



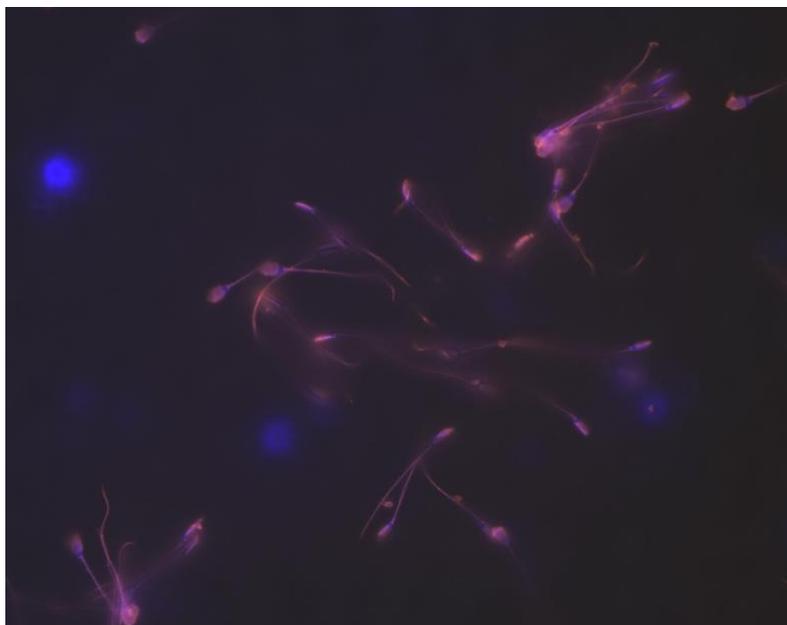
Proceedings of the 9th FARAH-Day

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

December 15, 2022

One Health

L'Animal et l'Homme, une même santé



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December 15, 2022

Edited by C. Bayrou, C. Delguste, C. Douny, J. Eppe, V. Frisée, C. Gatez, L. Gille, T. Jauniaux, V. Jergeay, N. Korsak, L. Martinelle, J. Ponthier, I. Tosi, D.M. Votion

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

COVER PICTURE CREDITS:

"Welcome to the dancefloor"

@Sophie Egyptien

Welcome to the 9th 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 9^{ème} 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

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Irene Tosi, Dominique-M. Votion

Dept of Functional Sciences

TECHNICAL SUPPORT

**Christine Bal
Jean-Michel Dusoulier
Anne Wlasowski**



Program

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

09:00 - Opening and Welcome Speech

Prof. Tania Art, Dean of the veterinary faculty

09:15 Invited speaker

Etienne Baise award

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

Chair: Bénédicte Machiels & Véronique Delcenserie

I. Tosi - First steps in discipline-specific canine exercise physiology: a field study on canicross dogs.

M. Gong - AIHV-1 infection causes oligoclonal expansion and activation of CD8+ T lymphocytes resulting in bovine malignant catarrhal fever via interaction with T cell signaling pathway.

S. Pirotte - A fish herpesvirus highlights functional diversities among Z domains related to phase separation induction and A-to-Z conversion.

M. Dancot - Comparative imaging of the proximal third metatarsal bone at the suspensory ligament origin.

M. Meunier - Lung neutrophil targeting has long-term consequences on cellular trafficking and endothelium homeostasis.

M. Gérardy - Kinetic overview of the viral dissemination following Usutu virus peripheral inoculation in wild type 129/Sv pups.

F. Rivas - A new avian in vitro model for the study of Usutu virus infection.

J. Dupont - Retrospective study of the use of inhaled salbutamol administered to anesthetized horses suffering from impaired oxygenation: mechanisms of action and predictability of outcome.

P. Janssen - Neutrophil Extracellular Traps Are Found in Bronchoalveolar Lavage Fluids of Horses With Severe Asthma and Correlate With Asthma Severity

C. Kruse - Proteomic profiling in equine atypical myopathy: new metabolic insights.

J. Ledeck - Comparison of combined thickness of the uterus and the placenta between 2 breeds of mares and their potential relationship with estrogens during normal pregnancy.

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 & Linde Gille

11:15 **C. Loublier** - *DysbiHorSIRS - Effect of an antibiotic-induced intestinal dysbiosis on the systemic immune response to an experimentally-induced endotoxemia in horses*

11:20 **M. Levoz** - *Impact of an original freezing process for tendon mesenchymal stem cells on their immunomodulatory properties*

11:25 **M. Vandersmissen** - *Multi-modality diagnostic imaging of the equine proximal phalanx*

11:30 **H. Amory** - *EQUISALM - Portage de Salmonella spp. chez des chevaux hospitalisés pour des problèmes gastro-intestinaux en Belgique : fréquence, facteurs de risque, sérovars circulants, résistome et optimisation de la qPCR pour le diagnostic*

11:35 **E. Rizzoli** - *Investigation of common molecular signatures in dogs suffering from Canine idiopathic pulmonary fibrosis (CIPF) and from lung cancers*

11:40 **I. Gonza Quito** - *FibroGut - Rôle des additifs alimentaires sur le développement des maladies inflammatoires de l'intestin et de la fibrose intestinale*

11:45 **O. Donohoe** - *Study polymerase III promoter activity in salmon models for their potential future use in the expression of immunostimulatory RNA*

11:50 **L. Martinelle** - *PIGEON-LEAD: Evaluation des schémas de vol précoces en tant qu'estimateurs de performances futures chez les pigeons voyageurs*

11:55 **V. Gronsfeld** - *The urogenital microbiota in dogs: ongoing studies in healthy and pathological dogs*

12:00 **J. Ledeck** - *PE2C2 - Placental Cells Culture for Pregnant Equine Estrogens Complex Comprehension during Placentitis*

12:05 **G. Parisi** - *ZooVet - Survey on selected zoonoses and associated risk factors in veterinary students and supervisors, Southern Belgium*

12:10 **L.F. Ludwig-Begall** - *Here comes the sun – methylene blue in combination with sunlight as an equitable decontamination method for surgical masks contaminated with viruses of varying tenacities*

12:15 **M. Bonhomme** - *Early blood biomarkers associated with the risk of severe musculoskeletal injuries in Thoroughbreds jump racing. Phase I: Longitudinal follow-up of horses in training*

12:20 **C. Kennedy** - *Prospective study on lactate concentrations and kinetics, cardiac dysfunction and coagulation disorders in canine trauma patients*

12:25 **H. Machiels** - *Utility of focused cardiac ultrasound and NT-proBNP level to detect canine pre-capillary pulmonary hypertension*

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: Nicolas Korsak Koulagenko & Irene Tosi

B. He - *Illumination of Cyprinid Herpesvirus 2 Infectious Cycle Using In Vivo Bioluminescent Imaging.*

C. Wouters - *Zebrafish larvae model to screen for the risk of *Acer pseudoplatanus* intoxication in grazing animals.*

F. Laforêt - *Impact assessment of vB_KpnP_K1-ULIP33 bacteriophage targeting *Klebsiella pneumoniae* on the human gut microbiota using an in vitro gastrointestinal model.*

I. Gonza Quito - *Effect of food additives on gut microbiota of healthy donors and IBD patients.*

C. Fang - *Genome-wide patterns of population structure and genetic diversity in Xinjiang indigenous sheep.*

14:25 Questions

A. Snoeck - *Serum 25-hydroxyvitamin D and 24,25-dihydroxyvitamin D in dogs with sinonasal aspergillosis.*

N. El Khalfaoui - *Longitudinal clinical follow-up of ovine caseous lymphadenitis in Settat province in Morocco.*

L. De Maré - *Relationship between the cycle threshold value (Ct) of a *Salmonella* spp. qPCR performed on feces and clinical signs in horses.*

M. Bonhomme - *Metabolomic investigation of exercise-induced response to moderate and high intensities training sessions in Thoroughbred flat racehorses*

14:55 Questions

15:00 Invited speaker

Prof. Etienne Thiry

From one emergence to another. Itinerary of a veterinary virologist (1980-2022)

15:30 - Coffee break and poster session 3 (Room P, building B45)

16:00 - Oral session 4: Awardee from CSR (lecture hall C, building B45)

Chair: Louisa Ludwig & Frédéric Farnir

16:00 **Lijing Tang** - *Uncovering genetic factors underlying de novo transposition rate variation in the cattle germline*

16:05 **Georgios Petrellis** - *Defining how coinfection with helminths shape the lung immunity to respiratory viral infections*

16:10 **Aude Blanquer** - *Rôle de la salive de moustique et de son microbiote sur la pathogénie du Flavivirus Usutu chez l'oiseau et la souris*

16:15 **Jean-Marc Lassance** - *Logique moléculaire de la détection de la progéniture par des gpcrs olfactifs*

16:20 **Claire Gourzones** - Impact des infections virales sur les ILC2 pulmonaires

16:25 **Alexis Balthazar** - Les infections par les gammaherpesvirus modifient à long terme l'immunité de l'hôte en déclenchant des réponses inflammatoires intestinales délétères.

16:30 **Stéphane Schurmans** - Effects of INPP5K knockdown/knockout on the constitutive activation and oncogenicity of mutated IL7Ra chains associated with B- and T-ALL in man

16:35 **Salomé Desmecht** - Construction, characterization and efficacy assessment of Recombinant Phage Endolysins as enzymatic against *Aeromonas salmonicida*, the causative agent of furunculosis (ASAPHLR)

16:40 Questions

16:45 Invited speaker

Prof. Laurent Gillet, Vice-Dean for Research

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

17:15 – Closing session

Prof. Michel Moutschen, Vice-Rector for Research

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

18:15 – Live music with EMPI, drinks and appetizers

19:00 – Food: Pick-up your Poke Bowl

19:30 – 20:00 – Live music with EMPI

FOOD BOX  Distributeurs d'Alimentation Saine 



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Short talks

First steps in discipline-specific canine exercise physiology: a field study on canicross dogs.

Bonhomme M.¹, Votion D.¹, François A-C.¹, Caudron I.², Perrot C.³, Art T.⁴, Tosi I.⁴

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Canicross and its derivatives are burgeoning sports activities for the dog-master duo; nonetheless, scientific knowledge in this field is lacking. Our aim was to work on this gap, thus helping owners and veterinarians for their sports and profession. On December 2021 (5°C, 80% humidity) and May 2022 (20°C, 54% humidity) we recruited 9 and 11 dogs covering a distance of 5.7 and 4 km, respectively. We collected physical and blood parameters as heart rate (HR), respiratory rate (RR), rectal temperature (RT), hematology, biochemistry, blood lactate and glucose, at rest (T0), immediately (T1) and 1 hour after exercise (T2). We performed a one-way ANOVA on repeated measures. On December as on May, we observed a significant increase in RT and HR. Mean RT at T1 was more than 1°C higher on May (40.49±0.88) than on December (39.29±1.06). Hematocrit increased significantly with exercise only in December. Concerning blood electrolytes, only Mg decreased with exercise on December, whereas also other electrolytes (Cl, K, Ca, P, Mg) changed with exercise on May. Biochemistry was unchanged, except for creatinine, and only on May. Interestingly, lactate was not affected by exercise on December, whereas it increased significantly on May from T0 to T1. In conclusion, few parameters were modified in cold racing conditions, while other electrolytes and lactate significantly changed in warmer conditions. The difference in the increase of RT between December and May is a crucial parameter when considering animal welfare. Hypomagnesemia with exercise was a common finding in both situations. These data have to be further investigated and explained in the light of comparative exercise physiology.

