalomas were located in the spine (n=22), followed by renal and respiratory ones (n=9). The male/female ratio was 1.06 in patients with incidentaloma and 1.12 in patients undergoing CT. Incidentalomas were found in patients aged between 7 months and 15 years, with the highest average number of incidentalomas in 13 - and 5-year-old dogs (3 and 2.6 incidentalomas/dog, respectively) and 12-year-old cats (n=2). Based on age groups, the types of incidentalomas were hiatal hernia and congenital anatomical anomalies (0-5 years); oral, ear, spinal, and neoplastic incidentalomas (6-9 years); variably-located findings, adrenal, respiratory, prostatic, renal and hepatic incidentalomas (10-13 years). The 56% of the patients were lost to follow-up or died and the remaining returned for other reasons than incidental findings. No patients returned for a follow-up of the incidentalomas. The results showed that incidental findings in veterinary CT were a phenomenon to be reckoned with; that the finding of incidentalomas was not influenced by species -even if the number of cats was too low- or sex of the patient; that there was no linear correlation between the number of incidentalomas and age of the patients, but the latter was rather related to the type of incidentalomas. Previous studies about CT incidentalomas in veterinary medicine evaluated their prevalence in particular anatomical sites, describing their typologies and tomographic characteristics [1-3]; other researches described the prevalence and tomographic features of various lesions of certain organs or region, mentioning also the ones found accidentally [4, 5]. Otherwise, this study reported all incidental findings in CT and it's not limited to a single site or pathology. While some results could depend on the higher sensitivity than other imaging techniques, and many were not clinically relevant, results on follow-up showed that there isn't enough knowledge about how to handle incidentalomas. Therefore, shared and evidence-based guidelines about the correct procedures for managing incidentalomas would be a useful tool to clinicians.

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EVALUATION OF URINALYSIS AND ANTIMICROBIAL SUSCEPTIBILITY AMONG DOGS REFERRED TO THE VETERINARY TEACHING HOSPITAL, UNIVERSITY OF MILAN

Filippo Tagliasacchi (1), Jari Zambarbieri (2), Tiziana Vitiello (2), Guido Grilli (2), Paola Scarpa (1)

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

Corresponding author: F. Tagliasacchi (filippo.tagliasacchi@unimi.it)

Suspecting a Urinary Tract Infection (UTI), urine culture and antimicrobial sensitivity tests represent the gold standard for the diagnosis and the starting point for a proper treatment. A complete urinalysis is recommended in all the patients with Lower Urinary Tract Signs (LUTS) (1, 2).

In a population of dogs with recurrent LUTS, this study aims to compare signalment, history and data obtained from urinalysis, between subjects with positive or sterile urine culture. Furthermore, the study reports the prevalence of uropathogens implicated in UTIs and the rate of resistance (RR) to the main antibiotics routinely tested. From the database of the Veterinary Teaching Hospital (University of Milan), were selected all dogs (n=154) referred for an history of rUTIs between January 2019 and February 2022, for which urine culture was performed. Urine samples by cystocentesis (n=148) and bladder wall biopsies (n=6) were taken for diagnostic purposes, under signed consent of the owners, in agreement with the ethical committee statement of the University of Milan number 2/2016. Urine samples were plated on blood and MacConkey agar. The bacterial species were identified using MALDI-TOF MS. The antimicrobial sensitivity tests were obtained by Kirby-Bauer disk diffusion method. Statistical analysis was performed by Chi-square, Wilcoxon or Kruskall-Wallis test using JMP 16 (SAS Inc., Cary, USA).

In the study population crossbreeds (28%) and intact males (36%) were prevalent, the mean age was 8 years (std=3.8). Chronic Kidney Disease (CKD) was the most represented comorbidity (26%), followed by urolithiasis, urethral sphincter mechanism incompetence, prostatic disorders and neurologic disorders of micturition. Patients with positive urine culture (group "cases") were more likely to be dysuric ($p=0.002$) than subjects with sterile urine (group "controls"), who, however, had a higher probability of been previously treated with an antibiotic ($p=0,01$). Statistically significant differences between the two groups were identified for White Blood cell count (WBC) and urinary pH, both higher in cases than in controls ($p<0.0001$ and $p=0.02$ respectively). No statistical difference was identified for other variables investigated, including Age, Sex, Breed, Red Blood cell Count (RBC), Urine Protein Creatinine ratio (UPC), USG.

Of the 154 urine cultures, 42% were positive, with *Escherichia coli* being the first uropathogen by frequency (43.7%), followed by *Proteus* spp. (14%) and *Enterococcus* spp. (14%), *Staphylococcus* spp. (9,3%), *Klebsiella* spp. (6.2%), *Pseudomonas* spp. (4.6%), *Streptococcus* spp. (3.1%), *Pasteurella* spp. (3.1%), *Acinetobacter* spp. (1.5%). A predominance of single isolates (96,4%) was observed compared to polymicrobial infections (4.6%). The highest total RR were identified for Clindamycin (91.4%), followed by Ampicillin (71.9%), Amikacin (60%), Doxycycline (57.6%). On the contrary, the maximum efficacy was found for Marbofloxacin, with a total sensitivity rate (SR) of 60%, followed by Gentamicin (58.7%), Ceftriaxone (55.6%) and Trimethroptim/Sulfamethoxozole (50.7%).

As prescribed by the 2019 Guidelines of the International Society for Companion Animals Infectious Disease (ISCAID), this study provides clinicians with local data for a proper empirical choice of the antibiotic in case of UTIs (3). Based on our preliminary results, Ampicillin is no longer to be considered an elective drug for empirical therapy in our region, given the high RR (71.9%). On the contrary, Trimethroptim/Sulfamethoxozole is one of the most effective molecules among those tested. A rather distinct behavior, in terms of SR and RR was observed between the different fluoroquinolones tested, with Marbofloxacin showing clearly superior efficacy to Enrofloxacin and Pradofloxacin.

Among our patients, 38% of dogs with negative urine culture were dysuric, while 36% of dogs with positive urine culture were asymptomatic. These cases represent the tricky patients for clinicians. According to ISCAID guidelines analgesic and antinflammatory treatment is recommended in both categories.

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SICV

EFFICACY OF BUPRENORPHINE ADMINISTERED TRANSDERMALLY, COMPARED TO THE INJECTABLE ROUTE, IN THE MANAGEMENT OF INTRA- AND POST-OPERATIVE PAIN IN DOGS UNDERGOING UNILATERAL MASTECTOMY: PRELIMINARY DATA

Caterina Di Bella, Pietro Toniolo, Alessandro Troisi, Luca Pennasilico, Margherita Galosi, Angela Palumbo Piccionello

School of Bioscience and Veterinary Medicine, University of Camerino.

Corresponding author: P. Toniolo (pietro.toniolo@studenti.unicam.it)

Transdermal opioid administration offers an attractive alternative to maintaining analgesia for a long time in veterinary patients, avoiding the disadvantages of parenteral or oral administration (invasiveness, fluctuating plasma plateau, need for use in a hospital setting) [1]. The aim of this study was to evaluate the efficacy of buprenorphine transdermal patches compared to intravenous (IV) administration of the same drug, in the treatment of intra- and post-operative pain in dogs undergoing unilateral mastectomy. Our hypothesis is that the two different routes of administration ensure equal analgesia throughout the perioperative period. Ten dogs were selected for this prospective clinical study. All dogs with mammary tumors were included in this study, however, the exclusion criteria were: ulcerated tumors, lung metastases, cardiovascular and respiratory diseases, aggressive behavior and other causes of pain. Dogs were randomly divided in two groups. In the BupreP group (5 dogs), transdermal patches were placed on the thoracic skin 36 hours before surgery and kept up to 24 hours after mastectomy. The dosage used was standardized on body weight (6 µg/kg/h) [1]. Instead, the BupreI group (5 dogs), received 20 µg/kg of IV buprenorphine, 30 minutes before surgery. The same administration was repeated every 6 hours for the 24 hours following the mastectomy. In the pre-operative period, all patients were premedicated with acepromazine (10 µg/kg) IM, and induced with propofol (5 mg/kg) IV. General anesthesia was maintained with isoflurane in pure oxygen. Ten minutes before the induction, heart rate (HR), respiratory rate (RR) and mean arterial pressure (MAP) were registered. Moreover, the sedation score (SS) was evaluated based on the modified Gurney Scale. During the intraoperative period, the main hemodynamic and respiratory parameters were registered 10 minutes before the start of surgery (BASE) and during the incision of the skin (SKIN), mastectomy (MAST) and suture of subcutis (SUTURE). Intraoperative nociception was assumed if HR or MAP increased by >20% from baseline, in which case costant rate infusion of lidocaine (30-50 µg/kg/h) was started. Following recovery from anesthesia, signs of postoperative pain were assessed 1, 2, 4, 6, 8, 10, 15, 20 and 24 hours from the extubation, using both Glasgow Composite Pain scale (GCPs) and the modified University of Melbourne Pain Scale (UMPS). Score of 6/24 for the first and 7/27 for the second scale was indicative of pain, therefore, sore dogs were withdrawn and pain was treated. Data were compared at each time of the study with the one-way ANOVA and Friendman test ($P < 0.05$). The BupreI group showed a significantly higher sedation score [median = 10 (7-11)] than BupreP group [median = 1 (1-2)]. Instead, the results of the intra- and post-operative evaluations, there were no statistically significant differences between the two study groups. Our results, therefore, prove that the analgesic efficacy of buprenorphine administered via transdermal patches is comparable to the injectable one. In addition, the greater sedation induced by IV administration, probably, is due to the achievement of a high plasma drug concentration in a short time, associated with a greater diffusion gradient between plasma and central nervous system, compared to slow and gradual release of buprenorphine from the patch [3]. In conclusion, the administration of buprenorphine via transdermal patches could be a valid alternative to injectable administration in the management of peri-operative pain in dogs. We should consider the small number of patients ad a limiting factor.

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EVALUATION OF THE EVOCATIVE IMMUNOREACTIVITY PROPERTIES OF TISSUE ENGINEERING PRODUCED DECELLULARIZED MEMBRANES, FROM HUMAN FORESKIN OF DONORS: IN VIVO EXPERIMENTAL STUDY IN AN ANIMAL MODEL

Luca Pennasilico, Margherita Galosi, Valentina Riccio, Caterina Di Bella, Giacomo Rossi, Angela Palumbo Piccionello

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

Corresponding author: L. Pennasilico (luca.pennasilico@unicam.it)

Millions of people suffer from physical and psychological damage caused by circumcision. To date, there is not yet therapeutic and effective surgery for foreskin restoration. Recently, a group of Italian researchers have developed a novel decellularized method for epithelial tissue [1]. In addition this method could be used in the development of heterologous dermal substitutes for the treatment of skin defects in veterinary and human medicine. The aim of our study was to evaluate the evocative immunoreactivity properties of decellularized membranes produced by tissue engineering, starting from human foreskin of donors, when implanted in the host (rat). The study, approved by Ministry of Health (n. 424/2021-PR), involved twenty-six Wistar breed male rats. After the induction of general anaesthesia, the animals were positioned in sternal recumbency and a square infrascapular area of about 6 cm^2 was clipped and prepared for surgery. Under complete asepsis, an infrascapular skin incision of about 1 cm was made and a decellularized membrane (about 2 cm of diameter) was implanted in hypodermal layer. Skin incision was sutured with USP 4/0 resorbable monofilament thread. Immediately after surgery the heart and respiratory rate, temperature, grade of sedation were evaluated and the Rat Grimace Scale was used to assess the state of discomfort of the implanted animals. The rats received antibiotic, antinflammatory and analgesic post-surgery therapy for 5 days. To evaluate different stages of inflammatory response, rats were subdivided in two groups: Group A (13 rats) that had a follow up of 30 days; Group B (13 rats) that had a follow up only of 5 days. The infrascapular region was monitored in attempt to score key signs of inflammation: calor, rubor and tumor, scoring from 0 (no alterations) to 3 (severe alterations). After 5 days for group B and 30 days for group A, the subjects underwent to general anaesthesia and euthanized. A tissue explant centered on the surgical scar was performed and sent to the pathology lab for histological and immunohistochemical analyzes, in attempt to assess the presence and degree of the inflammatory infiltrate, related to the host's immune response to the implant. Friedman test was used for the analysis of non-parametric data and they were expressed as median (min -max). The subjects showed no severe signs of inflammation for the parameters of calor, rubor and tumor. The greatest alterations occurred in the first day's post-surgery, and they returned to a value close to zero at T5. Histologically, all samples taken from group B showed a scaffold-demarcating mild to moderate inflammatory reaction. In the samples taken from group A in 61.5% of rats, a mild neoangiogenesis of the scaffold was seen, consisting in a colonization by some, CD 31+ small capillaries. In the same samples a slight cellularization of the scaffold by vimentine + fibroblasts was also documented. In 5/13 subjects of group B, the organization of a granulomatous reaction circumscribing the scaffold was observed (characterized by foreign body multinucleated or epithelioid giant cells), this reaction was not observed in the samples of group A. Finally, in 30% of cases no trace of the scaffold was found, but only a moderate inflammation in the implant area. Our results highlighted that these decellularized scaffolds can be moderately cellularized and neovascularized if properly maintained in the implant site. Taken together, these preliminary data show that tissue engineering produced decellularized membranes from human foreskin, represent a promising scaffold for the reconstruction of human foreskin. The subsequent phase of the study will plan to evaluate, in an ovine model, the effective engraftment and rapid revascularization of the decellularized membrane on the receiving tissue.

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CAN THE ARTERIAL OXYGEN BE ESTIMATED NON-INVASIVELY BY THE OXYGEN RESERVE INDEX in dogs? A PILOT STUDY.

Luca Bellini, Martina Cardinali , Giulia Maria De Benedictis

Università degli Studi di Padova, Dipartimento di Medicina Animale, Produzioni e Salute.

Corresponding author: L. Bellini (luca.bellini@unipd.it)

Clinically, arterial oxygen content (CaO_2) and oxygen tension indexes quantify the pulmonary oxygen transfer and monitor the effectiveness of mechanical or assisted ventilation in critically ill patients. Calculation of those parameters requires the partial pressure of oxygen (PaO_2) measurement in an arterial blood sample, but this procedure is invasive and challenging. Moreover, the sampling is intermittent, and the insertion of a catheter in an artery could lead to haematoma and bacterial contamination. New medical tools introduced in clinical practice try to overcome those limitations. In human medicine, the oxygen reserve index (ORI), a non-invasive parameter, estimates continuously the oxygen reserve defined as PaO_2 between 100 and 200 mmHg and ranging from 0 (no-reserve) to 1 (full reserve), respectively [1]. The aim of the study was to evaluate whether the CaO_2 and the tension index, PaO_2/FiO_2, calculated using the blood gas analysis are comparable to those estimated non-invasively with the ORI in dogs.

