



# **Proceedings of the 10<sup>th</sup> FARAH-Day**

**Faculty of Veterinary Medicine  
(University of Liège - Belgium)**

**December 21, 2023**

*One Health*

L'Animal et l'Homme, une même santé





# **Proceedings of the 10<sup>th</sup> FARAH-Day**

**Faculty of Veterinary Medicine  
(University of Liège - Belgium)**

**December 21, 2023**

**Edited by** C. Cesarini Latorre, C. de Meeüs d'Argenteuil, C. Delguste, C. Douny, J. Eppe, L. Fiévez, V. Frisée, C. Gatez, T. Jauniaux, J. Ledeck, M. Levoz, L. Martinelle, I. Tosi, D.M. Votion, C. Wouters

**Presses de la Faculté de Médecine vétérinaire de l'Université de Liège**  
4000 Liège, Belgique

**COVER PICTURE CREDITS:**

"Upside-Down Ballet: Zebrafish in a Blue Elegance. A Captivating Dance under the Intense Glow, Defying Gravity in a Well Plate"  
@Clovis Wouters

### ***Welcome to the 10<sup>th</sup> FARAH Day***

In 2012, the Scientific Staff of the Faculty of veterinary Medicine organised its first annual meeting. Each annual meeting has been a great success with an average of 100 abstracts submitted, among which about twenty were selected for an oral presentation by an independent scientific committee.

In 2013, an interdisciplinary structural research centre was created at the University of Liège. It has been named FARAH for "Fundamental and Applied Research for Animals & Health".

The founding principles of the FARAH incorporate the notion of interaction between scientists of the Centre and, as such, the annual meeting of the scientific staff gives us the opportunity to share our knowledge. Also, it is now under the auspices of the FARAH that the annual meeting will be held with the same organizers (i.e. members of the Scientific Staff). This edition gathers about 60 abstracts dedicated to fundamental, clinical and or applied researches.

**Daniel Desmecht, Jean-Luc Hornick & Dominique Votion.**

### ***Bienvenue à la 10<sup>ème</sup> journée du FARAH***

En 2012, le Personnel Scientifique de la Faculté de Médecine vétérinaire organisait sa première journée scientifique annuelle. Chaque réunion annuelle a été un grand succès avec, en moyenne, une centaine de résumés de recherche soumis dont une vingtaine était sélectionnés pour une présentation orale par un comité scientifique indépendant.

En 2013, un centre structurel interdisciplinaire de recherche a été créé au sein de l'Université de Liège. Ce centre est désigné par l'acronyme FARAH pour « Fundamental and Applied Research for Animals & Health ».

Les principes fondateurs du FARAH intègrent la notion d'interaction entre les Scientifiques du Centre et à ce titre, la réunion annuelle du personnel scientifique nous donne l'opportunité de partager nos connaissances. Aussi, c'est dorénavant sous l'égide du FARAH que s'organise, avec les mêmes forces vives (i.e. les membres du Personnel scientifique), la réunion annuelle des scientifiques. Cette édition inclut une soixantaine de travaux ayant trait à la recherche fondamentale, clinique et/ou appliquée.

**Daniel Desmecht, Jean-Luc Hornick & Dominique Votion.**

# Organisers of the meeting

## ORGANISING COMMITTEE

**Justine Eppe, Vincent Frisée**  
*Clinical Dept of Animal Production*

**Carlota Cesarini Latorre, Joy Ledeck**  
*Equine Clinical Sciences Dept*

**Ludovic Martinelle**  
*Care FEPEX*

**Catherine Delguste**  
*General Services*

**Caroline Douny**  
*Dept of Food Sciences*

**Constance de Meeüs d'Argenteuil, Thierry Jauniaux, Marine Levoz**  
*Dept of Morphology and Pathology*

**Laurence Fiévez, Carine Gatez, Irene Tosi, Dominique-Marie Votion, Clovis Wouters**  
*Dept of Functional Sciences*

## SCIENTIFIC COMMITTEE

**Carlota Cesarini Latorre,**  
*Equine Clinical Sciences Dept*

**Ludovic Martinelle**  
*Care FEPEX*

**Catherine Delguste**  
*General Services*

**Caroline Douny**  
*Dept of Food Sciences*

**Constance de Meeüs d'Argenteuil, Thierry Jauniaux**  
*Dept of Morphology and Pathology*  
*Dept of Infectious & Parasitic diseases*

**Laurence Fiévez, Irene Tosi, Dominique-Marie Votion, Clovis Wouters**  
*Dept of Functional Sciences*

## TECHNICAL SUPPORT

**Frédérique Damseaux  
Jean-Michel Dusoulier  
Siléna Mathieu**







# Program

**08:30 - Registration (lecture hall C, building B45)**

**09:00 - Opening and Welcome Speech**

**Prof. Anne-Sophie Nyssen, Rector of ULiège university**

**Prof. Tania Art, Dean of veterinary faculty**

**09:15 Invited speaker**

**Prof. Benjamin Dewals**

*Parasitic worms: friends or foes in the context of viral coinfections?*

**09:45 - Oral session 1: Short talks (lecture hall C, building B45)**

**Chair: Calixte Bayrou & Bénédicte Machiels**

**09:45 Georgios PETRELLIS** - *IL-4 receptor- $\alpha$  signaling regulates lung macrophages during helminth coinfection resulting in enhanced gammaherpesvirus permissiveness.*

**09:50 Brunette KATSANDEGWAZA** - *Helminth exposure protects against pneumovirus infection in an IL-4R $\alpha$ -independent and monocyte dependent manner.*

**09:55 Ophélie PIEDFORT** - *Modulation of effector memory T lymphocyte responses to vaccination by helminth infections.*

**10:00 Julien DUPONT** - *Neutrophil extracellular traps release following exploratory laparotomy in horses.*

**10:05 Benoît RENAUD** - *Beyond "On/Off": understanding the gradual nature of hypoglycin A poisoning in atypical myopathy.*

**10:10 Questions**

**10:15 Céline ANTOINE** - *In-vitro/in-vivo characterization of phage resistant E. coli K1 and impact on Bacterial Fitness and Biofilm Production*

**10:20 Anne-Sophie VAN LAERE** - *Towards a new, glyco-humanized rabbit line.*

**10:25 Maxime VANDERSMISSEN** - *MRI appearance of medial subchondral bone injuries of the proximal phalanx glenoid in Warmblood horses.*

**10:30 Salomé DESMECHT** - *In vitro characterization and preliminary efficacy assessment in galleria mellonella larvae of four newly isolated bacteriophages targeting aeromonas salmonicida.*

**10:45 Fanny LAFORÊT** - *Bacteriophage Efficacy Assessment for Decolonizing Hyper-Virulent Klebsiella pneumoniae K1 ST23 in the Intestinal Microbiota: An In Vitro Study using the SHIME® Model.*

**10:40 Questions**

**10:45 - Coffee break and poster session 1 (Room P, building B45)**

**11:15 - Oral session 2:** Awardee from FSR (lecture hall C, building B45)

**Chair: Bernard Taminiau & Irène Tosi**

**11:15 Hélène AMORY** - EQUIMICROLIC.

**11:20 Elodie RIZZOLI** - Réalisation de FAPI-PET scans pour investiguer l'expression de la « fibroblast activation protein » dans les poumons de chiens : une étude pilote.

**11:25 Nazaré STORMS** - Étude clinique de l'implication des produits dérivés des neutrophiles (MPO et NETs) dans la pathogénie des fourbures naturelles et de l'évolution structurelle et ultra-structurelle des lésions lamellaires chez les équidés.

**11:30 Caroline DOUNY** - Investigation de la formation de composés néoformés dans les produits de viande, de leur devenir dans le tractus gastro-intestinal et de leur impact sur le microbiote intestinal.

**11:35 Albane RIVES** - Analyse des expressions faciales de douleur des bovins à l'aide de l'intelligence artificielle.

**11:40 Justine EPPE** - Functional investigation of the hypothalamic-pituitary-thyroid axis in relation to energy metabolism and immunology in cattle.

**11:45 Nicolas KORSAK** - "Impact of organic selenium supplementation on the rumen microbiota and productivity of Belgian Blue cattle (BBB) during the finishing period"

**11:50 Questions**

**11:55 Margaux LEGRAND** - The predictive value of clinical, 2D and 3D echocardiographic variables for assessing risk of cardiac death in dogs with precapillary pulmonary hypertension: a longitudinal study.

**12:00 Margot STIPULANTI** - Sexing bovine fetus using sexual steroids assay with Mass Spectrometry coupled to Liquid Chromatography.

**12:05 Elodie ROELS** - Chronic idiopathic lymphoplasmacytic rhinitis - The use of Lokivetmab (Cytoint®, Zoetis) in client-owned dogs with chronic idiopathic lymphoplasmacytic rhinitis (CILPR): a masked, randomized, placebo-controlled pilot study.

**12:10 Constance WIELICK** - Dynamics of hepatitis E virus infection during the porcine life cycle: the influence of co-circulating pathogens.

**12:15 Charlotte SANDERSEN** - DEXmedetomidine to preserve tissue OXYgenation during general anesthesia of septic DOGS.

**12:20 Maëlle BONHOMME** (presented by Clovis Wouters) - Jump'Safely: Monitoring young athletes to prevent racehorse injuries

**12:25 Didier SERTEYN** - LAMITRANS

**12:30 Questions**

**12:45 - Lunch and poster session 2** (Room P, building B45)

**14:00 - Oral session 3:** Short talks (lecture hall C, building B45)

**Chair: Antoine Clinquart & Marie-Louise Scippo**

**14:00 Mélodie DE TREZ** - Potential transmission of *Brucella* sp. throughout respiratory parasites in Harbour porpoise.

**14:05 Nazaré STORMS** - Neutrophil extracellular traps and active myeloperoxidase concentrate in lamellar tissue of equids with naturally occurring laminitis.

**14:10 Meijiao GONG** - AIHV-1 infection causes oligoclonal expansion and activation of CD8+ T lymphocytes resulting in bovine malignant catarrhal fever via phosphorylation of a viral transmembrane protein.

**14:15 Thi Kim Duyen HUYNH** - Depletion and withdrawal times of doxycycline in white leg shrimp (*Litopenaeus vannamei*) after oral administration.

**14:20 Chiara DEBIE** - Prevalence and risk factors for *Mycoplasma* spp. positivity in cat blood donor units from Portugal, Spain and Belgium in 2022: retrospective study on 7573 blood donations from 4121 healthy donor cats.

**14:25 Bo HE** - In Vivo Imaging Sheds Light on the Susceptibility and Permissivity of *Carassius auratus* to Cyprinid Herpesvirus 2 According to Developmental Stage.

**14:30 Sébastien CZAPLICKI** - Optimizing heat stress detection in dairy cattle: leveraging datamining and unsupervised analyses to explore individual-level impact through behavior and meteorological factors.

#### **14:35 Questions**

**14:40 Mailis HUMBEL** - Welfare and resting behaviour of group-housed horses kept in loose housings: on farm assessment using the AWIN welfare protocol and time budget evaluation.

**14:45 Qianqian YAO** - 2'-Fucosyllactose ameliorates inflammatory bowel disease by modulating gut microbiota and promoting MUC2 expression.

**14:50 Shi Fang LI** - A systems immunology approach reveals distinct roles of genetic and non-genetic factors in shaping variation of immune responses in cattle.

**14:55 Clovis WOUTERS** - Zebrafish embryos as alternative model to study intoxication by *Acer pseudoplatanus* toxins: Hypoglycin A, Methylenecyclopropylglycine and Methylenecyclopropylacetate.

**15:00 Célia DARIMONT** - Development of a Systems Immunology Approach to Explore Factors Influencing Vaccination Response in Belgian Blue Cattle.

#### **15:05 Questions**

#### **15:10 Invited speaker**

##### **Jérôme Eeckhout (RISE)**

*RISE support for researchers and research projects.*

#### **15:40 - Coffee break and poster session 3 (Room P, building B45)**

#### **16:10 - Oral session 4: Awardee from CSR (lecture hall C, building B45)**

#### **Chair: Nicolas Korsak & Aline Fastrès**

**16:10 Elisa MARTINEZ** - Etude des interactions entre la bactérie pathogène *Clostridioides difficile* et un modèle de biofilm mucosal composé d'un microbiote intestinal défini.

**16:15 Irma Elizabeth GONZA QUITO** - Role of food additives on the development of inflammatory bowel diseases and intestinal fibrosis.

**16:20 Mazarine GERARDY** - FlaviAGE: Determinants of the age-dependent pathogenesis of the Usutu Flavivirus in mice.

**16:25 Jérôme BAIWIR** - Investigation de la lymphoprolifération induite par les gammaherpèsvirus par le biais du séquençage du répertoire des lymphocytes B.

**16:30 Eléa HENRARD** - Viruses Influence Tumor Aggressiveness in the Lung.

**16:35 Sébastien PIROTTE** - *Les domaines Zalpha du Cyprinid herpesvirus 3 et du virus de la fièvre porcine africaine sont-ils des homologues fonctionnels acquis par évolution convergente?*

**16:40 Questions**

**16:45 Invited speaker**

***Prof. Siska Croubels, Ghent University***

*Research at the Faculty of Veterinary Medicine of Ghent University*

**17:15 – Closing session**

***Dominique CASSART***

*Expiry date : 31-03-2024*

***Prof. Laurent Gillet***

**18:00 – FarahDay awards** (Room P, building B45)

**18:30 – Live music, drinks and appetizers**

**19:30 – Food and Fun (Games, Blind Test, Music, ...)**



# **Table of Contents**



# Short Talks

- L-4 receptor- $\alpha$  signaling regulates lung macrophages during helminth coinfection resulting in enhanced gammaherpesvirus permissiveness* **Petrellis G. et al.** p. 24
- Helminth exposure protects against pneumovirus infection in an IL-4Ra-independent and monocyte dependent manner* **Katsandegwaza B. et al.** p. 24
- Modulation of effector memory T lymphocyte responses to vaccination by helminth infections* **Piedfort O. et al.** p. 25
- Neutrophil extracellular traps release following exploratory laparotomy in horses* **Dupont J. et al.** p. 25
- Beyond "On/Off": understanding the gradual nature of hypoglycin A poisoning in atypical myopathy* **Renaud B. et al.** p. 26
- In-vitro/in-vivo characterization of phage resistant E. coli K1 and impact on Bacterial Fitness and Biofilm Production* **Antoine C. et al.** p. 26
- Towards a new, glyco-humanized rabbit line* **Van Laere A.S. et al.** p. 27
- MRI appearance of medial subchondral bone injuries of the proximal phalanx glenoid in Warmblood horses* **Vandersmissen M. et al.** p. 27
- In vitro characterization and preliminary efficacy assessment in galleria mellonella larvae of four newly isolated bacteriophages targeting aeromonas salmonicida* **Desmecht S. et al.** p. 28
- Bacteriophage Efficacy Assessment for Decolonizing Hyper-Virulent Klebsiella pneumoniae K1 ST23 in the Intestinal Microbiota: An In Vitro Study using the SHIME® Model* **Laforêt F. et al.** p. 28



# Short Talks

- Potential transmission of Brucella sp. throughout respiratory parasites in harbour porpoise* **p. 29**  
**De Trez M. et al.**
- Neutrophil extracellular traps and active myeloperoxidase concentrate in lamellar tissue of equids with naturally occurring laminitis.* **p. 29**  
**Storms N. et al.**
- AIHV-1 infection causes oligoclonal expansion and activation of CD8+ T lymphocytes resulting in bovine malignant catarrhal fever via phosphorylation of a viral transmembrane protein* **p. 30**  
**Gong M. et al.**
- Depletion and withdrawal times of doxycycline in white leg shrimp (Litopenaeus vannamei) after oral administration* **p. 30**  
**Huynh T.K.D. et al.**
- Prevalence and risk factors for Mycoplasma spp. positivity in cat blood donor units from Portugal, Spain and Belgium in 2022: retrospective study on 7573 blood donations from 4121 healthy donor cats* **p. 31**  
**Debie C. et al.**
- In Vivo Imaging Sheds Light on the Susceptibility and Permissivity of Carassius auratus to Cyprinid Herpesvirus 2 According to Developmental Stage* **p. 31**  
**He B. et al.**
- Optimizing heat stress detection in dairy cattle: leveraging datamining and unsupervised analyses to explore individual-level impact through behavior and meteorological factors* **p. 32**  
**Czaplicki S. et al.**
- Welfare and resting behaviour of group-housed horses kept in loose housings: on farm assessment using the AWIN welfare protocol and time budget evaluation* **p. 32**  
**Humbel M. et al.**
- 2'-Fucosyllactose ameliorates inflammatory bowel disease by modulating gut microbiota and promoting MUC2 expression* **p. 33**  
**Yao Q. et al.**
- A systems immunology approach reveals distinct roles of genetic and non-genetic factors in shaping variation of immune responses in cattle* **p. 33**  
**Li S.F. et al.**
- Zebrafish embryos as alternative model to study intoxication by Acer pseudoplatanus toxins: Hypoglycin A, Methylenecyclopropylglycine and Methylenecyclopropylacetate* **p. 34**  
**Wouters C. et al.**
- Development of a Systems Immunology Approach to Explore Factors Influencing Vaccination Response in Belgian Blue Cattle* **p. 34**  
**Darimont C. et al.**

