



Proceedings of the 8th FARAH-Day

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

November 19, 2021

One Health

L'Animal et l'Homme, une même santé



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Edited by C. Bayrou, C. Cesarini Latorre, C. Delguste, N. Delrez, C. Douny, J. Eppe, A.L. Etienne, V. Frisée, C. Gatez, L. Gille, C. Hupperts, T. Jauniaux, V. Jergeay, N. Korsak, C. Kruse, P. Loos, L. Martinelle, N. Moula, S. Parrilla Hernandez, J. Ponthier, D. Thiry, I. Tosi, D.M. Votion

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COVER PICTURE CREDITS:

"Vet Rescue Team during the floods that hit Liège in July 2021 "

@Hugues Guyot

Welcome to the 8th 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.

Véronique Delcenserie, Frédéric Farnir & Dominique Votion.

Bienvenue à la 8^{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.

Véronique Delcenserie, Frédéric Farnir & Dominique Votion.

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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

Prof. Damien Thiry

Phage Therapy as alternative to fight against antimicrobial resistant bacteria

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

Chair: Bénédicte Machiels & Véronique Delcenserie

09:45 **S. Li** - *A systems immunology approach reveals distinct roles of non-genetics and genetics factors in shaping variation of immune responses in cattle*

09:55 **J. Eppe** - *Technical and physiological testing of an ELISA kit for the determination of b-TSH in cattle: preliminary results*

10:05 **C. Antoine** - *In vitro characterization and in vivo efficacy assessment in Galleria mellonella larvae of newly isolated bacteriophages against Escherichia coli K1*

10:15 **S. Djebala** - *Antimicrobial susceptibility profile of several bacteria species identified in the peritoneal exudate of cows affected by parietal fibrinous peritonitis after caesarean section*

10:25 **C. Loublier** - *Freezing does not impact equine fecal bacterial viability on a specific fecal pre-transplantation processing method: a step towards equine stool bank*

10:35 **S. El Otmani** - *Chemical composition of cactus cladodes (Opuntia ficus indica) during the growing season in Northern Morocco*

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

11:15 - Oral session 2 (lecture hall C, building B45)

Chair: Bernard Taminiau & Linde Gilles

11:15 **T. Biénès** - *Gentamicin concentration in nasal lavage in healthy dogs after inhalation therapy*

11:25 **J. Baiwir** - *Influence of gammaherpesvirus infections on the antibody repertoire of their host*

11:35 Short talks

A. Demil - *Genetic and epidemiological analysis of BRSV strains that circulate in the Wallonia region of Belgium*

D. Nagy - Vitamin and antioxidant supplementation in the prevention and treatment of atypical myopathy - a pilot study to establish relevant tolerance intervals and assess the feasibility of supplementation

E. Goya-Jorge - In vitro evaluation of the aryl hydrocarbon receptor transactivity induced by human intestinal metabolites

G. Petrellis - A novel role for CXCR2-dependent neutrophil recruitment in the skin and lung in the anti-helminth type 2 immune response

Y. Gao - Virus-induced inhibition of superinfection as a means for accelerating fitness-based selection of cyprinid herpesvirus 3 single nucleotide variants in vitro and in vivo

A. Aberbour - The effect of in ovo administration of Rosemary and Artemisia Essential Oil on hatchability, hatch weight, weight loss and post hatch mortality rate on Japanese quail (*Coturnix coturnix japonica*)

N. Bernard - Jurassic Virology as a means for studying the evolution of a glycoprotein encoded by Cyprinid Herpesvirus 3

M. Servaty - The impact of grey seals on the mortality of other marine mammal species in the North Sea between 2011 and 2020

12:20 Questions

12:30 - Lunch and poster session 2 (Room P, building B45)

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

Chair: Nicolas Korsak Koulagenko & Irene Tosi

14:00 **C. Porsmoguer** - Congenital urethral sphincter mechanism incompetence in 11 bitches: a retrospective preliminary study

14:10 **E. Martinez** - Understanding the growth of *Clostridioides difficile* in vitro