The study enrolled eight dogs undergoing general anaesthesia for elective procedures requiring the insertion of an arterial catheter and mechanical ventilation with a fraction inspired of oxygen set between 0.21 and 0.4. ORI probe, wrapped around the tongue, was connected to a co-oximeter (Rad 97, Masimo Corp.). After a stable signal, an arterial sample was collected, and concurrently the value of ORI was recorded. PaO_2 and haematocrit were immediately measured in the arterial blood sample and corrected for the temperature of the patience. The relation between matched values of PaO_2 and ORI was assessed with a linear regression; the model assumed that ORI was the response variable and that a value of 0 indicated a PaO_2 of 100 mmHg. The equation obtained was used to estimate the value of PaO_2 to calculate the CaO_2 (CaO_2est) and the PaO_2/FiO_2 (PaO_2/FiO_2est) [2]. The agreement between variables calculated using the blood gas measurement or those estimated with ORI was evaluated with Bland-Altman's method [3].

Sixteen measurements of PaO2 and corresponding ORI were obtained, and the equation of the curve that fitted the data was ORI = (0.008957 PaO_2) – 0.8957 with a correlation coefficient of 0.32. For oxygen content, CaO_2–CaO_2est showed a bias of –0.01 ml/dl with a limit of agreement between –0.47 to 0.46 ml/dl, while PaO_2/FiO_2–PaO_2/FiO_2est showed a bias of –35 mmHg and a limit of agreement between –200 to 269 mmHg.

In conclusion, ORI mildly correlated with the measured PaO_2, agreement bias for arterial oxygen content was minimal, but the limit of agreement was wide. More paired observations are needed to support the use of ORI as a non-invasive alternative to calculate CaO_2 and PaO_2/FiO_2.

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SURGICAL MANAGEMENT OF CONGENITAL BILATERAL LATERAL PATELLAR LUXATION IN A FOAL

Gianluca Basso, Parastoo Memarian, Maurizio Isola

Università degli Studi di Padova, Dipartimento di Medicina Animale, Produzioni e Salute.

Corresponding author: P. Memarian (parastoo.memarian@phd.unipd.it)

Patella luxation is a rare orthopedic condition in equines. Most luxation are congenital in origin, with the lateral patellar luxation being the prevalent type (1,2). Treatment of equine lateral patellar luxation with a combination of soft tissue and hard tissue procedures have been presented sporadically in the literature (1-4). This case report describes a surgical technique modification for treating bilateral lateral patella luxation in a foal by reinforcing the imbricated medial tissues with mesh placement. A 2-month-old foal with mobility difficulties, geno valgus, and toe-out stance was presented. Orthopedic examination revealed congenital bilateral lateral patella luxation (grade IV in right, grade III in left stifle) associated with angular deformities in both hindlimbs. Surgery was planned with one-week distance, first on the right stifle and then on the left using the same surgical procedure. Intraoperatively, hypoplastic lateral trochlear ridge and shallow trochlear groove were confirmed on both limbs. The procedure included trochlear block recession, lateral release, and medial imbrication. A polypropylene mesh was then used to reinforce the medial imbrication sutures. The quadriceps mechanism alignment and function were validated intra-operatively and post-operatively. The foal was helped to stand and walk with external aids in the first few days after surgery, and gradually improved thereafter. Post-operatively, a self-limited seroma formed in the right limb that was managed conservatively. Two weeks following surgery, the orthopedic evaluation still shows satisfactory limb alignment and overall good outcome. The report suggests the application of polypropylene mesh for reinforcing the medial soft tissues, in addition to trochleoplasty and soft tissue reconstruction, could be considered as an alternative approach to manage high-grade patellar luxation in equines.

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ORAL TRANSMUCODAL SEDATION FOR MINOR PROCEDURES IN TWO LARGE FELIDS

Giulia Maria De Benedictis (1), Martina Cardinali (1), Luca Bellini (1), Francesca Zanusso (2)

(1) Università degli Studi di Padova, Dipartimento di Mediicna Animale, Produzioni e Salute. (2) Università degli Studi di Bologna, Dipartimento di Scienze Mediche Veterinarie.

Corresponding author: G.M. De Benedictis (giuliamaria.debenedictis@unipd.it)

Drug administration via oral transmucosal (OTM) route is considered simple, non-invasive, stressless, well tolerated and very helpful for patients who are fearful, anxious, or difficult to inject [1]. OTM route of administration has been widely studied in domestic cats but never described in large felids [1, 2].

A 21-year-old 62 kg male captive leopard (*Panthera pardus*) with an history of severe right hindleg osteoarthritis was sedated to perform clinical examination, blood sampling and nail clipping. To avoid restlessness, anxiety and fractious reaction to dart injection, sedation was administered by OTM route, spraying a combination of midazolam (0.2 mg/kg) and dexmedetomidine (0.005 mg/kg) from outside of the cage. After 30 minutes, the leopard vomited, relaxed in sternal recumbency and 10 minutes later a supplemental dose of dexmedetomidine (0.005 mg/kg) and butorphanol (0.3 mg/kg) was given OTM. Ten minutes later the animal was in lateral recumbency and safely approached. No reactions were observed at manipulation or blood sampling. Physiological parameters remained within the normal limits during the procedure. Sedation was adequate to safely manipulate the animal and only sporadic muscle twitches were observed. Atipamezole (0.1 mg/kg) was administered intramuscularly 38 minutes after the second dose and the leopard was able to stand within 10 minutes.

A 12-year-old 155 kg female trained tiger (*Panthera tigris*) was sedated for blood analysis and abdominal ultrasound check 5 days after ovariectomy. Thanks to the friendly nature of the animal and the relationship with the trainer, a combination of midazolam (0.13 mg/kg) dexmedetomidine (0.01 mg/kg) and butorphanol (0.16 mg/kg) was easily administered OTM. After 15 minutes the tiger vomited and at 30 minute was in lateral recumbency and safely approached. Physiological parameters remained within the normal limits. No reaction was noted at blood sampling and only a slight change in respiratory rate and pattern was seen in response to the increased ultrasound probe pressure on the abdominal wall. At the end of the procedure the animal was able to rise its head and started to walk within 68 minutes from OTM administration.

In both animals, the drug combinations used allowed satisfactory level of sedation, safe handling of the patients and calm and smooth recovery.

This is the first report describing chemical immobilization through OTM route in large felids; it resulted to be feasible, effective, and reliable although the onset of sedation is delayed compared to intramuscular administration [3].

In trained large felids, OTM route may represent an alternative approach for sedative-analgesic administration with lesser impact on animal welfare.

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MANAGEMENT OF BLADDER RUPTURE IN TWO COLTS: A CASE REPORT ABOUT COMBINATION OF SURGICAL AND CONSERVATIVE TREATMENTS

Chiara Montano (1), Giulia Forni (2), Jole Mariella (2), Aliai Lanci (2), Chiara Del Prete (1), Maria Pia Pasolini (1) and Riccardo Rinnovati (2)

(1) Università degli Studi di Napoli "Federico II", Dipartimento di Medicina Veterinaria e Produzioni Animali.

(2) Università degli Studi di Bologna, Dipartimento di Scienze Mediche Veterinarie.

Corresponding author: C. Montano (chiara.montano@unina.it)

Congenital or traumatic defects along any portion of the urogenital tract are the most frequent cause of uroperitoneum in foals. Surgical correction is the first choice of treatment even if complications are not uncommon. Non-surgical methods, such as urine removal via urinary catheters and abdominal drains, have been successfully performed in foals as well. The management of bladder/urethral rupture in two colts through the combination of surgical and conservative techniques is reported in the present study. The foals were referred to the Equine Perinatology Unit of the University Hospital "Giuseppe Gentile" for suspicious uroperitoneum. Haematology, serum biochemistry, arterial gas analysis and ultrasonography confirmed the diagnosis, therefore cystorrhaphy and cystoplasty were planned. Surgeons found a lesion in the dorsocranial margin of the bladder (Case 1) and a tear in the pelvic urethra (Case 2); the last one was impossible to repair due to its localization. A Foley urinary catheter was left in place in both cases and connected to a urine bag, anchored to the patient's body. In both colts, uroperitoneum recurred 72 hours after the surgery. In Case 1, the cystoscopy showed a tear in the dorsal wall of the bladder, caudal to the suture. A second surgery was not recommended in both cases, due to the localization of the tears, and conservative treatment was preferred. A 32F chest tube was placed at the most ventral aspect of the abdomen and the peritoneal cavity was lavaged twice daily with 2 litres of saline solution. Drains were removed on day 2 (Case 1) and 7 (Case 2) after the surgery due to poor return of abdominal fluid. The colts improved during hospitalization and were discharged 29 (Case 1) and 17 (Case 2) days after the admission. Two months after, one foal (Case 1) underwent exploratory laparotomy due to suspicious of bowel strangulation. Euthanasia was performed due to the presence of devitalized segments of small intestine and multiple adhesions between the small intestine and the abdominal wall. The second foal (Case 2) showed no complications and was still alive one year post-operatively. Complications following primary surgical repair of uroperitoneum may be related to surgical failure, latent tissue necrosis, over-pressurization of the bladder or an additional urinary tract defect. In the present study, conservative management consisted of continuous urine decompression with an indwelling Foley urinary catheter associated to peritoneal drainage and lavage. Even if the short-term prognosis was favourable, caution in the long-term is necessary due to risk of adhesions. Further studies are necessary to determinate the validity of surgical/no-surgical combination and primarily to assess the incidence of short and long- term postoperative complications.

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SIFTVET

ADENO-ASSOCIATED VIRUS VECTOR AS NEW THERAPEUTIC APPROACH IN RARE KIDNEY DISEASES

Anna Iervolino (1, 2), Consiglia Longobardi (2, 3), Sara Damiano (4), Antonio Miele (2), Valeria Russo (4), Luigi Navas (4), Giovambattista Capasso (2), Roberto Ciarcia (4)

(1) Università degli Studi della Campania "Luigi Vanvitelli", Dipartimento di Scienze Mediche Traslazionali. (2) Biogem S.c.ar.l., Istituto di Ricerche genetiche "Gaetano Salvatore". (3) Università degli Studi della Campania "Luigi Vanvitelli", Dipartimento di Salute mentale e Fisica e Medicina preventiva. (4) Università degli Studi di Napoli "Federico II", Dipartimento di Medicina veterinaria e Produzioni animali.

Corresponding author: C. Longobardi (consiglia.longobardi@unicampania.it)

The potential of gene therapy is compelling and a conspicuous number of progress have been made, especially in the field of rare genetic diseases. Through this strategy, it is possible to use genetic material as therapeutic molecule to repair a defective gene inducing very severe diseases [1].

Recent studies show promising advances in gene therapy demonstrating the efficiency of transduction of adeno-associated viruses (AAVs) in the cells of several organs [2]. Regarding renal diseases, further investigations are necessary as the kidney has sophisticated filtering mechanisms and the dimensions of the retroviruses (about 200 nm) are larger than those of the slit diaphragms within the glomeruli (10 nm). This physiological condition would not allow the passage of most of the viruses normally used in gene therapy, including AAVs [3].

This research foresees the in vivo administration of a recombinant AAV serotype 9 (AAV9) that has demonstrated to provide DNA transfer in all segments of renal nephron [4]. In particular, we have investigated the potential of the AAV9 vector against Fanconi-Bickel syndrome (FBS), a congenital renal tubulopathy disease which causes a dysfunction of the Slc2a2 gene encoding the GLUT2 (glucose transporter 2) protein, mainly expressed at the proximal tubule (PT) level [5]. Since the translational laboratory of nephrology of Biogem has just developed a mouse model mimicking this syndrome, the Glut2lox/lox Pax8cre strain, this mice model was subjected to retro-uretral administration of AAV9 vector that will deliver the mutated gene in the targeted cells (authorization number 354/2021-PR).

In the first phase of the research, AAV9 was designed and engineered with an appropriate cell-specific promoter that would allow the gene-therapeutic sequence to reach the portion of nephron affected by the rare renal disease. For this purpose, Sglt2 (sodium-glucose cotransporter type 2) was chosen to deliver the expression of GLUT2 only in the PT cells, since this cotransporter performs its function at this level. Also, the AAV9 was tagged with a dTomato orange fluorescent protein to trace and verify the correct expression of the carried target gene.

The obtained vector was transfected into murine proximal tubule cells, i.e. TKPTS cell line (ATCC No. CRL-3361™), and, through dTomato tag fluorescence, good transfection efficiency was observed under the confocal microscope.

These preliminary results will allow to test the in vivo potentiality of the therapeutic DNA strategies in the rare diseases affecting kidneys and could open a new era of research into gene therapy for these threatening disorders, since nowadays there are not resolutive strategies in the field for these rare renal condition.

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A NEW METHOD TO ANALYSE FUSARIUM MYCOTOXINS IN FOOD BY LIQUID CHROMATOGRAPHY AND HIGH RESOLUTION MASS SPECTROMETRY

Pasquale Gallo, Ida Duro, Maria Giovanna Buonomo, Olga Arace, Valeria Urbani, Ilaria Di Marco Pisciottano

Istituto Zooprofilattico Sperimentale del Mezzogiorno, Dipartimento di Chimica.

Corresponding author: P. Gallo (pasquale.gallo@izsmportici.it)

Fusarium mycotoxins are a large group of mycotoxins including more than 140 known metabolites of *Fusarium* spp. fungi. These mycotoxins are ubiquitous in nature and can be detected as contaminants in several vegetables and feed, representing a health risk both for humans and animals because of their high toxicity. Among *Fusarium* mycotoxins, deoxynivalenol (DON), its modified forms DON-3-glucoside (DON3G), 3-AcetylDON (3AcDON) and 15-AcetylDON (15AcDON) are some of the most occurring in cereals and derived products. DON is also known as vomitoxin, due to its activity as dopaminergic agonist, causing a strong emetic effects after consumption. Its occurrence in food and feed represents a potential marker of the occurrence of other mycotoxins [1]. Among the trichothecenes, also fumonins are noteworthy mycotoxins, because of their proved toxic effects on the liver and the nephron in animals. There are more than 15 fumonisin homologues, characterized as A, B, C, and P, but the fumonisin B1 (FB1), B2 (FB2), and B3 (FB3) are the most abundant and therefore the main food contaminants. In particular, the FB1 is the most toxic form acting as s promoter of hepatocarcinoma, causing stimulation and suppression of the immune system, defects in the neural-tube and nephrotoxicity [2]. Because of these health risks, the European Commission (EC) required monitoring activities to all the Member States and set maximum tolerable levels in many food: for DON in cereals and derived products, such as flour, bran, germ, pasta, bread, biscuits, pastries, breakfast cereals, snacks and baby foods; for the sum of FB1 and FB2 in maize and maize-based snacks, breakfast cereals, baby foods and processed maize-based food [3]. Moreover, the monitoring of DON related compounds DON3G, 3AcDON and 15AcDON was required for food safety assessment. In the frame of the Monitoring National Plans issued by the Italian Ministry of Health the monitoring of trichothecenes in cereals, maize and derived product was introduced for official control activities, requiring for accredited test methods. The aim of the present study was to develop and validate a rapid, selective and reliable method for the simultaneous analysis of DON, DON3G, 3AcDON, 15AcDON, FB1 and FB2 by ultra high-performance liquid chromatography (UHPLC) coupled to high resolution mass spectrometry (HRMS). The detection was performed on a Q Exactive Plus Orbitrap mass spectrometer, using Parallel Reaction Monitoring (PRM) mode in both positive and negative ionization. The chromatographic separation was achieved on a XB-C18 Kinetex stainless steel column, using buffered water and methanol as mobile phases. Sample extraction is carried out by a simple solid/liquid partition by acidified water/methanol mixture. The calibration curves were prepared in solvent and in matrix-matched, to evaluate the possible enhancement/suppression effects on ion signals. The use of Q Exactive Plus Orbitrap allowed for selective and simultaneous detection of several trichothecenes in a single run, thanks to accurate mass analysis, rapid sample preparation, quantification quite below the maximum levels set by the EU, applicability to a wide panel of food. The method performance parameters were evaluated in terms of trueness, precision, ruggedness for slight changes, linearity of the instrumental response. The results proved the method is reliable and effective, allowing us to quantify and unambiguously identify 2.0 µg/kg of 3AcDON, 15AcDON, FB1 and FB2 and 10.0 µg/kg of DON and DON3G in the sample. The mean percentage recoveries and relative standard deviations (RSD%) for all mycotoxins were in the range of 82.3%-94.1% and 6.9%-20.5%, respectively, accounting for good method trueness and precision. The ionization enhancement/suppression effects due to matrix in mass spectrometry was evaluated by matrix-matched calibration curves. The linearity of the instrumental response, expressed as determination coefficient, was higher than 0.95 for all the analytes and in all the analytical sessions.