# Posters

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## Veterinary public health

- 1 *Phytates in cereals and legumes: comparison of archeological plant remains from Graeco-Roman Egypt with their modern counterparts from various origins*  
**Ali A. et al.** p. 39
- 2 *Establishment of a COVID-19 murine model using the artificially mutated-adapted variant SARS2-N501Y MA30*  
**Callens J. et al.** p. 39
- 3 *Screening of carbapenemase-producing *E. coli* and *K. pneumoniae* in the aquatic environment and characterization by antibiogram, real-time PCR, immunochromatographic tests and whole genome sequencing*  
**Crettels L. et al.** p. 40
- 4 *Detection of *Brucella* spp. in lung nematode larvae from small cetaceans: a potential new transmission route?*  
**De Trez M. et al.** p. 40
- 5 *Implementing AI in digital histopathology: towards quantitative analysis and machine learning integration in diagnostics and research*  
**de Meeûs C. et al.** p. 41
- 6 *Stability and lytic activity assessment in milk of bacteriophages targeting *Staphylococcus aureus* causing bovine mastitis*  
**Diderich J. et al.** p. 41
- 7 *Study of the importance of the ORF63 expression products in the biology of Murid Herpesvirus 4 infection*  
**Gillard L. et al.** p. 42
- 8 *Consumption of secondary peanut-based food products in Benin*  
**Iko Afé H. et al.** p. 42
- 9 *Characterization of conjunctival microbiota in healthy people or people suffering from ocular disease*  
**Kamdougha H. et al.** p. 43
- 10 *Deciphering how gammaherpesvirus infection modulates the development of pro-fibrotic responses in the lung using multispectral cytometry and imaging technologies*  
**Kempen LJAP. et al.** p. 43
- 11 *Comparative genomic analysis of five new *Pseudomonas aeruginosa* phages*  
**Leonard C. et al.** p. 44
- 12 *Effect of a prolonged interval between exsanguination and evisceration on the microbiological contamination of carcasses following on-farm slaughtered cattle*  
**Maldague A. et al.** p.44
- 13 *Influence of gut microbiota on the growth and the gene expression of *Clostridioides difficile* in an in vitro co-culture model*  
**Martinez E. et al.** p. 45
- 14 *Enterotoxin gene and toxin production profiles of *Staphylococcus aureus* strains isolated from artisanal cheeses in Belgium*  
**Minutillo R. et al.** p. 45

<b>15</b>	<i>Survey on consumer preference for fish products consumption in Cambodia</i> <b>Mom V. et al.</b>	<b>p. 46</b>
<b>16</b>	<i>Analysis of the neurotoxic <math>\beta</math>-ODAP and its non-toxic <math>\alpha</math>-isomer in modern and archaeological grasspea remains using LC-MS/MS</i> <b>Mustafa A. et al.</b>	<b>p. 46</b>
<b>17</b>	<i>Development and validation of a new bovine teat apex epithelial cell (bTAEC) line</i> <b>Rota G. et al.</b>	<b>p. 47</b>
<b>18</b>	<i>Optimization of a microscopic dissection protocol of the intestine of zebrafish larvae for targeted screening and molecular analyses of the intestine</i> <b>Schiffers A. et al.</b>	<b>p. 47</b>
<b>19</b>	<i>Risk factors related to peanut production and processing in southern Benin</i> <b>Sogbossi Gbètokpanou C. et al.</b>	<b>p. 48</b>
<b>20</b>	<i>Effect of heat treatment on physicochemical, anti-nutritional, nutritional parameters and the colour of plantain fruit flours</i> <b>Takam Ngouno A. et al.</b>	<b>p. 48</b>
<b>21</b>	<i>Sampling and germline differentiation of avian mesenchymal stem cells: towards a relevant approach for genetic resources preservation?</i> <b>Vandenhove B. et al.</b>	<b>p. 49</b>
<b>22</b>	<i>Label Free Quantification of Milk Oligosaccharides from Different Mammal Species</i> <b>Yao Q. et al.</b>	<b>p. 49</b>

---

## Sustainable livestock production

23	<i>Potential of grass silage as a source of nutrients in poultry production</i> <b>Hamin et al.</b>	p. 53
24	<i>Characteristics of Rabbit Farming in Southern Benin</i> <b>ASSOUMA D. et al.</b>	p. 53
25	<i>Genetic Diversity of the Local Zebu Population in Benin</i> <b>Bio Seydou K et al.</b>	p. 54
26	<i>Genomic Tapestry Unveiled: A Journey into the Rich Diversity and population Structure of Xinjiang's Indigenous Sheep Populations in China</i> <b>Chao F. et al.</b>	p. 54
27	<i>Effects of Rumen-Protected Methionine supplementation on the fertility of lactating dairy cattle</i> <b>Kerbache I. et al.</b>	p. 55
28	<i>Survey on the consumption of milk and dairy products in the rural community of the Laghouat region in Algeria</i> <b>Laouadi M. et al.</b>	p. 55
29	<i>Consumption of milk and dairy products in urban region of Laghouat in Algeria</i> <b>Laouadi M. et al.</b>	p. 56
30	<i>Olive pomace: which impact on dairy cows' milk production and quality? (a systematic review)</i> <b>Merad I.A. et al.</b>	p. 56
31	<i>Characterization of the Hamra sheep breed in western Algeria</i> <b>Ould Ouelhadj H. et al.</b>	p. 57
32	<i>Using Artificial Intelligence to analyse cattle facial expressions and detect pain</i> <b>Rives A. et al.</b>	p. 57
33	<i>Effects of incorporating 3% and 6% nettle powder on zootechnical performance, oxidative status and physicochemical characteristics of commercial turkey meat</i> <b>Sadoudi A.. et al.</b>	p. 58
34	<i>Modulation of growth performance in Japanese quails by red pepper "Capsicum annum"</i> <b>Saidj D. et al.</b>	p. 58
35	<i>Socio-economic challenges of women's empowerment in the rabbit sector in southern Benin</i> <b>Karim Youssao D. et al.</b>	p. 59

---

## Comparative veterinary medicine

- 36 *Computed tomographic appearance of sinus pneumocele in two horses*  
**Charles A et al.** p. 62
- 37 *Reversible congenital bilateral laryngeal paralysis (grade IV) in a foal*  
**De Maré L. et al.** p. 62
- 38 *Ultrasonographic and CT-scan findings of splenic active chylous effusion in a cat with chylothorax associated with metastasized splenic angiosarcoma*  
**Roels E. et al.** p. 63
- 39 *Feasibility of US-guided cervical intervertebral disc injection to aid in intraoperative identification of herniation sites in dogs*  
**Ficherouille J. et al.** p. 63
- 40 *Diagnosing American Bison pregnancy: comparison between rectal palpation associated with ultrasonography, assay of Pregnancy Associated Glycoproteins and of steroids in feces or serum*  
**Frisée V. et al.** p. 64
- 41 *VET&PHARM pilot project: A case study focusing on the perceived value of a pilot seminar on interprofessional communication for students in Pharmacy and Veterinary Medicine in the rational delivery of drugs for veterinary use*  
**Gaspar A. et al.** p. 64
- 42 *Feasibility and reproducibility of 2d-shear wave elastography in evaluating meniscal stiffness in horses*  
**Guerra G. et al.** p. 65
- 43 *Longitudinal changes in fecal microbiota during hospitalization in horses with different types of colic and association with survival*  
**Loublier C. et al.** p. 65
- 44 *Thymic Carcinosarcoma With Melanocytic Differentiation In A Dog*  
**Pollet V. et al.** p. 66
- 45 *Ureteral diverticula as incidental findings in a 14-year-old dachshund*  
**Soliveres E. et al.** p. 66
- 46 *Histopathological characteristics of hoof lamellae of horses and donkeys with severe naturally occurring laminitis*  
**Storms N. et al.** p. 67
- 47 *Distal sagittal forelimb conformation in young Walloon sport horses: radiographic assessment and its relationship with osteochondral fragments*  
**Van Cauter R. et al.** p. 67
- 48 *Evaluation of pelvic and head movement asymmetries at trot in young horses and their correlation with intended use and suggestive forelimb conformation*  
**Van Cauter R. et al.** p. 68



## **Short talks**

### IL-4 receptor- $\alpha$ signaling regulates lung macrophages during helminth coinfection resulting in enhanced gammaherpesvirus permissiveness

Petrellis G.,<sup>1</sup> Rolot M.,<sup>1</sup> Preure A.,<sup>1</sup> Watthieu C.,<sup>2,3</sup> Chetty A.,<sup>4</sup> Chariot A.,<sup>2,3,5</sup> Horsnell W.,<sup>4,6</sup> Dewals B.G.<sup>1</sup>

1.Fundamental and Applied Research in Animals and Health (FARAH), Parasitology and Immunology-Vaccinology, ULiege ; 2.Interdisciplinary Cluster for Applied Genoproteomics (GIGA), ULiege ; 3.Laboratory of Medical Chemistry, GIGA Stem Cells, ULiege; 4.Institute of Infectious Disease and Molecular Medicine, Division of Immunology, University of Cape Town, South Africa ; 5.Walloon Excellence In Life Sciences And Biotechnology (WELBIO), Belgium. 6Institute of Microbiology and Infection, University of Birmingham, UK  
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Parasite nematodes migrating through the lung could condition the immune response to subsequent respiratory viral infections. Comparison of innate immune cell populations in the lung of *Nippostrongylus brasiliensis* (Nb)-infected BALB/c and C57BL/6 mice revealed pronounced phenotypic changes in macrophages that were associated with an increased type 2 airway inflammation in C57BL/6 mice. These changes consisted of a disappearance reaction of SiglecF<sup>+</sup> alveolar macrophages (AlVMs) and recruitment of CD11b<sup>+</sup> macrophages. Using *Ms4a3<sup>TdT</sup>* reporter mice, we demonstrated the monocytic origin of CD11b<sup>+</sup> macrophage. Using *in vivo* antibody-mediated IL-13 blockade and WT:*Il4ra*<sup>-/-</sup> bone marrow mixed chimeras, we found that IL-4/IL-13 macrophage signaling was required for the observed phenotypic changes during Nb infection. When Nb-exposed C57BL/6 mice were infected with murid gammaherpesvirus 4 (MuHV-4), we observed increased levels of acute replication in the lung compared to previously naïve mice. MuHV-4 has a natural tropism for AlVMs *in vivo* and we observed the increased permissiveness was restricted to AlVMs and monocyte-derived macrophages in coinfecting mice. Interestingly, intra-tracheal administration of recombinant IL-4 or IL-13 complexes reproduced the observed macrophage changes post Nb infection and the enhanced permissiveness to MuHV-4. Finally, airway macrophages isolated from Nb-infected C57BL/6 mice were more permissive to MuHV-4 infection *ex vivo* and IL-4 and IL-13 could potentiate their permissiveness to MuHV-4. In conclusion, we propose that type 2 inflammation, during Nb infection, causes phenotypical and functional *Il4ra*-dependent restructuring of the airway and lung macrophages, increasing their permissiveness to gammaherpesvirus coinfection.

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### Helminth exposure protects against pneumovirus infection in an IL-4Ra-independent and monocyte dependent manner

Katsandegwaza B.,<sup>1</sup> Rolot M.,<sup>1</sup> Dougall A.M.,<sup>1</sup> Dewals B.G.<sup>1</sup>

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Helminths have developed a reputation for being able to regulate bystander inflammatory disorders and the mechanisms for how they carry this out are beginning to be elucidated. In this study, we investigated the impact of helminth infection on immune control in a model of lethal viral pneumonia. BALB/c mice were infected with *Nippostrongylus brasiliensis*, and at either 6 or 35 days after *N. brasiliensis* infection, mice were intranasally infected with a wild-type pneumonia virus of mice (PVM, strain J3666) or a recombinant virus expressing the luciferase gene (PVM-luc) to track viral replication over time by *in vivo* imaging. PVM belongs to the *Pneumoviridae* family and is a murine model for the closely related human respiratory syncytial virus (hRSV), a respiratory virus of medical significance in neonates, geriatrics, and immunosuppressed patients. Based on daily weight changes and viral bioluminescence, we demonstrated that prior exposure to helminths protects against PVM-induced disease. Interestingly, protection was independent of IL-4Ra signaling, as both wild-type and IL-4Ra<sup>-/-</sup> mice were equally protected after helminth exposure. *N. brasiliensis*-induced protection was associated with reduced neutrophils in the airway after viral challenge and heightened Ly6C<sup>+</sup>CD64<sup>+</sup> monocyte recruitment to the lung. Further investigations are needed to determine how exposure to *N. brasiliensis* can control viral replication and/or regulate PVM-associated detrimental inflammation, as well as determine the role of Ly6C<sup>+</sup>CD64<sup>+</sup> cells in *N. brasiliensis*-induced protection.



### Modulation of effector memory T lymphocyte responses to vaccination by helminth infections

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Gastrointestinal helminth infections are a recurring issue in our pets and livestock, impacting viral coinfection and vaccination responses. Whereas most vaccines against viral diseases are designed to induce neutralizing antibodies, the humoral response is in general not sufficient to provide long term protection. However, eliciting an appropriate T cell-mediated response is important for long-lasting immunity. Modulation of the T cell mediated immune response could explain a lower efficacy of vaccination during parasitic worm infection. We used *Heligmosomoides polygyrus* (Hp) and a recombinant Vesicular Stomatitis virus (rVSV) vector as experimental mouse models to investigate how persistent helminth infection affects the establishment of effective memory T responses to vaccination. Mice were infected with Hp before intramuscular vaccination with rVSV-GP-OVA expressing the glycoprotein GP1 of LCMV and chicken ovalbumin. In addition, to investigate the antigen-specific response to vaccination, a recombinant strain of the murid gammaherpesvirus 4 expressing the ovalbumin antigenic peptide (MuHV-4-OVA) was used to recall the OVA-specific memory CD8<sup>+</sup> T cells. A significant systemic reduction in the number of total CD8<sup>+</sup> T cells and antigen-specific CD8<sup>+</sup> T cells was observed after helminth infection. In addition, a respiratory challenge of vaccinated mice with a Murid gammaherpesvirus 4 (MuHV-4) strain expressing the same antigenic ovalbumin peptide revealed a less effective recall of specific memory CD8<sup>+</sup> T lymphocytes in mice infected with Hp. The mechanisms remain to be investigated to understand how parasitic infection modulate the efficacy of vaccination.

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### Neutrophil extracellular traps release following exploratory laparotomy in horses

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Background: Excessive neutrophil activation is associated with poor outcome in surgical colic. Neutrophil extracellular traps (NETs) contribute to the pathogenesis of asthma, endometritis, recurrent uveitis, synovitis, and peritonitis in horses. Yet, NETs have not been studied in systemic inflammation or in colic surgery. Objective: To study the release of NETs following exploratory laparotomy in comparison to elective surgeries. Animals: 7 horses submitted to exploratory laparotomy for non-strangulated small intestine obstruction compared to 23 horses anesthetized for elective procedures. Methods: Free myeloperoxidase (MPO) and MPO bound to NETs were quantified in plasma before and up to 24 hours after the surgical procedure under general anesthesia. The patented SIEFED technology and an ELISA sandwich were used to measure free active MPO and free total MPO, respectively. The same techniques, preceded by histone-DNA complexes capture using anti-histone H3 antibodies, were applied to measure MPO bound to NETs. Results: Neither free MPO nor MPO bound to NETs were significantly affected by general anesthesia in horses undergoing elective procedures or colic surgery. However, levels of active MPO bound to NETs were higher in horses submitted to exploratory laparotomy at each time point. Conclusion: NETs release may contribute to the clinical presentation in horses undergoing colic surgery.

### **Beyond 'On/Off': understanding the gradual nature of hypoglycin A poisoning in atypical myopathy**

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**Introduction:** Atypical myopathy (AM) is a potentially lethal rhabdomyolysis syndrome in horses grazing near *Acer pseudoplatanus* trees. Hypoglycin A (HGA) and its metabolite, methylenecyclopropylacetyl-carnitine (MCPA-carnitine), serve as diagnostic markers for AM. This study examines the limitations and clinical importance of these blood markers.