14:20 **P. Loos** - A persistent gammaherpesvirus infection reprograms the alveolar macrophage niche for the long-term by dampening type 2 properties of group 2 innate lymphoid cells

14:30 Short talks

A. Fastrès - Assessment of SPP1 and FN1 in serum, bronchoalveolar lavage fluid and lung tissue samples from dogs affected with canine idiopathic pulmonary fibrosis

L. Ludwig-Begall - Analysis of Synchronous and Asynchronous In Vitro Infections with Homologous Murine Norovirus Strains Reveals Time-Dependent Viral Interference Effects

C. Streiff - Sensitivity and permissivity of zebrafish larvae to alloherpesviruses

H. Zhang - Anguillid herpesvirus 1 ORF35 is dispensable for viral replication in cell culture but essential for efficient infection of European eels (*Anguilla Anguilla*)

L. Crettels - Antimicrobial resistance of *Escherichia coli* isolated from freshwaters, hospital effluents and bathing water in Belgium

14:55 Questions

15:00 Invited speaker

Dr Bénédicte Machiels
***VI*ral **R**egulation **O**f **M**onocyte **E**ducation (**VIROME**)**

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

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

Chair: Louisa Ludwig-Begall & Antoine Clinquart

*16:00 **N. Delrez** - Social interactions between European eels mediate the transmission of Anguillid herpesvirus 1 through biting*

*16:10 **A. Lyssens** - Gentamicin concentrations in bronchoalveolar lavage and serum in healthy dogs after inhalation therapy*

*16:20 **M. Levoz** - Post-mortem mesenchymal stem cells: cryopreservation strategies*

*16:30 **H. Machiels** - Study of the use of IDEXX PROCYTE for total and differential cellular counts of bronchoalveolar lavage fluid in healthy dogs*

*16:40 **C. Maquet** - Ly6Chi monocytes balance regulatory and cytotoxic CD4 T cell responses to control virus induced-immunopathology*

16:50 Short talks

***M. Boumenir** - Does tourist pressure affect behaviour and feeding strategies of a commensal group of Barbary macaques, *Macaca sylvanus*, in Gouraya National Park (Bejaia, North-East Algeria)*

***B. Pirard** - Staphylococcal enterotoxins (SEs) genes screening of *Staphylococcus aureus* strains isolated from Walloon artisanal farm cheeses and assessment of their ability to produce SEs in vitro*

17:00 Questions

17:15 - Invited speaker (chaired by Prof. Laurent Gillet):

Prof. Peter Visscher (Dr Honoris Causa)

How genomic selection is a revolution and how it can impact both veterinary and human medicine?

17:30 – Cocktail and awards (Room P, building B45)

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Oral presentations

Oral presentations

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

Shifang Li¹, Françoise Myster¹, Gabriel Costa Moreira², Lijing Tang², José Luis Gualdrón Duarte², Justine Javaux¹, Rémy Sandor¹, Patrick Mayeres³, Michel Georges², Tom Druet², Carole Charlier², Laurent Gillet¹

1. Immunology-Vaccinology, Veterinary public health, FARA, Uliège

2. Unit of genomics animals, GIGA, Uliège

Corresponding author : fmyster@uliege.be

Immune system has primarily evolved to protect the host against infections. Given the breadth of the effects of immune responses on pathologies, it is necessary to understand the variability of immune responses in a given population and how this variability relates to susceptibility to infections and immune-mediated diseases. Recent studies have identified several host, environment, and genetic factors that influence the landscape of the immune system in human. However, such approaches have not yet been implemented in animal populations. In this study, the immune response of the Belgian White and Blue (BWB) breed was studied in a comprehensive manner by integrating environmental and genetic factors that lead to immune variation in cattle. A systemic immunophenotyping approach was established to quantify more than 200 immune parameters at steady state and after ex vivo restimulation of blood leukocytes. This approach revealed a distinct role of age, season, and genetic in shaping immune variation. Interestingly, based on genomic data from these animals, genome-wide association studies were able to identify several independent loci associated with the production of IL-8 and IL-10, two cytokines central to the development and regulation of immune responses, respectively. Overall, this approach may provide new information on the mechanisms underlying disease pathogenesis in cattle and will help us to better understand the genetic determinism of these mechanisms, allowing the application of genomic selection methods for these traits to improve these cattle breeds.