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FOURIER TRANSFORM INFRARED SPECTROSCOPY (FTIR) SPECTRAL PROFILING AND MACHINE LEARNING FOR THE DETECTION OF MILK MIXTURES FROM DIFFERENT BOVINE BREEDS

Antonella Spina (1, 2), Paola Roncada(1, 2), Bruno Tilocca(1, 2), Carlotta Ceniti(1, 2), Domenico Britti (1, 2), Valeria Maria Morittu (1, 2), Cristian Piras (1, 2)

(1) Department of Health Sciences-University of Catanzaro Magna Græcia, Viale Europa, 88100 Catanzaro, Italy.
(2) Interdepartmental Center Veterinary Service for Human and Animal Health, CISVetSUA, University "Magna Græcia" of Catanzaro, 88100 Catanzaro, Italy.

Corresponding author: C. Piras (c.piras@unicz.it)

Spectroscopic techniques are capable of generating a fingerprint of the analysed matrix or dairy product according to the absorption of various chemical components. The composition of the analysed material depends on the variety, season, location, and other characteristics. Virtually, such a fingerprint coupled with the correct machine learning algorithms and with a set of representative spectra or "standards" could be able to establish the quality or authenticity of many dairy products.

In this case, we applied Fourier transform infrared spectroscopy for the recognition of bovine milk from Podolica and Valdostana Pezzata Rossa breeds from the same farm.

The Podolica is a breed of domestic cattle from southern Italy renown for its valuable meat and dairy products. Podolica cows productivity is characterized by low yields and extraordinary quality linked to its wild nature that oblige farmers to breed these animals in a wild or semi-wild state. Most of the milk produced is used to be transformed into Caciocavallo Podolico cheese that is made with 100% Podolica milk. The valorization of this product needs cheap and easily applicable tools for the rapid recognition of this milk or of mixtures of this product with milk of other breeds.

Four different mixtures replicates were analyzed 4 times each with FTIR spectroscopy obtaining in total 16 different measurements of each mixture (100% Podolica; 95% Podolica-5% Valdostana Pezzata Rossa; 90% Podolica-10% Valdostana Pezzata Rossa; 80% Podolica-20% Valdostana Pezzata Rossa; 60% Podolica-40% Valdostana Pezzata Rossa; 40% Podolica-60% Valdostana Pezzata Rossa; 100% Valdostana Pezzata Rossa). The raw spectral profiles were analyzed through the Linear Discriminant Analysis module of jmpSAS (version 15). 6 out of 16 spectral profiles of each analyzed mixture were used for training the model and the remaining 10 were used as blind dataset to be analysed. FTIR spectroscopy detected Valdostana Pezzata Rossa milk as adulterant in Podolica milk down to amounts as low as 5% with 100% Sensitivity and 90% specificity on blind datasets. 10% Valdostana Pezzata Rossa milk as adulterant was detected with 100% Sensitivity and 100% specificity.

The results herein described demonstrate that FTIR spectral profiling coupled with machine learning could be capable of detecting milk from different breeds. In addition, the value of this study in further improved by the elimination of the environmental variable: the analyzed samples were collected from different breeds growing in the same environmental conditions (same farm) and the groups were formed by an homogenous population.

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A PRELIMINARY STUDY ON DDDAIT CALCULATION IN WATER BUFFALO FARMS

Gabriele Di Vuolo (1), Federico Scali (2), Giovanna Cappelli (1), Alborali Giovanni Loris (2), Bertocchi Luigi (2), Valentina Lorenzi (2), Francesca Fusi (2), Mario Orrico (3), Esterina De Carlo (1), Domenico Vecchio (1)

(1) CReNBuf Istituto Zooprofilattico Sperimentale del Mezzogiorno, Portici, Italy. (2) CReNBa Istituto Zooprofilattico Sperimentale della Lombardia e dell'Emilia Romagna Bruno Ubertini, Brescia, Italy. (3) Freelance Veterinarian, Calabria, Italy.

Corresponding author: G. Di Vuolo (gabriele.divuolo@izsmportici.it

The incresased development of antimicrobial resistance (AMR) is a problem of growing concern because of the associated risk of therapeutic ineffectiveness that equally jeopardizes human and animal health (1). Inappropriate use of antimicrobials (AMU) is reinforcing AMR (2), thus a prudent and responsible AMU is fundamental for fighting against this problem. The European Medicines Agency (EMA) has classified the antimicrobials according to their importance in human medicine. In particular, EMA category B refers to critical antimicrobials, which use should be allowed only when no valid alternatives are available (3). In the light of the multiple scientific evidences, the attention of the institutions is constantly increasing for implementing actions against AMR; starting from WHO, with the Global action plan on antimicrobial resistance (4), which reports the need for a close intersectorial collaboration between public and animal health (One Health approach).

The purpose of this work was to collect and analyze the first AMU data in Italian dairy water buffalo farms. Data on AMU and on production were collected during the 5-year period 2015-2019, in eight dairy water buffalo farms. AMU was estimated across three categories (adults, heifers and calves) using the Defined Daily Doses Animal for Italy (DDDAit) as a metric. This standard was established during the development of the ClassyFarm system (www.calssyfarm.it) owned by the Italian Ministry of Health. The farms involved in the study housed a median of 276 adults (range; 104-626), 182 heifers from 6 to 24 months (range; 46-401) and 254 calves (range; 92-579). Antimicrobials were used almost exclusively on adults (97.9% of total AMU) with a median of 2.62 DDDAit (range; 0.03-13.73). The median in heifers was 0 DDDAit (range; 0-0.68) and 0.10 DDDAit in calves (range; 0-2.72). In adults, antimicrobials belonging to EMA category B accounted for 39.3% of the total AMU. The five most commonly used classes, wich accounted for 91.4% of total AMU, were: third- and fourth-generation cephalosporins (38.8%), tetracyclines (33.5%), rifamycins (9.5%), first- and second –generation cephlasporins (5.8%), and macrolides (3.7%). Injectables were the most commonly used formulations (67.7%), followed by intramammary (23.5%), and intrauterine (8.8%). Treatments were mostly due to urogenital problems (46.6%), dry therapy (27.4%), and mastitis (23.5%). The model used for the following study allowed the development of a standardized method for measuring AMU in water buffalo farms. Although water buffalo farming is relatively widespread in Italy, information on AMU in this species is scarce. The study suggests that antimicrobial stewardship should not be neglected in the water buffalo species, especially considering the frequent administration of EMA category B antimicrobials. Although these results allowed an initial screening of AMU patterns, before drawing any conclusion on AMU in water buffaloes, it will be necessary to increase the sample size.

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PROTECTIVE ROLE OF PEA ON CANINE EPIDERMAL KERATINOCYTES (CPEK): IN VITRO STUDY

Fabio Bruno, Laura Messina, Patrizia Licata, Rosalia Crupi, Enrico Gugliandolo

Università degli Studi di Messina, Dipartimento di Scienze Veterinarie.

Corresponding author: F. Bruno (fabio.bruno@studenti.unime.it)

Autacoid Local Injury Antagonist amides (ALIAmides) are endogenous bioactive lipids, involved in biological and pathological processes as pain and inflammation. These amides, are produced by cells in response to tissue damage and ROS productions, with synthesis site local action. Their effects are aimed to contain tissue damage, through the anti-inflammatory and anti-hyperalgesic action, thanks to mast cells downregulation. Among these molecules, "Palmitoyl-ethanolamide" (PEA) has pro-homeostatic action (Melmon et al., 1981). The metabolism of these molecules involves primary degradation enzymes such as fatty acid hydrolase (FAAH) and N-acyl ethanolamine hydrolyzing acid amidase (NAAA). Already with PEA it has been shown, in studies conducted on dogs, that when the tissue concentration changes, there is a beneficial effect exerted on ischemic damage, in myocardium. The change in endogenous PEA levels, following cell damage, has also been found in other pathological conditions, such as in skin cells exposed to UV radiation. However, ALIAmides act on 3 types of receptors: PPAR-α activated by the peroxisome proliferator, GPR55 and GPR119 coupled to G proteins. These play an essential role in the recognition and signal transduction process of PEA, cannabinoid derivative, which do not bind the Cannabinoid Receptors 1 (CB1) and Cannabinoid Receptors 2 (CB2). However, they share metabolic pathways with the endocannabinoid system, which is useful for maintaining body homeostasis. Due to their lack of toxicity, their anti-inflammatory and analgesic effects, ALIAmides are ideal for long-term treatments of chronic diseases (Petrosino et al., 2010). These amides are currently used in various combinations, for example with antioxidant substances, in order to enhance their effect on target organs, for example at the gastrointestinal, urinary and skin levels. The application on experimental skin wounds and atopic dermatitis treatment in dogs, involved a reduction in mast cell degranulation, with a consequent slowing of the inflammatory process (Abramo et al., 2008). Based on this, the use of CPEK cell lines, immortalized canine epidermal cells, is useful for evaluating the inflammatory process. This is mainly carried out through in vitro research, following exposure to different concentrations of PEA in order to evaluate the anti-inflammatory response. The studies are aimed to understand the cytokine profiles trends as a function of treatment exposure, assuming that high levels of ALIAmides can determine positive effects in resolving skin inflammation. The expected protective effects, include downregulation of pro-inflammatory and inflammatory cytokines, with future implications also on the clinical side with the itching reduction in dogs. According to previously said, in order to improve the health and welfare of animals, ALIAmides appear to be a potential treatment in the near future for inflammatory skin diseases such as atopic dermatitis in dogs.

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ANTI-INFLAMMATORY EFFECT OF HELIX COMPLEX SNAIL MUCUS IN CANINE SKIN DAMAGE

Laura Messina, Fabio Bruno, Patrizia Licata, Rosalia Crupi, Enrico Gugliandolo

Università degli Studi di Messina, Dipartimento degli Studi di Messina.

Corresponding author: L. Messina (laura.messina@studenti.unime.it)

Atopic dermatitis (AD) is the most common chronic inflammatory skin disease. Genetic predisposition, epidermal barrier disruption and dysregulation of the immune system are some of the critical components of AD [1]. Canine AD exhibits a wide spectrum in its clinical phenotype, mirroring a complex and multidimensional interaction between components that represent potential fields of preventive and therapeutic interventions. Environmental and exposomal factors, the skin microbiome, the epidermal barrier; and fourth, the immune and inflammatory responses [2]. Atopic dermatitis treatment represents a challenge also in human medicine, given the high incidence and the substantial psychosocial burden involved; for these reasons, many studies are focused on characterizing the disease pathomechanisms in our and other species to find an optimal preclinical model for drug development, and the main cellular and soluble components involved in the pathophysiology of AD represent the key targets of current efforts in pharmacological intervention. Currently, pharmacological treatments involve the use of corticosteroids and antihistamines, while the most serious cases require the use of immunomodulating agents [3]. Thus, the research of new molecules that can represent a helpful therapy for conventional drugs is certainly a great resource that should not be underestimated. Based on this, we decided to investigate the role of snail secretion filtrate (SSF) in inflammatory response as new potential therapeutic compound through *in vitro* studies on skin inflammation models in order to analyze the anti-inflammatory, immunomodulatory and therapeutic activity against a globally widespread inflammatory disease in humans and veterinary fields, such as Atopic dermatitis [4].

Using an *in vitro* model of CPEK (long-term canine epidermal keratinocytes) cell culture, here we tried to observe the alteration of transcriptional regulation of skin barrier defense of a natural compound containing collagen, fatty acids, allantoine, glycolic acid, hyaluronic acid, which are already well-known for their applications within dermatology diseases, to identify the anti inflammatory effect of SSF as a new treatment that is capable of improving the conditions of veterinary patients. CPEK cells were treated with or without SSF in the presence or absence of Lipopolysaccharide (LPS). A cell viability assay was performed to test Snail Secretion Filtrate toxicity and the protective effect of SSF after LPS stimulation.

Our study demonstrated that SSF is able to decrease levels expression of mediators involved in inflammatory process such the secretion of pro-inflammatory cytokines IL-6, IL-8, IL-17A and the mRNA expressions of COX-1, COX-2 and TNF-α in cAD. These results lay the foundation for the use of this natural bioactive compound in veterinary medicine and provide a model for deeper understanding of its mechanisms of action, with potential translation to human research. Our findings suggest, at the end, that *Helix aspersa* extract snail mucus is a natural, safe and effective alternative treatment in canine cAD acting as new therapeutic agent.