AIHV-1 infection causes oligoclonal expansion and activation of CD8⁺ T lymphocytes resulting in bovine malignant catarrhal fever via interaction with T cell signaling pathway

Meijiao Gong^{1*}, Françoise Myster^{1*}, Abdulkader Azouz², Guillem Sanchez Sanchez^{2,3}, Shifang Li¹, Justine Javaux¹, Sylvain Leemans¹, Olivier Nivelles¹, Willem van Campe¹, Stefan Roels¹, Laurent Mostin⁴, Thierry van den Bergh⁴, Pierre Kerkhofs⁴, Laurent Gillet¹, Andrew J. Davison⁵, David Vermijlen^{2,3}, Stanislas Goriely², Tim Connelley⁶, Alain Vanderplasschen¹, Benjamin G. Dewals^{1#}

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Alcelaphine herpesvirus 1 (AIHV-1), a member of the Gammaherpesvirinae subfamily, induces malignant catarrhal fever (MCF), a fatal peripheral T-cell lymphoproliferative disease, in a diversity of ruminant species, including cattle. Here, we first confirmed in the bovine species that AIHV-1 latency-associated gene expression is essential for persistent infection of CD8⁺ T cells and MCF development. Next, we performed an in-depth characterization of peripheral CD8⁺ T cells during bovine MCF. T cell receptor sequencing of both CDR3α and β revealed oligoclonal expansion of CD8⁺ T cells, and we observed severe transcriptomic and epigenetic changes in CD8⁺ T cells using RNA-seq and ATAC-seq analyses. We also observed upregulation of effector, memory, and exhausted CD8⁺ T cell signature genes, and performing viral tracking at the single cell level, revealed that viruses are mainly enriched in effector, memory, and exhausted-like CD8T cells. Analysis of the viral genome transcription identified viral genomic regions being expressed in infected bovine CD8⁺ T cells, such as the region predicted to encode the gene A10. We demonstrated that A10 is phosphorylated in T cells *in vitro* and affects T cell signaling. Furthermore, impaired expression of A10 did not affect AIHV-1 replication *in vitro* but rendered AIHV-1 unable to induce MCF in the rabbit model. Overall, we provide a thorough description of CD8⁺ T cell responses during MCF to uncover a novel mechanism explaining how AIHV-1 dysregulates T cell signaling leading to MCF.

Short talks

A fish herpesvirus highlights functional diversities among Z domains related to phase separation induction and A-to-Z conversion

S. Pirotte^{1,†}, M.A. Diallo^{1,†}, Y. Hu¹, L. Morvan¹, K. Rakus^{1,2}, N.M. Suárez³, L. PoTsang^{1,4}, H. Saneyoshi⁵, Y. Xu⁵, A.J. Davison³, P. Tompa⁶, J.L. Sussman⁷, A. Vanderplasschen¹ ([†]Co-first authors)

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Double-stranded (ds) nucleic acids can adopt different conformations under natural conditions. DNA is mainly found under the right-hand B-conformation described by Watson and Crick in 1953. The equivalent conformation for dsRNA is called A-conformation. Both dsDNA and dsRNA can also exist under a left-handed conformation called Z. Z α domains found in few proteins bind to left-handed Z-DNA and Z-RNA. The Z α domain protein family includes cellular (ADAR1, ZBP1 and PKZ) and viral (vaccinia virus E3 and cyprinid herpesvirus 3 (CyHV-3) ORF112) proteins. These proteins are involved in innate immunity or immune evasion mechanisms. We studied CyHV-3 ORF112, which contains an intrinsically disordered region and a Z α domain. Genome editing of CyHV-3 indicated that the expression of only the Z α domain of ORF112 was sufficient for normal viral replication in cell culture and virulence in carp. In contrast, its deletion was lethal for the virus. These observations revealed the potential of the CyHV-3 model as a unique platform to compare the exchangeability of Z α domains expressed alone in living cells. Attempts to rescue the ORF112 deletion by a broad spectrum of cellular, viral, and artificial Z α domains showed that only those expressing Z-binding activity, the capacity to induce liquid-liquid phase separation (LLPS), and A-to-Z conversion, could rescue viral replication. For the first time, this study reports the ability of some Z α domains to induce LLPS and supports the biological relevance of dsRNA A-to-Z conversion mediated by Z α domains. This study expands the functional diversity of Z α domains and stimulates new hypotheses concerning the mechanisms of action of proteins containing Z α domains. This study has been published in the prestigious journal *Nucleic Acids Research*.

Comparative imaging of the proximal third metatarsal bone at the suspensory ligament origin

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Introduction: Metatarsal sclerosis has been reported on limbs with suspensory ligament enthesopathy and in limbs with normal proximal metatarsal Magnetic Resonance Imaging (MRI). The aim of this study was to describe morphological variations in a series of isolated limbs with no abnormalities at MRI. **Methods:** Ten hindlimbs were collected from 7 horses with no known history of lameness, euthanized for reasons other than the study (age 3-19 years, weight 386-635kg). Limbs underwent CT, radiographic and ultrasonographic examination. Standing MRI was used to confirm normalcy of the suspensory ligament and absence of subcortical sclerosis. **Results:** All limbs were normal at MRI examination. All limb showed more or less prominent sagittal linear crests on the proximal palmar metatarsal surface (1 or 2 crests in the midline, 1 or 2 abaxially located at the medial or lateral margin of the suspensory ligament). The midline crests were visible as linear increased opacities on DPI views and on transverse ultrasonographic images and were located facing a central small musculo-adipose bundle dividing the 2 suspensory ligament lobes. Because of the obliquity of the plantar metatarsal surface, delineation of the trabecular/cortical interface of the proximal metatarsus was sharper on the L5PI-MD oblique views. **Discussion/Conclusion:** Proximal metatarsal bone shows significant morphological variations in MRI normal limbs. Because these variations alter homogeneity of bone opacity on DPI radiographic views and may mimic sclerosis, a L5PI-LMD oblique view is suggested to better assess trabecular/cortical bone interface.

Lung neutrophil targeting has long-term consequences on cellular trafficking and endothelium homeostasis

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The lung exerts vital functions that are sustained by both structural and immune components. The lung microenvironment has indeed been shown to shape the functional identity of immune cells to ensure appropriate responses that maintain its immunophysiological functions under homeostasis and pathogenic conditions. In line with this, one intriguing feature of the lung is the presence, at steady-state, of a substantial pool of neutrophils located in the microvasculature, called marginated neutrophils (MarNeu). Lung MarNeu display a unique transcriptomic profile as compared to other neutrophils across the whole body. Interestingly, the gene signature of MarNeu is mainly associated with vascular growth and repair. Here, we show that targeting MarNeu during a few days has a substantial long-term impact on cellular trafficking and endothelial cell (EC) homeostasis. Indeed, two weeks after MarNeu depletion, the endothelium permeability was decreased, leading to an impairment of leukocyte transendothelial migration upon exposure to various inflammatory stimuli (allergen or lipopolysaccharide [LPS] exposures, infection with influenza virus). Moreover, while the adult lung was thought to be a relatively quiescent organ, recent studies have demonstrated an unexpected tissue-specific heterogeneity of ECs and the presence of lung-specific EC subsets that exert particular functions, like CD34⁺ ECs implicated in angiogenesis after tissue damage. Here, we show that targeting MarNeu is associated with a decrease in the number of CD34⁺ ECs and their ability to proliferate after a tissue damage. We are currently further investigating the MarNeu-EC axis and the long-term control of EC stemness, identity and functions by MarNeu.

Kinetic overview of the viral dissemination following Usutu virus peripheral inoculation in wild type 129/Sv pups

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Usutu virus (USUV) is an emerging mosquito-borne flavivirus closely related to West Nile Virus. Originating from Africa, both viruses are currently co-circulating all over our continent, causing more and more seasonal episodes of mass mortalities in birds as well as sporadic neurological diseases in both healthy and immunocompromised mammals. The host and pathogen's factors involved in these occurrences have not been described yet, and the increasing number of cases diagnosed in Europe over the past few years undoubtedly represent a serious threat for public health. Thus, the need to develop a relevant model to study USUV-mammals interactions has become crucial. Considering the occasional susceptibility of these dead-end hosts, the mammalian models used to study USUV pathogenicity are mostly immunocompromised mice. However, we recently succeeded to identify a relationship between the age at the time of infection and the outcome in 129/Sv wild-type pups. Indeed, the animals infected at 9 days-old by a footpad injection showed 100% lethality, while the 15 day-old group entirely survived to the infection without clinical symptoms. We then decided to compare the viral dissemination within the different body compartments for these two groups over time, in order to identify the main host-pathogen interface(s) involved in the difference of outcome that we observed. Various barriers appeared to have a role in the determination of the phenotype, as obvious differences were spotted in the viral loads measured in the blood and the central nervous system of both groups. Future studies will aim to characterize the host-pathogen factors responsible for these differences.

Short talks

A new avian *in vitro* model for the study of Usutu virus infection

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Usutu virus (USUV) is a RNA virus that belongs to the *Flaviviridae* family, transmitted by mosquito bites and affecting mainly birds, observing two phenotypes: species resistant to the infection like the domestic chicken (*Gallus gallus*) and species susceptible to infection, especially the Eurasian blackbird (*Turdus merula*). The mechanisms underlying these differences are still unknown. The objective of this work was to develop *in vitro* models for the study of these phenotypic differences. For this purpose, we used tendon-derived mesenchymal stem cells (MSCs) that can be isolated up to 48h post-mortem. These cells express similar markers as dermal fibroblasts, which play a key role in the pathogenesis of Flaviviruses. To validate blackbird MSCs as a model of infection, they were compared to Vero E6 cells, the gold-standard used in Flavivirus studies. Both cells showed a high permissivity to USUV infection and similar replication kinetics, blackbird MSCs showing slightly lower viral loads. Then, to validate MSCs as a model to study susceptibility/resistance phenotypes, USUV binding and replication kinetics were compared in blackbird and chicken MSCs. No significant differences were observed in the binding of USUV to cells of both species. In terms of replication kinetics, blackbird MSCs were more permissive to USUV infection, with higher viral loads, suggesting a role in the susceptibility/resistance phenotypes observed *in vivo*. In conclusion we isolated and validated avian MSCs as an interesting model for the study of USUV infection. In addition, these cells facilitate access to *in vitro* models in wild animals, which are difficult to sample due to their conservation status.

Retrospective study of the use of inhaled salbutamol administered to anesthetized horses suffering from impaired oxygenation: mechanisms of action and predictability of outcome

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Animals: 73 horses treated with salbutamol when arterial partial pressure of oxygen (PaO₂) < 100 mmHg during anesthesia. Methods: Horses were divided between responders (R), where PaO₂ after salbutamol ≥ 1.2 PaO₂ before, and non-responders (NR), where PaO₂ after salbutamol < 1.2 PaO₂ before. Demographic data and intraoperative variables before treatment were compared between R and NR. Dynamic compliance, arterial to end-tidal carbon dioxide difference (P(a-ET)CO₂), ratio of dead space over tidal volume (V_D/V_T), estimated shunt fraction (F-shunt), heart rate, systolic, mean (MAP) and diastolic (DAP) arterial pressure and dobutamine requirements were compared before and after treatment within R and NR. For each parameter, the difference between values after and before treatment was calculated (delta) and compared between R and NR. Numerical data were compared using univariate or bivariate analysis and categorical data were compared using chi-square test; *p* < 0.05. Results: 50 horses were classified as R while 23 horses were classified as NR. All the demographic data and initial intraoperative variables but body weight were similar between R and NR. Salbutamol significantly altered P(a-ET)CO₂, V_D/V_T and F-shunt in R only. Delta P(a-ET)CO₂, delta V_D/V_T and delta F-shunt were significantly different in R than in NR. MAP was significantly altered in R only while DAP significantly increased in both R and NR. Conclusions: Inhaled salbutamol is effective in improving venous admixture when successfully administered to anesthetized horses with PaO₂ < 100 mmHg. However, its outcome remains unpredictable.

Neutrophil Extracellular Traps Are Found in Bronchoalveolar Lavage Fluids of Horses With Severe Asthma and Correlate With Asthma Severity.

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Asthma encompasses a spectrum of heterogeneous immune-mediated respiratory disorders sharing a similar clinical pattern characterized by cough, wheeze and exercise intolerance. In horse, equine asthma can be subdivided into severe or moderate asthma according to clinical symptoms and the extent of airway neutrophil inflammation. Yet, the physiopathology of different phenotypes of equine asthma remains poorly understood and there is a need to elucidate the underlying mechanisms tailoring those phenotypes in order to improve clinical management and elaborate novel therapeutic strategies. In this study, we sought to quantify the presence of neutrophil extracellular traps (NETs) in bronchoalveolar lavage fluids (BALF) of moderate or severe asthmatic horses and healthy controls, and assessed whether NETs correlated with disease severity. To this end, we evaluated the amounts of NETs by measuring cell-free DNA and MPO-DNA complexes in BALF supernatants or by quantifying NETs release by BALF cells by confocal microscopy. We were able to unequivocally identify elevated NETs levels in BALF severe asthmatic horses. Moreover, we provide evidence that BALF NETs release was a specific feature seen in severe equine asthma, as opposed to moderate asthma, and correlated with disease severity. Finally, we showed that NETs could act as a predictive factor for severe equine asthma. Our study thus uniquely identifies NETs in BALF of severe asthmatic horses using three distinct methods and supports the idea that moderate and severe equine asthma do not rely on strictly similar pathophysiological mechanisms. Our data also suggest that NETs represent a relevant biomarker, a putative driver and a potential therapeutic target in severe asthma disease.