**Materials and Methods:** Blood samples were collected between 2016 and 2020 from horses with clinical signs of AM (diseased horses), their healthy pasture companions (cograzers), and a control group of healthy grazing horses with no detectable HGA or MCPA-carnitine in their blood. HGA levels were quantified using aTRAQ® kit and tandem mass spectrometry, and MCPA-carnitine was determined using liquid chromatography and mass spectrometry. Free carnitine and 21 acylcarnitines were measured in serum using mass spectrometry. Partial Least Squares regressions (PLS) compared acylcarnitine profiles.

**Results:** 245 sera were analysed, with higher HGA and MCPA-carnitine in diseased horses compared to co-grazers ( $p < 0.0001$ ). Diseased horses had higher free carnitine and most acylcarnitines, except C18, compared to control horses. PLS analysis separated "diseased horses" from other groups, while co-grazers displayed varied profiles, some resembling diseased horses and others controls. Co-grazers had intermediate levels of free carnitine and acylcarnitines.

**Conclusions:** Subclinical horses with detectable HGA and MCPA-carnitine, though outwardly healthy, showed elevated serum acylcarnitine profiles, indicating metabolic disturbances akin to AM cases. This study challenges the binary nature of HGA poisoning, suggesting it results from gradual alterations, including fatty acid metabolism.

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### **In-vitro/in-vivo characterization of phage resistant *E. coli* K1 and impact on Bacterial Fitness and Biofilm Production**

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Understanding bacterial phage resistance mechanisms has implications for developing phage-based therapies. This study aimed to explore human and avian *Escherichia coli* K1 isolates' resistance to K1\_ULINTec4, a K1-dependent bacteriophage. Resistant colonies were isolated from two different strains (APEC 45 and C5), both previously exposed to K1\_ULINTec4. Genome analysis and several parameters were evaluated, including growth capacity, phage adsorption, phenotypic impact at capsular level, biofilm production and virulence in the in-vivo *Galleria mellonella* model. One of the resistant isolates exhibited a significantly slower growth rate suggesting the presence of a resistance mechanism altering its fitness. Comparative genomic analysis revealed insertion sequences at various capsular gene sites. In addition, antigenic tests targeting the K1 capsule showed a very low positive reaction compared to the control. Nevertheless, microscopic images of resistant strains revealed the presence of capsules with a clustered organization and biofilm assessment showed increased biofilm production compared to the original strains. In the *G. mellonella* model, larvae infected with phage-resistant strains showed better survival rates than larvae infected with phage-sensitive strains. In conclusion, a phage resistance mechanism was detected at the genomic level, had an impact on capsular expression, biofilm production and was able to decrease the virulence of *E. coli* K1 in-vivo.

### Towards a new, glyco-humanized rabbit line

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Humans lack Neu5Gc and αGal moieties on glycosylated proteins due to mutations in the enzymes CMAH and GGTA1, respectively. For a variety of reasons, rabbits produce exquisitely effective antibodies that are suitable for use as human therapeutics. However, as they display these Neu5Gc and αGal epitopes, a strong humoral response is elicited upon injection in humans, which reduces clinical efficacy and shortens half-life. In this context and in accordance with the scope of our University, FEDER-backed EPI-SHIELD initiative, we aimed at inactivating CMAH and GGTA1, relying on the CRISPR/Cas9 technology. Six-month old, superovulated does were sacrificed and their zygotes harvested ~14 hours after mating. A mix of 2 ribonucleoproteins, each made of Cas9 and a specific guide-RNA, was injected in the pronucleus, and injected embryos were either cultured for 5 days (up to the blastocyst stage) or transferred in the oviducts of a synchronized, pseudopregnant doe. High proportions of five-day old blastocysts displayed indels at expected locations, with success rates of ~43 (3/7) and ~71% (5/7) for GGTA1 and CMAH, respectively. After transfer, a litter of 4 kits was born. Among the 4 kits, the CMAH of one is expected to be inactivated, due to insertion of a single G generating a premature stop codon.

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### MRI appearance of medial subchondral bone injuries of the proximal phalanx glenoid in Warmblood horses

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#### Introduction/purpose

There is growing evidence that medial glenoid subchondral bone (SCB) damage occurs in the proximal phalanx (P1) in Warmblood horses. This retrospective study aims to describe the magnetic resonance imaging (MRI) appearance of these lesions in a clinical case series examined in a standing low-field MRI unit.

#### Methods

Horses with MRI evidence of medial glenoid SCB injury of P1 were included. Lesions were characterized according to their configuration, including signal and extent. Concurrent lesions of the metacarpal condyle and periarticular soft tissues were recorded, as well as follow-up data when available.

#### Results

Ten horses (12 forelimbs) met the inclusion criteria. MRI changes included SCB plate thickening, trabecular sclerosis and enlarged vascular channels (12/12); bone marrow lesion (10/12), SCB resorption (10/12), and periosteal reaction (8/12). Distribution of SCB resorption in the transverse plane was linear (6/10), or ill-defined (4/10). All limbs had concurrent metacarpal condyle abnormality, osteophytosis and joint effusion. At recheck MRI (ranging 8 weeks to 2 years from initial examination), SCB lesions were static (3/6), improved (2/6) or worsened (1/6). One horse encountered a comminuted fracture of the affected P1 18 months after the last MRI examination.

#### Discussion/conclusion

The MRI appearance of medial glenoid SCB injuries of P1 in this case series is consistent with chronic bone overload or stress injuries, as observed in CT<sup>2</sup>. The linear configuration of some lesions suggests short incomplete stress fractures. Medial SCB overload may play a role in the development of P1 fractures and possibly be prodromal for catastrophic injury.

### **In Vitro Characterization And Preliminary Efficacy Assessment In *Galleria Mellonella* Larvae Of Four Newly Isolated Bacteriophages Targeting *Aeromonas Salmonicida***

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The Gram-negative bacteria *Aeromonas (A.) salmonicida* is a primary fish pathogen that causes furunculosis in salmonids as well as septicemia in a variety of fish. In one hand because this disease is responsible for significant losses in salmonid production worldwide and in the other hand because of the frightening tendency of this bacteria to exhibit antimicrobial (multi)resistances, phage therapy could represent a leading alternative to treat this infection in aquaculture. The aims of this study were to create a collection of *A. salmonicida* strains, isolate phages targeting these strains, phenotypically and genomically characterize these newly isolated phages and finally assess their potential for phage therapy in the preliminary *in vivo* model of *Galleria (G.) mellonella* larvae. Four new phages active against *A. salmonicida* were isolated from water samples collected in fish farms and natural aquatic environments in southern Belgium. Genomic analysis showed that 3 of these phages, named vB\_AsaM\_ULASA2 (170,823bp), vB\_AsaM\_ULASA3 (164,381bp) and vB\_AsaM\_ULASA4 (171,205bp), belong to the *Straboviridae* family while vB\_AsaM\_ULASA1 (47,813bp) stay in the unclassified part of the *Caudoviricetes* class. All 4 presented a myovirus morphotype. Fourday efficacy experiments in the preliminary *in vivo* model of *G. mellonella* larvae showed that 3 of these 4 phages were responsible for a significant extension in the larval survival time at the 2 treatment doses tested (MOI 10 and 100). In light of these results, these phages targeting *A. salmonicida* could represent potential new candidates for the development of antifurunculosis phage treatments in aquaculture.

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### **Bacteriophage Efficacy Assessment for Decolonizing Hyper-Virulent *Klebsiella pneumoniae* K1 ST23 in the Intestinal Microbiota: An *In Vitro* Study using the SHIME® Model**

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*Klebsiella pneumoniae* is an opportunistic enterobacterium found in the nasopharyngeal and intestinal tracts, which has developed various antibiotic resistance mechanisms. In a hospital setting, the primary source of this pathogen is the intestinal microbiota, and early detection often proves challenging. Thus, preventive intestinal decolonization appears to be a viable solution to curb the spread and consequences of this pathogen, particularly using phages. In this context, this study aims to assess the effectiveness of phage vB\_KpnP\_K1-ULIP33 in decolonizing an intestinal microbiota colonized by a hyper-virulent ST23 *K. pneumoniae* with K1 capsule type (SA12), using the *in vitro* Simulator of Human Intestinal Microbial Ecosystem (SHIME). Although each replicate of the triplicate exhibited different implantations, no significant impact of phage and bacterial additions were observed on the microbiome in terms of short-chain fatty acid production, diversity parameters ( $\alpha$  and  $\beta$ ), and qPCR targeting different genera associated with chronic or acute bowel inflammation. However, the emergence of phage-resistant bacteria and potential biofilm formation prevent drawing definitive conclusions regarding the efficacy of phage ULIP33 alone as a decolonizer of SA12 in a simulated *in vitro* gastrointestinal model.

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### Potential transmission of *Brucella sp.* throughout respiratory parasites in Harbour porpoise

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Some marine mammals harbor *Brucella sp.*, emerging zoonotic bacteria. The exact spread of these bacteria in the marine environment remains unclear. Identifying the sources of human contamination risk is challenging. However, scientists propose vector-borne transmission through lungworms infecting cetaceans, based on *Brucella* presence in nematode intestines and uteri. In northern Europe, these parasites contaminated flatfish as intermediate hosts, consumed by porpoises and seals. To investigate vertical transmission, a protocol was developed to collect lungworm larvae (*Pseudalidae spp*) during small cetacean autopsies. Larvae were isolated from lung samples using NaCl 0.9% shaking, decantation, filtration, and centrifugation. Subsequently, they are manually pipetted one by one using a 10 µL pipette. *B. ceti* presence in larvae was detected via PCR on PBS-washed samples from animals previously PCR-positive in lung, spleen, central nervous system, pre-scapular, and tracheobronchial lymph nodes. Four freshly autopsied small cetaceans tested negative for *Brucella sp.* by PCR. Furthermore, in the two lung samples from two porpoises carrying *B. ceti* detected by PCR and immunohistochemistry, sample A20 5053 allowed the isolation of *Pseudalidae spp* larvae. In two porpoises with *B. ceti* detected, sample A20 5053 yielded *Pseudalidae spp* larvae. PCR showed *Brucella sp.* presence in these larvae by the 32 Ct. This result is confirmed by sequencing. This result supports vector-borne transmission in North Sea porpoises, but limitations prevent confirming exclusive internal *B. ceti* carriage in larvae. Vector-borne transmission doesn't apply to all cetaceans; some carry only parasites or *B. ceti*.

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### Neutrophil extracellular traps and active myeloperoxidase concentrate in lamellar tissue of equids with naturally occurring laminitis

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Laminitis is a pathology of the equine digit ultimately leading to a failure of the dermo-epidermal interface. Neutrophil activation was recognized both in SIRS-associated and endocrinopathic laminitis evidenced by the presence of myeloperoxidase (MPO). Neutrophil extracellular traps (NET) are released with neutrophil activation. This study investigates the presence and activity of MPO and NET-bound MPO in the lamellar tissue of equids with naturally occurring laminitis.

Samples of lamellar tissue of 5 horses and 5 donkeys with laminitis as well as 8 control horses were collected. Lamellar tissue extracts were submitted to ELISA and specific immuno-extraction followed by enzymatic detection assays to confirm the presence and activity of both MPO and NET-bound MPO. Lamellar sections were also immunohistopathologically stained for MPO and NET.

Analysis of lamellar extracts revealed that laminitis cases had significantly higher levels of total MPO concentration, MPO activity, and NET-bound MPO activity compared to control horses. Moreover, a strong correlation was identified between the activity of NET-bound MPO and the total MPO activity, suggesting that MPO activity predominantly originates from NET-bound MPO. Immunohistochemical staining showed that MPO and NET labelling in laminitis cases was primarily localized in the epidermal lamellae and in inflammatory infiltrates containing neutrophils.

This article constitutes the first confirmation of the presence and activity of MPO and NET-bound MPO in the lamellar tissue of horses and donkeys with naturally occurring laminitis. Targeting these substances may provide new treatment possibilities for this debilitating disease.



### **AIHV-1 infection causes oligoclonal expansion and activation of CD8<sup>+</sup> T lymphocytes resulting in bovine malignant catarrhal fever via phosphorylation of a viral transmembrane protein**

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Alcelaphine herpesvirus 1 (AIHV-1) is a member of the *Gammaherpesvirinae* subfamily and establishes asymptomatic latent infection in its natural host species, the wildebeest. Cross-species transmission to various ruminant species including cattle can occur, resulting in the induction of malignant catarrhal fever (MCF), a deadly peripheral T cell lymphoproliferative disease. Here, we first confirmed in the bovine species that AIHV-1 latency-associated gene expression is essential for persistent infection of CD8<sup>+</sup> T cells and MCF development. Next, we performed an in-depth characterization of peripheral CD8<sup>+</sup> T cells during bovine MCF. T cell receptor sequencing of both CDR3 $\alpha$  and  $\beta$  revealed oligoclonal expansion of CD8<sup>+</sup> T cells, and we observed severe transcriptomic and epigenetic changes in CD8<sup>+</sup> T cells using RNA-seq and ATAC-seq analyses. MCF was associated with significant enrichment of gene expression involved in proinflammatory cytokine signaling, cell cycle, TCR signaling, chromatin remodeling but reduced expression of genes involved in adhesion. We observed upregulation of *MKI67*, as well as effector molecules like *GZMA*, *GZMK* and *GNL1*. Whereas *TCF7*, *CCR7* and *CD226* were downregulated, exhaustion genes like *PDCD1*, *EOMES* and *TOX2* were upregulated in MCF, confirmed by analysis of open chromatin. Such unique MCF-related transcriptomic program was confirmed in clusters containing infected CD8<sup>+</sup> T cells by single-cell RNA-seq analysis. Analysis of the viral genome transcription identified viral genomic regions being expressed in infected bovine CD8<sup>+</sup> T cells, such as the region predicted to encode the gene A10. A10 encodes a transmembrane protein containing an immunoreceptor tyrosine-based activation motif (ITAM) and a SRC homology 3 domain (SH3), suggesting interaction with intracellular T cell signaling. We demonstrated that A10 is phosphorylated in T cells *in vitro* and affects T cell signaling. Impaired expression of A10 did not affect AIHV-1 replication *in vitro* but rendered AIHV-1 unable to induce MCF in the rabbit model. Furthermore, AIHV-1 expressing mutated forms of A10 devoid of ITAM and/or SH3 domains induced MCF with a significant delay compared to a wildtype virus, whereas A10 knock-in mutant unable to phosphorylate tyrosine residues resulted in the absence of MCF development. Overall, we provide a thorough description of CD8<sup>+</sup> T cell responses during MCF to uncover a novel mechanism explaining how AIHV-1 dysregulates T cell signaling leading to MCF.

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### **Depletion and withdrawal times of doxycycline in white leg shrimp (*Litopenaeus vannamei*) after oral administration**

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The aims of the study are to determine the depletion and withdrawal times of doxycycline in white leg shrimp (*Litopenaeus vannamei*) after feeding medicated feed once a day and twice a day for three consecutive days through oral administration at a dose of 20 mg/kg shrimp body weight. Shrimp muscle was collected on day 1 and day 3 during medication and day 1, 3, 7, 14 and 21 after stopping medication. Seven days after stopping administration, doxycycline residues of both two treatments were eliminated completely from shrimp muscle. Considering a maximum residue limit (MRL) of 50  $\mu\text{g}/\text{kg}$  (set by Japan), the withdrawal times was 44.9 degree-days (corresponding to 40.6 h at 26.5°C) and 197 degree-days (178 h at 26.5°C) for once and twice a day feeding medicated feed, respectively. However, at the MRL of 100  $\mu\text{g}/\text{kg}$  (set by EU), those numbers decreased to 27.3 degree-days (corresponding to 24.7 h at 26.5°C) and 141 degree-days (128 h at 26.5°C), respectively.

### **Prevalence and risk factors for *Mycoplasma* spp. positivity in cat blood donor units from Portugal, Spain and Belgium in 2022: retrospective study on 7573 blood donations from 4121 healthy donor cats**

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This retrospective study assessed the prevalence and risk factors for *Mycoplasma* spp. positivity in cat donor units from Portugal, Spain and Belgium. A private blood bank database of donations performed in 2022 was reviewed. Studied risk factors for *Mycoplasma* spp. positivity included age, sex, breed, blood type, geographic area, season, and retroviral co-infection. 7573 blood donations from 4121 donor cats from Portugal (n=4034, 97.9%), Spain (n=70, 1.7%) and Belgium (n=17, 0.4%) were studied. 212 Portuguese cats tested positive for *Mycoplasma* spp., with an estimated prevalence of 5.3%. Two cats in Spain had tested positive, whilst all Belgian cats were negative. The small sample sizes in these countries prevented robust prevalence estimation. Among positive Portuguese cats, 30 cats donated blood >1 time in 2022: 26 cats were negative first then later tested positive, 3 cats were positive on two occasions, and 1 cat was initially positive and later tested negative. Blood units from male cats were at higher risk for *Mycoplasma* spp. positivity (OR 1.9,  $P<0.001$ ). Increased risk was also observed for units that tested positive for FeLV (OR 2.9,  $P=0.0018$ ) and for donations performed in winter (OR 2.5,  $P<0.0001$ ). None of the other studied risk factors was associated with *Mycoplasma* spp. positivity. Conclusion: European cat blood donors displayed a low prevalence of *Mycoplasma* spp. with an increased risk in cats affected with FeLV and male cats. The seasonality for *Mycoplasma* spp. positivity remains to be explained. Positive *Mycoplasma* spp. results identified in previously negative donors emphasizes the importance of testing on every donation instead of annually.