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SIRA

USE OF O3IL® TO FAST THE WOUND HEALING IN BITCH AFTER THE SURGICAL UNILATERAL MASTECTOMY

Vincenzo Cicirelli, Matteo Burgio, Giovanni M. Lacalandra, Giulio G. Aiudi
Department of Veterinary Medicine, University of Bari "Aldo Moro", Bari, Italy.
*Corresponding author: V. Cicirelli (vincenzo.cicirelli@uniba.it)

Unilateral mastectomy is one of the most painful surgical procedures in canine species. This surgery result in extensive tissue damage, severe postoperative pain and long healing time [Cicirelli et al., 2021]. This study aimed to compare conventional therapy (using only oral medications and cleaning the surgical wound) to topic application of O3IL® in order to determine which is better and faster for the healing process after canine surgical unilateral mastectomy. A total of 18 bitches were included in the present study, all of which were domesticated, healthy and 4-12 years of age. The animals were selected for unilateral mastectomy due to mammary tumours, diagnosed on clinical examination and which were staged according to the tumour node metastasis (TNM) classification system [Owen et al., 1980]. Informed consent was obtained from the dog owners prior to participation in the study and this protocol was approved by the Ethics Committee for animal testing–CESA of the Department of Veterinary Medicine of the University of Bari "Aldo Moro". The day before surgery, patients underwent general preoperative examinations and were randomly divided into two groups (n=9): a control group (C) treated in the post operatory with routine therapy and a group treated with O3IL® (O group) as an adjuvant for the scarring process. The same surgical team performed all surgeries, in full compliance with the *leges artis*. In the postoperative period, we assessed the wound status and healing process using the Bates-Jensen Wound Assessment Tool (BWAT) (Harris et al., 2010). The BWAT assesses the following nine items: size, depth, edges, necrotic tissue type, necrotic tissue amount, exudate amount, exudate type, granulation tissue, and epithelialisation. The total BWAT scores were divided into five severity categories: 0-8, healing; 9-19, minimal severity; 20-29, mild severity; 30-38, moderate severity; and 39-45, extreme severity. Statistical analysis was performed with SPSS Software using two-way ANOVA. Values were judged significant if $p < 0.05$. In the postoperative period, all patients had similar lesions with a BWAT score higher than 42 (extreme severity). The dog was considered cured when his BWAT score was ≤8. All animals were monitored daily after surgery until all patient injuries were completely recovered (for a total of 16 days). The same surgeon monitored the wound status each day by collecting data for the BWAT. All pain symptoms and post-operative discomfort gradually disappeared in both groups. In particular, in the C group, they disappeared from day 8 to day 10; in the O group, they disappeared from day 5 to day 6. In general, the healing process in both groups was similar until day 3. At the beginning of the experiment, the control group seemed to show a better response to the therapy, which confirmed by BWAT scores. On day 3 of therapy, the values of both groups were the same; however, the treated group showed a subsequent improvement in scores and achieved healing point by day 4. Total wound healing occurred on day 13 for group O, and on day 16 for group C. This study shows the use of O3IL® on traumatic lesions of the genital mucosa is an excellent therapeutic aid to be used in the canine species.

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PLEASE, DO(G)N'T SMOKE: BEWARE OF THE RISK OF SMOKE EXPOSURE IN PETS

Giulia Pizzi, Silvia Mazzola, Alessandro Pecile, Elisa Giussani, Valerio Bronzo, Debora Groppetti

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

Corresponding author: D. Groppetti (debora.groppetti@unimi.it)

Negative health effects of both active and passive tobacco smoke have been well known in humans for a long time [1, 2]. Also pets who share the owner's lifestyle intimately, may be exposed to the same risks. This study aimed to detect and quantify cotinine (i.e. a metabolite of nicotine) in canine serum. Sixteen dogs exposed (EX; 6 females and 10 males) and 16 dogs not exposed (NE; females and 10 males) to the owner's smoke were enrolled. All dogs were purebred belonging to 16 different breeds, aged 1.5 to 8 years (3.9±1.8), and weighing 14 to 77.7 kg (34.7±13.3). Serum cotinine significantly differed ($p<0.01$) between the EX and NE group with higher values in dogs exposed (10.2±6.8 ng/ml) than in those not exposed to smoke (1.9±0.7 ng/ml). A gender difference was recorded in exposed animals ($p<0.05$), with bitches showing higher serum cotinine concentrations (15.2±8.09 ng/ml) than male dogs (7.2±3.9 ng/ml).

These results confirmed that also pets are exposed to domestic secondhand smoke. Household pets, could be negatively affected by their owner's habits. Although owners and breeders do not seem to perceive the risk of smoke for their dogs, greater awareness should be advisable, especially in pregnant and growing animals.

This study is part of a general project on environmental factors affecting canine reproduction, and complies with ethical standards, under the approval of the Ethical Committee of the Università degli Studi di Milano (OPBA_161_2019).

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EVALUATION OF THE EFFECT OF OXIDATIVE STRESS ON UTERINE PATHOLOGIES IN POSTPARTUM DAIRY COWS

Sanjana Malledevarahalli Chandrappa (1), Gianguido Donato(1), Ahmed Elkhawagah(1)(2), Giorgia Meineri (1), Leila Vincenti (1), Alessandro Ricci (1)

(1) Università degli Studi di Torino, Dipartimento di Scienze Veterinarie. (2) Theriogenology Department, Faculty of Veterinary Medicine, Benha University, Egypt.

Corresponding author: S. Malledevarahalli Chandrappa (sanjana.malledevarahallichandrappa@unito.it)

Dairy cows diagnosed with metritis (METR) and endometritis (ENDO) may experience a greater degree of oxidative stress (OS) and a deficit in the antioxidative capacity compared to healthy cows. Serum OS markers can be used as a management tool to monitor the early stages of uterine diseases. This study aims to evaluate the effect of OS markers and the influence of metabolic status in postpartum cows affected by METR, ENDO, and combined form (COMB). In this study, 121 Holstein cows were subjected to a weekly clinical examination from 7±3 to 35±3 days postpartum (dpp). Among 121 cows, 21 were diagnosed with METR, 18 with ENDO, 24 with COMB, and 58 were healthy cows. Fetid vaginal discharge and T°>39.5 were considered (first 21 dpp) to diagnose METR, and vaginal discharge (more than 21 dpp) was scored to diagnose ENDO, and COMB cows if showed both diseases [1]. Blood samples for serum reactive oxygen metabolites (d-ROM), antioxidants (OXY), and oxidative status index (OSI) tests, evaluated via photometric determination of plasma thiols, were performed at 7, 14, 21, 28, and 35 dpp. Blood glucose and β-Hydroxybutyrate (BHB) were measured at day 7±3 dpp. If BHB>1.2 mmol/L, cows were considered ketotic (KET) [2]. For this analysis, the statistical program used is the R software version 1.41. Significance was considered with P<0.05. Serum concentrations of d-ROMs and OSI were greater in METR (116±28 Carratelli units (UCarr)), ENDO (94±26 UCarr), and COMB (110±27 UCarr) than in healthy (84±23 UCarr); *P*<0.05. OSI for METR (0.42±0.26), ENDO (0.36±0.24), and COMB (0.39±0.25) vs Healthy (0.18±0.05); *P*<0.05. The concentration of OXY was lower in METR (345±153 µmol/L), ENDO (380±170 µmol/L), and COMB (360±160 µmol/L) than in healthy cows (474±115µmol/L); *P*<0.05. The incidences of METR, ENDO, or COMB were 16%, 19%, and 19%. Moreover, the parturition to conception interval (PC) was higher than in healthy cows (189±20, 148±21, 170±15 vs 126±10; p<0.05). Milk yield decreased in METR, ENDO, and COMB compared to healthy animals (28, 32, 30 vs 39.2 kgs). There was no significant difference in blood glucose and BHB concentration between healthy and diseased cows (P>0.05). This study showed that cows with METR, ENDO, and COMB experience a greater degree of OS in comparison to healthy cows. These findings provide new avenues for research for prevention and potential supportive treatments for metritis and endometritis via the utilization of antioxidants *per os*.

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SOFIVET

HEMATOCHEMICAL AND ENDOCRINOLOGICAL PARAMETERS IN HORSES UNDER DIFFERENT MANAGEMENT SYSTEMS

Giovanna Marliani, Renzo Pedone Ferri, Francesco Dondi, Pier Attilio Accorsi

Università degli Studi di Bologna, Dipartimento di Scienze Veterinarie.

Corresponding author: G. Marliani (giovanna.marliani2@unibo.it)

Nowadays, horses are employed in different equestrian activities and the need of guaranteeing high standards of welfare to these animals has brought to the development of different kinds of management. Traditionally horses are stabled in single-stall, with little possibility of socializing. They follow a precise feeding schedule, wear horseshoes, and are ridden with mouthpieces. In contrast, Natural boarding tries to place the horse in a context as close as possible to the natural one. The natural management foresees the presence of large spaces with the possibility of socialization and movement, a varied and ad-libitum diet, absence of clipping, mouthpieces, and shoeing [1].

This preliminary work compares the cortisol level and hematobiochemical parameters in two different groups of horses: a) traditionally stabled horses (TS, n=8), most of which are showjumping horses employed in competitions, b) horses under natural boarding (NB, n=9), especially involved in school and walking activities. Cortisol level was measured from feces and horsehair using Radio Immuno Assay (RIA), and hematobiochemical parameters were analyzed through the Automated Hematology System ADVIA® 2120 (Siemens) and Automated analyzer OLYMPUS AU 480. A non-parametric test (Mann-Whitney test) was used for the statistical analysis.

Results have shown a higher level of cortisol with a significant tendency both in horsehair ($p=0.09$) and feces ($p=0.06$) for the TS group. Haematocrit has evidenced that the count of leucocyte/mm^3 ($p<0.05$) and the absolute value of monocytes/mm^3 ($p<0.05$), lymphocyte/mm^3 ($p<0.05$), and neutrophils/mm^3 ($p=0.06$) were higher in TS, while NB had higher values of eosinophil/mm^3 ($p<0.05$), platelets/mm^3 ($p<0.05$), Mean Corpuscular Haemoglobin pgr (MCH, $p=0.06$) and Mean Corpuscular Haemoglobin Concentration gr% (MCHC, $p<0.01$). According to biochemical results, NB recorded lower levels of fibrinogen (g/L) ($p<0.05$) and of total, direct and indirect bilirubin (mg/dl) (BIL-T, $p<0.05$; BIL-D, $p=0.07$; BIL-I, $p<0.05$).

Most of the hematobiochemical results could be justified by the different intensity of the activity to which horses are subjected. However, especially considering cortisol results, we cannot exclude that also a chronic stress status, possibly linked to the different management conditions [2], could be responsible for these results, especially for the higher level of leukocytes, neutrophils, and monocytes [3]. Both physical and psychological stress can influence immune conditions, and prolonged elevated levels of stress can result in impairment of the immune function, causing a major exposure to diseases [4]. This preliminary study evidenced interesting initial results, but it presents several limitations due to the small sample size. Further studies are needed to distinguish the influence of psychological and physical stress on the immune function of horses, in order to ameliorate the working and management conditions of these animals, enhancing general welfare.

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MONITORING OF THE PRESENCE OF TRACE ELEMENTS IN PASTURES THROUGH THE USE OF ROE DEER HAIR

Susanna Draghi (1), Federica Riva (1), Gabriele Brecchia (1), Daniele Vigo (1), Stella Agradi (1), Caterina Piantoni (1), Lorenzo De Giovanni (1), Duygu Tarhan(2), Bengü Bilgiç (3), Banu Dokuzeylül (3), Alev Meltem Ercan (2), Mehmet Erman Or (3), Giulio Curone (1)

(1) Università degli Studi di Milano, Dipartimento di Medicina Veterinaria e Scienze Animali. (2) Istanbul University-Cerrahpasa, Cerrahpasa Faculty of Medicine, Biophysics Department. (3) Istanbul University-Cerrahpasa, Faculty of Veterinary, Internal Medicine Department.

Corresponding author: S. Draghi (susanna.draghi@unimi.it)

A lot of global challenges are affecting the agricultural and zootechnical sectors and the necessity to address production towards a more environmentally sustainable approach becomes more evident every day. Examples of these global challenges include population increase, urbanization, increased environmental degradation and trend towards consumption of animal protein, and climate change. Moreover, the outbreak of war in Ukraine has led to a dramatic increase in the price of raw materials, cereals in particular, putting intensive animal farming in crisis and imposing a reflection on the use of animals that allow to maintain a good production even with diets less rich in energy and thus through the exploitation of pastures [1]. One of the challenge that might be encountered moving to a major use of grazing could be the lack of trace minerals or the excessive presence of some elements. Indeed, chromium, aluminum, iron, copper, selenium, nichel, and several other elements are essential for life because involved in physiological functions, but when they exceed safe concentrations for physiological functions, may become toxic. In the last decades, due to its potentiality of providing additional and different information, compared with blood, hair has been extensively used in ecotoxicological investigations. Indeed, trace elements are incorporated in hair in different quantity based on their affinity with hair structural elements. Mammalian hair has already proven to be suitable indicator of metal bioavailability and the strong correlation between metals within hair and other tissues has been proved [2]. Thus, consideringthat hair analysis can be utilized for both monitoring the nutritional status of the animal and the exposure to toxic elements, the aim of the work is to understand if hair of wild animals, roe deer in particular, is an adequate matrix to assess the presence of some trace and toxic elements Moreover we aim to study any differences of bioaccumulation based on age, sex and home ranges of the animals. To achieve the goals, hair from 39 roe deer were collected during the 2021 hunting season. Animals were hunted in two different areas (urban and rural) in the South area of Oltrepo Pavese (Italy) according to the regional hunting plan. The hair content of elements was assessed through the use of an inductively coupled plasma-optical emission spectrophotometer (ICP-OES; Thermo iCAP 6000series). Age, sex and geographic area were categorized into two different levels. The differences between groups (<3 y.o./ >3 y.o; male/female; urbanized/non-urbanized) were considered statistically significant when p value was <0.05. We found that mean concentrations of Fe, Mg, Mn, Al, Cr and Pb differed significantly between the two considered areas, higher in the urbanized. The mean level of Mg and Cr differed significantly between the two class of age resulting higher in older animals, while in the case of sex the differ ences between the mean levels of Cu, Fe, Mg, Cd an Cr showed a trend of higher accumulation in females. In agreement whit what has been reported by other researchers we found that animals belonging from different environmental compartments contain different deposition patterns of trace elements. Furthermore, in accordance with literature, our findings showed an age-related variation of elements, with higher concentrations in adult animals. Finally, as reported in bibliography, females showed higher accumulation patterns of these elements [3]. In conclusion, our findings prove that wild animals are good bioindicators for monitoring the presence of trace elements in pastures, thanks to their accumulation pattern similar to livestock species. Moreover, hair has proven to be a valid matrix for this type of surveys.

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WHAT'S THE INFLUENCE OF A SUPEROVULATORY TREATMENT ON PLASMATIC KISS-1?

Tanja Peric (1), Alessio Cotticelli (1, 2), Isabella Pividori (1), Antonella Comin (1), Susy Urli (1), Giuseppe Stradaioli (1), Alberto Prandi (1)

(1) Università degli Studi di Udine, Dipartimento di Scienze Agroalimentari Ambientali e Animali. (2) Università degli Studi di Napoli Federico II, Dipartimento di Medicina Veterinaria e Produzioni Animali.