Proteomic profiling in equine atypical myopathy: new metabolic insights

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Equine atypical myopathy (AM) is a seasonal intoxication of grazing equids. In Europe, this poisoning is linked with the ingestion of toxins contained in the seeds and seedlings of the sycamore maple tree (*Acer pseudoplatanus*). Once ingested, one of the incriminated toxins, hypoglycin A (HGA), is metabolized into methylenecyclopropylacetyl-CoA (MCPA-CoA) which inhibits several steps of the energy production from fatty acid β -oxidation. This toxin-induced energy deprivation may explain the 74% mortality rate observed. To date, the mechanism of toxicity is not completely elucidated. Indeed, in the same environmental context, some horses are poisoned and others, so-called "co-grazing horses", remain free of clinical signs while blood tests confirm the ingestion of toxins. With the purpose of improving our understanding of the pathological process, a large-scale study (60 horses) was conducted to highlight the metabolic pathways modified in the context of toxin ingestion by determining proteomic changes occurring in the blood of diseased (HGA+/MCPA+), healthy co-grazing (HGA+/MCPA \pm) and reference horses (HGA-/MCPA-) through a label-free quantitative proteomic study in UHPLC/MS by timsTOF Pro (Bruker). We identified 2736 proteins with 156 showing significant differences between the diseased and co-grazing groups. The proteomic study also reveals substantial changes in the apparently healthy co-grazing group assuming the existence of a subclinical status. The differentially expressed proteins were sorted according to fold enrichment in metabolic pathways such as glycolysis/gluconeogenesis, complement and coagulation cascades and biosynthesis of amino acids revealing new metabolic insights.

Short talks

Comparison of combined thickness of the uterus and the placenta between 2 breeds of mares and their potential relationship with estrogens during normal pregnancy.

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To the best of our knowledge, combined thickness of the uterus and the placenta (CTUP) has never been compared between different breeds of mares using the same settings. This study compares the CTUP in 4 to 11 months pregnant Spanish Pure-Breed (SPB) and showjumping Belgian Saddle-Breeds (SJ) mares. The relationships between CTUP and estradiol (E2), estrone (E1) or estrone sulfate (E1S) concentrations was also investigated. CTUP was monthly measured and blood was collected in 15 SPB and 11 SJ mares. The CTUP was measured by transrectal ultrasonography (Renaudin et al., 1997) and mares presenting signs of placentitis were excluded. Estrogens were assayed in serum using Liquid Chromatography coupled to Mass Spectrometry (Dufour et al., 2021). For the same month of pregnancy, no difference in CTUP was observed between breeds. The CTUP gradually increased during the pregnancy and was significantly larger ($p < 0.01$) at 11 months, showing a decreased and heterogenous echogenicity. No significant difference in CTUP was observed between 9th and 10th month, but they tended to be higher ($p < 0.1$) than those observed at previous months of pregnancy. No correlation was observed between CTUP and estrogens concentrations. Some previous reports led to think that there were differences in CTUP between breeds of mares. However, with this design, such differences were not observed. The CTUP was not related to E2, E1, E1S concentrations showing that morphologic and endocrinologic evolutions of placenta are not associated: maximal CTUP was observed at 11 months whereas estrogens peak is described between 5 and 6 months.

Illumination of Cyprinid Herpesvirus 2 Infectious Cycle Using *In Vivo* Bioluminescent Imaging

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During the past two decades, as a consequence of international trading, Cyprinid herpesvirus 2 (CyHV-2) reached a worldwide distribution. This virus causes outbreaks associated with high mortality rate in goldfish (*Carassius auratus*) and related fish species (*Carassius gibelio* and *Carassius carassius*). Its high economic impact on the aquaculture sector has subsequently stimulated an increasing number of fundamental and applied researches. In the present study, we aimed to use bioluminescent *in vivo* imaging to gain an understanding of some important fundamental aspects of CyHV-2 pathogenesis, such as, for example the portal of viral entry into the host. With that goal in mind, we produced a recombinant strain expressing luciferase and copepod GFP as reporter proteins. Several approaches were developed to compare the recombinant strain produced to its parental strain in term of genome structure, expression of genes flanking the insertion site, replication in cell culture and virulence. All together, these results suggest that the LucGFP recombinant produced exhibits adequate properties for the study of the pathogenesis of CyHV-2. Consequently, this recombinant was used to study CyHV-2 pathogenesis in various development stages goldfish using IVIS following different inoculation modes mimicking different epidemiological conditions. These experiments suggested that the skin is the main portal of entry of the virus into its host and that the digestive track does not act as a portal of entry but rather a portal of egress from the infected host.

Zebrafish larvae model to screen for the risk of *Acer pseudoplatanus* intoxication in grazing animals

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Invasive or newly encountered plants constitute an emerging threat of intoxication for grazing animals and humans by direct or indirect consumption. *Acer pseudoplatanus* poisoning is a well-known emerging pasture-associated intoxication leading to atypical myopathy (AM) in equids. In this study, the interest of zebrafish larvae to screen environmental intoxication was evaluated using *A. pseudoplatanus* poisoning as a model. Zebrafish larvae of 4 days post fertilization were exposed during 22 hours to several concentrations of methylene cyclopropyl acetate (MCPA), one of the toxic metabolites implicated in *A. pseudoplatanus* poisoning. The mortality and the median concentration (LC50), the morphological aspect, the behavior, the heartbeat as well as the acylcarnitines (AC) profile were evaluated. The LC50 of MCPA after 0.5, 1 and 22 hours were 180 µM, 100 µM and 22 µM, respectively. The gradual signs before the death were in order of appearance (i) loss of instinct to flee (ii) reduced motility (iii) alteration in head and ventral cavity/yolk sac (iv) spasms with paralysis leading to abnormal swimming. Heartbeat and movements were inversely proportional to MCPA concentration. Short-chain AC were increased in the profile as commonly found in AM-affected horses. This preliminary study suggests that zebrafish could be used to obtain rapid feedback on the toxic effects of a known, emerging or unknown source of intoxication. The intoxication model will be further developed using plant extracts rather than a pure toxic molecule to decipher the pathological process taking into account all the components of a natural intoxication

Impact assessment of vB_KpnP_K1-ULIP33 bacteriophage targeting *Klebsiella pneumoniae* on the human gut microbiota using an *in vitro* gastrointestinal model

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New control methods are needed to counter antimicrobial resistances and the use of bacteriophages as an alternative treatment seems promising. To that end, the effect on intestinal microbiota of the phage vB_KpnP_K1-ULIP33, whose host is the hypervirulent *Klebsiella pneumoniae* SA12 (SB4585) (ST23 and capsular type K1), was assessed, in triplicate, in an *in vitro* model: the SHIME system (Simulator of the Human Intestinal Microbial Ecosystem). This tool simulates the gastro-intestinal tract and in particular the colonic microbiota. Colons were inoculated with samples of faeces from a healthy donor. After 23 days of stabilization, the phage was inoculated once a day for 7 days and its persistence in the different colons was studied until its disappearance, using Petri dishes titrations. The concentration of short chain fatty acids, on samples collected three days a week, showed a good colonization of the bioreactors and non-significant variations related to the phage treatment. Diversity (α and β) and relative abundance of bacteria (obtained by metagenetic analysis) were studied on samples collected at "end points" and no significant variation was observed following phage administration. Further studies are needed to assess the efficacy of this phage against its bacterial host within the human intestinal ecosystem.

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Short talks

Effect of food additives on gut microbiota of healthy donors and IBD patients

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Some food additives have been related with adverse effects on health by the onset of microbial dysbiosis in animal models. However, the effects of food additives on intestinal microbiota of patients suffering from IBD (Inflammatory Bowel Disease) is less known. The aim of this work was to determine how food additives influence intestinal microbiota of healthy and "IBD" donors. Six food additives (polysorbate 80, maltodextrin, titanium dioxide, sodium nitrite, sucralose, and carrageenan) were tested *in vitro* in batch culture models of intestinal microbiota under controlled conditions. The tested concentrations were based on the acceptable daily intake (ADI) established by the Expert Committee on Food Additives (JECFA). Three groups of donors participated in this study: healthy persons, patients in remission of IBD and patients with an active IBD. Changes in microbiota were assessed using qPCR targeting bacterial groups involved in short-chain fatty acid (SCFA) production or inflammation. SCFA production was assessed using SPME- GC/MS methodology. Depending on the group of donors, the same compound had different effects on both metabolites production and changes in microbial communities. Polysorbate 80, decreased markedly the production of propionate and butyrate in healthy donors but not for donors in remission of IBD. This was related to a decrease of Bacteroidetes and *Clostridium coccooides* populations in healthy donors that was not observed for donors in remission of IBD. A decrease of many others bacterial populations was however observed for IBD donors when exposed to PS80. To our knowledge, this is the first study *in vitro* focusing on the impact of food additives on altered intestinal microbiota such as those of IBD patients.

Genome-wide patterns of population structure and genetic diversity in Xinjiang indigenous sheep

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Indigenous sheep breeds from Xinjiang are widely renowned for their high level of adaptation to a variety of ecological conditions and their diverse phenotypic characteristics. Due to this large phenotypic diversity, the genetic background of Xinjiang indigenous sheep populations is of considerable interest. The present-day Xinjiang indigenous sheep resource was shaped by a variety of historical occurrences, breeding programs, and climatic and topographical factors. For the purpose of government germplasm conservation and domestic animal genetics research, it is crucial to evaluate the genetic diversity and population structure of local sheep breeds in Xinjiang. In a first study, we have analyzed the genetic relationships between the breeds and the structuration of the populations. The Duolang, Chinese Xinjiang Merino, and Cele sheep are different from other traditional Chinese sheep lineages, according to what we have discovered. Our population structure analysis has also revealed the clustering of five Xinjiang indigenous sheep populations. A second study has examined the levels of inbreeding within these populations. The degree of inbreeding of Duolang and Cele sheep is a concern. Although these two breeds aren't often taken into account in large-scale breeding operations, some of their characteristics are economically valuable, and these sheep breed exhibit exceptional environmental adaptability. In conclusion, our research provides a foundation for subsequent breeding planning and genetic research in Xinjiang sheep, as well as lays out a direction for the sustainable use and upkeep of the region's indigenous sheep populations.

Serum 25-hydroxyvitamin D and 24,25-dihydroxyvitamin D in dogs with sinonasal aspergillosis

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Sinonasal aspergillosis (SNA) is a common cause of chronic nasal disease with a still poorly understood pathophysiology and which remains a challenge to treat. As convincing evidence emerge showing that vitamin D plays a role in immunity, we aimed to investigate a potential implication of hypovitaminosis D in the SNA pathogenesis. Twenty dogs with SNA, 12 healthy control dogs, 9 dogs with lymphoplasmacytic rhinitis (LPR), 10 dogs with nasal neoplasia were included. Nine dogs with SNA were available for follow up until cure. Serum vitamin D metabolites were measured by liquid chromatography tandem mass spectrometry. The vitamin D metabolite ratio (VMR) was calculated by dividing the 25(OH)D by 24,25(OH)₂D. Serum 25(OH)D and 24,25(OH)₂D were lower in dogs with SNA at the time of diagnosis (mean ± standard deviation = 23.5 ± 7,1 ng/ml – 10,5 ± 4,2 ng/ml, respectively) than in healthy dogs (34,1 ± 7,5 ng/ml; p=0,017 - 18,2 ± 5.4 ng/ml; p = 0,005) while there was no difference between healthy and dogs with tumor or LPR. There was no significant difference in serum 25(OH)D and 24,25(OH)₂D between dogs with SNA at the time of diagnosis and dogs achieving cure. The VMR was higher in SNA dogs (2,4 ± 0,7) than in control dogs (1,9 ± 0,3; p=0,031 t-test), indicating a decreased catabolic clearance of vitamin D in SNA dogs. These results further support the rationale that vitamin D could play a role in dogs with SNA as it does in human with aspergillosis. Whether hypovitaminosis D could contribute to the development of SNA or if oral supplementation could be a beneficial adjunctive therapy in affected dogs is unknown and warrants future investigations.