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### **In Vivo Imaging Sheds Light on the Susceptibility and Permissivity of *Carassius auratus* to Cyprinid Herpesvirus 2 According to Developmental Stage**

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Cyprinid herpesvirus 2 (CyHV-2) is a virus that causes mass mortality in economically important *Carassius* spp. However, there have been no comprehensive studies into host susceptibility or permissivity with respect to developmental stage, and the major portal of viral entry into the host is still unclear. To help bridge these knowledge gaps, we developed the first ever recombinant strain of CyHV-2 expressing bioluminescent and fluorescent reporter genes. Infection of *Carassius auratus* hosts with this recombinant by immersion facilitated the exploitation of various in vivo imaging techniques to establish the spatiotemporal aspects of CyHV-2 replication at larval, juvenile, and adult developmental stages. While less susceptible than later developmental stages, larvae were most permissive to CyHV-2 replication, leading to rapid systemic infection and high mortality. Permissivity to CyHV-2 decreased with advancing development, with adults being the least permissive and, thus, also exhibiting the least mortality. Across all developmental stages, the skin was the most susceptible and permissive organ to infection at the earliest sampling points post-infection, indicating that it represents the major portal of entry into these hosts. Collectively these findings provide important fundamental insights into CyHV-2 pathogenesis and epidemiology in *Carassius auratus* with high relevance to other related economically important virus-host models.

### **Optimizing heat stress detection in dairy cattle: leveraging datamining and unsupervised analyses to explore individual-level impact through behavior and meteorological factors**

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Dairy cows have various strategies for coping with heat stress, including a change in behavior. The aim of this study was to explore, via unsupervised learning, the behavior of cows and to relate it to their environment using the comprehensive climate index. A total of 8,928 observations, associated with behaviors known to be influenced by heat stress, per cow over the month of August 2020 were recorded from a herd of 28 grazing cows. The CCI was established for each day using radiation, relative humidity, ambient temperature and wind speed. Hopkins statistic was used to measure the clustering potential of the observations, with a value of 0.812 indicating strong clustering of the data. A principal component analysis was performed to determine the number of groups to be formed based on the data. Visualization of dimensions 1 and 2, which explain 58.81% of the variability in the data. The unsupervised learning method of k-means partitioning was implemented in order to form 4 distinct groups and outliers in each group were removed using the Mahalanobis distance method based on a p-value of less than 0.05. The interpretation of the groups was based on the average of the behaviors. A correlation of 0.44 was established between the first group and the increase of CCI. The potential prospects of this study are to provide a better understanding of the individual responses of cattle to heat stress and to improve health management. In addition to an approach based on behavior and not on an index, future predictive models could subsequently be implemented to enable early adaptation in the face of events unfavorable to animal welfare.

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### **Welfare and resting behaviour of group-housed horses kept in loose housings: on farm assessment using the AWIN welfare protocol and time budget evaluation**

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The welfare of 156 horses kept in bare outdoor areas, with and without a Track design, was evaluated during the winter 2020 in Belgium using the AWIN welfare protocol (4 environment-based, 22 animal-based indicators) and a time budget evaluation. Data analysis included descriptive analysis, non-parametric tests and regression ( $p < 0.05$ ). Assessed horses had sufficient access to water and forage and a mean body condition score of  $3.2 \pm 0.5$  (scale of 5), nearly 30% were slightly overweighted. All horses could move freely and interact with conspecifics. The time budget indicated that a Track design and more surface significantly increased movement (walk and trot), but a Track design also increased alert posture, a potential stress indicator. Rest might be a concern, with insufficient shelter area (53.8%) and lack of bedding (75%) as a dry soft substrate is necessary to lie down and reach REM sleep. Episodes of excessive daytime sleepiness (EDS) were observed in 7 horses, some to the point of partial collapse, with less lying area provided, the more likely to observe EDS episodes (Spearman:  $S=1021.7$ ,  $\rho = -0.82$ ,  $p\text{-value} < 0.001$ ). Wounds, coughing and lameness were rare. Alopecia were present in 50.6% of horses. Serous ocular and nasal discharges were seen in 37.2 and 14.1% of horses, which is higher than in pastured horses. Stereotypy were rare (1.3% of horses). Horses showed positive human-animal relationships with high scores on approach (98.1%) and forced (87.8%) tests. In conclusion, outdoor group housings were adequate for the horses even during the winter, providing adequate health and covering basic behavioural needs. An appropriate resting area should be provided.



### **2'-Fucosyllactose ameliorates inflammatory bowel disease by modulating gut microbiota and promoting MUC2 expression**

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Gut microbiota dysbiosis, together with goblet cells dysfunction has been observed in ulcerative colitis cases. 2'-fucosyllactose (2'-FL) is the most abundant member in human milk oligosaccharides (HMOs) and is known for its multiple beneficial functions. In this study, 2'-FL was orally administered to C57BL/6J mice daily (400 mg/kg b.w.) for 21 d, and 5% dextran sulfate sodium (DSS) was used to induce the colitis in the last 7 days. Meanwhile, fecal microbiota transplantation (FMT) was conducted to test the roles of gut microbiota in the remission of colitis by 2'-FL, and bacteria alteration was analyzed through 16S rRNA sequencing. The results showed that the DSS+2'-FL mice were found to have a slower rate of weight loss, lower disease activity index (DAI) scores, and longer colon lengths than the DSS group, so as in the FMT recipient mice which received fecal microbiota from the DSS+2'-FL group. In addition, the data revealed that 2'-FL relieved the disorder of DSS-induced gut microbiota, as well as altered mucin-utilizing bacteria. PAS and immunofluorescence staining showed that 2'-FL treatment promoted the recovery of goblet cells and enhanced MUC2 and NLRP6 expression, which was also observed in the FM (D +2'-FL) group. Moreover, NLRP6, which is a potential negative regulator for TLR4/MyD88/NF- $\kappa$ B pathway, was upregulated by 2'-FL in colon tissue. In conclusion, this study suggests that 2'-FL ameliorates colitis in a gut microbiota-dependent manner. The underlying protective mechanism associates with promoting the recovery of goblet cells number and MUC2 secretion.

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### **A systems immunology approach reveals distinct roles of genetic and non-genetic factors in shaping variation of immune responses in cattle**

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One of the major challenges facing agriculture is to increase its production capacity to meet global demand while minimizing the reliance on synthetic inputs like antibiotics. These observations are particularly true for beef cattle breeding, notably Belgian White Blue (BWB) cattle which is the most muscular breed in the world. Historically, the selection process for these animals has prioritized production traits, often at the expense of health and disease resistance. However, recent studies in humans indicate that a significant portion of the variability of immune responses can be explained by genetic determinants. Applied to BWB, this indicates that there could be a considerable opportunity to increase the resistance of those animals to infectious diseases. Using systems immunology, we explored genetic and environmental factors driving immune variation in BWB. While immune variation was largely influenced by non-genetic factors such as seasonality, we identified at least six loci with major effect on distinct immunophenotypes by genome-wide association studies. Moreover, a computational predictive model based on these genetic data was able to forecast cytokine responses to immune stimulations, offering new health management avenues. Taken together, our data have established a resource for understanding immune variability in ruminants that may pave the way to select animals with improved immunity. In the future, integrating such approaches across species could advance not just animal health but also our understanding of mammalian immune responses in general.

### **Zebrafish embryos as alternative model to study intoxication by *Acer pseudoplatanus* toxins: Hypoglycin A, Methylene cyclopropylglycine and Methylene cyclopropylacetate**

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Hypoglycin A (HGA) and methylenecyclopropylglycine (MCPrG) are protoxins synthesised by plants of the *Sapindaceae* family notably *Acer pseudoplatanus*. *A. pseudoplatanus* poisoning is a recognised emerging pasture-associated intoxication leading to atypical myopathy (AM) in equids. Once ingested the protoxins are metabolised into toxic compounds including methylenecyclopropylacetate-CoA (MCPA-CoA) that impairs lipid metabolism and induces a severe rhabdomyolysis syndrome. Currently, there are limited laboratory models for studying AM under controlled conditions, with few existing cell culture models utilising MCPA alone. In this study, zebrafish embryos were used to evaluate the toxicity of AM protoxins (*i.e.* HGA and MCPrG) and the toxic metabolite MCPA. Zebrafish embryos of 1 day post fertilisation were individually exposed during 72 hours to several concentrations of HGA, MCPrG and MCPA. The experiment was performed in triplicate using 20 larvae per concentration, E3 medium and 3,4 dichloroaniline as negative and positive control, respectively. Every 24 hours, four endpoints based on OECD guidelines were recorded as indicator of lethality as well as sublethal effects to determine the LC<sub>50</sub> and EC<sub>50</sub> using a four parameters log-logistic model. Markedly, MCPrG did not induce mortality or sublethal effects, even after 72h at 5 mM. Conversely, HGA-induced toxicity appeared after 48 hours with mainly a reduced heartbeat. After 72h of intoxication, the LC<sub>50</sub> and EC<sub>50</sub> of MCPA were 0.98 and 1.33 µM, respectively, while those for HGA were 1.66 and 2.07 µM. This study suggests that zebrafish embryos could be used as an alternative intoxication model for studying HGA toxicity, particularly as cell culture models did not exhibit cytotoxicity with this protoxin.

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### **Development of a Systems Immunology Approach to Explore Factors Influencing Vaccination Response in Belgian Blue Cattle**

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Response to vaccination varies significantly among individuals and is influenced by various factors, including genetic and environment. In particular, persistent viruses, such as herpesviruses, appear to profoundly impact the immune response of their host. While these factors have been largely studied in humans, nothing is known in domestic animals. Here, we investigated the effect of genetics and of Bovine Herpesvirus 4 (BoHV4) infection on the responses of calves to vaccination. Briefly, 227 Belgian Blue calves, aged 1 to 6 months and housed under uniform conditions at the Ciney cattle selection center, were vaccinated against *Mannheimia haemolytica*, BRSV, BPI3, and *Clostridium perfringens* ( $\epsilon$  and  $\beta$  toxins). Antibody levels were measured four weeks post-vaccination using commercial ELISA kits. BoHV-4 infection status was determined through serology and qPCR. Genotyping was conducted for all calves, and a genome-wide association study (GWAS) was performed to identify genetic factors associated with variation in the responses to vaccination. The findings unveiled significant variations, particularly in response to *Clostridium perfringens*  $\epsilon$  and  $\beta$  toxins vaccination, with BoHV4-infected calves exhibiting a more robust response. Moreover, the GWAS pinpointed a specific region on chromosome 23 linked to the response to *Clostridium perfringens*  $\epsilon$ -toxin. This region encompassed genes related to the TNF family and MHC classes I and III. Further research is essential to establish causal links between these identified factors and response to vaccination. Nevertheless, this study paves the way for a detailed understanding of immune response variability in Belgian Blue cattle.

# Posters



# **Veterinary Public Health**



### 1. Phytates in cereals and legumes: comparison of archeological plant remains from Graeco-Roman Egypt with their modern counterparts from various origins

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Cereals and legumes, including wheat, barley, pea, lentil, lupine, and grass pea, are considered the oldest domesticated crops. The nutritional quality of such crops is dictated by their antinutritional compounds, e.g. phytates. Phytate is a primary storage form of phosphorus in plants. The concern with phytates in animal nutrition is related to their interaction with proteins and minerals, which reduces their bioavailability resulting in major health issues. In this study, phytate levels were measured for the first time in archaeological remains of plants dating from the Graeco-Roman period in Egypt (Karanis) and compared to their modern equivalents. The (anti)nutritional composition of modern foods could have evolved, compared to ancient foods, particularly due to the Green Revolution. Determining the phytate content in ancient foods is a first step to learn more about the nutritional status of ancient crop populations compared to modern times. A colorimetric Wade assay was developed to quantify the total phytate in raw cereals and legumes. The method was validated, and the recovery % of phytic acid ranged between 87.2-108.8%, repeatability ranged from 2.9% to 7.3%, and intermediate precision was 10.5%. The phytate content found in modern samples (expressed in g of phytate per 100 g of the sample) was as follows: barley (0.76-1.04%), wheat (0.59-0.98%), grass pea (0.65-0.82%), lentil (0.45-0.83%), lupine (0.70-1.11%), and pea (0.61-1.01%). In ancient ones, the contents were 0.81, 0.97, 0.90, 1.01, 1.41, and 0.95% for barley, wheat, grass pea, lentil, lupine, and pea, respectively. To conclude, there were no significant differences between ancient and modern samples regarding phytate content.

### 2. Establishment of a COVID-19 murine model using the artificially mutated-adapted variant SARS2-N501Y MA30

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Until now, there was no preclinical model capable of recapitulating the severe human form of Covid-19. This absence has contributed to delaying the identification of the key mechanisms involved in this severe form. Recently, a genetically modified SARS-CoV-2 virus, made capable of binding to the mouse ACE2 receptor, appears to fill this void. Here, following on from our previous work with Sendai (Faisca et al., 2005) and PVM (Anh et al., 2006) viruses, and expecting to catch genetic determinants possibly underlying disease severity, we started a project aimed at dissecting the possible variation in responses to SARS-CoV-2 among mouse inbred lines. Our poster describes the very first results of this project. Said mouse-adapted virus, named SARS2-N501Y-MA30, was inoculated to a set of sex- and age-matched BALB/c, SJL and 129/Sv mouse lines in a set up designed to establish the dose of virus causing half of the infected cohort to reach one of the defined endpoints (formerly, the DL<sub>50</sub>). As expected, we found that BALB/c mice are indeed very susceptible to the modified SARS-CoV-2 virus and develop a severe form of the disease. On the contrary, the SJL line did not show noticeable symptoms except a minute weight loss after inoculation. Surprisingly, the 129/Sv line, which had always displayed the weakest resistance phenotype to respiratory viruses in the past, was found to be far more resistant than the BALB/c line. In the coming months, we want to screen a set of ~6-7 additional strains that are phylogenetically as distant as possible in an attempt to identify the possible genetic determinants of one or more Covid-19-resistant phenotypes.

## Posters

### 3. Screening of carbapenemase-producing *E. coli* and *K. pneumoniae* in the aquatic environment and characterization by antibiogram, real-time PCR, immunochromatographic tests and whole genome sequencing

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Antimicrobial resistance (AMR) is recognized by the WHO as one of the greatest threats to global health. Carbapenem resistance is a major concern, as this class of antibiotics, belonging to the  $\beta$ -lactam family, is used as a last resort molecule in hospitals in human medicine. Enteric bacteria, including *E. coli* and *K. pneumoniae*, from humans and animals can be released in aquatic environment and be a vector for AMR. This study focused on carbapenemase (CPE)-producing *E. coli* and *K. pneumoniae* in the aquatic environment, based on screening of 3 hospital continuums (Hospital-Wastewater Treatment Plant (WWTP)-receiving river), 20 surface waters and 29 bathing waters in 2022. No *E. coli* and *K. pneumoniae* CPE was isolated from bathing waters, while 24 *E. coli* CPE and 27 *K. pneumoniae* CPE were isolated from 6 of the 20 surface water samples and hospital continuums. Phenotypic tests including disk combination tests to identify the Ambler class, genotypic tests (real-time PCR) and immunochromatographic tests were used to characterize each isolate. Of the 24 *E. coli* CPE strains, 20 had the *bla*<sub>KPC</sub> gene (83.3%) and 4 the *bla*<sub>OXA</sub> gene (16.6%), while of the 27 *K. pneumoniae* CPE strains, the number of strains with CPE genes was: 11 *bla*<sub>KPC</sub> (40.7%), 9 *bla*<sub>OXA</sub> (33.3%), 1 *bla*<sub>VIM</sub> (3.7%), 1 *bla*<sub>NDM</sub> (3.7%) and 5 (18.5%) had another gene than those tested. The genomes of 11 *E. coli* CPE and 15 *K. pneumoniae* CPE were sequenced, and all possessed CPE genes - *bla*<sub>KPC-3</sub> (n=14), *bla*<sub>OXA-48</sub> (n=9), *bla*<sub>OXA-244</sub> (n=1), *bla*<sub>VIM-1</sub> (n=1) and *bla*<sub>NDM-5</sub> (n=1) - mostly coupled to ESBL-encoding genes. This study demonstrated that *E. coli* and *K. pneumoniae* CPE are disseminated in the Belgian aquatic environment.