Corresponding author: T. Peric (tanja.peric@uniud.it)

KISS1 neurons of the hypothalamus are involved in crucial stages of maturation and reproductive function, such as sexual differentiation in the brain, the onset of puberty, folliculogenesis, spermatogenesis, steroidogenesis and ovulation [1, 2]. Specifically, animal studies suggest that kisspeptin is involved in generation of the luteinizing hormone surge, which is required for ovulation [3]. Thus, the aim of this preliminary study was to evaluate if a superovulatory treatment could have an influence on the kisspeptin surges described in the literature for the ovulatory cycle. A total of four healthy non-lactating P.R.I. (*Simmenthal*) heifers that were in regular cyclicity were selected for the experiment and maintained under a similar managing system. Heifers were subjected to the synchronization of estrus by the administration of 0.150 mg of Dexcloprostenol (Veteglan, Calier Italia S.r.l.; day -3) followed by the insertion of CIDR at day 5 and from day 9 to day 13 by the administration of a total 500 IU of FSH-LH (Pluset, Calier Italia S.r.l.) in 9 decreasing dosage. At day at 12, 0.150 mg of Dexcloprostenol were administered and CIDR removed. Blood samples were collected in EDTA tubes by coccigeal venipuncture from day -3 (1^{st} $PGF_{2\alpha}$ injection) to day 22 of the reproductive cycle to estimate plasma kisspeptin with a commercially available ELISA kit (Cow Kisspeptin 1 (KISS1), Catalogue No: abx257481, Abbexa Ltd, UK) validated for bovine plasma. The kisspeptin concentrations reached two peaks (188.0±21.7 pg/mL and 215.6±7.6 pg/mL; mean±SD) before the first (physiological) and the second (induced by the superovulatory protocol) ovulation. Between these two rises the lowest kisspeptin concentration (121.3±16.8 pg/mL; mean±SD) was recorded at day 10 of the ovulatory cycle. Although this is the first investigation about kisspeptin and the application of superovulatory treatments it seems that the protocol doesn't interfere with the physiological kisspeptin surges.

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SAME-SEX PAIRING IN THE GRIFFON VULTURE (GYPS FULVUS)

Marta Satta (1), Marco Muzzeddu (2), Gian Nicola Frongia (1), Valentina Satta (1), Giovanni Giuseppe Leoni (1), Salvatore Naitana (1)

(1) Department of Veterinary Medicine, University of Sassari. (2)) Ente Forestas, Sardinia Region Council. Corresponding author: S. Naitana (snaitana@uniss.it)

Sexual behavior directed toward the same sex has been evidenced in a variety of animals, including Mammals and Aves classes, manifold in captivity but also in wild animal populations. Into Gyps, homosexual pair relationships has been described in a colony of the Cape Vulture (Gyps coproteres9 [1]. Male homosexual pair in the Amsterdan and Nordhorn zoos has been evidenced to incubate an egg and both take care of the chick [2, 3]. Some hypothesize that same-sex pairing performs a function of strengthening ties and reducing social tensions. Our aim was to verify the reproductive behavior of a small group of griffons kept in an aviary in Bonassai (Sassari).

We studied for 3 years the reproductive behavior of 6 griffons which were regularly fed with sheep carcasses. All individuals were identified as belonging to the male gender via nuclear DNA from the feathers. We found the typical reproductive activity of the species of two individuals who at the end of December collected twigs and together build a nest in a corner of the aviary. During the nesting they proceeded to mating always respecting their role as male or female, attacking any other individual who approached the nest, but naturally without reaching a deposition. We therefore introduced into the aviary an adult female with previous reproductive experience who was very interested to the nest but who was generally attacked by the "male" of the couple. On March 20 we introduced a goose egg from a window which immediately induced in the couple the behavior of the brooding and the cessation of the couplings. The egg intake and overturning times were similar to heterosexual couples with an extension of the intake of air and after 68 days it stopped to brooding but maintained the nest area defense behavior.

It seems very difficult to explain in evolutionary terms the same-sex pairing as it does not produce any genetic gain. We can think that the mating of individuals of the same-sex pairing may also represent a particular reproductive strategy under certain conditions (4) probably induced by hormonal influence. In conclusion, the same-sex pairing in the griffon vulture could be considered, according to Bailey et al. [5], as an adaptive response of the species to life in captivity represented by individuals of the same genus. (Supported by Fondo di Ateneo per la Ricerca 2019-Naitana).

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INFLUENCE OF OOCYTE VITRIFICATION PROCEDURES ON THE EXPRESSION OF MATERNAL EFFECT GENES

Monica Pirastru (1), Sara Succu (2), Valentina Satta (2), Paolo Mereu (1), Luisa Bogliolo (2), Salvatore Naitana (2), Giovanni Giuseppe Leoni (2)

(1) Department of Biomedical Science, University of Sassari. (2) Department Veterinary Medicine, University of Sassari.

Corresponding author: S. Naitana (snaitana@Uniss.it)

In a previous work we demonstrated that oocyte cryopreservation negatively affects mitochondrial activity, energy production and antioxidant activity and that these abnormalities were restored during 4-6 hours of in vitro culture [1]. The aim of the present study was to assess the impact of the oocyte cryopreservation on the expression of several maternal effect genes (MEG) involved in epigenetic chromatin reprogramming in the embryo such as DNA methyl transferases (DNMT1 which maintain methylation, DNMT3A and 3B involved in the de novo methylation of most loci imprinted in the preimplantation embryo) and DPPA3, a maternal factor required for protecting DNA methylation in early embryos. Cumulus-oocyte complexes (COCs) obtained from slaughtered ewes were matured in vitro for 24 hours. After collection, the MII oocytes were randomly divided into a vitrified and a control, not-vitrified group. In vitrified group, the MII oocytes were subjected to vitrification procedure [1]. Vitrified/warmed oocytes were then cultured in PBS/10% FCS and used for subsequent analyses (0-2-4 hours post-warming). The control group was directly subjected to developmental competence evaluation and gene expression analyses. Developmental competence was assessed by fertilization using cryopreserved ram spermatozoa of the same batch. Presumptive zygotes were cultured in vitro until blastocyst stage and cleavage and blastocyst rates were calculated [1]. For gene expression, mRNAs were extracted from pools of 10 oocytes using Poly-T conjugated microbeads (Dynabeads™ mRNA Purification Kit; Invitrogen) and retro-transcribed into cDNA (SuperScript™ III Reverse Transcriptase; Invitrogen) [2]. Quantitative expression of the specific genes was made by Real-time PCR (RT-PCR) in a StepOne RealTime-PCR system (Applied Biosystems) and data were compared according to Livak and Shmittgen [3]. Data were espressed as fold of expression of each gene in vitrified relatively to control group.

Our results evidenced that cryopreservation procedures negatively affect oocyte developmental competence. Cleavage and blastocyst rates were lower ($p<0.01$) in vitrified group (19±4 and 2±1% respectively) compared to control (68±5% and 62±1% respectively) . Vitrification influence the expression of analyzed MEG genes. At 0h after warming the expression of all genes was lower in vitrified group compared to control. At 0h post-warming the expression of DNMT1, DNMT3A, DNMT3B and DPPA3 was respectively 2, 2.2, 6.2 and 3.8 fold decreased, respectively. After 2 hours of culture, the pattern of DNMT3B and DPPA3 expression between fresh and vitrified groups was nearly identical, whereas DNMT1 and DNMT3A were overexpressed (3.7 and 4.1 fold increased respectively). The maximum expressions of all genes were observed in vitrified groups after 4 hours of incubation. The relative expressions of DNMT1, DNMT3A, DNMT3B and DPPA3 were 4.5, 5.8, 2.2 and 2.4 fold higher than in controls, respectively.

Our study demonstrated that, although vitrification negatively affects the expression of MEG genes, the plasticity of oocyte allows to recover all these abnormalities into 2/4 hours of in vitro culture. Our data could be used to determine the fertility window in order to improve the developmental competence of vitrified/warmed oocytes.

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CAN TRANSPORT STRESS BE ASSESSED AT THE SLAUGHTERHOUSE?

Aloma Zoratti, Isabella Pividori, Antonella Comin, Edi Piasentier, Tanja Peric, Alberto Prandi

Università degli Studi Udine, Dipartimento di Scienze Agroalimentari, Ambientali e Animali.

Corresponding author: A. Zoratti (zoratti.aloma@spes.uniud.it)

Fecal biomarkers are widely used in assessing individual's physiological status, and between them the concentrations of 11,17-dioxoandrostanes in feaces is an indicator of adrenocortical activity in horses [1,2]. The aim of the study was to evaluate the applicability of faecal sampling at the slaughterhouse to evaluate the HPA axis activity of horses transported over a long (over eight-hour) road journey in an easy and standardized way. This preliminary study included 9 Trotter horses (2 females and 7 males, aged 1 to 18 years old) which were transported on trucks for 10-12 hours and two pregnant mares. The two mares have been considered as animals with a high activation of the HPA axis since they were close to delivery. Feces were collected from the large intestine following the evisceration phase of the animal at the slaughterhouse and they were immediately frozen until lab extraction. Samples were extracted as described by Merl et al. [3]. The extraction was done in two phases: the first with methanol and the second with diethylether. A commercially available competitive ELISA kit (11-oxoetiocholanolone ELISA kit, Cayman chemical, No. 501420, Ann Arbor, MI, USA) validated for ungulates has been used for the first time on equids. Interestingly, the 11-oxoetiocholanolone concentrations in long-distance transported animals ranged between 11.0 and 64.0 ng/g (26.3±15.5 ng/g; mean±SD) while the mares at delivery have both shown a concentration of 30.0 ng/g. Even if preliminarily, it has been interesting to observe as, compared to the delivery concentrations, the long road journey has strongly solicited the HPA axis of some animals while others shown a low level of HPA axis activity. Morever, the feaces sampling at slaughterhouse looks promising in providing an easily collecting and standardizable sample to monitor the stress transport by public authorities or animal dealers.

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DOES HORMONAL PROFILE INFLUENCE BEHAVIOURS OF PUBESCENT EWES?

Isabella Manenti (1), Elisabetta Macchi (1), Mario Baratta (2), Silvia Miretti (1), Irene Viola (1), Paola Toschi (1), Paolo Cornale (3)

(1) Università degli Studi di Torino, Dipartimento di Scienze Veterinarie. (2) Università degli Studi di Parma, Dipartimento di Scienze Chimiche, Scienze della vita e Sostenibilità Ambientale. (3) Università degli Studi di Torino, Dipartimento di Scienze Agrarie, Forestali e Alimentari.

Corresponding author: I. Manenti (isabella.manenti@unito.it)

Small ruminants farming systems are essential part of mountain ecosystems and rural economy, contributing to management, care, and conservation of a unique landscape [1]. Therefore, research aims at improving the economic value of breeding, welfare, and productivity of animals identifying new examples of sustainable supply chain [2]. The study presented here is part of the SmartSheep project, which wants to contribute in developing new efficient and sustainable systems [3]. The purpose of this work is to better evaluate behaviour modification and adrenal response in relation to the stage of the first oestrous cycle.

Twenty ewes of Frabosana-Roaschina breed (10-12 months old) were checked during the first oestrus. The animals were synchronized with a standardised protocol [4]. During two months of sampling, animals were monitored continuously with video camera and sampled for saliva and blood samples.

Behavioural and hormonal data were analysed in relation to the oestrous cycle phases obtained from hormonal patterns (progesterone and 17β-estradiol) and ultrasound assessments (follicular waves, presence of corpus luteum). Video data, registered through scan sampling, were eventually related to an adaptive endocrine response (salivary cortisol).

Parametric (hormone data) and non-parametric (behaviours analyses) tests were used to analyse the data in relation to the phases of the oestrus cycle. Statistical difference was determined if $P \leq 0.05$. Data are presented as mean±SEM.

Significant difference was observed between progesterone concentration (ng/mL) at luteal and follicular phases. These hormonal levels, supported by ultrasonographic imagines analysis, confirmed proper cyclic activities in the ewes at first oestrus. However, despite this evidence, no differences were observed in the regular behavioural patterns (e.g., feeding, resting, and standing) as well as in social (i.e., displacing from resources, grooming, nudging, and sniffing) behaviours. Furthermore, the ewes didn't show any sexual behaviours during the reproductive cycle. On the contrary, "play" activities (i.e., frontal butt, reciprocal butt, and side/rear butt) were observed. Finally, no significant differences were detected in the cortisol levels during the phases of cycle.

Although these animals reached the age of puberty, like other Italian dairy breeds [5], our findings showed a silent ovulation. Play behaviours and specific features in breed of the animals may be reflected in reproductive functionality. In literature it is reported that young ewes show less reproductive behaviour and has a shorter oestrus compared to mature ewes [6]. Lastly, Frabosana-Roaschina is an autochthonous and rustic breed in West North Alpine bioregion and a milder selection has been performed. Thus, even minor changing in animal welfare or managing may delay the ovulatory activity like wild sheep species [7].

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DIFFERENTIATION OF RABBIT LEUKOCYTES BASED ON SCATTER PROPERTIES: PRELIMINARY RESULTS USING A FLOW CYTOMETRIC APPROACH

Majlind Sulce (1), Albana Munga (1), Giulio Curone (2), Stella Agradi (2), Laura Menchetti (3), Gabriele Brecchia (2)

(1) Università Agraria di Tirana, Facolta di Medicina Veterinaria. (2) Università degli Studi di Milano, Dipartimento di Medicina Veterinaria e Scienze Animali. (3) Universita degli Studi di Bologna, Dipartimento di Scienze Mediche Veterinarie.

Corresponding author: M. Sulce (msulce@ubt.edu.al)

The first laboratory approach for veterinary clinicians implicates the performance of a complete blood count (CBC). Information gained from such analysis may play an important role on the initial diagnosis and the follow up of animals. As well as other mammals, rabbits have a set of leukocytes; Granulocytes, Monocytes, Eosinophils, Basophils and two classes of Lymphocytes (small and large) in the peripheral blood circulation [1]. The manual cell count is considered as the classic and most accurate method to perform. Recently automatic techniques such as ADVIA 2120 have been proposed for the CBC in rabbits showing a good correlation with manual count [2]. In best of author knowledges no other techniques have been used to differentiate rabbit leukocytes based on scatter properties. The aim of this prospective study was to evaluate the ability of Flow Cytometry (FC) to identify different leukocyte populations based on their scatter properties. In veteriary medicine the FC technique is mainly used to diagnose and stage different neoplastic disorders such as lymphomas and leukemias especially in dogs and cats [3,4]. Cases were included when the flollowing criteria were present: vaccinated against Myxomatosis and Hemorragic disease of rabbits, no previous diseases, clinically healthy rabbits one week before sample collection. One mililiter of pheripheral blood was collected from the central auriculary artery and placed in EDTA tubes. A blood smear was done for all samples and stained with May-Grunwald Gimsea, while FC analysis took place immidiatly afer collection. Fifty microliters of each sample were placed in FC tubes and lysis was performed for 10 minutes in order to lyse red blood cells. Samples were than centrifugated in 1200 rpm/min for 5 minutes and supernatant was discarded. Propidium Iodide was used in order to ecxlude the possibility of the calculation of debris as live cells. Gates of analysis were designed for each leukocyte populations based on their scatter properties. Cytological smears were evaluated from one expert cytologists blinded regarding the FC data. Percentages of each leukocyte populations were calculated for both methods. In total eighteen samples from helathy rabbits were included in this prospective study. Flow cytometry showed a good correlation with the manual count method, especially for specific populations. Granulocytes showed the highest correlation ($r=0.988$, $p<0.01$), followed by big ($r=0.971$, $p<0.01$) and small Lymphocytes ($r=0.877$, $p<0.01$) and Monocytes ($r=0.615$, $p<0.01$). A low correlation between FC and manual count was observed for monocytes. In conclusion flow cytometry succesfully reached to identify different leukocytes populations using only scatter properties showing a good correlation with manual count mehtod. Taking into consideration these results flow cytometry appears a relaible and feasible method to evaluate the percentave of leukocytes in rabbit peripheral blood samples. However comparison of FC with other automatic methods would be of great interest while these preliminary results have to be confirmed in a larger number of cases.