Longitudinal clinical follow-up of ovine caseous lymphadenitis in Settat province in Morocco

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Caseous lymphadenitis (CL) is an important infectious disease caused by *Corynebacterium pseudotuberculosis*. CL is clinically characterized by suppurative necrotic inflammation in the superficial and internal lymph nodes and in organs. In Settat province in Morocco, a mean clinical prevalence of 34.6% of CL superficial abscesses in sheep flocks was previously reported (El Khalfaoui N. et al., 2020), however incidence rate of CL new cases and its variation over time are not known. To estimate the clinical incidence and its variation over time in sheep in Settat province, a longitudinal follow-up of 274 clinically healthy sheep was conducted between February 2021 and July 2022. Recruited animals were clinically examined every 2 months to detect superficial abscesses by palpation of superficial lymph nodes. Information about the number and location of abscesses was recorded. The clinical incidence rates of new cases were calculated based on 2-month periods. Statistical analysis is in progress.

During one year of longitudinal follow-up, 185 new CL cases appeared among the 274 recruited animals, leading to a clinical incidence rate of 0.67. The proportion of new CL clinical cases during the period between April and September was higher than during the period between October and March. The higher clinical CL incidence between April and September could be explained by the indoor intensive rearing management of young animals. By combining this result with previously identified risk factors (non-published data), it is aimed to provide precise advice and guidelines to reduce and control CL in sheep flocks in Settat province in Morocco.

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Short talks

Relationship between the cycle threshold value (Ct) of a *Salmonella* spp. qPCR performed on feces and clinical signs in horses

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Background: Salmonellosis is a zoonosis and a recurrent problem in equine clinics. Fecal shedding of *Salmonella* spp. is confirmed by culture on repeated samples, but the delay until result obtention limits its clinical usefulness. PCR-tests provide faster and more sensitive results, but its use as diagnostic confirmation of clinical salmonellosis in horses is controversial. **Objectives:** To evaluate the clinical significance of quantitative real-time PCR (qPCR) results when taking the cycle threshold values (Ct) into account. **Animals:** Eighty-four horses with ≥ 1 positive *Salmonella* spp. fecal qPCR. **Methods:** Retrospective study on 120 *Salmonella* spp. qPCR-positive fecal samples over 2 years. The mean Ct value of the qPCR test was compared in regard to (1) clinical signs of salmonellosis, (2) outcome, and (3) SIRS status (no, moderate or severe SIRS). A ROC analysis was performed to establish the optimal cut-off Ct value associated with severe SIRS. **Results:** The mean \pm SD Ct value was significantly lower in samples (1) from horses with fatal outcome (27.87 \pm 5.15 cycles) than in surviving horses (31.75 \pm 3.60 cycles) (unpaired t-test, $p = 0.022$) and (2) from horses with severe SIRS (27.87 \pm 2.78 cycles) than from horses with no (32.51 \pm 3.59 cycles) or moderate (31.54 \pm 3.02 cycles) SIRS (ANOVA test, $p < 0.001$). ROC analysis showed an optimal cut-off value of Ct associated with severe SIRS of 30.40 cycles, with an area under curve of 0.84 [95% CI: 0.76-0.91] and an OR of 0.64 [95% CI: 0.51-0.79]. **Conclusion:** Results suggest that including the Ct value in the interpretation of fecal qPCR results could improve the diagnostic value of this test for clinical salmonellosis in horses.

Metabolomic investigation of exercise-induced response to moderate and high intensities training sessions in Thoroughbred flat racehorses

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The training program of Thoroughbred (TB) flat racehorses includes exercise sessions of low to high intensities, short to long distances and rest periods. This field study aimed at assessing the metabolic responses induced by two training sessions of different intensity, widely used in the training of TB. Blood samples were collected in two groups of six and five TB at rest and 30 minutes after a high-intensity (gallop, 1.400m) and a moderate intensity (canter, 2.500m) exercise, respectively. Plasma samples were analyzed using an untargeted metabolomic approach based on liquid chromatography coupled with mass spectrometry. Exercise intensity was evaluated by monitoring heart rate and speed as well as exercise-induced changes of targeted biochemical parameters (e.g. lactatemia, hematocrit, etc.). Unsupervised principal component analysis performed on all detected features revealed changes in metabolome from pre- to post-exercise plasma samples with a greater extent following high-intensity exercise. In addition, supervised analyses revealed changes of 172 features by high-intensity exercise, among them 35 metabolites were identified using standard metabolites comparisons. These metabolites were mainly related to the chemical class of amino acids, nucleic acids and vitamins. Moderate intensity exercise induced changes of only a few metabolites, related to lipids and amino acids. In conclusion, TB training sessions induce differential changes in energy pathways depending on the intensity and the distance of the exercise.

Posters

Veterinary Public Health

1. Study of nutritional and anti-nutritional profiles of cereals and pulses from Greco-Roman Egypt, and analysis of chemical hazards resulting from ancient food processing techniques

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Were ancient foodstuffs more or less nutritious than modern counterparts? This question is a historical debate on ancient diet and nutrition for a long time. Present foodstuff nutritional data are based on components and processing practices influenced by the 20th century Green Revolution. Therefore, in this study, we will update this debate based on empirical data obtained from unique ancient plant samples originating from the from Graeco-Roman site of Karanis in Egypt. In our methodology, firstly, we will perform nutritional analyses on the ancient plant remains from Karanis in order to reconstruct the nutritional profiles of the raw ingredients. The focus will be on the main staple crops, being the cereals (6-row hulled barley and hard wheat) and pulses (lentil, chickpea, lupine, pea, bitter vetch, and grass pea). For the nutritional profile, our focus will be on the macronutrients (carbohydrates, proteins and lipids), gluten content in cereals, vitamin E, fatty acid, amino acid profiles, antinutrients and (taxon-specific) toxins such as phytates, tannins, saponins, alkaloids in lupine and the neurotoxin 3-(N-oxalyl)-L2,3diamino propionic acid in grass pea. The results will be compared to, and interpreted within the framework of, modern results from both literature and analyses on modern reference materials from Karanis, as well as comparison with modern samples from various origins. Secondly, the relative changes that occur during historical food processing will be elucidated by assessing the (anti)nutritional alterations at each phase of the production processes of at least one major foodstuff/production process from each food category.

2. Phage targeting *E. coli* K1 in the intestinal microbiota of pregnant donors to fight neonatal meningitis

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Escherichia coli capsular type K1 is a leading cause of human neonatal meningitis. The asymptomatic carriage of these strains in the maternal intestinal and vaginal microbiota constitutes a risk of vertical transmission to infant at birth. The aim of this work was to evaluate the efficacy of phage therapy against *E. coli* K1 in an intestinal environment. For this purpose, 3 independent experiments were conducted in triplicate on the SHIME® system (*Simulator of Human Intestinal Microbial Ecosystem*) inoculated with intestinal microbiota of 3 pregnant donors. After two weeks of stabilisation of the microbiota, experiments were conducted. In the first experiment, only the phage vB_EcoP_K1_ULINTec4 was inoculated. In the second experiment, only *E. coli* O18:K1:H7 was inoculated and in the last experiment, *E. coli* was inoculated together with the phage. Phage and *E. coli* K1 concentrations were measured 3 times a week for 6 weeks. The results showed that phage K1_ULINTec4, when inoculated alone, was progressively cleared by the peristaltic system. In the presence of the target bacterium, after a decrease in the first days, the phage titers tended to stabilize between 4 and 5 log PFU/mL in both proximal and distal colons. Comparison of bacterial concentrations in experiments 2 and 3 showed that *E. coli* K1 persisted in the microbiota but with lower titers in presence of the phage. In conclusion, those experiments showed that the phage administered was able to affect the survival of *E. coli* K1 in the system but did not completely eliminate it. However, further research is needed to investigate the hypothesis that the phage K1_ULINTec4 is able to decrease the virulence of this strain.

3. New insights on Usutu virus infection in a susceptible bird model : the domestic canary (*Serinus canaria*)

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Usutu virus (USUV) is a mosquito-borne Flavivirus pathogenic to avian species. It is often associated with massive die-off events, some of which occurred during the winter, a vector-free period. The main hypothesis to explain how USUV “overwinters” is a bird-to-bird transmission, as shown for the closely-related West Nile virus (WNV). To assess this question, we first experimentally challenged canaries with intranasal inoculation of USUV, which led to a systemic dissemination of the virus comparable to that observed after parenteral inoculation, provided the inoculated dose was sufficient ($> 10^2$ TCID₅₀). Next, we co-housed infected birds (after intranasal or intradermal inoculation) with naive sentinels, to determine if an horizontal transmission could be reproduced experimentally. However, we failed to prove such a transmission, and although we highlighted the oronasal excretion of infectious viral particles in infected birds, the shed titres and/or the direct contacts between birds seemed insufficient to allow a direct transmission, in our experimental conditions. To complete our study, we infected *ex vivo* tracheal explants of canaries to determine the viral tropism at the inoculation site, but we observed only very limited signs of viral replication or amplification. Contrary to its human counterpart, the avian tracheal epithelium seem thus to not be permissive to USUV infection. Further research on the cell tropism is needed to identify the cell type(s) involved in the initial replication and dissemination of USUV after intranasal inoculation and to understand how the bird-to-bird transmission might occur in wildlife.

4. Prevalence and characterization of extended-spectrum β -lactamase-producing *Escherichia coli* in freshwaters, hospital effluents and wastewaters in Belgium

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Antimicrobial resistance (AR) is recognized by the WHO as one of the greatest threats to global health. The enteric bacteria coming from humans and animals (One Health approach) can be found in aquatic systems and be a vector for the spread of AR into the environment. The purpose of this work was to evaluate the prevalence of extended-spectrum β -lactamase-producing *E. coli* (ESBL-EC) in freshwaters, hospital effluents and wastewaters during two sampling campaigns (winter/summer) in 2021 and to characterize them. A total of 24 stations were sampled including 17 freshwaters, three hospital effluents and the entry/output of two wastewater treatment plants (WWTPs) in the Ourthe watershed. A total of 615 *E. coli* strains were isolated from Brilliance ESBL agar medium. Disk-diffusion assays were performed following the EUCAST's recommendations. All the ESBL-EC isolates were tested for the presence of blaCTX-M-1, blaCTX-M-2 and blaCTX-M-9 gene's group by PCR. Genes belonging to blaCTX-M-1 and CTX-M-9 groups were detected respectively in 72% and 15% of the isolated strains. No gene of blaCTX-M-2 group was found. A subset of isolates (n=40) were selected for whole genome sequencing. *E. coli* serotype O18:H7 with sequence types ST1463 was predominant (n=14). β -lactamase genes identified were blaCTX-M (n=21), with blaCTX-M-15 the most represented (n=15), as well as blaTEM (n=6), blaOXA (n=9) and blaSHV (n=9). One of the most observed concerns was the large number of strains containing carbapenemase genes- blaKPC-3 (n=19), blaNDM-1 (n=1) and blaVIM-1 (n=2) -even in freshwaters. This study shows that hospital effluents and WWTPs contribute to the dissemination of AR into the environment.

5. Isolation and preliminary characterization of four new lytic bacteriophages against *Aeromonas salmonicida*, the causative agent of furunculosis

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Aeromonas (A.) salmonicida, a Gram-negative bacteria belonging to the *Aeromonadaceae* family, is a primary fish pathogen that causes furunculosis in salmonids, carp and perch, as well as septicemia in a variety of fish. This species is considered as one of the main bacterial pathogens responsible for important economic losses in aquaculture industry. Large amounts of antibiotics such as oxytetracycline, quinolones and sulfonamides are used to treat this infection, which highly contributes to the emergence of antibiotic-resistant strains. The application of bacteriophages (phages) in aquaculture seems to be a promising solution to control pathogenic bacteria in this field because these organisms are well adapted to aquatic environments. The aim of this work was to isolate and characterize new lytic phages against *A. salmonicida*. Phage isolation was performed by the enrichment method from water samples collected in different fish farming ponds and natural aquatic environments in southern Belgium in early 2022. From these water samples, four new lytic phages were isolated. A preliminary host spectrum test showed that three of these four phages were active against other wild *A. salmonicida* strains while the fourth one showed a narrower host spectrum. These four phages were not active against any of the *A. hydrophila* strains tested. After having determined temperature and pH stabilities, adsorption times and kinetics of these four new phages, further studies are needed to analyse their genomes and to assess the *in vivo* safety and efficacy of these phages.