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### 4. Detection of *Brucella* spp. in lung nematode larvae from small cetaceans: a potential new transmission route ?

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Cetaceans are frequently infected by *Brucella* spp., an emerging zoonotic bacterial pathogen, human cases of marine *Brucella* spp. infection being described. The cycle of the bacteria in the marine environment remains unclear. Identifying the sources of human contamination is challenging, direct contacts could be one way of infection as well as through the food. However, vector-borne transmission between cetaceans through lungworms has been suggested, based on *Brucella* spp. identification by immunohistochemistry in the uterus of nematode within airways of harbor porpoises (*Phocoena phocoena*). In Europe, these parasites contaminate fish as intermediate hosts of their lifecycle, fish being consumed by cetaceans and potentially by humans. During necropsies of cetaceans, samples have been collected as well as nematodes (*Pseudalidae* spp.) and larvae in airways. The first step was to detect by PCR the presence of *Brucella* spp. in tissues. In the second step, larvae were tested by PCR for the presence of *Brucella* spp.. All tested tissues from three porpoises and one common dolphin (*Delphinus delphi*) were negative. Larvae from one porpoise lung sample proved PCR positive. The genus *Brucella* spp. was confirmed by sequencing of the amplicon. Together with the detection of *Brucella* spp. by immunohistochemistry in nematodes in previous studies, the PCR-detection of *Brucella* in larvae strongly suggests that they might be involved in the transmission of *Brucella* between porpoises, warranting further investigations. As the *Pseudalidae* life-cycle includes fish species consumed by cetaceans but also by humans, carriage of *Brucella* spp. by larvae could in addition be of zoonotic importance.



## 5. Implementing AI in digital histopathology: towards quantitative analysis and machine learning integration in diagnostics and research

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Histopathological analysis of tissue specimens is a cornerstone in diagnostics and research. The traditional approach for histopathological analysis involves manual examination by a pathologist, which is time-consuming, with certain degree of subjectivity, intra- and inter-observer variability. To counteract this, transition to digital pathology integrating artificial intelligence (AI) has been made, because of its potential to enhance accuracy, efficiency, depth of analysis, and reproducibility, while minimizing the risk of error. It offers a range of different features facilitating and automating image analysis using machine learning and deep learning. Some examples include object detection and classification, cell count and positive cell detection, and even classification of cell types in an inflammatory process, which are critical tasks in histopathological image analysis. More complex analyses, such as tumor margin detection, and measuring the degree of fibrosis or necrosis can be performed as well, as AI can be trained to improve its performances. The aim of this presentation is to give an overview of how machine learning and deep learning can be integrated in research projects and diagnostic applications and to discuss the benefits of AI in histopathology, which include time savings, reduction of variability and the ability to extract relevant information from large-scale histopathological datasets.

## 6. Stability and lytic activity assessment in milk of bacteriophages targeting *Staphylococcus aureus* causing bovine mastitis

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Bovine mastitis is a major production disease in dairy cattle and complementary treatments to antimicrobials are urgently needed. Intramammary phage therapy is a promising approach but milk components can affect the phages properties. The objective of this study was to compare the stability and lytic activity of phages targeting *Staphylococcus aureus* in raw and pasteurized milk. A total of 28 bacteriophages previously isolated against *S. aureus* were spotted on 44 *S. aureus* strains isolated from bovine mastitis to evaluate the phage host range. The phage stability in milk was assessed after 6h incubation at 37°C. The lytic activity was established by inoculating milk with *S. aureus* and phages at a MOI of 1000 and titrating the phages at different timepoints to highlight their replication. The optimal MOI was assessed for each phage and used for further lytic tests where bacteria were titrated. A broad host spectrum was observed for 24 out of 28 phages. Stability analysis showed that all phages presented a decrease in titer which was significantly higher in raw milk. However, phages were still active in raw and pasteurized milk with stability rates of respectively 7% and 10%. Regarding the lytic activity, half of the phages were able to replicate in milk with a lower rate in raw milk. When using optimal MOI, bacterial reduction was observed. In conclusion, heat-sensitive components in milk alter the phages properties. Further tests in milk components should be performed to assess their effect on phages. The resistance of bacteria against phages should be investigated and the use of phage cocktails should be evaluated to circumvent the phage resistant bacteria emergence.

## Posters

### 7. Study of the importance of the ORF63 expression products in the biology of Murid Herpesvirus 4 infection.

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Gammaherpesviruses ( $\gamma$ HVs) are widespread viruses that cause lifelong infections in many mammalian species and represent a significant cause of diseases. Thus, in humans, Kaposi's sarcoma-associated herpesvirus (KSHV) is associated with several cancers and is therefore a major subject of research. Among the different constituents of the  $\gamma$ HVs virions, tegument proteins play major roles in viral cycle. In particular, a potential role in immune evasion has been proposed for the tegument protein encoded by KSHV open reading frame 63 (ORF63). However, KSHV study is limited by the absence of established animal model and by the lack of permissive cell lines. In this work, we used Murid herpesvirus 4 (MuHV-4), a phylogenetically related virus, to decipher the roles of ORF63 in  $\gamma$ HV lifecycle. Surprisingly, our study revealed the existence of two distinct MuHV4 pORF63 isoforms, a long one, and a short one. In order to investigate the specific functions of these two proteins during infection, we generated three different knock-out mutants; one expressing only the short form (STOP), one coding only for the long isoform (LIO) and one neither expressing the long nor the short isoform (double-STOP). While we previously showed that the long pORF63 isoform is essential for the migration of viral capsids toward the nucleus during entry, a comparative analysis of the different MuHV-4 ORF63 mutants revealed that in absence of ORF63 a dramatic growth deficit was observed *in vitro*. These results suggest therefore an important role for the short isoform of pORF63 and provides new insights into MuHV-4 lifecycle.

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### 8. Consumption of secondary peanut-based food products in Benin

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Worldwide, several studies reported aflatoxins and furan in peanut products. In Benin, peanut represent the second oilseed quantitatively produced after soybeans (177 876 tonnes in 2021), but the contribution of peanut to the exposure of Beninese to chemical contaminants has not been extensively assessed so far. This study aims at paving the way to exposure evaluation by firstly assessing the consumption patterns of five secondary peanut-based products. To achieve this goal, four hundred adult consumers were randomly surveyed during a face-to-face interview in six different municipalities of Benin using a digital semi-structured questionnaire. The results showed that most of the consumers were male (60%) and mainly belong to sociocultural groups Fon (58%), Adja (13%) and Goun (12%). Most (88%) of them received formal education (at least primary school) and were mainly either single (51.5%) or married (47.3%). They were aged between 18 and 95 years with 50% aged below or above 28 years with a mean body weight of 65.6 kg. No significant difference ( $p > 0.05$ ) was recorded between female and male regarding their age and body weight. Among these 400 interviewed consumers, 68.5%, 67.8%, 25.2%, 22.5% and 19.0% consumed caramelized peeled peanut (kohouncada), boiled peanut, peanut-based nougat, peanut ball (andou) and sugar-coated peanut (cacahuete), respectively. These products are consumed either alone, with another food or both. The daily consumption of these products ranged from 0.4 g to 354.8 g. These findings will be used for risk assessment study.

## 9. Characterization of conjunctival microbiota in healthy people or people suffering from ocular disease

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Ocular surface microbiota plays a significant role in maintaining eye homeostasis, and its alteration can contribute to the development of various ophthalmic diseases. The aim of this study was to investigate potential association between the ocular surface microbiota and two ophthalmic diseases: glaucoma and dry eyes disease. Conjunctival swab samples are obtained from both eyes of 32 healthy volunteers (n=64), 12 glaucomatous patients (G, n=24), 14 patients with dry eyes disease (DE, n=28) and 14 glaucomatous patients suffering also from dry eye syndrome (GDE, n=28). Using 16S ribosomal RNA gene amplicon sequencing of the V1-V3 region, we have performed a bioinformatic and biostatistic analysis using respectively Mothur tools and R language to elucidate the taxonomic composition of the microbiota and assess community structures and next to investigate correlations and differences between groups. Our data revealed a very diverse bacterial community with 560 different genera where *Corynebacteriaceae*, *Propionibacteriaceae*, and *Staphylococcus* spp. were predominant in all groups. Samples from DE group had significantly greater alpha-diversity compared to non-dry eye samples ( $P < 0.05$ ). Permutational multivariate analysis of beta-diversity variance showed a statistically significant differences in ocular surface microbiota between glaucoma versus non glaucoma group and between dry eye and non-dry eye group ( $P < 0.05$ ). Our findings expand previous studies which proved the domination of ocular surface microbiota by Gram positive bacteria and supports other research that displayed the association between ocular surface microbiota alteration and both glaucoma and dry eye diseases.

## 10. Deciphering how gammaherpesvirus infection modulates the development of pro-fibrotic responses in the lung using multispectral cytometry and imaging technologies

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Pulmonary fibrosis is a very complex multifactorial and ultimately fatal lung disease whose pathophysiology is not well understood and for which various risk factors have been identified, such as Epstein-Barr Virus. Previous research has shown an association between Epstein-Barr Virus, or its murine ortholog Murid herpesvirus-4 (MuHV-4), and exacerbated pulmonary fibrosis. Here, we aim to investigate the mechanisms of  $\gamma$ -herpesvirus-mediated exacerbation of bleomycin-induced lung fibrosis in C57BL/6 mice using complementary approaches such as flow cytometry and multispectral imaging. Sirius Red staining and quantitative assays showed that prior MuHV-4 infection led to an increase in collagen deposition at day 66 post-bleomycin, confirming the MuHV-4-triggered exacerbation. In addition, we have identified different polarisations of immune responses following the administration of bleomycin at early and late stages. One notable difference was the significant infiltration of activated memory CD8 T cells in both MuHV-4 infected groups. Lastly, using a 13-plex marker combination in a multispectral imaging technology, we were able to distinguish cell types based on (co-)expression of surface markers and visualise them for future analyses. Altogether, while many avenues remain to be explored, this work confirms the exacerbation phenotype of prior-MuHV-4 infection in the bleomycin-induced pulmonary fibrosis model. The results obtained so far support that the abundant infiltration of activated T cells, and especially CD8+ T cells within the airways and parenchyma, could have a deleterious effect on the development of bleomycin-induced fibrosis.

# Posters

## 11. Comparative genomic analysis of five new *Pseudomonas aeruginosa* phages

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The rise of multidrug-resistant *Pseudomonas aeruginosa* infections necessitates the exploration of alternative therapeutic options, such as bacteriophages. However, the safety and efficacy of phage therapy depend on selecting phages free from lysogeny. In this study we evaluated five lytic *Pseudomonas* phages previously isolated and characterized from wastewater samples in Belgium for their potential in combating *Pseudomonas* spp. strains. Host spectrum and efficiency of plating were assessed using about fifty *Pseudomonas* spp. strains isolated from different body site localizations and different animal species. The phages showed a wide spectrum of lysis only on *Pseudomonas aeruginosa*. Subsequently, the isolated phages were subjected to whole-genome sequencing to gain insights into their genetic makeup. The complete genomes of five lytic *Pseudomonas* phages were sequenced, revealing their genetic composition and classification. All phages were found to belong to the *Uroviricota* phylum and *Caudoviricetes* class. Specifically, two phages were classified under *Bruynoghevirus* genus (45118bp (base pair) and 45772bp), two under the *Nankokuvirus* genus (85265bp and 85231bp), and one under the *Pakpunavirus* genus (92476bp). Importantly, none of these phages carried lysogenic genes. The genomic analysis of these phages provided valuable insights into the presence of virulent genes necessary for infecting *Pseudomonas* species. These results suggest the potential use of these phages in the treatment of *Pseudomonas aeruginosa* infections. Further evaluation of their efficacy and safety through future in vivo tests is warranted before considering their application in the ear canals of dogs.

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## 12. Effect of a prolonged interval between exsanguination and evisceration on the microbiological contamination of carcasses following on-farm slaughtered cattle

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The uncertainties related to microbiological risks associated with an extended exsanguination-to-evisceration interval have resulted in a limited implementation of on-farm slaughter in some European countries. The microbiological standards for bovine carcasses in the European Union are currently governed by Commission Regulation (EC) No 2073/2005, which establishes microbiological process hygiene criteria applicable to aerobic colony count and *Enterobacteriaceae*. In this study, five carcasses of Holstein dairy cattle were bled but not eviscerated and sampled for *Enterobacteriaceae*, aerobic colony count, and *E. coli* up to 4 hours *post-mortem*. None of the samples exceeded the limit thresholds defined by Belgian health authorities for non-destructive swabbing method, and most of the samples were completely free of *E. coli* and *Enterobacteriaceae*. These preliminary results suggest that meat from cattle slaughtered on-farm and eviscerated up to 4 hours *post-mortem* does not appear to pose increased microbiological risks to human health. However, it is important to note that these conclusions should be substantiated by further analyses conducted during warmer months and on a more diverse sample set to explore the potential impact of factors known to influence microbiota composition.

### 13. Influence of gut microbiota on the growth and the gene expression of *Clostridioides difficile* in an *in vitro* co-culture model

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*Clostridioides difficile* is an anaerobic spore-forming Gram-positive pathogenic bacterium. The aim of this study was to analyze the impact of gut microbiota on *C. difficile* gene expression using co-culture *in vitro* model. In compartment "in", a nutritive matrix, bile salts and  $10^3$  UFC/ml of *C. difficile* spores were placed. In compartment "out", a nutritive matrix, bile salts and feces, if applicable, were placed. Three trans-well plates were realized: one without feces (WOF); one with feces from donor 4 (F04) and one with feces from donor 3 (F03). Total DNA and RNA were extracted. RNA seq analysis at 12h and at 24h were realized on the compartment "in" to characterize the transcriptome dynamic of this pathogen. RT-qPCR targeting *rnfG* (electron transport complex protein), *eutA* (ethanolamine ammonia-lyase reactivating factor), *eutB* (ethanolamine ammonia-lyase subunit *flaA* (electron transfer flavoprotein subunit alpha), *gluD* (housekeeping genes), *tcdB* and *tcdA* (toxins genes) were realized to confirm RNA seq results. The plate without feces was the control. The growth rate of *C. difficile* was similar in the three tested conditions. Differences were observed in gene expression in the 3 conditions. At the end of exponential phase (at 12h), when bacterial microbiota was present, the gene expression was lower in the categories of germination, sporulation, production of toxins and growth. In the middle of the stationary phase (at 24h), *C. difficile* set-in place iron metabolism genes to opposite the iron limitation. Between 12h and 24h, the gene expression was higher in the categories of germination and sporulation with donor microbiota 4. This work confirmed previous work which described that *C. difficile* can use ethanolamine as primary source of nutrient. To study the interaction between *C. difficile* and the gut microbiota, a co-culture model with a bacterial consortium consisting of a mix of characterized intestinal strains will be used instead of fecal samples.

### 14. Enterotoxin gene and toxin production profiles of *Staphylococcus aureus* strains isolated from artisanal cheeses in Belgium

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*Staphylococcus aureus* is an opportunistic pathogen which plays a role in many human illnesses and one of the worldwide leading causes of food poisoning outbreaks. *S. aureus* coagulase positive strains are known to produce staphylococcal enterotoxins (SEs). Staphylococcal food poisoning (SFP) is induced by the ingestion of food containing a sufficient amount of SEs and commonly leads to a rapid and self-limited offset of symptoms of acute gastroenteritis. Currently, 33 SEs and SE-like toxins have been described but only five, named "classical"-SEs, are commonly investigated in SFP due to lack of specific routine analytical techniques. The aim of this study was to have an overview of the genetic and toxin production profiles of strains isolated from artisanal cheeses in Belgium (n=30) and to perform a comparison between results given by several analytical tools. Forty-nine isolates of *S. aureus* from positive culture batches (n= 10) and their toxin production were analyzed by immunological, liquid chromatography-mass spectrometry, genetic and genomic methods. The results showed that strains harboring classical-SEs genes were not dominant in Belgian artisanal cheeses studied, but all strains harbored at least one gene coding for one of the new enterotoxins. Among the "new" SE/ls and SE/ls-gene detected, strains producing SEs with demonstrated emetic activity in SFP outbreaks, as SE type Y and *ecg*-coding SEs, were found. Furthermore, this study highlighted the wide diversity of *S. aureus* genetic profiles in dairy products and the importance of the use of new specific techniques for the investigation of SFP outbreaks.