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CANINE CHRONIC ENTEROPATHIES AFFECT DOG QUALITY OF LIFE AND BEHAVIOUR

Veronica Marchetti, Eleonora Gori, Valeria Mariotti, Angelo Gazzano, Giacomo Riggio, Chiara Mariti
Università di Pisa, Dipartimento di Scienze Veterinarie.
Corresponding author: C. Mariti (chiara.mariti@unipi.it)

Quality of Life (QoL) is based on positive and negative aspects of an animals' life, that make it better or worse, referred to that specific animal. Its evaluation can be relevant in the euthanasia choice process or in the evaluation of both therapy and necessity of intervention, especially in ill animals. This research aimed at assessing the impact of canine chronic enteropathies on dogs' QoL and behaviour. A group of dogs suffering from primary chronic enteropathies (n=44; 59.3±45.0 months of age) were assessed on the first visit with a veterinary gastroenterologist and on the first follow-up visit using: a 1–10 visual scale to evaluate dosg' QoL; the Canine Chronic Enteropathy Clinical Activity Index; and the Canine Behavioral Assessment and Research Questionnaire. They were compared to a control group of healthy dogs (n=49; 64.0 ±44.9) using Mann–Whitney U-test. Correlations between scores were evaluated using Spearman's test. Enteropathic dogs on the first visit had a lower QoL than healthy dogs ($p<0.001$), and dogs with a clinically insignificant–mild illness dogs had a statistically higher QoL than moderate-to-very severely ill dogs ($p=0.001$). The reduction of severity on the follow-up visit was associated with an improvement in dogs' QoL ($p=0.004$). QoL was negatively associated with sensitivity to touch at the first visit ($r=-0,314$; $p=0.038$) and severity was negatively associated with aggression towards familiar dogs at follow-up ($r=-0.505$; $p=0.020$). Chronic enteropathies seem to have a strong negative impact on the quality of life, as well as on the behaviour, of affected dogs.

UNTANGLING THE INTERACTIONS BETWEEN ERBB RECEPTORS AND THEIR LIGANDS IN THE PORCINE MAMMARY GLAND AS A MODEL FOR BREAST MORPHOGENESIS

Alessia Morato (1), Josephine F. Trott (2), Eugenio Martignani (1), Paolo Accornero (2), Russell C. Hovey (2)

(1) Department of Animal Science, University of California Davis, CA 95616, USA. (2) Department of Veterinary Sciences, University of Torino, Italy.

Corresponding author: A. Morato (amorato@ucdavis.edu)

The mammary glands develop after puberty in response to the circulating reproductive hormones, ultimately becoming a milk-producing organ that supports lactation. The ErbB family of transmembrane receptors serves as the effector of hormone action in the mammary glands and are often mutated in breast cancers. While ErbB1 and its ligand amphiregulin (AREG) mediate the actions of estrogen (E) in the mammary glands in pubescent mice [1, 2], their role in other species is undefined. Here we analyzed the mammary glands of pigs as an authentic model for the human breast, where we hypothesized that different ErbB receptors and their ligands would be alternately expressed in the epithelial and stromal compartments of the mammary glands and would be differentially regulated by various combinations of exogenous E, progesterone, and prolactin (PRL) administered to ovariectomized, pubescent female pigs [3]. Mammary epithelium was isolated from the surrounding stroma in the mammary gland of pubescent female pigs and analyzed by quantitative RT-PCR. Except for EGFR, which was prevalent in fat, all ErbB receptors were mainly expressed in the epithelium. Among ErbB-binding factors prevalently detected in the epithelium, while AREG, epiregulin and epigen were exclusively upregulated by E, neuregulin 1 (NRG1) and epidermal growth factor (EGF) were under the dual positive effect of E and PRL; namely EGF was uniquely upregulated by E+PRL, which is also the hormone treatment inducing maximal morphological development in the porcine mammary gland [3]. Conversely, high levels of two ErbB-binding factors (betacellulin and neuregulin-4) were expressed in the adjacent adipose tissue, and were not induced by E. We are currently cultivating explants of porcine mammary gland ex vivo to confirm our in vivo data about the hormonal regulation of ErbB receptors and their ligands. We are also optimizing a protocol for confocal imaging of entire mammary explants stained for BrDU and STAT5, in order to correlate ErbB-mediated signaling with both proliferation and differentiation of the mammary epithelium. Our data highlight that a diversity of receptors and ligands serves as effects of E action on the mammary gland, are regulated by interactions between E and PRL, and are tissue specific. Untangling the endocrine regulation of ErbB-pathways in the porcine mammary both in vivo and ex vivo can also help to understand the endocrine basis of human breast cancer.

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SOIPA

CRYPTOSPORIDIUM AND GIARDIA IN BUFFALO CLAVES FROM CAMPANIA REGION

Giovanna Cappelli, Domenico Alfano, Lucrezia Lucchese, Domenico Vecchio, Alessandra Martucciello, Giorgio Galiero

Centro di Referenza Nazionale per l'Igiene e la tecnologia dell'allevamento delle produzioni bufaline, Istituto Zooprofilattico Sperimentale del Mezzogiorno.

Corresponding author: G. Cappelli (giovanna.cappelli@izsmportici.it)

Cryptosporidium and *Giardia* are genera of protozoan parasites that infect animals and humans worldwide (1). Transmission occurs via the oro-fecal route, through contact with people, animals, water, food and contaminated environments (2). *Cryptosporidium and Giardia* infections in water buffaloes (*Bubalus bubalis*) are less studied than in other bovine species. However, molecular studies have detected *C. parvum* , *C. bovis* , *C. ryanae* , *C. andersoni* and a genotype similar to *C. suis* in water buffalo (3). These parasites can cause high morbidity and mortality in buffalo calves in the Campania Region herds. Some species of *Cryptosporidium* and *Giardia* are able to infect gastrointestinal cells, even of humans, and have since been counted among the aetiological agents causing zoonoses (4). In this study, a high prevalence of the diseases was observed and it was established that, in the presence of diarrheal syndromes of varying severity, *Cryptosporidium* and *Giardia* can be found in buffaloes in association with other aetiological agents, such as toxin-producing *E. coli*, Rotavirus and/or *Salmonella* (5). We investigated the presence of *Cryptosporidium* sp. and *Giardia* in buffalo calves that died as a result of gastrointestinal episodes and that came from farms in the Campania region that were conferred to the Istituto Zooprofilattico Sperimentale del Mezzogiorno in the last three years. Faecal samples were collected during necropsy investigations, diagnosis was performed using an immunochromatographic kit on the intestinal contents of individual subjects for a total of *n*=299 samples from *n*=135 buffalo farms. Overall, out of 299 faecal samples collected from buffalo calves, 66 (22.7%; 95% Confidence Interval [CI]=17.7-27.1) were positive for *Cryptosporidium* sp. and 25 (8.4%; 95% [CI]=5.7-12.05) were positive for *Giardia*.

These results revealed the high presence of *Cryptosporidium* sp. and *Giardia* in buffalo calves, demonstrating that both are significant pathogens for this species and that their specific life cycle and extreme resistance in the farm environment must be considered for their containment.

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LUNGWORMS IN WILD RUMINANTS IN THE ITALIAN ALPS: THE CASE OF DICTYOCAULUS CERVI IN RED DEER (CERVUS ELAPHUS)

Alessandra Cafiso (1), Chiara Bazzocchi (1), Perla Tedesco (2), Clelia Buccheri Pederzoli (1, 2), Daniele Bonato (1), Serena Robetto (3), Riccardo Orusa (3), Luca Corlatti (4, 5), Giovanni Poglayen (2), Camilla Luzzago (1)

(1) Università degli Studi di Milano, Dipartimento di Medicina Veterinaria e Scienze Animali (DIVAS). (2) Università degli Studi di Bologna, Dipartimento di Scienze Mediche Veterinarie (DIMEVET). (3) Istituto Zooprofilattico Sperimentale Piemonte, Liguria e Valle d'Aosta, Centro di Referenza Nazionale Malattie Animali Selvatici (CeRMAS). (4) Stelvio National Park, ERSAF Lombardia. (5) University of Freiburg, Wildlife Ecology and Management.

Corresponding author: A. Cafiso (alessandra.cafiso@unimi.it)

Nematodes of the genus *Dictyocaulus* are the causative agents of parasitic bronchopneumonia in various domestic and wild ruminants. Among these, *Dictyocaulus eckerti* (DE) was previously described as a collective species infecting cervids [1], and the sole species characteristic of red deer and reindeer [2]. However, *Dictyocaulus cervi* (DC) was recently described as a new species in red deer based on light microscopy and ultrastructural observations, as well as molecular data [1]. The high morphological similarity between the two species suggests that misidentification of DC with DE could have occurred in the past. In the Italian Alps area current and up-to-date data available on *Dictyocaulus* spp. are scant, especially in red deer. Exact species identification may be useful for wildlife management and conservation, as well as for investigating potential interactions between wild and domestic ruminants during the grazing season. The aim of this work was to investigate the presence of *Dictyocaulus* spp. in red deer in two Italian Alpine areas (Valle d'Aosta – VdA, and the Lombardy sector of the Stelvio National Park – SNP), and determine molecularly the lungworm species.

The respiratory tracts of 250 individuals of red deer (VdA=104; SNP=146), sampled in the culling seasons of 2017-19, were dissected. Adult lungworms were collected, morphologically observed using light microscopy, and molecular analyses were performed on a subsample of one randomly selected nematode per host (21=VdA; 17=SNP). Partial 18S rDNA, ITS2 and cytochrome *c* oxidase I (*coxI*) genes were PCR amplified and phylogenetic analyses were performed. Parasitological examination showed an overall lungworms prevalence of 22% (95% CI = 14-30) and 21.9% (95% CI = 15.2-28.6), mean abundance of lungworms (±SD) 1.2±3.6 and 1.6±5.6, mean intensity of lungworms infection (±SD) 5.6±5.7 and 7.3±8.9 in VdA and SNP respectively. Morphological parameters most closely resembled those of DC, although some parameters overlapped with measures of DE. 18S rDNA and ITS2 gene sequences confirmed the identification of DC in 35 out of 38 analyzed lungworms (VdA=15/17; SNP=20/21), the remaining ones were classified as *Dictyocaulus* sp. Based on *coxI* gene phylogenetic analyses, SNP DC isolates clustered together with already available DC isolates, while VdA isolates were more closely related to putative DE *coxI* gene sequences. Finally, undetermined *Dictyocaulus* sp. specimens were confirmed as a separate lineage from DC and DE. These results update the knowledge of DC occurrence in red deer in the Italian Alps, showing the presence of separate geographic isolates of DC, thereby leading to a reconsideration of *Dictyocaulus* spp. occurrence in Italy. Further analyses should be performed to better understand the epidemiology of dictyocaulosis in red deer and should also be focused on evaluating the risk of cross-transmission between red deer and other ruminant species, including those undetermined *Dictyocaulus* sp. identified in both study areas.

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A TEN-YEAR PARASITOLOGICAL MONITORING IN KENNELS IN CAMPANIA REGION

Saverio Pennacchio (1), Maria Paola Maurelli (2), Sergio Illiano (2), Antonio Bosco (2), Lavinia Ciuca (2), Giuseppe Cringoli (2), Marina Pompameo (1), Laura Rinaldi (2)

(1) National Health Service (ASL NA1), Naples, Italy. (2) Department of Veterinary Medicine and Animal Production, University of Naples Federico II, CREMOPAR Campania Region, Naples, Italy.

Corresponding author: M.P. Maurelli (mariapaola.maurelli@unina.it)

Endoparasitic infections are a problem of great health and welfare relevance for owned and stray dogs, and particularly for those housed in kennels. Intestinal and pulmonary parasites, in fact, are frequently recorded in dogs and can be responsible for severe clinical symptoms [1]. The life in kennel is considered a risk factor for several canine intestinal parasites, especially when there isn't an area for the quarantine for the new arrives and there are a high number of animals. Indeed, living outdoors and with other dogs may represent a great risk of acquiring parasites and may require special consideration [2]. Moreover, dogs play an important role in the transmission of zoonotic parasites, e.g., *Toxocara canis*, *Ancylostomidae*, *Echinococcus* spp. and *Giardia duodenalis*. The aim of this study was to perform a ten-year (2011-2021) parasitological monitoring in kennels of the Campania region (southern Italy). The surveillance activities were performed in 84 public kennels regularly monitored annually. In each kennel 20 boxes were examined *at random*; if less, all the boxes were tested. Fresh faecal samples were collected from the ground of each box (pooled sample) and preserved in formalin 5%. Each pooled faecal sample was then examined using the *FLOTAC dual technique* with two flotation solutions, a sodium chloride based solution (specific gravity= 1,200) and a zinc sulphate based solution (s.g. = 1,350). All the kennels were positive for at least one parasite. Among intestinal parasites, *Trichuris vulpis* showed the highest prevalence (89.2%; 95% CI = 79.9-94.6) in the kennels examined, followed by hookworms (84.3%; 95% CI= 74.3-91.1) and *Toxocara canis* (80.7%; 95% CI= 70.3-88.3). Lungworms as *Angiostrongylus vasorum*, *Crenosoma vulpis* and *Oslerus osleri* were found respectively in 28.9% (95% CI= 19.8-40.1), 10.8% (95% CI= 5.4-20.1) and 2.4% (95% CI= 0.4-9.2) of the 84 kennels. Finally, protozoa as *Isospora* spp. and *Giardia* were found in 33.7% (95% CI= 23.9-45.0) and 10.8% (95% CI= 4.8-21.5) of the kennels, respectively. Among positive samples, only 3.8% of kennels examined (95% CI= 1.0-11.5) had a single species of parasites, whilst 7.6% (95% CI= 3.1-16.4) were positive for two and 25.3% (95% CI= 16.5-36.6) for three parasites. The 63.3% of kennels (95% CI= 51.6-73.6) were positive for more than three parasites.

The findings of the present survey showed a high prevalence of helminths (including many zoonotic agents) in kennel dogs from southern Italy, although a reduction was registered during the ten years for all the above-mentioned parasites. Therefore, preventive measures, regular parasitological surveillance and appropriate treatment strategies are efficient and strongly needed to guarantee the health and welfare of pets, and to enhance public health.