6. Development of a method of embedding and high-throughput histology of 8-day-old zebrafish (*Danio rerio*) larvae.

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The need for high-throughput histological analysis methods of large numbers of zebrafish (*Danio rerio*) larvae came up to meet the increasing demand for zebrafish as a reference model in the laboratory. This method has become a useful tool in the study of modern clinical medicine, histopathology and research. Because of the small size of zebrafish larvae, it is valuable to develop an accurate method to perform histological sections of many larvae simultaneously in the same cutting plane. This reduces the laboratory work but also the time needed to identify individual changes. Here we describe the details of a method for embedding and sectioning histological slides using larval matrices. This work aims to optimize a histological method to detect changes and deleterious effects in tissues, especially in the digestive tract, of 8-day old zebrafish under simultaneous exposure to microplastics and a bacterial pathogen. High-quality histological images will give us useful information on how early exposure to microplastics can affect the response and resistance of zebrafish larvae to an infectious agent. Until today, there is no standardized or commercialized solution for this type of analysis, it must be developed and manufactured in the laboratory. The mold developed in our laboratory is designed to perfectly fit the shape of an 8-day old larvae to facilitate the inclusion process and allows to set 24 larvae per slides. In this way, we managed to obtain good quality and reproducible sagittal histological slides where the digestive tract and other tissues are clearly visible.

7. Assessment of the health effects of a fruit extract through an *in vitro* dynamic simulation of the human gastrointestinal system

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INTRODUCTION: There are numerous medicinal plants and fruits traditionally used to treat gastrointestinal disorders. However, the effects caused by these vegetal products on the intestinal microbial populations are poorly understood. **METHODS:** An *in vitro* gut model (SHIME®) was used in this work to analyze the intestinal effects of two weeks of treatment with three increasing doses of a vegetal extract. Gut microbiota community and metabolites were studied on ascending (AC), transverse (TC), and descending (DC) colon regions using qPCR and chromatographic methods, respectively. The effects of the mixture of metabolites upon the host intestinal cells and the impact of the test products on relevant molecular pathways were evaluated through cell culture assays. **RESULTS:** A significant increase of acetic acid in TC, and of butyrate in all colons was observed by the end of treatment, while propionate levels remained unchanged. Of 12 targeted microbial populations, some health biomarkers such as *Bifidobacterium*, *Roseburia*, and *Bacteroides-Prevotella* genera significantly increased in at least one of the colon sections due to treatment. The highest dose of the vegetal extract induced an increase of *Akkermansia muciniphila* in AC and TC as well as an increase of *Faecalibacterium prausnitzii* in all colon regions. No cytotoxic effects of the plant extract were identified when exposed to human intestinal cell lines. The fermentation-derived metabolic pool activated molecular pathways linked to intestinal health and host immune defense. **CONCLUSIONS:** Overall, the studied fruit extract induced the growth of health-promoting bacteria and the production of metabolites that potentially contribute to gut barrier integrity and have a beneficial impact on human gastrointestinal health.

8. Phage-mediated Shiga-toxin gene transduction from O80:H2 Shiga toxigenic *E. coli* (STEC) to non-STEC strains and *in vivo* virulence assessment

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Shiga toxin-producing *Escherichia coli* (STEC) are major foodborne pathogens causing human diseases ranging from diarrhea to life-threatening complications such as hemolytic-uremic syndrome (HUS). Virulence of STEC strains and their ability to cause severe diseases are linked to the activity of prophage-encoded Shiga toxins (Stxs). Stx phage acquisition and stability studies are crucial in terms of public health. The first objective of this work was to isolate and characterize the Stx2d phage from STEC O80:H2, an emerging serotype in humans and calves, and then study the transfer of this phage in non-STEC strains. The second objective was to assess the survival of *Galleria mellonella* larvae inoculated with these transduced strains. Three temperate phages were induced and isolated from a bovine STEC *E. coli* O80:H2 under UV radiation. One of them was used to infect six non-STEC strains and its genome was analyzed. The three successfully transduced strains (K12-MG1655, K12-DH5 α and O80:H26) were confirmed by *stx2d* PCR and whole genome sequencing. A stability study was performed and showed that this phage was stable in the new STEC strains after three successive subculturing steps. This phage belongs to the *Caudoviricetes* class (unclassified genus and family) and presents resistance to moderate temperature (45°C) and low pH (up to pH2). *Galleria mellonella* experiments showed that convertant strains caused significantly higher mortality rates than the corresponding non-STEC strains. In conclusion, this study showed that Stx2d phage can be transferred to non-STEC strains and contributes to their virulence.

9. The N-terminal region of Cyprinid herpesvirus 3 ORF104 inhibits the formation of stress granules induced by various stress stimuli

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The molecular mechanisms that trigger stress granules (SGs) formation in eukaryotic cells occur through eIF2 α -dependent and eIF2 α -independent pathways depending on different types of stress. Cyprinid herpesvirus 3 (CyHV-3) is a member of the *Alloherpesviridae* family infecting common and koi carp. Recently, we identified CyHV-3 ORF104 as one of the two proteins responsible for the inhibition of SG formation induced by arsenite. ORF104 protein consists of an N-terminal (Nter) intrinsically disordered region and a C-terminal kinase domain. The N-terminal of ORF104 contains a NLS and a NES signal. Both domains were shown to be essential for viral replication in cell culture.

We studied ectopic expression of ORF104 or its recombinant forms in Hela cells. These experiments led to the following observations: (i) ORF104 localizes both in the cytoplasm and the nucleus. (ii) Cytoplasmic expression of ORF104-Nter domain is essential for SG inhibition. (iii) Expression of ORF104-Nter domain fused to two NES (ORF104-2NES-Nter) increases the cytoplasmic localization and the associated inhibition of SG formation. (iv) Expression of ORF104-Nter domain in which the NLS has been deleted was no longer able to inhibit SG formation by arsenite. (v) Hela cells expressing ORF104-2NES-Nter inhibited SG formation induced by various stress stimuli through eIF2 α -dependent pathway and eIF2 α -independent pathway. Taken together, our observations suggest that the ORF104-Nter inhibits SG formation either through different mechanisms acting on different pathways and/or through a common mechanism operating at the point of convergence between these pathways.

10. The effects of cefotaxime on hepatopancreas, pharmacokinetic and antibiotic residues in white-leg shrimp (*Litopenaeus vannamei*)

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The aims of the study are to determine the effects of cefotaxime on hepatopancreas, pharmacokinetic data, and withdrawal time of antibiotic in white-leg shrimp (*Litopenaeus vannamei*). The content of the research included (i) determination of minimum inhibition concentration of cefotaxime on *Vibrio parahaemolyticus* isolates by dilution method and plating (ii) cefotaxime distribution in shrimp hepatopancreas and plasma and their pharmacokinetic data when feeding at a concentration of 25 mg/kg shrimp, sampling was done at 0.5, 1, 2, 4, 8, 12, 24 hours after feeding medicated feed (iii) determination the withdrawal time of cefotaxime after feeding once and twice a day for three consecutive days, shrimp muscle was collected 1 and 3 days during medication and 1, 3, 7, 14 and 30 days after stopping the medication. Shrimp hepatopancreas was also observed the change in structure by histology.

The selected *Vibrio parahaemolyticus* strains were susceptible to cefotaxime through the low MIC values and large inhibition diameter, 28-32 mm. Cefotaxime maximum concentration (19446 \pm 2099 μ g/kg) reached in hepatopancreas after one hour while it was relatively low in shrimp plasma, 184 \pm 61 μ g/L, after 4 hours. Feeding cefotaxime once a day for three consecutive days at a concentration of 25 mg/kg shrimp, the withdrawal time was 54 degree-day at 29,5°C while it was 205 degree-day when feeding cefotaxime twice a day at the same concentration. Results from histology analysis revealed that cefotaxime did not affect shrimp hepatopancreas even feeding twice a day for three consecutive days.

11. Understanding the mechanisms underlying gammaherpesvirus- triggered modulation of immune and pro-fibrotic responses using a multispectral imaging model

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The lung is continuously exposed to different environmental insults like microbial products, smoke, pollutants and pathogens that can damage the tissue. Therefore, a large reparative capacity is required. When inadequately controlled, this repair process can lead to pathology such as seen in pulmonary fibrosis. Many studies have shown that idiopathic pulmonary fibrosis is associated with environmental risk factors. Epstein bar virus, a γ -herpesvirus or its mouse homologue, murid herpesvirus 4 (MuHV-4), is highly prevalent and is responsible for persistent latent infection. While these infections are associated with lymphoproliferative disorders in immunocompromised individuals, there is also evidence that γ -herpesviruses correlate with the aggravation of various immunopathologies such as lung fibrosis. While lung immune cells may play a beneficial or detrimental role in regulating tissue repair responses, it is not clear how this occurs in a spatio-temporal manner. A better understanding of the mechanisms by which environmental factors shape the lung immune system to promote or prevent lung regeneration is a pressing unmet need that may open new avenues to improve lung regeneration. Thus, to study the mechanisms of MuHV-4-induced exacerbated pulmonary fibrosis and the interactions between immune cells and their microenvironment, an innovative multiplex immunofluorescence technique will be employed. This technique uses a multispectral Nuance camera that can visualize up to 15 markers in a single tissue section, which can then be overlapped with classical histopathological analyses. As a first step, respiratory infection by MuHV-4 followed by bleomycin intratracheal administration one month later is characterized. Lung cryosections are employed to quantify collagen deposition, using Sirius Red stainings, as well as for multispectral imaging.

12. Interest of a diet enriched with organic selenium and omega-3 fatty acids during finishing period for bbb beef production

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Selenium (Se) is an essential micronutrient for human and animal health by improving antioxidant status and immunity. In Belgium, where meat is one of the main source of Se, consumers and cattle are often deficient. Enriching the fattening diet could improve cattle health and meat quality. This study aimed to assess the impact of a diet enriched with L-selenomethionine at 0.5 mg/kg (SeMet) and omega-3 fatty acids on the fattening performance, slaughter parameters and meat quality of BBB. In breeding-fattening system, 18 bulls and 11 cows were studied during the finishing period. Average daily gain (ADG) was measured and health status was assessed by clinical examination. Blood samples were taken to monitor the status of vitamins (A, E, B12, β -carotene), erythrocyte glutathione peroxidase activity (GSH-Pxe) and trace elements (Cu, Zn, Se). At the slaughterhouse, the body weight and hot carcass weight (HCW) were recorded. Five days later, *Rectus abdominis* (RA), *Longissimus thoracis* (LG) and *Rectus femoris* (RF) were sampled and subjected to technological (pH, color, drip loss, tenderness) and chemical (fatty acid profile) analyses. ADG was higher in cows (1.5 kg/day) than bulls (0.9 kg/day). Blood analyses showed a continuous increase of Se in plasma as well as GSH-Pxe activity, higher in cows than bulls throughout 90 days. HCW was higher to that one reported in literature for both bulls and cows. According to our observations, other differences (blood parameters evolution, ADG and meat quality) were attributed to different breeding practices, genetic, as well as to the gender and age of cattle.

13. A Comparative HPLC-MS/MS and HPLC/DAD study of neurotoxin β -ODAP and Its non-toxic α -isomer in modern and ancient archaeological remains of grass pea

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Lathyrus sativus (grass pea) is a high-yielding, drought-resistant high protein legume consumed as a food in Northern India and neighboring countries as well as in Africa. Its development into an important food legume, however, has been hindered by the presence of the antinutritional neurotoxin β -N-oxalyl-L- α , β -diaminopropionic acid (β -ODAP) in seeds which, if consumed in large quantities for prolonged periods, can cause irreversible paralysis. Recently, some low-toxin lines have been developed that may prove safe for both animal and human foods. HPLC has been the most used for β -ODAP quantification. However, the analysis of ODAP by HPLC is a challenge due to its high polarity and weak UV absorption. The aims of the current study are to perform for the first time: 1) a comparative HPLC-MS/MS and HPLC/DAD study of α - and β -ODAP; and 2) to compare α , β and total ODAP contents in modern samples and ancient archaeological remains of grass pea collected from Greco-Roman Egypt. The current study will help to monitor the level of the toxic β -ODAP so that the consumption of grass pea can be optimized. In addition, this work contributes a new and complementary piece of fundamental information to the puzzle of ancient diet and nutrition in comparison to modern diet as it will provide for the first time empirical data on the antinutritional factor ODAP in ancient raw ingredients.