## Posters

### 15. Survey on consumer preference for fish products consumption in Cambodia

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Fish is one of the primary sources of food consumption in Cambodia. It is consumed as fresh or processed fish (smoked, fermented) and various products are currently sold in Cambodian market. Cambodians cook fish products differently and consume them in the main meal. This survey was conducted to assess the preferences of consumption of 250 local people in some provinces and cities for various fish products. Phnom Penh and four provinces located around Tonle Sap Lake are the main sources of raw and processed fresh water fish : Battambang, Siem Reap, Kampong Thom, and Kampong Chhnang. Smoked fish, sun-dried fish, salted fish (named Trey Broma and Trey Herm) and fermented fish (including Prahok, Mam and Phak-ak) would be the targets of this study. People preferred to consume smoked fish, sun-dried fish, fermented fish (Prahok, Mam, Pha-ak) and salted fish (trey broma, trey herm) were 19,2%, 68,4%, 43,6%, 3,2%, 8,8%, 4%, and 3,6%, respectively. The consumption of fish products was significantly different ( $p < 0,05$ ) in each province. Smoked fish and Prahok were most consumed in Battambang, Siem Reap, and Kampong Thom, while sun-dried fish and salted fish tended to be mostly consumed in Phnom Penh compared to other provinces. 71% of consumers consumed sun-dried fish (mainly made from the species "trey Ros" (*Channa Striata*), 32% consumed Prahok (mainly made from the species "Trey Kompleanh" (*Trichopodus microlepis*)), and 27,5% consumed smoked fish (mainly made from trey An deong (*Clarias macrocephalus*)). Consumption of different fish species or fish products is influenced by the availability of the fish species or the product in each province and by its price.

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### 16. Analysis of the neurotoxic $\beta$ -ODAP and its non-toxic $\alpha$ -isomer in modern and archaeological grasspea remains using LC-MS/MS

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Grasspea is a high-yielding, drought-resistant high protein legume consumed as a food in many countries. Its development into food, however, has been hindered by the presence of the antinutritional  $\beta$ -N-oxalyl-L- $\alpha,\beta$ -diaminopropionic acid ( $\beta$ -ODAP). The aim of the current study is to determine the levels of  $\alpha$ ,  $\beta$ , and total ODAP in different parts of modern grass pea seeds to be compared to archaeological specimens from Greco-Roman Egypt to assess diachronic trends in the presence of  $\beta$ -ODAP. To fulfil this goal, a new, simple, fast, and accurate LC-MS/MS method was developed. The results revealed that the seed cotyledons showed the highest levels of  $\alpha$ ,  $\beta$ , and total ODAP, followed by entire seeds and seed coats. The cotyledons of Italian, Spanish modern grass pea and archaeological samples showed 86.36%, 88.19% and 86.65% of  $\beta$ -ODAP amounts found in the entire seeds, respectively; and 86.94%, 90.57% and 83.92% of  $\alpha$ -ODAP content, respectively. In fact,  $\beta$ -ODAP content was 3.91, 4.45, and 4.90 times higher than  $\alpha$ -ODAP in Spanish, Italian modern grasspea, and archaeological entire seeds respectively, and  $\alpha$ -ODAP represented 20.38%, 18.34%, and 16.96% of their total ODAP contents, respectively. Contents of  $\beta$ -ODAP in entire seeds of modern Spanish, Italian and archaeological grass pea were 6759.75 and 5843.21 and 52.01 mg/kg, respectively. In general, modern grasspea seeds showed high levels of  $\alpha$ ,  $\beta$ , and total ODAP, while archaeological samples demonstrated extremely low amounts. Overall, the current study provides the first empirical data on the levels of  $\alpha$ -,  $\beta$ -, and total ODAP in archaeological material and on the distribution of these compounds in different parts of grasspea seeds.

## 17. Development and validation of a new bovine teat apex epithelial cell (bTAEC) line.

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The teat sphincter and canal serve as the primary physical barrier against the entry of pathogens into the mammary gland, playing a crucial role in the udder's defence. Although mastitis-causing pathogen interactions with secretory mammary epithelial cells are extensively studied, there's a gap in understanding interactions with teat canal keratinocytes. To address this challenge, we are developing a new bovine teat apex epithelial cell line, termed bTAEC, designed to deepen our understanding of the role this unique extramammary region plays in the development of intramammary infection. Two isolation protocols, utilizing collagenase and trypsin, respectively, were investigated to compare the efficacy of the isolation of keratinocytes from dairy cows' teat canals and assess their immunophenotype. Collagen-coated plates were used to culture tissues, and two protocols were employed: one with collagenase type III and another with trypsin 0.25%. Immunophenotyping involved the application of Cytokeratin Type I Monoclonal Antibody (AE-1) and BD Horizon™ BV421 Rat Anti-Mouse IgG1. Viability of the keratinocytes was measured using 7AAD, and FlowJo analyses were conducted. The trypsin protocol demonstrated superior results, yielding 23.9-28.0% keratinocytes compared to 16.0-18.0% in the collagenase protocol. However, total viability was slightly lower in the trypsin protocol (74.1% vs. 76.6%), and keratinocyte viability was low at approximately 9.1%. Moving forward, the following steps will be taken: firstly, enhancing keratinocyte viability over time using two specific keratinocyte cell mediums; secondly, minimizing fibroblast contamination; and finally, cultivating keratinocytes for subsequent sorting and immortalization. The resulting innovative cell line will then be used as a valuable tool for advancing our understanding of bovine mastitis pathogenesis.

## 18. Optimization of a microscopic dissection protocol of the intestine of zebrafish larvae for targeted screening and molecular analyses of the intestine

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Microplastics (MP) (5mm-1µm) are ubiquitous pollutants gaining interest in the ecotoxicological field and widespread in marine ecosystems. Microbial communities can colonize MPs, forming what is referred to as the "plastisphere". These microbially colonized MPs can have adverse effects on aquatic organisms once ingested. There is extensive research related to the hazards of MP exposure in zebrafish, particularly in early developmental stages. Our research focuses on the effect of MPs on the gut of zebrafish larvae and the associated alterations at the tissue, cellular and molecular levels. Most of the studies that have so far investigated the effect of MP exposure on the gut of zebrafish larvae have used whole larvae for molecular analyses. However, these studies do not allow targeted molecular screening of the gut response and may mask valuable gut-specific information. Some studies have used fluorescent intestinal epithelial cell biomarkers or transgenic zebrafish lines to allow precise isolation of larval intestines, but such applications require sophisticated and expensive equipment, and allow only incomplete assessment of the gut response. The aim of this study was to develop a protocol allowing for the rapid dissection and isolation of a large number of larval intestines. This microdissection technique has been validated through histological processing of dissected intestines and provides insight for future research in the field of ecotoxicology of zebrafish larval ontogeny, as it can be further combined with subsequent applications such as transcriptomics, metagenomics and proteomics.

## Posters

### 19. Risk factors related to peanut production and processing in southern benin

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In Benin, peanut pre- and post-harvest practices could lead to chemical contamination of peanuts and peanut products by toxic compounds which could be hazardous for consumers. This study aims to identify the endogenous practices associated with peanut production and processing, which are potentially sources of chemical hazards in food. A survey was carried out with 395 peanut farmers and 362 peanut processors in six municipalities of southern Benin, through individual interviews. Most of the peanut farmers interviewed (53%) used mainly non-approved pesticides (which represented 83.3% of all the pesticides reported), purchased from illegal distributors. Twenty-four commercial products, mainly glyphosate-based, were identified, and used by farmers during peanut production. After harvest, 58% of farmers dried their seeds in two steps: first at the farm and then at home. After drying, the peanuts were stored in shells (100%), mostly conditioned in polyethylene bags (76%). The intensive use of pesticides, the drying of peanuts at the farm, in contact with the soil, and the storage of seeds in polyethylene bags could be sources of chemical contamination of peanut seeds during both their cultivation and their post-harvest storage. During peanut processing, roasting with a diversity of heat transfer materials, the use of shared corn mills for seed grinding and peanut paste production, the use of wood as a combustible material and the high temperatures reached during peanut processing are potential sources of the presence and formation of chemical hazards in the derived products. The characterization of these risk factors and their importance in the Beninese context will enable to foreseen adequate mitigation techniques to ensure the safety of consumers.

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### 20. Effect of heat treatment on physicochemical, anti-nutritional, nutritional parameters and the colour of plantain fruit flours.

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Banana fruit flours represent a form of preservation with economic and health benefits. These flours can undergo various alterations depending on the treatment applied. To this end, the effect of two heat treatments (blanching and pre-cooking) on the qualities (colour, physico-chemical, nutritional and anti-nutritional) of flour from pulp and whole fruit produced at stages of ripening 1, 3 and 5 of two banana cultivars, BATARD and CARBAP K74, was studied. The physicochemical, nutritional and anti-nutritional characterization of treated and untreated banana plantain flours was carried out using standardised AOAC methods, while minerals were determined by atomic spectrophotometry and the fatty acid profile by GC-MS. The L\*a\*b\* parameters of the flours differed significantly according to the heat treatments. The water content, pH, TESS and ATT of the flours varied respectively from 6.84-11.92%, 5.56-7.04, 4-49.2%, 1022.2-8622.2 meq/100g. The protein, lipid and fiber content of the flours ranged from 2.51-4.36%, 0.57-2.08% and 1.59-8.12%, respectively. The fatty acid profile of the flours showed that the main fatty acids were palmitic acid (0.17-1.01%), linoleic acid (0.18-0.68%) and alpha-linolenic acid (0.05-0.35%). The highest levels of phytates, oxalates and tannins in flour were 0.41%, 0.31% and 0.97%, respectively. Although flours are usually produced at stage 1, stages 3 and 5 can be used in the production of flours because of their high soluble solids and mineral composition. To produce pulp-based flours, pre-cooking can be used, or no treatment at all can be applied. As for whole fruit flours, pre-cooking should be used to improve the L\* a\* b\* parameters, especially for the CARBAP K74 variety.



## 21. Sampling and germline differentiation of avian mesenchymal stem cells: towards a relevant approach for genetic resources preservation?

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The preservation of avian genetic resources uses methods that remain unsatisfying. Indeed, cryopreservation of sperm is poorly effective and physically impossible for oocytes. Regarding primordial germ cells, collection, culture and cryopreservation require high level technical skills and requires the sacrifice of embryos, impeding effective availability. Contrarywise, mesenchymal stem cells (MSCs) are abundant and can be isolated from various tissues (bone marrow, fat, skin, etc.). Several teams report their transformation into gametes by induced transdifferentiation *in vivo* and *in vitro*. Recently, our laboratory has succeeded in isolating MSCs from feather follicles from living animals, and from *post-mortem* chicken tendons. These cells were cultured, amplified and we were able to induce differentiation towards a germline phenotype. This promising proof of concept raises questions of (i) its generalization to other avian species, (ii) confirmation of the germline competence of differentiated MSCs, (iii) maintenance of this competence after cryopreservation, and (iv) the determination of key mechanisms involved in germline-oriented transdifferentiation. This project therefore aims at answering these questions, by implementing the technological suite developed in chickens to other species, by confirming the germline competence of differentiated MSCs and by identifying *in vitro* key players in this differentiation. This study should thus contribute to defining the best options for safeguarding the avian genomes of healthy, but also sterile, debilitated or even deceased animals and could have immediate practical consequences on biobanking policies in these species.

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## 22. Label Free Quantification of Milk Oligosaccharides from Different Mammal Species

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Milk oligosaccharides (MOs), a collection of complex carbohydrates in milk, function as prebiotics. Their concentrations and patterns vary significantly among different species. However, there is limited knowledge about MOs in domestic animals and the impact of heat treatment on them. Here, we developed a LC-ESI-MS/MS method to analyze 11 MOs (2'-FL, 3'-FL, 3-GSL, LNT, LNnT, 3'-SL, 6'-SL, LNFP I, LNFP II, LNDFH I and LNDFH II) in milk samples from 7 distinct species. The results showed that human milk (6.285 g/L for total) presented a completely different composition pattern of MOs compared to animal milk, with concentrations 11.7, 15.8, 10.7, 9.0, 7.6, 7.7 times higher than dairy cow (0.535 g/L), camel (0.399 g/L), yak (0.589 g/L), buffalo (0.699 g/L), sheep (0.832 g/L), and horse (0.818 g/L) respectively. In particular, animal milk predominantly contained sialylated MOs, and human milk had high levels of fucosylated neutral MOs. Notably, sheep milk exhibited similarities to human milk in terms of MOs composition. Then, the dairy cow milk samples were submitted to two common industrial heat treatments, and 2'-FL, 3'-FL, LNT+LNnT, 3-GSL, 3'-SL, and 6'-SL concentrations were analyzed. We found that a treatment of 30 min at 65 °C had no significant effect on the concentration or distribution of MOs, whereas 135 °C heating for 60 seconds was associated with their decline. Collectively, these findings provide new insights into the concentrations and patterns of MOs in milk of various species, and suggest to bring more attention to temperature control in milk products processing.

## Posters

**Sustainable livestock production**



### 23. Potential of grass silage as a source of nutrients in poultry production

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Feed costs constitute over 60% of total expenses in organic layer poultry production, with feed protein supply being a significant concern. Alfalfa-based dehydrated silage pellets (ABSP) are non-conventional protein source that could enhance profits by reducing feed costs and ensuring consistent availability. Two experiments studied the effects on the performances of Novogen Brown light layers of a commercial control diet replaced with either 10% or 5, 10, 15 and 20% ABSP respectively in trial 1 and 2. After a 21-day trial feeding in trial 1, significant differences were observed in weight gain, feed conversion ratio, consumption, yolk color, egg polyunsaturated fatty acids (PUFA) and  $\omega-3$  contents, and  $\omega-6/\omega-3$  ratio ( $P<0.05$ ). Laying rate showed a tendency to increase ( $P=0.06$ ). After 35 days of feeding in trial 2, there was significant differences between groups on yolk/albumen ratio ( $P<0.01$ ), yolk color, egg yolk weight ( $P<0.05$ ),  $\omega-3$  content and  $\omega-6/\omega-3$  ratio ( $P<0.05$ ), but laying rate remained similar. Preservation of egg was significantly higher in ABSP eggs ( $P<0.01$ ) after 15<sup>th</sup> and 30<sup>th</sup> days of storage of eggs at ambient temperature. These findings suggest that ABSP can replace at least between 15 to 20% of the feed in organic layer diets without compromising production parameters negatively and with better egg quality preservation.

### 24. Characteristics of Rabbit Farming in Southern Benin

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The level of biosecurity applied in rabbit farms in Benin is highly diverse, impacting animal productivity. The aim of the study was to characterize rabbit farming in Southern Benin. Eighty (80) rabbit farms were visited for data collection, which were subsequently analyzed. Three (03) groups of breeders were identified and characterized following the analyses. Group 1 consisted of individuals with no formal education, literate up to secondary level, raising rabbits in wooden structures with acceptable biosecurity conditions. Group 2 comprised mostly primary-level educated breeders with wooden, earthen, or tin habitats and poor biosecurity practices. Group 3 were university-educated breeders raising rabbits in solid structures with good biosecurity practices. Breeders used cages within the habitats to house the rabbits. The majority used metal cages (93.7%) with nest boxes inside (95%). They fed rabbits complete diets, sometimes supplemented with forages (especially *Moringa oleifera*). Breeders faced challenges related to pathological and nutritional issues, with fewer issues observed in Group 3. Pathologies that significantly affected farm productivity included diarrheal diseases, mange, and viral hemorrhagic disease (VHD), especially in Groups 1 and 2. These diseases continuously reduce herd numbers, resulting in production falling short of demand. This situation facilitates the sale of products (live rabbits, rabbit carcasses) for breeders. In conclusion, farming practices need further improvement in Groups 1 and 2 to better address the obstacles hindering their development.

## Posters

### 25. Genetic Diversity of the Local Zebu Population in Benin

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The genetic characterization of the local zebu population in Benin has received very little attention in the scientific literature, which hinders the genetic improvement of zebus. This study aims to analyze the genetic diversity of zebus in Benin, focusing on the Azawak, Goudali, and Yakana breeds. Of the 172 samples collected (55 Yakana, 57 Goudali, and 60 Azawak) in the peri-urban area of Parakou, DNA was extracted and genotyped using Illumina BovineHD BeadChip 50K SNP. After quality controls, 169 individuals were genotyped. The analysis was performed using PLINK v1.07, PERL, and R (packages: Factominer, Factoextra, and RZooRoH). The results show an observed heterozygosity ( $H_o$ ) of around 34%, slightly lower in Goudali (34.14%) and Yakana (34.25%) compared to Azawak (34.72%). Average inbreeding coefficients range from 0.10 in Azawak to 0.12 in Yakana and Goudali. The RZooROH algorithm revealed longer homozygous segments in Goudali and Yakana, indicating more recent inbreeding in these breeds. Inbreeding classes, based on the number of meioses separating homozygous haplotypes, demonstrated that inbreeding primarily originated from distant ancestors, beyond what a pedigree could capture. Overall, the study reveals considerable genomic diversity in the three zebu breeds. Principal Component Analysis (PCA) to visualize intra- and inter-breed genomic variability indicates close genetic proximity between Yakana and Goudali, suggesting a potential genetic mixture between these two breeds. A plausible explanation could be uncontrolled crossbreeding practices by farmers, contributing to the observed genetic similarity.