Technical and physiological testing of an ELISA kit for the determination of b-TSH in cattle: preliminary results

J. Eppe^{1*}, P. Petrossians², P. Bossaert¹, F. Rollin¹, H. Guyot¹

1. Clinical department of ruminant, FARA, University of Liege, Quartier Vallée 2, Avenue de Cureghem 7A-7D, Liège, 4000, Belgium

2. Department of Endocrinology, CHU of Liege, University of Liege, Chemin de l'Hôpital 1, Liège, 4000, Belgium

Corresponding author: justine.eppe@uliege.be

The thyroid gland plays an important role regarding health and production of cattle. Nevertheless, the metabolism and the functions of this gland remain poorly understood compared to other animal species. Moreover, diagnostic tools to assess the thyroid status in cattle are lacking while thyrotropin hormone is often used in other species.

This study tested an ELISA kit for the determination of bovine thyrotropin (b-TSH) via a thyrotropin releasing hormone (TRH) stimulation test and a b-TSH inhibition test performed on 4 young Holstein calves.

The ELISA kit had an intra-assay coefficient higher than the 9% announced in the kit notice (17.5±5.2%) and did not respond consistently to the inhibition test (differences between extreme values: $p > 0.1$). Nevertheless, this kit seems sensitive to the stimulation test and showed significantly different ($p < 0.05$) b-TSH concentrations when basal values (T-60, T-30, T0) are compared to T+5, T+10 and T+100 minutes after intravenous TRH injection. Further studies are needed to determine whether this ELISA kit can be used routinely. For example, the intra- and inter-assay coefficients could be calculated in the same way as in the kit notice or b-TSH could be measured on serum instead of plasma. Also, in view of the involvement of thyroid metabolism in milk production of high producing cows, this analysis could be studied as an additional tool to ration analysis. Finally, acute respiratory distress syndrome with a thyroid metabolic component implies that b-TSH analysis could be investigated to provide diagnostic but also prognostic value.

***In vitro* characterization and *in vivo* efficacy assessment in *Galleria mellonella* larvae of newly isolated bacteriophages against *Escherichia coli* K1**

Antoine C.^{1,2}, Laforêt F.^{1,2}, Blasdel B.³, Fall A.⁴, Duprez J.N.¹, Mainil J.¹, Delcenserie V.^{2*}, Thiry D.^{1*}

1. Dept of Infectious and Parasitic Diseases, Bacteriology and Pathology of Bacterial Diseases, FARAH, ULiège 2. Dept of Food Science, Quality management in the food chain, FARAH, ULiège 3. Vésale Bioscience, Vésale Pharmaceutica, Noville sur Mehaigne, Belgium 4. Genalyse Partner SA, En Hayeneux 62, Herstal, Belgium,

*these authors equally co-supervised the work

This research is funded by the Walloon Public Service, BIOWIN project: Inteliphages

Corresponding author: celine.antoine@uliege.be

Extra-intestinal *Escherichia coli* express several virulence factors that increase their ability to colonize and survive in different localizations. The K1 capsular type is involved in several infections including meningitis, urinary tract and bloodstream infections. The aims of this work were to isolate, characterize and assess the *in vivo* efficacy of phages targeting avian pathogenic *E. coli* (APEC) O18:K1, sharing many similarities with human strains responsible for neonatal meningitis. Eleven phages were isolated against APEC O18:K1 and four of them presenting a narrow spectrum targeting *E. coli* K1 strains were further studied. The newly isolated phages vB_EcoS_K1-ULINTec2 were similar to the *Siphoviridae* family and vB_EcoP_K1-ULINTec4, vB_EcoP_K1-ULINTec6 and vB_EcoP_K1-ULINTec7 to the *Autographiviridae* family. They are capsular type (K1) dependent and present several advantageous characteristics of lytic phages such as short adsorption time and latent period. vB_EcoP_K1-ULINTec7 is able to target both K1 and K5 strains. This study shows that these phages replicate efficiently both *in vitro* and *in vivo* in the *Galleria mellonella* model. Phage treatment increase the larvae survival rates even while none of the phages were able to eliminate the bacterial load.