14. Pollen preferences of stingless bees in the Amazon region and southern highlands of Ecuador by scanning electron microscopy and morphometry

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Stingless bees are effective pollinators of native tropical flora. Their environmental service maintains pollen flow through pollination. Meliponiculture, management of stingless bees, is an activity limited to the countryside in Ecuador. The lack of knowledge of their managers about pollen resources can affect the correct production of nests. The objective is to identify botanical families and genera of pollen grains collected by stingless bees by morphological features and differentiate potential species using geometric morphometry. Thirty-six pot pollen samples were collected from three Ecuadorian provinces located in two climatically different zones. Pollen type identification was based on the Number, Position, Character system. Using morphological features, the families and genera were established. Abundance, diversity, similarity and dominance indices were established by counting pollen grains, as well as spatial distribution relationships by means of Poisson regression. Forty-six pollen types were determined in two study areas, classified into 27 families and 18 genera. 1148 ± 799 (max 4211; min 29) pollen grains were counting in average. Families Melastomataceae and Asteraceae, genera *Miconia* and *Bidens*, were found as the main pollen resources. The results showed that stingless bees do not collect pollen from a single species, although there is evidence of a predilection for certain plant families. The diversity indexes showed high richness but low uniformity in the abundance of each family identified. These information are meaningful to the Ecuadorian meliponiculture as there is a need to improve management practices to preserve the biodiversity.

Sustainable livestock production

Posters

15. The effect of *in-ovo* administration of Rosemary Essential Oil on hatchability, relative hatching weight and embryo mortality rate in Japanese quail (*Coturnix Coturnix Japonica*)

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The *in-ovo* technique consists in injecting exogenous substances inside hatching eggs to improve embryonic development and hatching parameters. This technique can also limit antimicrobial resistance to antibiotics by administering up-stream alternative substances as plant extract. The aim of this study was to determine the effects of quail eggs air sac injection of different concentrations of rosemary essential oil, on hatching rates, weight of chicks at hatching, and embryonic mortality rates. A total of 800 Japanese quail eggs were divided into four groups: negative control (non-injected), positive control (30µl/distilled water egg), and both treatment groups (T1 and T2) injected on the ninth day of incubation with 30µl/egg of rosemary essential oil solution at concentrations of 1µl/egg and 3µl/egg, respectively. The results showed that the *in-ovo* injection of 3 µl/egg rosemary essential oil has a toxic effect on embryonic development, revealed by 18.21% post-injection mortality rates compared to control groups. The group that received 1µl/egg showed 8.32% mortality rates. A beneficial effect of 1µl/egg concentration was observed on hatching rates with 69.1% compared to the 3µl/egg which showed rates of 44.48% but these results remain similar to those of control groups. For hatch chicks weight, treatment with essential oils showed a higher result with rates of (65.39%) and (67.44%) for chicks from eggs treated with a 1µl/egg and 3µl/egg, respectively. In conclusion, this study showed that injecting eggs with 3µl/egg of rosemary essential oil proved to be toxic to the quail embryo compared to the 1µl/egg injection which showed a beneficial effect in terms of hatching.

16. Thermal manipulation during Post-Hatch and acetic acid supplementation on the physiological response of broiler chickens exposed to chronic heat stress

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The effects of post-hatch thermal manipulation (TM), Acetic acid supplementation (AA) of drinking water, and their interactions (TM-AA) on respiratory rate, body temperature, blood biochemical and hematological parameters, and thyroid hormones of broiler chickens reared under high ambient temperatures were determined. A total of 1100 1-day-old chicks were divided into four treatments: the "control" which were non-thermal manipulated and non-supplemented; "TM" which were exposed to 38 ± 1 °C for 24 h at 5 days of age; "AA" which were given drinking water supplemented with 0.2% of commercial vinegar from 28 to 49 days of age; and "TM-AA" which were both Thermal manipulated and Acetic acid supplemented. All groups were exposed to the natural fluctuations of summer ambient temperature (average diurnal ambient temperature of about 30 ± 1 °C and average relative humidity of 58 ± 5%). TM did not affect the respiratory rate or body temperature of chronic heat-exposed chickens. AA changed the body temperature across time (d35, d42, d49) (linear and quadratic effects) without changing respiratory rate. Thermal manipulated chickens exhibited lower levels of glycemia and higher hematocrit and red blood cell counts. Furthermore, the greatest effects of AA, alone or associated with TM, were the lowering of cholesterol and triglyceride blood concentrations. A significant effect of TM on T3:T4 ratio was observed. Finally, some beneficial physiological responses induced by TM and AA, separately or in association, on chronically heat-stressed chickens were observed. However, the expected cumulative positive responses when the two treatments were combined were not evident.

17. Pilot study on caudal vena cava size by fast ultrasonography through different views in healthy calves.

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Introduction: Ultrasonographic measurements of caudal vena cava (CVC) and aorta (Ao) are known as reliable tools to assess intravascular volume status in humans and companions' animals. The objective of this study was to perform a pilot study on the feasibility to obtain ultrasonographical measurements of the CVC and the aorta (Ao) in two different views, assess intra- and interobserver variability, and study the effect of age, sex, breed, or body weight on measurements in healthy calves. **Methods:** We performed a single observer prospective observational study in standing healthy calves from beef and dairy herds. All calves aged less than six weeks and weighted less than a hundred kilos and were deemed healthy based on history and clinical evaluation. Two anatomic sites were assessed by the investigator, to obtain three views of CVC and Ao (longitudinal view in subxiphoid site and transversal and longitudinal views in the paralumbar site). **Results:** 48 calves were enrolled, 22 males and 26 females, from 4 different herds. Subxiphoid view was tried on every calf but cineloop was not recorded in 20%. In the paralumbar site, cineloops were recorded on 94 % of the calves, and interpretable in 92%. A high significant linear correlation was found between the age of the calves and CVC and Ao measurements made at paralumbar views except for CVC area in transversal PV. **Conclusions:** Caudal vena cava size assessment by point of care ultrasound can be easily performed at paralumbar site in calves under 4 months of age and could be used to assess intravascular volume status. Current study is ongoing to compare CVC and Ao measurements between healthy and hypovolemic calves.

18. Assessing forest and pastureland cover in northern Morocco using remote sensing

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Northern Morocco harbors the large-scale oak forests in the country, surrounded by agricultural and pastureland mosaics. Despite the socio-economic role and the predominance of pastoral activities, especially in Western Rif (Chefchaouen), there is little information about the current land cover mapping of forests and pasturelands. The objective of this study was to spatially delineate the forest and pastureland based on remote sensing data. The available data are an image scene from the operational land imager/ thermal infrared sensor (OLI/TIRS) images, with 30 m of spatial resolution on board of Landsat 8 satellite, on July 18th, 2019. To better describe the LULC change types and to increase the probability that these changes closely reflect the ground reality based on our prior knowledge of land use in the study area, a total of seven major land cover classes were used: forest; pasture grassland under matorral, pasture grassland; grassland mixed with cultivated areas; cropland; built-up area; and water. Forest, pasture grassland, and cropland cover an area of 39, 3.9, and 3.1%, respectively. The most prominent cover land was recorded by pasture grassland mixed with cropland (51.5%). The land cover results of the forest class agree with the statistics data reported in the official reports, except for the land cover of classes including pasture grasslands, because they have never been studied before. Overall, this research contributes to knowing Western Rif's current land cover types for future interventions to develop and manage forest and pasture areas.

Posters

19. Feeding behavior of grazing goat kids in forest rangeland of Bouhachem Natural Park, Northern Morocco

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This work was conducted in a forest rangeland of Bouhachem Natural Park (northern Morocco) to understand the feeding behavior of local goat kids. The direct observation method was used to estimate, the diet composition and dry matter intake (DMI) of local goat kids during three grazing seasons (spring, summer, and autumn). In addition, the palatability of plant species was determined. The results showed that *Cistus* and *Quercus* species are considered among the most palatable plant species for goat kids during the two grazing seasons of summer and autumn. The DMI of goat kids was greater in the summer and autumn, while it was lower in spring. During spring, the diet of goat kids was composed of 63% herbaceous, 32% shrub, and 5% tree species. For the summer and autumn, their diet was composed of more than 80% of woody species. The results underline the high adaptability and ability of goat kids to select a woody species across seasons. Knowledge about the feeding behavior of goat kids could be used as the first guide for rangeland managers to ensure herd and forest sustainability.

20. Valorization of some red berries by-products in ruminants' diet

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Goat species, such all ruminants, is known by their capacity to valorize resources with low nutritive values to produce milk and meat. In harsh environment, the diet of this herd is based essentially on rangelands. Thus, goat diet should be diversified by incorporating unconventional resources. The red berries cultivations are practiced in Northern Morocco, and during their crop cycle, large quantities of leaves are generated as by-products. Their incorporation in ruminant diet requires the knowledge of their composition and metabolizable energy. This work aims to evaluate the chemical composition, digestibility, and the energy of three red berries cultivation by-products to be introduced in goat diet. Three samples of strawberry, raspberry and blackberry were collected during pruning or uninstallation from farms located in Larache in Northern Morocco. The studied parameters were dry matter, ash, ether extract, fibers (NDF, ADF and Lignin), condensed tannins, dry and organic matter digestibility and metabolizable energy. Results showed that all studied parameters were similar for all by-products, except dry matter, ash, and ether extract. Strawberry leaves contained the lowest dry matter, and ash, and the highest ether extract. In conclusion, the red berries by-products provide a similar nutritive value and could present an alternative feed to diversify ruminant's diet.

21. Chemical composition, digestibility and metabolizable energy of some legume's cultivation

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In Northern Morocco, forest rangelands are the main feed resource for grazing goats. These rangelands are characterized by annual and seasonal variation, causing the low herds' productivity. Thus, it is necessary to improve goat herd feeding by alternative feed resources incorporation. Legume cultivations are widely practiced in Northern Morocco. After primary products harvest, these cultivations generate important quantities of by-products (straw and hay) that could take place in ruminant diet. To introduce them, it is crucial to characterize their nutritional value. Thus, this work aims to determine the chemical composition, digestibility, and energy of four legumes practiced in Northern Morocco. Six samples of bean, faba bean, lentil and chickpea were collected from several places in Tangier and Larache, during summer after harvest. The studied composition parameters were dry matter, ash, ether extract, fibers (NDF, ADF and Lignin), condensed tannins, dry and organic matter digestibility and metabolizable energy. Results showed that dry matter, ether extract, ADF and Lignin were similar in all studied by-products. However, ash, NDF, condensed tannins, digestibility and metabolizable energy were variable ($P < 0.05$). Highest values of ash, digestibility and metabolizable energy were recorded in beans by-products. However, high contents of NDF and condensed tannins were observed in faba bean and chickpea by-products, respectively. In conclusion, the legumes by-products present an alternative feed to diversify ruminant's diet and bean by-product seems to be more nutritive among the studied by-products.

22. Potential use of some vegetables by-products as an alternative feed resource

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Nowadays, the substitution of conventional resources by agricultural by-products widely available could be used in the ruminant diet. In northern Morocco, vegetable farms are widely present and generate large quantities of by-products. However, it is necessary to know their nutritional quality to be incorporated into animal diet. This work aims to evaluate the chemical composition and metabolizable energy of two vegetables by-products mostly available in the study area. Three samples of onion and watermelon were collected from different places in Tangier and Larache, during summer after harvest, and dry matter, ash, ether extract, fibers (NDF, ADF and Lignin), condensed tannins, dry and organic matter digestibility, and metabolizable energy parameters were determined. According to the results, ether extract, fibers, and condensed tannins were similar in both of by-products. However, watermelon contained high water, ash, and metabolizable energy, and was more digestible compared to onion ($P < 0.05$). In conclusion, the vegetable by-products present an alternative feed to diversify ruminant's diet and watermelon by-product seems to be more nutritive among the studied by-products.