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### 26. Genomic Tapestry Unveiled: A Journey into the Rich Diversity and population Structure of Xinjiang's Indigenous Sheep Populations in China

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Xinjiang's indigenous sheep, integral to the local economy, embody a complex genetic landscape influenced by historical events, breeding strategy, and ecological diversity. Our study, sequencing 170 individuals across 13 populations, meticulously explores the intricate genomic tapestry. Distinct breeds, including Duolang, Chinese Xinjiang Merino, and Cele, are identified through structure analysis. The first study analyzes genetic relationships and population structure, highlighting unique characteristics of breeds and historical genetic exchange with Merino sheep. The second study delves into inbreeding levels, revealing concerns for Duolang and Cele, each exhibiting distinctive patterns, especially in the face of Duolang's rapid expansion.

Our research provides a foundation for subsequent breeding plans and genetic research on Xinjiang sheep and lays out a direction for sustainable use and conservation of the region's indigenous sheep populations.

## 27. Effects of Rumen-Protected Methionine supplementation on the fertility of lactating dairy cattle

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High-producing dairy cows may have lower fertility due to an energy deficit at the beginning of lactation, linked to diet, housing, and environmental-genetic interactions. The influence of nutrition on the fertility of high-producing dairy cows is complex and multifactorial, requiring further work exploring the roles of certain fatty acids, amino acids and carbohydrates in dairy cow reproduction. The aim of this study, carried out in north-central and northeastern Algeria (Algiers, Setif), was to estimate the effects of rumino-protected methionine supplementation on the reproductive performance of lactating dairy cows in early lactation. 32 primiparous and 68 multiparous early lactation cows (the first 12 weeks of lactation) were randomly assigned to one of two dietary treatments based on their expected calving date: 1) a basal diet (group control); or 2) a basal diet supplemented with Rumino-Protected DL-Methionine (group Met). Both groups were watered ad libitum and cows are milked two times per day (4h and 16h). Milk production and feed consumption were tracked on a daily basis. A body condition (BCS) was scored at early lactation. Reproduction parameters were evaluated on the basis of the following intervals: interval to first post-partum heat (VC1), calving to first service interval (VIA1), open days (VIF) and interval from first service to conception (IA1-IAf). Statistical analysis was performed with SAS software (Version 9.3). The results indicate that supplementing with RP Met has a significant impact ( $p > 0.05$ ) on several reproductive parameters. The group Met demonstrated improvements in the VIF (131.86 vs. 143.96 days), first insemination success rate (39.02% vs 29.27%), and fertility index (1.88 vs. 2.05). The findings underscore the potential positive effects of supplementing with rumen-protected methionine on reproductive performance in high-producing dairy cows, contributing valuable insights for optimizing their reproductive efficiency.

## 28. Survey on the consumption of milk and dairy products in the rural community of the Laghouat region in Algeria

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The main objective of this study is to analyze the consumption of milk and dairy products within rural families in the Laghouat region, as well as the associated consumption behaviors. Data was collected through a questionnaire administered between November 2022 and April 2023, involving 60 rural households. The results highlight the significance of milk and its derivatives in the diets of rural populations in Laghouat, with a consumption rate of 100%. The average *per capita* consumption levels, expressed in kilograms of milk per person per year, amount to  $298.05 \pm 135.04$  kg/person/year. A wide variety of dairy products, both industrial and traditional are consumed, such as raw milk (96.67%), pasteurized or sterilized milk (50.00%), powdered milk (45.00%), rayeb or curdled milk (65.00%), Lben or babeurre (100.00%), yoghurt (55.00%), cheese (50.00%), and butter (85.00%). Traditional processing of milk and its derivatives plays a significant role within the rural community (participation of 100% in dietary practices). However, the marketing of fresh milk appears to be limited, as over half of the surveyed farmers (78.33%) do not have a local sales channel for their milk in rural areas. A comprehensive study of consumption characteristics in rural areas is crucial for revitalizing the dairy sector in the Laghouat region and Algeria.

## Posters

### 29. Consumption of milk and dairy products in urban region of Laghouat in Algeria

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This study aims to investigate the consumption patterns of milk and dairy products among urban families residing in the Laghouat region. Data was gathered through surveys conducted between December 2022 and May 2023, involving a sample of 80 families. The results indicate that milk and dairy product consumption is a vital component of the Laghouat residents' diets, with a 100% participation rate. Individual consumption levels, measured in kilograms of milk equivalent per person per year, averaged  $108.30 \pm 59.92$  kg. A diverse array of dairy products, encompassing both industrial and traditional varieties, was identified, including raw milk (82.50%), pasteurized or UHT milk (87.50%), powdered milk (90.00%), lben or babeurre (88.75%), rayeb or curdled milk (50.00%), cheese (90.00%), yogurt (96.25%), and butter (76.25%). The traditional processing of milk and dairy products was relatively limited, accounting for only 7.5% of the total. Conducting a comprehensive study on consumption trends holds promise for reinvigorating the dairy industry in the Laghouat region particularly and Algeria as a whole.

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### 30. Olive pomace: which impact on dairy cows' milk production and quality? (a systematic review)

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The olive oil extraction process generates substantial amounts of olive cake, laying disposal challenges and potential environmental concerns. Olive pomace can be a beneficial supplement for ruminants, particularly in enhancing milk quality. This systematic review assesses the impact of incorporating olive pomace into the diet of lactating dairy cows on milk yield and quality. Using the Science Direct database, relevant research from 2014 to 2022 was identified. Following title and abstract relevance screening, articles meeting inclusion criteria, such as a clear experimental design on dairy cows and the presence of a control group, were selected, resulting in 7 studies from an initial pool of 119 articles. The studies revealed olive pomace inclusion levels in lactating dairy cow diets ranging from 5 to 15%. While there were no significant variations in fatty acids, proteins, casein, lactose, urea, and pH, olive pomace inclusion notably changed the fatty acid composition of milk. Monounsaturated fatty acids increased by 15%, while, saturated fatty acids decreased by 5%. Medium-chain fatty acids experienced a significant reduction (7 to 12%) with olive pomace inclusion, whereas long-chain fatty acids increased (19 to 21%). Short-chain fatty acid levels remained unaffected. Despite these changes, milk production was not impacted by olive pomace. In conclusion, including olive pomace in the diet of dairy cattle is safe and can provide additional nutritional and health benefits to cow's milk.



### 31. Characterization of the Hamra sheep breed in western Algeria

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The present work is a part of a National Research Project (PNR Algeria 2022) dedicated to the development of the Hamra sheep breed in his cradle western Algeria. The aim of this study is to characterize the morphology, growth and reproductive performance of this ovine breed. A sample of 15 rams and 30 ewes were used for the morphological characterization versus a total of 1,328 head for the growth and reproduction performance carried out at the technical livestock institute in Saida area located in the western Algeria. Hamra sheep breed is small compared to other Algerian breeds. It has a dark brown (mahogany) head and legs, a blackish-blue tongue, white wool, spiral horns, and a fine, medium-length tail. Total body length is 124.5 cm for males and 110.5 cm for females, with a trunk length of 78.6 cm for males and 67.7 cm for females. Depth, width and circumference of chest are respectively 40.3 cm; 30 cm; 116.4 cm for males, and 34.2 cm; 22 cm; 99.8 cm for females. Growth monitoring showed an average birth weight of 3 to 3.6kg, depending on sex (male or female) and litter size (single or double). Live weights reached an average of 17.9kg for males and 16.2kg for females at 90th day of age. The ewes' reproductive performances are as follows: 82.2% for fertility, 97% for fecundity and 118% for prolificacy at birth and weaning. In conclusion, the Hamra breed has shown promise performances in terms of growth, but its butchering potential needs more assessments. Further studies are required to establish a conservation strategy becoming urgent by the uncontrolled introduction of genetics from other sheep breeds.

### 32. Using Artificial Intelligence to analyse cattle facial expressions and detect pain

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Pain is one of the many factors that can negatively impact animal welfare. However, it remains difficult to quantify because 1) some species, such as cattle, show few apparent signs of pain and 2) pain detection can be subjective and observer dependent. New digital tools, including Artificial Intelligence (AI), offer new solutions to these limitations. For example, automated analysis of facial expressions is increasingly being developed in various species, often using muscular action units and/or geometric morphometric. Identifying pain through these approaches is a priority, especially in cattle. The objective of our study is to develop an early detection and gradation system of cattle pain using Artificial Intelligence. Our study model is calves disbudding, a common and well-documented practice, defined by a legal framework. The first step of the study involves acquiring images (using cameras) and labelling them into pain-free situations (before disbudding) or painful situations (after disbudding) to define a "learning set". Subsequently, a "validation set", consisting of images where the situation (pain or pain-free) is known by the experimenter but not the algorithm, will confirm the analysis made by the algorithm (which is an artificial neural network) or allows the experimenter to correct it. Other parameters will also be measured to properly categorize the images and verify the results obtained by the machine: the respiratory rate, a pain score, an ethogram, and the eyes temperature through infrared thermography. Ultimately, this automated analysis system could be applied to various animal ages, speculations, and farming practices.

## Posters

### 33. Effects of incorporating 3% and 6% nettle powder on zootechnical performance, oxidative status and physicochemical characteristics of commercial turkey meat.

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The aim of this study was to investigate the impact of incorporating nettles (*Urtica dioica* L.) on the zootechnical performance, biochemical and physicochemical parameters of the meat and the oxidative status of the turkey (*Meleagris gallopavo*) reared under intensive conditions. The evaluation of the potential of this ingredient was carried out by comparing two rates of dietary incorporations, 3 and 6%, of nettles powder in three commercial feeds corresponding to three growth phases, during 12 weeks of rearing. The feed was distributed *ad-libitum* during the whole rearing phase. A total of 72 one-day-old turkeys were assigned to 3 dietary treatments, consisting of three replicates of eight birds each. Results showed that nettle leaves significantly improved the growth performance, with live weight at 12 weeks of age ( $P < 0.05$ ), which is higher in the group fed with 3% nettle powder (NP3%) than in the groups fed with 0% (NP0%) and 6% (NP6%) nettle powder (NP3%: 10534g; NP0%: 9829g; NP6%: 9233g;  $P < 0.05$ ). Over the rearing period, the NP0% group recorded a better feed conversion ratio (NP0%: 2.86; NP3%: 3.03; NP6%: 3.06;  $P = 0.03$ ). Mortality was similar between the three groups (8% average). Oxidative status was significantly affected by supplementation among the three groups ( $P < 0.001$ ). The antioxidant capacity, measured by the way of Total Antioxidant Capacity, Glutathione Peroxidase, Superoxide dismutase, Catalase and Malondialdehyde, was linearly improved with the level of incorporation of nettle powder ( $P < 0.001$ ). No significant differences were recorded between the 3 groups for the chemical composition (water, protein, Fat and ash) and physicochemical parameters (pH24, WHC, Cooking loss, Redness, yellowness and brightness) of the meat.

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### 34. Modulation of growth performance in Japanese quails by red pepper "*Capsicum annuum*"

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"*Capsicum annuum*" can be a quality phytobiotic in poultry farming as an alternative solution to the use of antibiotics. In this context, the objective of this study was to evaluate effects of the spicy red pepper incorporation "*Capsicum annuum*" in to the diet on growth performance and their ingested in Japanese quails during their growth period. A total of 162, one-day old quails (*Coturnix coturnix*),  $8.6 \pm 0.19$ g weight, were individually identified and randomly divided into two groups, consisted of 27 quails in each of three replications during a growth period of forty days. The control group received a basic diet containing 0% "*Capsicum annuum*" (T) and other group (E) received 2 % of spicy red pepper "*Capsicum annuum*" supplementation. Live weight and feed intake were recorded on a weekly basis and feed conversion efficiency was calculated. The results obtained showed that quails receiving red pepper in their rations have recorded better zootechnical performances (average daily gain, average body weight and the feed conversion ratio) in comparison with the sample group. The live weight at the forty days age is  $204.04 \pm 23.44$ g for the T group and  $216.6 \pm 20.38$ g for E group with insignificant daily and total feed intake. Mortality rates were similar in the two groups throughout the monitoring period, with a high rate during the first days of implementation. In conclusion, the present study demonstrated that the red pepper incorporation "*Capsicum annuum*" on diets of quails has positive effect on growth performances and can be one of the interressant phytobiotic in the quail livestock. the data will soon be supplemented by the results of histometry, as well as the health status of these birds and can be continue the experimentation to evaluate the red pepper incorporation effect on egg production.

### **35. Socio-economic challenges of women's empowerment in the rabbit sector in southern Benin.**

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The empowerment of women is seen as a crucial driver aimed at improving their living conditions as well as those of their families. Faced with stereotypes associated with women's emancipation, the rabbit farming sector emerges as an opportunity for women to realize this aspiration. How does the rabbit farming sector contribute to women's empowerment? This study aims to analyze the challenges surrounding the rabbit farming sector in the context of the socio-economic empowerment of women in Southern Benin. Qualitative in nature, this research targets women and men active in the rabbit farming sector in Southern Benin. In total, 45 participants were selected using along a purposive sampling and were interviewed using an interview guide. The approach adopted is inspired by Guy Rocher's theory of social change. The results highlight that rabbit farming activities, carried out on a small scale by women, contribute to strengthening their financial independence. This change stems from efforts made at both individual and collective levels, as well as the institutional support provided to women. This empowerment is notably manifested through their contribution to family expenses. However, it appears that women's empowerment faces challenges due to the persistence of social norms and institutions that perpetuate gender inequalities. Faced with obstacles such as access to land, credit, capacity building, and their low representation in the sector, most of the women we met are involved in rabbit processing and marketing. This reality questions the institutional role in this process and underscores the constraint women face in making activity choices by default. However, from a capability perspective, it is conceivable to grant women more equitable access to economic opportunities, thus enhancing their contribution to poverty reduction and improving their situation.

# **Comparative veterinary medicine**



## Posters

### 36. Computed Tomographic Appearance of sinus pneumocele in two horses

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Pneumocele is a rare slow-growing paranasal sinus expansion reported in humans and horses associated to bone thinning or erosion of the sinusal walls. The rarity of the condition and the paucity of reports in horses give relevance to the knowledge of its imaging features. Two 15-year-old equids, a pony mare (Case1) and a Selle-Français gelding (Case2) were presented for CT investigation of a firm progressive left-sided facial swelling (maxillary in Case1, orbital in Case2). In Case2 there was an associated ipsilateral epiphora. CT identified an expansile sinus deformity (maxillary in Case1, conchofrontal in Case2) with lateral displacement, irregular thinning and scalloping of the lateral bony sinus wall. There were thin supernumerary sinus septa. The lacrimal canal was deviated medially. No mass was present and nasomaxillary opening was pervious. Histopathology performed in Case1 identified bone lysis, granulation tissue and a flat epithelium overlying the normal respiratory sinus epithelium. Etiopathogenesis of sinus pneumocele remains obscure. In humans, several causes have been proposed, including a one-way valve mechanism with air-flow obstruction, trauma, and drainage of mucocele. No sinonasal obstruction was identified in the two cases, though additional septa may have caused partial sinus compartmentalisation. Spontaneous drainage of a sinus mucocele has been proposed to explain a pneumocele in an equine case report with histopathological findings similar to Case1.

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### 37. Reversible congenital bilateral laryngeal paralysis (grade IV) in a foal

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Laryngeal paralysis, often idiopathic, is the second most common laryngeal abnormality in babies and a well described congenital disease in multiple dog breeds. However, it has rarely been reported in foals. This report details the case of a 3-hour old warmblood stallion that presented inspiratory stridor, dyspnea and cyanosis immediately after a mild dystocic birth. The referring veterinarian placed a nasotracheal tube and send the foal to the clinic. It arrived in lateral recumbency, showing a weak suckle reflex and mild signs of dehydration. Blood analyses revealed increased PCV, inversed neutrophil:lymphocyte ratio, severe hyperlactatemia, and high serum creatinine and creatin kinase levels. Airway endoscopy showed a bilateral grade IV laryngeal paralysis and a recurrent dorsal displacement of the soft palate without signs of inflammation. A primary diagnosis of neonatal dysmaturity and/or maladjustment syndrome was strongly suspected. A nasotracheal tube was kept in place and the foal was hospitalized, receiving multiple supportive treatments, including iv fluid therapy, glucose, DMSO, broad-spectrum antimicrobials, NSAIDs, low-molecular-weight heparin, colostrum, plasma and vitamin E/selenium supplementation. Clinical evolution was good and laryngeal function recovered progressively, allowing tube removal 24h after arrival and becoming normal one month later. Laryngeal paralysis should be considered as a differential diagnosis of neonatal stridor in foals. This case shows that a grade IV bilateral laryngeal paralysis in a foal may be reversible without the need for invasive surgical procedures, and should not necessarily imply a poor vital or sportive prognosis.