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RETROSPECTIVE LONGITUDINAL SURVEY CONCERNING CANINE VECTOR-BORNE DISEASES: TRENDS AND CHALLENGES OF 10 YEARS OF ACTIVITIES OF A BLOOD BANK

Fabrizia Veronesi, Giulia Morganti, Iolanda Moretta, Arianna Miglio Maria Teresa Antognoni

Università degli Studi di Perugia, Dipartimento di Medicina Veterinaria

Corresponding author: F. Veronesi (fabrizia.veronesi@unipg.it)

Canine vector-borne diseases (CVBDs) represent a challenge for veterinary transfusion medicine, since some of these could be potentially transmitted by blood transfusion and show a zoonotic potential [1]. Aim of the present study was to provide information on some CVBDs (i.e. *Leishmania infantum*, *Ehrlichia canis, Anaplasma phagocytophilum, Babesia canis, Rickettsia conorii* and *Dirofilaria* spp.) occurring in Umbria region (Central Italy) exploit the retrospective results obtained in 10 years of activity (2012-2021) of the blood bank of the Veterinary Medicine Department of Perugia (Emovet-Unipg). Parasitological data on CVBDs obtained at the time of blood donation from 1260 voluntary canine blood donors were used to conduct a retrospective longitudinal survey. The results were achieved by indirect immunofluorescence assay (IFA) conducted on sera in order to assess presence and titre for IgG antibodies against *L. infantum, E. canis, A. phagocythophilum, B. canis* and *R. conorii*, by using commercial kits (Diagnostik MEGACORE GmbH, Austria). Serological data on *R. conorii* were available only for the period 2012-2014. Serum circulating antigens for *D. immitis* were determined by Dirochek Heartworm Antigen Test (Zoetis Inc., USA) and one ml of blood in EDTA was used for the modified Knott's test. Prevalence with confidence intervals (CI) at 95% was calculated for each CVBD referring to overall dogs. Inferential analysis was performed to evaluate differences on the positive rates across the studied period ($p<0.05$). Three hundred twenty-four (25.71%, CI 95% ±2.41) on the 1260 dogs screened for CVBDs, were found to be seropositive to at least one pathogens. The highest overall positive rate was detected for *L. infantum* (n. 154 dogs, 12.22%, CI 95% ±1.81), followed by *E. canis* (n. 29 dogs, 2.30%, CI 95% ±0.83), *A. phagocytophilum* (n. 15 dogs, 1.19%, CI 95% ±0.60), *D. repens* (n. 12 dogs, 0.95%, CI 95% ±0.54), *D. immitis* (n. 4 dogs, 0.32%, CI 95% ±0.31) and *B. canis* (n. 2 dogs, 0.16%, CI 95% ±0.22). From 2012 to 2014, a prevalence of 20.11% (CI 95% ±.39) was recorded for *R. conorii* (n. 108 dogs). Mixed infections were recorded in 21 dogs. No mix infection between filarids were detected. Over the 10 years of longitudinal survey, prevalence of: i) *L. infantum* ranged from 3.6% to 17.3%; ii) *E. canis* ranged from 0% to 8%; iii) *A. phagocytophilum* ranges from 0% to 3.6%; iv) *B. canis* ranged from 0% to 1%; v) *D. immitis* ranged from 0% to 0.9%, while *D. repens* from 0.92% to 1.02%. For all the CVBDs investigated no statistically significant difference ($p> 0.05$) was observed across the studied period. None prevalence trend was evaluated for *R. conorii*. The present retrospective longitudinal study evidenced a moderate, but not negligible, prevalence of CVBDs in selected canine donors. Obtained data resulted in line with previous local epidemiological survey [2, 3], confirming canine blood donors and blood bank as a reliable local epidemiological observatory. Serology for *R. conorii*, even if not mandatory for the Italian guidelines [4], should be encouraged since dogs represent sensitive sentinels to assess the infective pressure of this zoonotic pathogen, that is the highest detected [3]. Thus the results confirm the necessity of continuing and also implemented screening protocols recommended by the experts of the Transfusion Study Group (GSTVet), considering possible implications in transfusion veterinary medicine and on public health.

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EFFICACY OF FENBENDAZOLE AND EMODEPSIDE AGAINST HOOKWORM INFECTION IN DOGS

Sergio Illiano, Lavinia Ciuca, Ruggero Amato, Giuseppe Cringoli, Laura Rinaldi

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

Corresponding author: L. Ciuca (lavinia_vet1@yahoo.com)

Hookworm infection is a common parasitic disease in both owned and stray dogs worldwide. Its clinical importance consists in causing severe states of anemia and diarrhea, especially in puppies, and in impacting public health due to the zoonosis caused by *Ancylostoma caninum* [1]. There are several molecules on the market registered for the treatment of this parasitic infection. However, few field studies have been conducted in Italy on the efficacy of the different anthelmintics available on the market. Therefore, the aim of this study was to compare the efficacy of two different molecules (fenbendazole and emodepside) for the treatment of hookworm infection in dogs. A field trial was carried out from February to March 2022 on 16 dogs naturally infected with hookworms in an Australian Cattle dogs breeding farm in Sant'Apollinare (FR), central Italy. Dogs were randomly allocated in two groups of 8 dogs each: dogs in Group 1 (6 months aged) were treated with fenbendazole (Panacur®, Intervet Italia Srl) administered at the dose of 50 mg/kg orally once a day for 3 consecutive days, dogs in Group 2 (4 months aged) were treated with emodepside (Procox®, Vetquinol Italia Srl) administered at the dose of 0.45 mg/kg (0.5 ml/kg) orally at once. All the dogs were screened before the treatment (Day-3, Day-2, Day-1) and then tested at Days 7, 14, 21, 28 and 35 after the treatment (Day 0) by the FLOTAC technique using NaCl (specific gravity 1200) as flotation solution and with an analytic sensitivity of 2 eggs per gram (EPG) of faeces [2]. The efficacy of the treatment (%) for each group was determined by adapting a formula according to Geurden et al. [3]. Before treatment, the mean EPG of hookworms for Group 1 was 365.3 (min=284; max=482), while for group 2 was 478 (min=78; max= 922). The results showed an efficacy of the treatment against hookworm infection of 100% in Group 1 and ranging from 91.4% to 100% in Group 2. Despite the effectiveness of the molecules on the market for the treatment of hookworm infection, regular parasitological surveillance, standardized treatment strategies and high-quality standard of hygiene are always needed for a holistic and comprehensive approach to hookworm infection control, according to guidelines from ESCCAP [4].

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EPIDEMIOLOGICAL SURVEY ON Toxoplasma gondii INFECTION IN CATTLE SLAUGHTERED IN NORTHERN ITALY

Alessia Libera Gazzonis (1), Luca Villa (1), Daniela Tripolini (2), Massimo Sinelli (2), Annalisa Guida (2), Sergio Zanzani (1), Maria Teresa Manfredi (1)

(1) Università degli Studi di Milano, Dipartimento di Medicina Veterinaria e Scienze Animali. (2) Distretto Veterinario Basso Lodigiano.

Corresponding author: A.L. Gazzonis (alessia.gazzonis@unimi.it)

Toxoplasmosis represents an important public health issue, with the consumption of raw or undercooked meat being a major way of human infection. The role of beef in the transmission of the parasite to humans is questioned due to lower quantity of tissue cysts compared with other meat-producing species. However, the habit of consuming raw beef has been increasing in recent years, and the risk posed by Toxoplasma gondii infection in cattle should not be overlooked. *T. gondii* antibody detection in cattle does not strictly correspond to the presence of parasite cysts in their tissues [1]. Therefore, the use of both serology and molecular methods may allow to better assess the risk of T. gondii infection transmission from the consumption of meat originating from infected animals [2]. To update information on *T. gondii* infection in bovines, an epidemiological survey combining serological and molecular tests was planned in cattle slaughtered in northern Italy. Steers were selected for the study: indeed, their meat is frequently commercialized fresh and potentially consumed raw.

In the period comprised between January 2020 and March 2021, 144 steers (age: 6-36 months) were sampled from a slaughterhouse in northern Italy. Dairy and dual purpose breeds or crossbreeds from 37 farms located in northern Italy were sampled. Data on gender, age, breed and movements were noted. Approximately 50 g of diaphragm was collected to obtain meat juice and muscle homogenate samples. Meat juice samples were analyzed with a commercial ELISA to detect specific anti-*T. gondii* antibodies. DNA extracted from muscle homogenate samples was subjected to B1 real-time PCR. Statistical analysis of obtained data was performed by means of generalized linear models (GLMs).

Anti-T. gondii antibodies were found in 17 (11.8%) examined animals, whereas parasitic DNA was detected in 41 diaphragm muscle samples (28.5%). Only nine samples scored positive in both test: a fair agreement between ELISA and B1 real-time PCR results was achieved (κ value = 0.172). Nevertheless, higher ELISA S/P% values were recorded in diaphragm samples scoring positive to PCR (80.9 S/P%) vs. those scored negative to PCR (61.03 S/P%). Higher number of positive samples were found in younger than older animals considering both ELISA and B1 real-time PCR results. Similarly, considering the provenience, animals that have been acquired from other holdings scored more frequently positive to both ELISA and B1 real-time PCR compared to animals that have never left the holding of origin until slaughter. Statistical analysis showed an effect of ELISA S/P% values on B1 real-time PCR results, increasing the risk of parasitic DNA detection when increasing the S/P% values. Animal trade was also statistically associated with positivity (ELISA or PCR results), with animals purchased from other Italian farms more at risk than animals that have never been moved from the farm of origin. The study confirmed the role of beef meat as a potential source of *T. gondii* infection for humans. Considering the consumption of raw beef preparations in many regions of northern Italy, the zoonotic importance of *T. gondii* from beef should not be neglected.

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SEROLOGICAL SURVEY ON SARCOPTES SCABIEI IN WILD BOARS (Sus scrofa) HUNTED IN AN ANTHROPIZED AREA IN NORTHERN ITALY.

Carolina Allievi (1), Luca Villa (1), Alessia Libera Gazzonis (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 e Scienze Animali. (2) Istituto Zooprofilattico Sperimentale della Lombardia e dell'Emilia-Romagna, Sede di Cremona.

Corresponding author: C. Allievi (carolina.allievi@unimi.it)

Wild boar populations are now expanding in terms of both number of animals and range of habitats. In addition to the ecological impact and the conflicts with human activities, the increased frequency of contacts among wild boars, livestock and humans could also influence the transmission of zoonotic and animal-specific pathogens [5].

Sarcoptic mange is caused by *Sarcoptes scabiei*, an obligate ectoparasitic arthropod which is responsible for significant morbidity and mortality in wild, domestic and farm animals. Further, this mite has a zoonotic impact being transmitted from several animal species to humans and the World Health Organization (WHO) listed the scabies among the neglected tropical diseases [4]. Transmission between hosts can occurr both directly (by prolonged skin to skin contact) and indirectly, indeed mites may apparently survive for some time off their hosts remaining infective in humid and cold environments [3]. Sarcoptic mange can affect free-ranging populations of wild ungulate and carnivore species in many European countries, including wild boars (*Sus scrofa*) [1]. Little is known about the pathogenicity and the disease signs in this species, which seems to be subjected to a high morbidity and a low mortality. Scavenger habits expose wild boar to an increased risk of infestation, especially in winter when mite-associated mortality in preys increases. Another risk is represented by the interactions between wild boars and domestic pigs, which facilitate the transmission of sarcoptic mange. A recent survey conducted in Switzerland witnessed a seroprevalence in wild boars between 0.1 and 12.7%, depending on the different cantons. In northern Italy are reported values of seropositivities among 6.2 and 9.4%, but further studies are needed [3]. For this reason, the aim of the study was to investigate the serological exposure to *Sarcoptes scabiei* in wild boars hunted in an anthropized area in northern Italy. Blood samples were collected from 128 wild boars 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 and separate generalized linear models (GLMs) with negative binomial distribution were performed to verify the influence of epidemiological data on seropositive results. Sera were analyzed with the commercial indirect kit SARCOPTES-ELISA 2001® Pig (AFOSA GmbH, Dahlewitz bei Berlin, Germany), which uses *Sarcoptes* mites from pigs as antigen and is validated also for wild boars, following the manufacturer's instructions [2].

Nine out of 128 wild boars (7.03%) resulted positive to *Sarcoptes scabiei* antibodies. Seropositivity values did not differ considering age, gender, and killing place categories, by the way positive wild boars were mainly young animals (2 <1 year old, 5 between 1 and 2 and 2 between 2 and 3 years old), which suggests a first exposure to mites early in life, including both male (n=3) and female (n=6) exemplars.

This study highlights that *S. scabiei* is quite distributed in free-ranging wild boar populations. The awareness of hunters and wildlife professionals should be further promoted to obtain an overview of the distribution of clinical cases and to monitor the disease occurrence, considering the zoonotic impact of this pathogen.

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PREVALENCE OF SARCOPTES SCABIEI ANTIBODIES IN FATTENING PIGS AND SOWS FROM INTENSIVE FARMS IN NORTHERN ITALY

Carolina Allievi, Alessia Libera Gazzonis, Luca Villa, Sergio Aurelio Zanzani, Maria Teresa Manfredi

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

Corresponding author: C. Allievi (carolina.allievi@unimi.it)

Sarcoptic mange is caused by the mite *Sarcoptes scabiei*, an obligate ectoparasitic arthropod, responsible in pigs for intense itching with skin irritation and lesions, and consequently for a decrease in growth rate, feed efficiency, and great economic losses. In addition, as the mite has a zoonotic potential, domestic pig is involved in the "pig handler's itch" [2].

Even if *Sarcoptes scabiei* is usually undergoing to control programs in pig farms, data on the distribution of this ectoparasite in pigs are scarce and fragmentary worldwide. In this respect, the use of a serological diagnostic method allows to conduct an epidemiological survey on a large number of animals. For this reason, a study aimed to investigate the serological exposure to *Sarcoptes scabiei* in pigs from intensive farms in northern Italy was planned.

Two-hundre-nineteen fattening pigs and 151 sows from 23 conventional farms in Lombardy were sampled. Data on farm management were collected and a "biosecurity score" (1=poor, 2=moderate, 3=optimal) was determined for each farm. Separate generalized linear models (GLMs) with negative binomial distribution were performed to verify the influence of farm management on parasite infection.

Serum samples were analyzed using the commercial indirect SARCOPTES-ELISA 2001® Pig (AFOSA GmbH, Dahlewitz bei Berlin, Germany), according to the manufacturer's instructions.

At the farm level, 69.6% (16/23) of the selected herds, 90.9% of which housed sows and 40% fattening pigs, tested positive. At the individual level, 43 animals (43/370, P=11.6%) were positive for *Sarcoptes scabiei* antibodies; a higher seroprevalence was found in sows (35/151, P=23.2%) than in fattening pigs (8/219, P=3.6%). In addition, higher seroprevalence was recorded in herds with low and moderate health scores (P=100% and P=64.3%, respectively) than in herds with higher health scores (P=44.4%).

By univariate analysis, only the variable "productive category" was significantly associated with *Sarcoptes scabiei* infection (OR=0.7, p-value=0.02), indeed sows were at a higher risk of infection than fattening pigs.