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23. Utilization of peanut cultivation by-products as a valuable source of animal feed

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The use of unconventional feed such as agricultural by-products seems to be a solution to diversify ruminant's diet and reduce human-ruminant competition. Peanut cultivation produces, in addition to seeds, large quantities of by-products that could be used as alternative feed resources. To be incorporated in ruminants' diet, this by-product should be evaluated. Thus, the aim of this work is to determine the chemical composition, the digestibility and metabolizable energy of this by-products. Six samples of peanut cultivation by-product were collected after harvest in Larache in Northern Morocco. The studied parameters were dry matter, ash, ether extract, proteins, fibers (NDF, ADF and Lignin), condensed tannins, dry and organic matter digestibility and metabolizable energy. Results showed that peanut by-product contains high dry matter (89%), and fibers (52, 31 and 9% for NDF, ADF, and Lignin, respectively). Ash was estimated at 8%, and proteins was about 8%, which satisfy the ruminant maintenance requirement threshold. The condensed tannins were low with 0.6%. This by-product is characterized by a high digestibility of 77% for dry matter and 81% for organic matter, which is reflected in the high value of metabolizable energy with 11 MJ/Kg DM. In conclusion, peanut by-product presents a high nutritional value that should be valorized as alternative feed resources.

24. Understanding of antibiotic resistance and use of the antibiotics by veterinary students at the Faculty of Veterinary Medicine, University of Liege.

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Antibiotic resistance renders one or more antibiotics ineffective against a bacterial infection. This can make it difficult or impossible to treat certain infections. One of the main reasons for the increase in antibiotic resistance is the excessive and inappropriate use of antibiotics by physicians and veterinarians. Proper training of veterinary students on the use of antibiotics in animal husbandry can reduce antibiotic resistance. For this purpose, a survey was conducted among the 2nd cycle veterinary students of the University of Liege. In a survey, a total of 94 structured questionnaires were completed by 4th (11), 5th (56) and 6th (27) year students.

The majority of students surveyed were female (71.3%). Career interests in the context of future work as a veterinarian was very diverse, with 35.1% of respondents considering mixed practice in veterinary medicine (large animals and companion animals). For the impact of the veterinary curriculum on the knowledge of antibiotic resistance, 54.3% of the students reported a real and positive impact. Students responses to the questions on practical knowledge of antibiotics demonstrated good basic knowledge. Students responded that antibiotics are not effective against viruses (89.4%) and that they should be used against bacteria (92.6%). The main factors favoring the emergence of antibiotic resistance cited by the students were the overuse of antibiotics by health professionals (88.3%) and the misuse of antibiotics in veterinary medicine (63.8%).

In conclusion, this preliminary study suggests the need to increase the level of knowledge of antibiotics among veterinary students, especially regarding their practical use in their future work as practitioners.

25. Valorization of olive cake in the feed of japanese quail

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Olive cake (*Olea europaea L.*) is a by-product of olive industries that can be used as an alternative resource in animal feed in order to minimize the cost of importing raw materials in Algeria.

This work aims to study the effect of the incorporation of olive cake in the diet on the zootechnical performance of Japanese quail. A total of 370 one-day-old quails were reared on the ground and randomly divided into three groups T, E1 and E2, receiving ad libitum feed containing increasing levels of olive cake incorporation: T (0 %), E1 (10%) and E2 (20%). Subject weight and feed intake were measured weekly. Animal health status and mortalities were recorded daily.

The results show that the incorporation of olive cake in the diet does not cause any negative effect on the health status of quails. Mortality rates were similar in the three experimental groups throughout the monitoring period, with a high rate during the first days of implementation. Nevertheless, the weight gain of the animals decreases with the incorporation of olive cake (161.9g and 151.1g for E1 and E2 groups respectively), while the daily intake increases (43.70g for E1 group and 44.31g for E2 group).

In conclusion, olive cake can be considered as an important feed source for quails. The valorization of this raw material in poultry feed requires additional work to determine the optimum rates of incorporation.

26. Post-mortem examination of the genitalia of dairy cows from northern Algeria

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The objective of our study was to identify the different pathologies of the genital tract of the cow encountered in the slaughterhouse of El-Harrach located in the city of Algiers. A total of 301 bovine genital tracts were collected after slaughter. The results obtained showed that 75.4% of the genital tracts were free of any detectable pathology, of which 61.1% were non-pregnant and 14.3% were pregnant during the first third of gestation. However, 24.6% of matrices were affected by pathologies. After anatomopathological study, the frequencies of the different pathologies were as follows: for ovarian pathologies, 7.6% of ovarian cysts, 2% of smooth ovaries and 8.9% of adhesions. Regarding tubal pathologies (1.33% of salpingitis and hydrosalpinx). Uterine pathologies were represented by 1.7% of uterine infections and 0.33% of tumors. 0.7% of cases of cervical inflammation and 0.33% of curved cervix were also reported. At the vaginal canal level, macroscopic examination revealed 1% Gartner's duct cysts and 0.7% Bartholin's gland cysts. It is also important to mention the presence of 0.33% of congenital pathologies translated by Hermaphroditism and unilateral segmental aplasia (unicornis uterus). At the end of this study, the ovarian pathologies were the most answered, certainly because of the nutritional disorders and the bad ovarian manipulations of the practitioners.

Posters

27. In vitro screening of feed additives to maintain ruminal fermentation levels under the heat stress conditions

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This study aimed to evaluate the potential use of different feed additives to maintain ruminal fermentation levels in vitro, under the heat stress (HS) conditions. Six additives (clay, sodium bicarbonate, betaine, protected fat, yeast and lemon balm) were selected based on a literature review, their availability on the market, their cost and their effectiveness against heat stress based in previous studies. In vitro gas production dynamics, pH, redox potential and the quantity of protozoa were assessed for each additive and compared to a control devoid of additive. These measurements were carried out during fermentations of rumen fluid in a carbonate buffer solution submitted to a temperature of 39°C and 40.5°C, simulating respectively a rumen under normal temperature and under HS conditions in a batch fermentation system. The additives were tested in combination with 2 diets: winter diet (confinement) and summer diet (grazing system). Gas production dynamics and pH did not differ (p-value > 0.05) between additives and the two diets at 39°C and 40.5°C. The redox potential was higher (p-value 0.03) for the control fermentation at 40.5°C. The total number of protozoa was higher for the samples that were not under HS conditions. Although the impact of a rise in temperature has not demonstrate difference in this work, the fermentation has made it possible to hypothesize that these additives would allow the maintenance of normal ruminal levels under higher temperatures. An increase in the temperature of the water bath simulating conditions of ST is strongly recommended in order to conclude the efficacy of the additives in maintain ruminal fermentation levels in vitro.

Comparative veterinary medicine

28. Venous intravasation of contrast medium during retrograde urethrography in a dog with urethral stricture and urethritis

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Retrograde urethrography is a commonly used imaging modality to investigate urethral diseases. Complications are uncommon and include iatrogenic urinary tract rupture and infection. Extravasation of contrast medium has been described into the peritoneal space, cavernous tissues of the penis or soft tissues adjacent to the urethra. A 12-year-old castrated pug was referred for a suspicion of urethral stricture. The dog was presented with a history of hyporexia, vomiting, dysuria and stranguria for 3 weeks, having required multiple urethral catheterisations. The patient underwent an abdominal ultrasound, caudal abdominal radiographs, retrograde urethrography, followed by an urethroscopy. A retrograde urethrography showed a focal luminal narrowing with an irregular mucosal margin of the penile urethra. Contrast medium was observed within the adjacent penile tissue, venous sinuses of the bulbus glandis, ascending venous pathway as well as in the external iliac and urethral veins. Urethroscopy confirmed a marked urethral stricture associated with signs of severe urethritis in the region of the bulbus glandis. Cytology samples taken by cytobrush showed mixed inflammatory cells associated with bacterial infection. Scrotal urethrostomy was performed along with a medical treatment for concurrent prostatitis. Urethro-venous contrast medium intravasation is a rare complication of retrograde urethrography. It is due to the alteration of the integrity and permeability of the urethral wall with contrast medium entering the surrounding penile tissue, venous plexus and ascending venous pathway. It can therefore be observed concomitantly with stenosis and/or urethritis.

29. Histological characterization of the proximal suspensory entheses in the normal equine hindlimb

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The enthesal morphology of the equine suspensory ligament (SL) origin has not been fully characterized. This experimental pilot study is intended to describe the preliminary results of histological evaluation of the proximal SL entheses in the normal equine hindlimb. The entheses of a hindlimb from a horse, euthanized for reasons other than the study, has been divided in 6 sagittal compartments based on prior CT examination (proximal-distal, each lateral-sagittal-medial). After freezing and cutting, the histological slices were decalcified, cut and prepared for hematoxyline-eosine and trichrome staining. All slices passed histological quality control, although 1 slice did not contain the entheses and was considered too distal. The remainder of the entheses revealed a 4-layered appearance, consisting of compact bone, calcified fibrocartilage (CF), uncalcified fibrocartilage (UF) and ligamentous collagen fibers. The fibrocartilage layer showed multiple aligned columns of chondrocytes, with a tidemark clearly separating CF and UF. Contrary to this rather smooth tidemark, the plantar cortical and/or dorsal ligamentous outline was markedly irregular with several variable interdigitations. The preliminary results of the current study show that the equine hind proximal SL entheses are predominately fibrocartilaginous in nature¹. Such fibrocartilaginous entheses are often found in biomechanically complex structures and are considered to play a key role in stress dissipation². The fibrocartilaginous nature of the entheses may affect imaging criteria for the diagnosis of proximal SL enthesopathy in different imaging modalities.

30. Isolation and preliminary characterization of five lytic bacteriophages against *Pseudomonas aeruginosa* causing canine otitis externa

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Multi-drug resistant (MDR) *Pseudomonas aeruginosa* is a common bacteria isolated in canine chronic suppurative otitis externa. The presence of this bacteria is often correlated with a non-response to the medical treatment. Its virulence factors, including biofilm formation, make it a fearsome pathogen. The use of bacteriophages seems to be a promising alternative to treat MDR infections. The objectives of this study were to isolate and characterize lytic *P. aeruginosa* phages from wastewater. The enrichment method was used to isolate bacteriophages from Belgian wastewaters active against *P. aeruginosa*. Host range and efficiency of plating were performed on about fifty strains of *Pseudomonas* spp. coming from different species (dogs, cats, horses and reptile) and organs (ear canal, skin, tracheal and broncho-alveolar lavage, nose, fluid, eye). An efficiency of plating was performed for phages showing total and partial lysis. A total of five lytic *P. aeruginosa* phages were isolated from Belgian wastewaters. The phages showed a wide spectrum of lysis on *Pseudomonas* spp. Further studies are required to analyze the genome of these phages, but also their effect on the biofilm, before considering using a phage cocktail in MDR *P. aeruginosa* suppurative otitis externa.

31. What is behind? Accidentology in racehorses in jump races

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Jump races are known as having the highest incidents and fatal injuries rates amongst equestrian disciplines. However, what is really behind those numbers? The racing industry has been granted a social license for now, but animal welfare compels us to better understand the different processes that lead to a horse being injured. We hypothesize that the risk of accidents associated to jump racing can be modelled using (1) the data of all horses entered in racing, in France, (2) the input obtained by reviewing the integrality of the races occurring all over the country for two years and, (3) spatio-temporal analysis. Racehorses' accidents during a race can result from cardiovascular conditions, spontaneous fractures, tendinopathies, a fall or a pathology inherent to a previous fall, etc. Determining the temporality of an accident with its causal or predisposing factors is of utmost interest for defining preventive measures. This research uses new technologies such as video analysis and GIS (Geographic Information System) combined with statistical scrutiny in order to better consider the context of occurrence of accidents/incidents at jump racing. Each horse fall and fatalities were analyzed using open-source software allowing to work in stop motion and multiple plans enabling to focus on each momentum. Subsequently, the information's were linked with veterinarians' files when available. Our preliminary results highlight the value of this new process to answer fundamental questions: which kind of incidents/pathologies can occur before a fall? What are the direct consequences of a fall, or do the incident at racing result from lesions developed during training? Etc.