### 38. Ultrasonographic and CT-scan findings of splenic active chylous effusion in a cat with chylothorax associated with metastasized splenic angiosarcoma

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A 12-year-old domestic shorthair cat was presented with relapsing chylothorax causing mild tachypnoea, moderate dyspnoea, inappetence and lethargy for 3 weeks. Echocardiography excluded a cardiac origin for the chylous effusion. Thoracic and abdominal CT-lymphangiograms were performed and confirmed bilateral pleural effusion without evidence for an underlying intra-thoracic origin. CT-lymphangiography also revealed a 2.6 cm diameter splenic mass surrounded by a chylous effusion, as well as splenic and hepatic nodules. The chylous effusion around the splenic mass was actively collecting during ultrasonographic evaluation after ultrasound-guided drainage. Exploratory laparotomy confirmed splenic masses and subcapsular effusion adjacent to the masses. The liver had an abnormal mottled appearance. Splenectomy and liver biopsies were performed. Histopathology diagnosed a splenic angiosarcoma, a tumour arising from the endothelial cells lining the walls of blood vessels and/or lymphatic vessels, with multifocal metastases to liver. The chylothorax observed in the present case was most likely due to distant microscopic intra-thoracic metastasis to either the lung or the pleura. Post-operatively, the cat was palliatively treated with prednisolone and routine for 1 month before euthanasia due to recurrent pleural effusion.

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### 39. Feasibility of US-guided cervical intervertebral disc injection to aid in intraoperative identification of herniation sites in dogs

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Intervertebral disc (IVD) herniation commonly affects dogs and may require surgery. Whilst the affected IVD is identified on imaging modalities, intraoperative orientation for cervical IVD herniations is mostly based on digital palpation which is sometimes misleading. We therefore studied the feasibility of US-guided contrast medium and methylene blue injection to aid in intraoperative IVD identification. Twenty canine cadavers were used. For each cadaver, one IVD (C2-C3 to C6-C7) was randomly selected. The ventral cervical region was ultrasonographically examined from C1 to the targeted site, pushing the trachea to the left. A 50/50 mixture of contrast medium and methylene blue (0,2-0,3 mL) was injected under US-guidance in the IVD and in the ventral soft tissues. X-rays and CT-scans were performed before and after the injection. Each cadaver was dissected as if performing a ventral slot procedure. The success rate in ultrasonographically identifying the right IVD, the time taken to inject, numerical "contrast scorings" (x-ray, CT-scan) as well as a numerical "visibility scoring" (surgical) were recorded. IVD injections were successfully performed in the correct IVD in all cadavers. Mean time to injection was 4 minutes 30 seconds (range: 2 minutes 17 seconds – 8 minutes 30 seconds). "Contrast" and "visibility" scores were optimal for all cadavers besides a suboptimal "visibility" score in one that was not injected in the ventral soft tissues. Ultrasonographical cervical IVD identification and US-guided injection of IVD and ventral soft tissues are feasible and helpful for intraoperative cervical IVD identification. These results should be confirmed in a clinical setting.



## Posters

### **40. Diagnosing American Bison pregnancy: comparison between rectal palpation associated with ultrasonography, assay of Pregnancy Associated Glycoproteins and of steroids in feces or serum**

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The study aimed to propose sensitive, specific and easy pregnancy diagnosis for American Bison. For 2 years, 40, 27 and 15 pregnancy diagnoses were performed on American Bison, respectively, non-pregnant, in second and third trimester of gestation. Pregnancy diagnosis consisted of trans-rectal palpation and ultrasonography (TRPUS), assay of Pregnancy Associated Glycoproteins (PAG), Progesterone (P4), Estrone (E1), Estrone-Sulfate (E1S) in serum, and P4 and E1S assay in feces. Immuno-assay was used to determine PAG concentration. Steroids were assayed in serum and feces using Liquid Chromatography coupled to Mass Spectrometry. Sensitivity and specificity of TRPUS was determined after *Fisher's* test. Thresholds leading to best sensitivities and specificities for each hormonal assay were determined using Receiver Operating Characteristic curves. Despite TRPUS requires contention, palpation skills and was inconclusive in 29.37% of cases, good sensitivity (97.50%) and decent specificity (83.33%) were observed. Concentrations of PAG, P4, E1S and E1 in serum were higher in Pregnant (P) animals, with thresholds giving decent specificity and sensitivity (lower sensitivity for E1 and E1S: 92.11%; lower specificity for P4: 93.48%). In feces, P4 and E1S were higher in P animals: pregnancy diagnosis using E1S assay in feces showed 100% of sensitivity and specificity. In conclusion, TRPUS is useful for pregnancy diagnosis in American Bison, but requiring contention and skill. Serum hormonal assays are of interest and can help evaluating the pregnancy period. However, E1S assay in feces is a highly sensitive and specific pregnancy diagnosis that can be performed on wild American Bisons.

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### **41. VET&PHARM pilot project: A case study focusing on the perceived value of a pilot seminar on interprofessional communication for students in Pharmacy and Veterinary Medicine in the rational delivery of drugs for veterinary use**

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Antibiotic resistance has gained attention, prompting changes in prescribing practices in human and veterinary medicine. However, this hasn't extended to over-the-counter drugs like antiparasitic medications. The current public health landscape emphasizes interdisciplinary approaches in healthcare. Strengthening community pharmacists' proficiency in veterinary pharmacotherapy is crucial, especially in addressing antiparasitic resistance and environmental impact. The VET&PHARM pilot project aimed to evaluate the perceived value of an interdisciplinary healthcare simulation in education, involving pharmacy and veterinary students. Methods: This study assessed the perceived value of an interdisciplinary healthcare simulation focusing on rational pharmacy-based veterinary drug dispensing. Students engaged in role-playing, simulating the rational delivery of veterinary medications at an educational pharmacy counter. Scenarios included a pharmacist receiving a veterinary drug request from a pet owner. Unable to fulfill it without consulting a veterinarian, a telemedicine sequence ensued between the pharmacist and the vet. Debriefings emphasized professionals' actions and their impact on patient and animal care. The activity, with 40 student volunteers, featured two VET&PHARM sessions in May 2022. Post-activity, an online questionnaire assessed perceived value for both pharmacy and veterinary students. Results: With an 82.5% response rate, post-intervention questionnaires highlighted the educational interest of the pilot seminar. Students reported improved collaboration and confidence in providing veterinary medicine services and counseling. Discussion and Conclusion: Beyond developing communication and collaboration skills, students exceeded expectations, embracing the One Health vision. This initiative addresses the urgent need for improved rational dispensing practices in veterinary medicines, contributing to the overarching goal of enhancing healthcare delivery in pharmacy.



## 42. Feasibility and reproducibility of 2d-shear wave elastography in evaluating meniscal stiffness in horses

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Stifle injuries are a major cause of hindlimb lameness in horses. Soft tissues of the joint are the most affected, therefore ultrasonography is more sensitive and specific than radiography. Elastography is an ultrasonographic technique that allows measurement of tissue stiffness. The aim of this study was to determine feasibility, reproducibility, and repeatability of two-dimensional Shear wave elastography (2D-SWE) and to evaluate its diagnostic efficiency to discriminate between sound and degenerative menisci in horses.

2D-SWE was independently performed by two operators with a Logiq S8 (GE Healthcare) and a high frequency linear probe (10 MHz) under sedation after owner consent. Elastographic images were blindly analyzed by two observers, manually drawing 3 regions of interest (ROI) of 1 cm in diameter at 3 different sites of each meniscus. Shear waves velocity (m/sec) and Young's Modulus (kPa) were calculated three times at each selected ROI. Statistical analysis was performed with SPSS 27.0 ( $P < 0.05$ ).

25 horses were enrolled and divided in 3 groups. Intra and interobserver agreement were excellent. Interoperator variability was fair. The middle/axial site of the meniscus was significantly stiffer than the abaxial. Healthy menisci were significantly stiffer than degenerative menisci.

Due to its excellent repeatability, but fair reproducibility, 2D-SWE should be applied with caution in clinical settings, especially in less experienced operators. Findings from our study, however, support future clinical application of 2D-SW elastography as a noninvasive imaging modality which could provide additional information about meniscal elasticity.

## 43. Longitudinal changes in fecal microbiota during hospitalization in horses with different types of colic and association with survival

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Changes in fecal microbiota during hospitalization of horses with colic have been poorly studied. This study aimed to investigate changes in the fecal microbiota during hospitalization in horses with different types of colic and its association with outcome. A prospective observational study was carried out, including 23 horses with colic (9 inflammatory, INFL, 9 simple obstructions, SIMPLE and 5 strangulated obstructions, STR) with fecal samples collected on days 1 (admission), 3 and 5 of hospitalization. Bacterial taxonomy profiling was obtained by V1V3 16S amplicon sequencing. Data were statistically compared between groups (2-way ANOVA) and LEfSE analysis was used to identify bacteria significantly associated to a group ( $p$ -value  $< 0.05$ ). Horses from the INFL group showed greater richness ( $p = 0.0075$ ) and diversity (Shannon,  $p = 0.0001$ , Simpson,  $p = 0.0137$ ) than other colic types on day 5. Alpha diversity indices did not change significantly during hospitalization within each colic group. Beta diversity analysis of bacterial membership was significantly different in the INF group in regard to SIMPLE and STR ( $p < 0.001$ ). Comparisons within colic groups revealed a difference between day 1 and 5 in the group SIMPLE ( $p < 0.001$ ). *Treponema* was more abundant in the INFL group and *unclassified Acidaminococcaceae* in the SIMPLE group. Furthermore, beta diversity membership was statistically different in survivors ( $p = 0.001$ ). Increased relative abundances of *Bacilliculturomica* and *Saccharofermentans* were associated with survival. Results of this study suggest that in horses with colic fecal microbiota seems more influenced by the nature of the digestive disease than by hospitalization.

## Posters

### 44. Thymic Carcinosarcoma With Melanocytic Differentiation In A Dog

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Carcinosarcomas are very rare tumors in dogs. While carcinosarcomas with melanocytic differentiation arising from the uterus or urethra have been described in humans, this type of tumor has not been reported in dogs. We observed such a tumor in the cranial mediastinum of an 11-year-old spayed female dachshund. The dog was admitted for cough, sporadic regurgitations, and dyspnea. Thoracic ultrasonography and computed tomography revealed a large mediastinal mass, that was surgically removed via sternotomy. The tumor was possibly of thymic origin and demonstrated three distinct components: an epithelial component positive for pancytokeratin (AE1/AE3) and high molecular weight cytokeratin (CK5/CK6) with some cystic spaces; a mesenchymal component positive for vimentin; and in association with the epithelial part, a minor melanocytic component positive for Melan A. Histologic metastasis of the epithelial and melanocytic components was present within a tracheobronchial lymph node. The dog died 105 days post-operatively after an episode of acute dyspnea.

To the authors' knowledge, this is the first report of thymic carcinosarcoma with melanocytic differentiation.

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### 45. Ureteral diverticula as incidental findings in a 14-year-old dachshund

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A 14-year-old, male neutered dachshund underwent computed tomography of the spine because of suspicion of medullary compression localised in the T3-L3 spinal cord segment. Pre-anaesthetic biochemistry, blood gas analysis and haematology were within normal limits. The owners have reported no urinary signs.

CT scan showed a compressive L1-L2 discal hernia. Both ureters were tortuous with multiple focal dilations filled with contrast medium.

Lateral radiography of the spine was performed. Ureters appeared tortuous in their caudal part, with multifocal outpouchings. The proximal two-thirds of the ureters showed heterogeneous filling, and the contrast medium seemed to accumulate only in outpouchings.

On ultrasonography, the ureters were visible at their departure, the wall was mildly thickened, and the ureters were tortuous. No outpouching was observed.

Right L2-L3 hemilaminectomy was performed. The dog went home 48 hours post-surgery.

Acquired diverticula are characterised by multifocal, small outpouchings of the ureters and are due to protrusion of the mucosa through the muscularis because of urothelial hyperplasia. In human patients, the diagnosis is often made based on intravenous urography, antegrade urography, or retrograde pyelography. Patients are usually asymptomatic. Calculi have been described as a cause of ureteral diverticulum. It is important to recognise ureteral diverticula because they are described as a potential risk factor for urothelial carcinoma.

#### 46. Histopathological characteristics of hoof lamellae of horses and donkeys with severe naturally occurring laminitis

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Laminitis is a common, severe and multifactorial condition of the hoof lamellae of equids. The mechanisms involved in its pathophysiology have been investigated using histopathology in the developmental phase of the disease in equine models. However, information regarding lesions in naturally occurring laminitis is lacking both in horses and in donkeys. This study describes histopathological lesions in equids with naturally occurring laminitis.

Lamellar samples were collected in 9 horses and 5 donkeys euthanized due to severe laminitis and in 8 control horses. Samples were processed for histology and stained with haematoxylin-eosin, periodic acid-Schiff and Masson's trichrome.

Histopathological analysis in laminitis cases exhibited typical characteristics, including narrow and elongated secondary epidermal lamellae and an acute primary-secondary epidermal lamellae angle. Additional observations in the epidermal tissue included hyperkeratosis, keratin pearls, cytoplasmic vacuolization, nuclear abnormalities, phantom cells, mitotic figures and basement membrane detachment. Dermal tissue displayed elevated collagen content, mild edema, distended lymphatic vessels and increased cellularity. In some cases, tissue destruction was so extensive that its architecture was completely lost. Focal inflammatory cell infiltration was noted in destructed areas and consisted mainly of degenerate neutrophils. All control horses presented normal lamellar anatomy.

This study constitutes the first to provide a detailed description of histopathological lesions in horses and donkeys with naturally occurring laminitis, thereby providing additional insights in the progression of the disease.

#### 47. Distal sagittal forelimb conformation in young Walloon sport horses: radiographic assessment and its relationship with osteochondral fragments:

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Equine osteochondrosis is a pathological condition characterized by focal ischemic chondronecrosis within epiphyseal growth cartilage and formation of irregularity, flattening, cyst or fragment within the articular cartilage, the subchondral bone and the joint. Although the underlying cause is yet to be clearly identified, three main factors leading to this condition seem implicated: heredity, metabolic and traumatic. To date, few studies have evaluated the impact of conformation traits and their correlation with osteochondrosis. This study, based on the radiographic screenings of young horses (145 individuals, 290 forelimbs), tends to show a correlation between hoof and fetlock conformation and distal limb osteochondral fragments. In fact, fragments located at the dorso-proximal margin of the proximal phalanx in forelimbs were significantly associated ( $p < 0.05$ ) with increased palmar angle of the distal phalanx and a tendency toward decrease in the dorsal fetlock joint angle and upright foot conformation. The metacarpal III diameter of the left forelimb is furthermore significantly ( $p < 0.05$ ) correlated with the presence of osteochondral fragments within the tarsus and/or stifle. This study provides information about factors predisposing individuals to certain types of osteochondral fragments or early changes in limb conformation secondary to their presence. Further studies are required to determine whether the conformation led to osteochondral fragmentation or the opposite.

## Posters

### **48. Evaluation of pelvic and head movement asymmetries at trot in young horses and their correlation with intended use and suggestive forelimb conformation**

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Orthopedic pathologies and associated lameness constitute a major cause of retirement in sport horses. In order to assess gait asymmetries, recent years have seen the appearance of gait analysis systems based on wireless sensors allowing precise quantification of asymmetries of the head and pelvis at trot. The clinical significance of these asymmetries, sometimes very mild, remains poorly understood and the influencing factors little known. The study presented here was carried out on a population of young Walloon horses ( $3.95 \pm 0.5$  years), in the process of breaking-in or starting light work, intended for equestrian sports (eventing (28), dressage (20), endurance (23) and jumping (24)) and considered "sound" by their owner. All individuals were evaluated at trot by gait analysis system (equinosis© lameness locator) and different conformational traits were reported. It was possible to demonstrate a high prevalence of asymmetries (79%) within the population studied. In addition, horses intended for endurance (Arabians and Arabians part-bred), of small size, presented significantly more vertical asymmetries of the head at trot than the rest of the population ( $p < 0.05$ ). Although trends were present, forelimb deviations and asymmetries did not significantly influence the appearance of vertical asymmetries during locomotion. This study demonstrates and confirms the high prevalence of trot asymmetries in young horses destined to different disciplines, particularly individuals oriented towards endurance, as well as a limited effect of front legs conformation. Additional studies are required to confirm and evaluate the long-term impact of these observations.