Obtained results evidenced that *S. scabiei* circulates in intensive pig industry in Lombardy region. Breeder animals were more exposed to the infestation and higher seropositivity was evidenced in farms with scarce herd management biosecurity measures. Previously, a seroprevalence of 28.2% was reported in Austrian pig farms [1], whereas a few studies both in Europe and worldwide using direct diagnostic methods performing a deep ear scraping, subsequently analyzed under a microscope, reported values of positivity between 1.2% and 45.4%. Even if, the study revealed that sarcoptic mange is rather well controlled in fattening pigs, sows remain the most frequently infested subjects responsible for the maintenance and persistence of the mite in farmed animals. Furthermore, some farms still present high prevalence of infestation demostrating that adopetd strategies are not suitable for controlling the circulation of the parasite in the breeding stock. Thus, data obtained in this study suggest raising awareness among farmers and veterinarians to monitor the occurrence of S. scabiei infestation, considering both its economic impact and zoonotic potential.

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SEROLOGICAL PREVALENCE OF NEOSPORA CANINUM AND EFFECTS ON HERD EFFICIENCY IN DAIRY CATTLE FARMS IN NORTHERN ITALY

Luca Villa (1), Carolina Allievi (1), Alessia Libera Gazzonis (1), Sergio Aurelio Zanzani (1), Emanuele Fumagalli (2), Giorgio Gelati (3), Riccardo Zanchetta (4), Marco Colombo (4), Maria Teresa Manfredi (1)

(1) Università degli Studi di Milano, Dipartimento di Medicina Veterinaria e Scienze Animali. (2) Buiatra libero professionista, Bergamo. (3) Buiatra libero professionista, Crema. 4) Buiatra libero professionista, Milano.

Corresponding author: L. Villa (luca.villa@unimi.it)

Among infectious agents triggering reproductive disorders in cattle, *Neospora caninum*, an obligate intracellular protozoan parasite, is a major cause of bovine abortion and neonatal mortality worldwide [1]. Transplacental transmission is retained the predominant and most efficient transmission route. The outcome of infection in pregnant cows can be the abortion of the fetus, or the birth of a still-born calf, a calf with neurological clinical signs, or a clinically healthy persistently infected calf [2]. Besides, a few reports suggested an adverse effect of *N. caninum* in early pregnancy manifested as a return to service, increased time to conception or infertility, and increased calving intervals in seropositive cows [3]. Furthermore, some studies also reported a reduction in milk yield in dairy cattle and reduced weight gain and feed efficiency in beef calves and steers [4, 5].

Considering the relevance of the dairy industry in nothern Italian regions, the aim of this study was to investigate on the seroprevalence of *N. caninum* and the effects of parasite infection on herd efficiency in selected dairy cattle farms in Lombardy region.

Six dairy cattle farms located in the provinces of Bergamo, Lodi, Milano and Cremona were selected. All cows above 24 months of age present in the farms at the time of sampling were included in the study. Blood samples were collected to obtain sera for serological analysis. Individual data and information regarding reproductive and productive parameters of cattle were collected by the farm managerial software. Serum samples were analyzed for the detection of anti-*N. caninum* specific antibodies by using a commercial immunofluorescence antibody test (MegaScreen® FLUO NEOSPORA caninum, Megacor, Hoerbranz, Austria). Seroprevalence of *N. caninum* was calculated considering the six farms. The effects of the parasite infection on the herd performances in the two farms were also investigated through univariate generalized linear models (GLMs) with linear distribution.

Overall, out of 1133 animals, 363 cows scored positive for *N. caninum* antibodies with a prevalence of 32.04%. Seroprevalence varied between the six farms from 15.5% to 38.65%.

Considering reproductive parameters for all farms, the number of inseminations necessary to make an animal pregnant was higher in seropositive animals than in seronegative animals (Insemination mean: 1.9 in seronegative vs 2.1 in seropositive cows). The number of days in milk of not-pregnant cows was higher in seropositive cows (DIM mean: 199 in seronegative vs 217 in seropositive cows). Concerning productive parameters, both the daily production (Daily milk production in kg mean: 36.7 in seronegative vs 34.9 in seropositive cows) and the mature equivalent milk yield (MEM mean: 11341 in seronegative vs 10567 in seropositive cows) were lower in seropositive than seronegative cows. Statistical analysis by GLMs did not show any association between *N. caninum* infection and the considered reproductive and productive variables.

This preliminary data showed that *N. caninum* circulates in dairy herds in Lombardy and may suggest an impact of this parasite on herd performances.

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LOCALIZATION OF DIROFILARIA REPENS IN DOGS TESTIS: A RESEARCH NOTE

Stefania Di Giorgio, Davide Mazzara, Giada Giambrone, Alessandra Sfacteria, Gabriele Marino, Emanuele Brianti, Ettore Napoli

Università degli Studi di Messina, Dipartimento di Scienze Veterinarie.

Corresponding author: S. Di Giorgio (sdigiorgio@unime.it)

Canine subcutaneous filariasis caused by *Dirofilaria repens* is wide-spread mosquitoes borne disease, that generally affects domestic carnivores, and sporadically humans. Adult nematodes of *D. repens* are mainly found in subcutaneous or intramuscular connective tissue and most affected animals are asymptomatic; however, they may rarely present dermatological complaints or other symptoms related to the unusual localization.

In fact, recently, some authors have reported unusual localizations of the adult *D. repens* worm, in particular, adults have been observed in the pelvic cavity, mesentery, bulbar conjunctival mass, and, also in the testis; some authors hypnotized that this latter *D. repens* localization could be related to neoplastic processes. Therefore, this study aims to investigate the presence of *D. repens* in testis and its possible correlation with the occurrence of testicular neoplasms. A total of 100 male dogs that underwent castration, preferring the "closed technique", either for pathological conditions (testicular degeneration, cryptorchidism and testicular tumours) or regular castration procedures were enrolled. The dogs were included in the study from different provinces of Southern Italy regardless of their breed, attitude and/or age. At the inclusion, all the animals underwent a physical examination and anamnestic data were recorded. For each animal, the testicles were collected along with a blood sample (1 mL) stored in a tube containing K3EDTA. The testicles were examined under a stereomicroscope, assessing the possible presence of the parasite, macroscopic pathological changes on the vaginal tunics or the cut surface of the testicle, in particular the presence of testicular neoplasms. The presence of circulating microfilariae was assessed using a Knott's test. Nematodes classified morphologically and molecularly as *D. repens* were detected in 3 out of 100 examined dogs (i.e., 3%). *Dirofilaria repens* adults were localized in the tunica vaginalis in testes, which were not associated with any pathological changes, and two of these were positive for the presence of microfilariae in the blood. In 4 out of 100 dogs, testicular neoplasms were observed, however, they tested negative for both *D. repens* micro and macrofilarie. This study highlights the asymptomatic nature of *D. repens* infection and supports the lack of correlation between the presence of *D. repens* in the testes and testicular neoplasms [1]. In fact, in no case, the testis neoplasm was associated with *D. repens* presence. In another similar study, conducted in Kerala (India), a similar prevalence was observed (i.e., 3.3%), however in this study in all the *D. repens* infected dogs thickened and enlarged epididymis along with interductal fibrosis, congestion of veins and widening of the cavernous spaces of testes and epididymis were observe [2]; however, the localization of the parasite, in the beneath of the tunica vaginalis, in any way explain the onset of the pathological alterations.

Dirofilaria repens is normally observed in the subcutaneous tissue of dogs, however, another localization of the parasite has been highlighted in the last years, being the testicular localization more frequent compared to other unusual localization such as the pelvic and mesentery cavities, the eyes and subconjunctival [3]. Also, in humans, the localization of *D. repens* in the gonads and in the breast are common localization.

Dogs living in endemic areas are silent carriers of the disease and given the zoonotic potential and the rapid spread of *D. repens* in endemic areas, it is essential to implement adequate chemoprophylaxis for all animals to preserve animal and human health, while in non-endemic areas, all dogs from endemic countries should be tested with the modified Knott's test and further confirmed by PCR.

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[2] Ravindran et al. *Dirofilaria repens* in scrotum of dogs, Trop Biomed, 33: 842-846, 2016.

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AUTOCHTHONOUS AND ORNAMENTAL CHICKENS REARED IN NORTHERN ITALY: GASTROINTESTINAL PARASITES IN BREEDING FACILITIES

Sergio A. Zanzani, Alessia L. Gazzonis, Luca VIlla, Giuseppe Rauseo, MariaTeresa Manfredi

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

Corresponding author: S.A. Zanzani (sergio.zanzani@unimi.it)

Biodiversity and its conservation represent global issues. This concern addresses domestic poultry too: reared worldwide for thousand years with more than 1500 different breeds, nowadays poultry industry is based on highly selected commercial lines of poultry. However, small-scale breeders are becoming more aware of the interest not only for autochthonous breeds for backyard farming but also for breeds reared as pets and selected for aesthetics. The management and health status of these small-scale breeding facilities are not well known. Hence, the present study aimed to investigate their features and parasitological status in northern Italy.

Between November 2019 and February 2020, thirty-two breeders adhered to the survey. In their facilities, they reared 47 different autochthonous and ornamental breeds. Out of 32 facilities, 9 were classified "small" (rearing up to 75 chickens), 13 "medium" (76-150), 10 "large" (more than 150). In 14/32 facilities, other rural breeds/hybrids of chicken were also reared for eggs or meat production, while the remaining 18/32 breeders reared only autochthonous and ornamental breeds; 18/32 also reared other Galliformes species and 15/32 reared Anseriformes. In the 32 surveyed facilities, pooled faecal samples were collected from all the chickens' groups composed of autochthonous and ornamental breeds. Overall, 185 groups of chickens were sampled; 114 groups out of 185 were classified “small” (<10 animals) and 71 “large” (≥10). In 34 groups out of 185, young animals (<6 months old) were present. Groups composed of males, females, and mixed sexes groups were 26, 24, and 135, respectively. Information about cleaning practices and disinfection was also collected and used to produce a score ranging from 0 (worst hygiene) to 5 (best hygiene). The 185 pooled faecal samples were analyzed by FLOTAC dual technique [1]. Values of eggs/oocysts per gram of feces (UPG/OPG) of most prevalent parasites were logarithmically transformed and introduced as dependent variables in three generalized linear models. Farm and group features were introduced in the models as the independent variables. Final models were obtained by backward elimination of not significant variables and best Akaike Information Criterion (AIC) (SPSS 20.0, IBM).

Eggs of Ascaridia/Heterakis, Capillaria spp., Trichostrongylidae, Strongyloides spp., Raillietina spp., and oocysts of Eimeria spp. were detected in 66.5% (123/185), 57.8% (107/185), 9,7% (18/185), 1.6% (3/185), 2.7% (5/185), and 81,6% (151/185) of samples, respectively. Ascaridia/Heterakis, Capillaria spp., and Eimeria spp., the endoparasites presenting the highest percentages in the analyzed samples, were found with means UPG/OPG values of 70 (min-max: 0-4,668), 48 (0-1,233), and 968 (0-28,848) respectively.

Ascaridia/Heterakis eggs excretion was higher in “medium” facilities than in “large” ones, in groups reared on wood shaving litters than in those reared on a straw litter, and in farms with worst hygienic conditions. Moreover, medium size breeds excreted significant higher levels of eggs than heavy, light, and dwarf breeds. EPG of Capillaria spp. were higher in groups exclusively composed of females; they were lower in groups housed in chicken cops with an aviary when compared with groups reared exclusively in chicken cops or in an aviary. EPG of both Ascaridia/Heterakis and Capillaria spp were lower in groups of chicken that received anthelmintic treatments in the past six months. Eimeria spp. OPG were higher in larger groups of animals, in groups with young animals, and in groups of medium size and dwarf breeds. Gastrointestinal parasites are widespread in small-scale breeding facilities of autochthonous and ornamental chickens in northern Italy; EPG and OPG of most common endoparasites can be affected both by management, farm and animal features and should be considered to improve health status of reared chickens.

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RISK OF LYME BORRELIOSIS AND IXODES RICINUS BITES IN DOGS: A SEROLOGICAL SURVEY IN A HIGH-RISK CLUSTER FOR RESIDENT HUMAN POPULATION IN LOMBARDY REGION

Alessandra Cafiso, Luca Villa, Chiara Bazzocchi, Alessia L. Gazzonis, Donatella Scavone, Chiara Raffa, Stefania Lauzi,, Maria Teresa Manfredi, Sergio A. Zanzani

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

Corresponding author: S.A. Zanzani (sergio.zanzani@unimi.it)

Tick-borne diseases are spreading worldwide, and this trend has been widely documented for Lyme borreliosis, a bacterial disease that in western Europe is mainly transmitted by the hard tick *Ixodes ricinus*. In Italy, Lombardy was considered a low-incidence region, but recently a high-risk cluster for the resident human population has been identified (Zanzani, 2019).

To evaluate the risk of exposition to *Borrelia burgdorferi* s.l. (the causative agent of Lyme borreliosis) and *I. ricinus* bites for dogs living inside the described cluster, a serological survey was planned to detect antibodies anti-*B. burgdorferii* and *Midichloria mitochondrii*, an alphaproteobacterium symbiont of *I. ricinus*.

From May to October 2019, 149 blood samples were collected from owned dogs living in the described cluster (Sondrio and Lecco provinces). For each dog enrolled in the survey, data about age sex and size were collected. In dogs' sera, the presence of anti-*B. burgdorferi* s.l. IgM and IgG was evaluated by a commercial indirect immunofluorescence assay (MegaScreen Fluoborrelia, MegaCor Diagnostik GmbH, Austria; cutoff titer, 1:64). To assess the possible exposition to I. ricinus bites in dogs in which transmission of *B. burgdorferi* s.l. did not occur, the presence of anti-*M. mitochondrii* IgG was investigated by an ELISA assay [2]. Statistical analysis to determine the significant risk factors of *B. burgdorferi* s.l. seroprevalence and of *I. ricinus* bites was implemented by SPSS 20.0 (IBM, Chicago, Il).

In the present study, 9.3% of dogs (14/149) showed detectable anti-*B. burgdorferi* s.l. IgM, and in 6.7% of dogs (10/149) anti-*B. burgdorferi* s.l. IgG were detected; overall prevalence of antibodies anti-*B. burgdorferi* s.l. was 12.1% (18/149). Out of the 149 tested dogs, 26.8% (n=40) were classified as positive for the presence of anti-*M. mitocondrii* IgG. Considering seropositivity to *B. burgdorferi* s.l. and/or seropositivity to *M. mitochondrii* as a consequence of at least one tick bite by *I. ricinus*, in the present study 34.9% of dogs (52/149) have been bitten. Considering seasonality, seropositivity to *B. burgdorferii* s.l. and/or *M. mitochondrii* significantly peaked in July (57.1%, 8/14) and October (83.3%, 15/18), and this result was consistent with *I. ricinus* phenology in temperate climates. Other features (province, age, sex, size) were not significantly related to *B. burgdorferi* s.l. seroprevalence and to *I. ricinus* bites risk.

Results of our study showed that in the previously described high-risk cluster of human Lyme borreliosis, the seroprevalence of *B. burgdorferi* s.l. in owned dogs was 12.1% and from spring to autumn the risk for tick bites was high. This risk coincided with *I. ricinus* seasonal dynamics in western Europe, and probably it was uniform in owned dogs population; in fact, the province in which the dogs lived, their age, sex, and size were not significant risk factors.

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