32. Tgfb-Tgfr receptor signaling is essential for lung interstitial macrophage differentiation and identity

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In the lung, interstitial macrophages (IM) spontaneously produce the immunosuppressive cytokine interleukin (IL)-10, thereby maintaining lung homeostasis and preventing the development of allergic inflammation to aeroallergens. Recently, we discovered two distinct IM subsets and found that MafB was an important transcription factor that restricted local monocyte proliferation and mediated IM differentiation and identity of both subsets. While macrophage colony-stimulating factor (M-CSF) acts as a local signal contributing to this process, it remains to be determined whether additional factors from the lung microenvironment are imprinting the identity of IM. We performed single cell RNA-sequencing of whole lung cells in steady-state and performed NicheNet analysis, allowing us to identify the *Tgfb-Tgfr* axis as a promising ligand-receptor interaction mediating IM identity. Both IM subsets expressed high protein levels of Tgfb-RII, and BMDM stimulation with Tgfb triggered expression of IM-associated genes. Then, we generated myeloid-restricted *Tgfr2*-deficient mice (i.e., *LysM^{Cre}Tgfr2^{fl/fl}* mice) and found that numbers of IM were lower in *LysM^{Cre}Tgfr2^{fl/fl}* mice as compared to littermate controls. Of note, IM differentiation from monocytes seemed to be impaired and blocked in a monocyte-to-IM transition state in those mice. We also found that MafB directly or indirectly regulated IM-specific Tgfb receptor expression in myeloid-restricted *Mafb*-deficient mice. This work adds to our understanding of IM biology by showing how the lung-specific microenvironment shapes IM identity, thus providing foundations for future IM-targeted therapeutic interventions in the context of lung chronic inflammatory disorder.

33. Confirmed hypoglycin A toxicosis in two gnus

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In spring 2021, two gnus (*Connochaetes taurinus taurinus*) exposed to *Acer pseudoplatanus* seedlings in their zoo enclosure were found in decubitus with signs of depression and tremors. Blood samples collected from these animals at that time were tested positive for hypoglycin A (HGA) and its toxic metabolite methylenecyclopropylacetyl-carnitine (MCPA-carn). These observations highlight (i) exposition to HGA, (ii) HGA absorption and, (iii) HGA metabolization into its active metabolite. In addition, biochemical findings (i.e., increased muscle enzyme activity in blood) indicated an ongoing rhabdomyolysis process. Hypoglycin A metabolization into MCPA-CoA is known to induce a severe alteration in lipid metabolism resulting in an energy deficit in type I muscle fibers. To confirm *A. pseudoplatanus* poisoning in the diseased gnu, free carnitine and twenty-one acylcarnitines (C2, C3, C3DC, C4, C5, C5-OH, C5DC, C6, C8, C8:1, C10, C10:1, C10:2, C12, C12:1, C14, C14:1, C16, C16:1, C18 and C18:1 -carnitine) were quantified in serum and compared to non-exposed gnu as controls. In the diseased gnu, HGA appears to have similar consequences on fatty acid catabolism than the one described in equids with a severe modification of the acylcarnitines profile. This study confirms HGA poisoning in Bovidae associated with exposure to *A. pseudoplatanus* and so, discloses a potential risk of toxicity for domestic ruminants and a potential risk for food safety.

34. Genetic diversity of Bovine Viral Diarrhea virus in cattle in France between 2018 and 2020.

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Bovine Viral Diarrhea Virus (BVDV) is one of the main pathogens that affects ruminants worldwide, generating significant economic losses. Like other RNA viruses, BVDV is characterized by a high genetic variability, generating the emergence of new variants, and increasing the risk of new outbreaks. The last report on BVDV genotypes in France was in 2008, since which there have been no new information. The goal of this study was to determine the genetic diversity of BVDV strains currently circulating in France. To this aim, samples of cattle were taken from different departments that are part of the main areas of livestock production during the years 2018 to 2020. Using the partial sequence of the 5'UTR region of the viral genome, we identified and classified 145 samples corresponding to *Pestivirus A* and one sample corresponding to *Pestivirus D*. For the *Pestivirus A* samples, the 1e, 1b, 1d and 1l genotypes, previously described in France, were identified. Next, the 1r and 1s genotypes, not previously described in the country, were detected. In addition, a new genotype was identified and was tentatively assigned as 1x genotype. In conclusion, these results indicate an increase in the genetic diversity of BVDV in France, which may be a risk to the health of cattle in France. In addition, the new 1x genotype was identified, increasing the variability of *Pestivirus A* worldwide.

35. Expression of fibroblast activation protein in dogs with idiopathic pulmonary fibrosis or lung cancer

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Canine Idiopathic Pulmonary Fibrosis (CIPF) is a progressive fibrotic interstitial lung disease affecting predominantly the old West Highland White Terrier (WHWT). Fibroblast activation protein (FAP) is a cell surface protease specifically expressed in areas of active tissue remodelling and upregulated in various types of cancers. The aim of this study was to assess the expression and localization of FAP in the lungs of WHWTs affected with CIPF and in canine lung tumours, in comparison with healthy lungs. FAP expression was investigated by immunohistochemistry on lung biopsies from 22 WHWTs with CIPF, 8 dogs with lung cancer (6 adenocarcinomas, 1 histiocytic sarcoma, and 1 metastasized mammary carcinoma) and 15 old dogs (including 4 WHWTs) without lung disease. A staining index (absent, low, intermediate or high) was attributed according to the percentage of positive cells combined with the staining intensity. FAP was identified in the lungs from 20 WHWTs with CIPF (staining index high, intermediate, or low in respectively 11, 6 and 3 dogs), in 7 lung tumours (high and intermediate in respectively 6 and 1 dogs) but in 2 control WHWTs only (1 of each intermediate and low). FAP was expressed by fibroblasts in areas of active fibrosis in CIPF and by cancer-associated fibroblasts (all types of cancer) and cancer cells (5 adenocarcinomas only) in lung tumours. We hypothesize that positive results in 2 predisposed dogs may represent a very early-stage disease. FAP overexpression in lungs from dogs with CIPF and lung neoplasia opens new perspectives in the fields of diagnosis and therapy for both diseases, since FAP could be investigated as a biomarker or a therapeutic target.

36. Biomolecular investigation for *Capillaria* spp. infections on bronchoalveolar lavage fluid of owned domestic dogs presented for chronic cough in Belgium

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The trichuroid parasitic nematode *Capillaria aerophila* is responsible for lower respiratory infections and *Capillaria boehmi* for sino-nasal infections in wild and domestic carnivores. The aim of this study was to assess the prevalence of *C. aerophila* infection in coughing, client-owned, domestic dogs in Belgium. Stored bronchoalveolar lavage fluid (BALF) from 125 dogs was retrieved. All dogs had history of chronic cough (> 2 weeks duration) and underwent BALF collection for microbiologic testing of common respiratory pathogens. A conventional polymerase chain reaction targeting a region internal to the *cox1* gene of *C. aerophila*, a Capillarinae consensus sequence, was performed on BALF samples in duplicate and in batch analysis using previously published primers sequences. DNA of adult *C. boehmi* specimens was included as a positive control. Molecular grade water was used as a negative control. Neither DNA of *C. aerophila* nor *C. boehmi* were detected in the BALF samples. Sixty-seven dogs (54%) had a recent history of deworming against lungworms with either moxidectin or fenbendazole (deworming protocol not standardized), 9 dogs (7%) were not up to date with deworming therapy, and the remaining 49 dogs (39%) had unknown deworming status. Result of this study suggest that *C. aerophila* infection is not prevalent in Belgium in dogs with chronic cough. This might be explained by recent deworming therapy in half of the included dogs. Epidemiosurveillance of capillarid infection may be considered in wild canids, such as foxes, to determine whether these parasites are a potential risk for domestic animals.

37. Atypical myeloid cells orchestrate epithelial repair by promoting AT2 expansion following respiratory viral infection

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Lung tissue repair mechanisms are critical components of the host immune response to respiratory viral infections in order to restore tissue integrity and homeostasis. As a corollary, dysregulated immune responses can be deleterious and lead to severe disease phenotypes. While myeloid cells are thought to substantially contribute to tissue repair, their complexity, diversity and fine-tuned functions remain under-investigated. Here, we used a sublethal mouse model of influenza virus infection (strain PR8) to investigate the mechanisms underlying lung tissue recovery. By analyzing lung immune cell dynamics post-infection, we observed the emergence of an as-yet-unknown atypical myeloid cell population peaking during the early recovery phase of infection, which shared expression of macrophage markers such as CD11b, F4/80 and CD64 and of neutrophil markers like Ly-6G and CXCR4. Electron microscopy analyses highlighted unique ultrastructural features, including a multilobular nucleus, granules, lysosomes and autophagy vacuoles, all delimited by a microvilli-rich cell membrane. These hybrid cells arose in the lung in a CCR2-dependent manner, and their presence and unique ability to produce high levels of Arginase-1 were dependent on IL-4 receptor signaling, a feature shared with alternatively activated macrophages. Furthermore, we found that they were located in alveolar spaces, at the edges of injured, hepatized areas, in the vicinity of alveolar epithelial cells. We have accumulated evidence *in vivo* and *in vitro* that one critical function of such atypical myeloid cells is to promote epithelial repair and lung re-epithelialization by physically interacting with alveolar type 2 epithelial cells. Ongoing work is investigating this myeloid-epithelial cell axis and the mechanisms behind their central role in tissue healing and lung function recovery.

38. MafB-restricted local monocyte proliferation precedes lung interstitial macrophage differentiation

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Resident tissue macrophages (RTM) are differentiated immune cells populating distinct niches and exhibiting important tissue-supportive functions. RTM maintenance is thought to rely on either monocyte engraftment and differentiation, or RTM self-renewal. Two main RTM populations have been identified in the lung: alveolar macrophages (AM) and interstitial macrophages (IM). Previously, we have demonstrated that lung IM exhibit a tolerogenic profile and are able to limit the development of aberrant immune responses against allergens, thus underscoring their role as crucial regulators of lung homeostasis. To further understand the contribution of IM subpopulations to lung development, homeostasis, inflammation and repair, it is fundamental to investigate the mechanisms underlying IM development and differentiation. To this end, we developed an inducible mouse model of IM niche depletion and repopulation to investigate IM development *in vivo*. Using time-course single-cell RNA-sequencing analyses, bone marrow chimeras and gene targeting, we generated a real-time transcriptional atlas of monocyte-to-IM differentiation and found that engrafted Ly6C⁺ classical monocytes could proliferate locally in a CSF1R-dependent manner before their differentiation into RTM. We further showed that the switch from monocyte proliferation towards IM subset specification was controlled by MafB, while c-Maf specifically regulated the identity of the CD206⁺ IM subset. Our data shed new light on the transcriptional regulation of IM development and provide evidence that, in the mononuclear phagocyte system, cell proliferation is not merely restricted to myeloid progenitor cells and mature macrophages, but is also a tightly regulated capability of mature monocytes developing into RTM *in vivo*.

39. Computed-tomography assessment of the main abdominal vessels in dogs

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Computed tomography (CT) characterization of abdominal vessels is poorly studied. The aims of this study were to assess during growth the evolution of the aorta (Ao), caudal vena cava (CVC), portal vein (PV), length of second lumbar vertebra (L2) and different ratios between these structures; and to determine the influence of dog size and age on the vessels size and ratios. The main abdominal vessels were assessed on CT angiography retrospectively. The first group included 6 Beagles scanned every month from 4-12 months old, then every trimester up to 24 months old. The second group included 26 dogs of different breeds and weights that were considered hemodynamically normal. Those dogs were divided in 3 weight sub-groups: <10kg, 10 to 30kg and >30kg. The measurements were repeated 3 times by 3 operators. The diameter of the Ao, CVC and PV, and the length of the Ao and L2, were significantly different from 4 to 7 months in age group. The diameter of the Ao, CVC, PV, and L2 length, were variable between weight sub-group but stable within the same sub-group. In contrast, the different ratios, such as CVC/Ao, Ao/L2 and CVC/L2 were stable whatever the parameter studied, such as age or weight in this study. For inter- and intra-agreement statistics, there were no significant differences between the 3 measurements by the same operator. However, significant differences were observed between the different operators. The student measurements were most often significantly different from the resident and board-certified radiologist measurements. The adult vascular standards published in the literature should not be used in medium dogs younger than 7 months old. As variability is observed between the weight sub-groups, pure measurements should be used with care in very small or very large dogs. Ratios can be used to erase the variability influencing those measurements.