Antimicrobial susceptibility profile of several bacteria species identified in the peritoneal exudate of cows affected by parietal fibrinous peritonitis after caesarean section

Djebala S.¹, Gille L.¹, Eppe J.¹, Casalta H.¹, Bayrou C.¹, Evrard J.², Gregoire F.², Thiry D.³, Moula N.⁴, Sartelet A.¹, Bossaert P.¹

1. Clinical Dept of Ruminants, ULiège 2. Gestion et Prév. de Santé, Reg. Assoc. of Health & Animal Ident., Ciney 3. Bacteriology, Dept of Infectious and Parasitic Diseases, ULiège 4. Dept of animal production, ULiège

Corresponding author: sdjebala@uliege.be

This study aimed to evaluate the antimicrobial susceptibility of bacteria strains identified by bacterial culture in the peritoneal exudate of **156** cows affected by parietal fibrinous peritonitis (PFP) after caesarean section.

A total of **185** strains belonging to **21** species were identified. The most common isolated pathogen was *Trueperella pyogenes* (*T. Pyogenes*) (**107** strains) followed by *Escherichia coli* (**38** strains), *Proteus mirabilis* (**6** strains), *Clostridium perfringens* (**6** strains), *Fusobacterium necrophorum* (**3** strains) and *Streptococcus uberis* (**3** strains). Various other species were identified either **twice** (*Helcococcus ovis*, *Mannheimia varigena*, *Staphylococcus aureus*, *Streptococcus dysgalactiae*, *Providencia rettgeri*, *Proteus sp*) or **once** (*Proteus vulgaris*, *Helcococcus sp.*, *Salmonella typhimurium*, *Streptococcus mitis*, *Pseudomonas aeruginosa*, *Actinobacillus rossii*).

The antimicrobial susceptibility (disk diffusion assay), was tested for several isolated strains (**59**), but not for anaerobic bacteria and *T. pyogenes*, since susceptibility tests for these bacteria are technically complicated and beyond the routine expertise of the laboratory. Antibiotic resistance was commonly observed, even against molecules of critical importance (cefquinome **10/59**, ceftiofur **10/59**, enrofloxacin **17/59** and marbofloxacin **13/59**). Isolated bacteria were classified as weakly drug resistant (**22/59**) (resistant to Fewer than 3 classes), multidrug resistant (**24/59**) (resistant to more than 3 classes), extensively drug resistant (**12/59**) (resistant to several tested classes except one or two) or pan-drug resistant (**1/59**) (resistant to all the antibiotics tested).

This study highlights the level of antimicrobial resistance in cows suffering from PFP, and provides new insights in the therapy of PFP. Ideally, antimicrobial treatment of PFP should be based on bacterial isolation.

Oral presentations

Freezing does not impact equine fecal bacterial viability on a specific fecal pre-transplantation processing method: a step towards equine stool bank

Loublier C.^{1,3}, Taminiou B.^{2,3}, Amory H.^{1,3}, Daube G.^{2,3}, Lecoq L.^{1,3}, Cesarini C.^{1,3}

1. Equine Clinical Department, Faculty of Veterinary Medicine, ULiège 2. Department of Food Sciences – Microbiology, Faculty of Veterinary Medicine, ULiège 3. Fundamental and Applied Research for Animals & Health (FARAH), Faculty of Veterinary Medicine, ULiège

Corresponding author: ccesarini@uliege.be

Equine fecal microbiota transplantation (FMT) has been used empirically for decades but evidence based information on this technique is scarce. This *in vitro* study aimed to assess the impact of a common pre-FMT processing method and short-term freezing on bacterial composition and viability of the final fecal inoculum.

A fresh equine fecal sample was divided in 3 aliquots, to be processed identically as follows: feces (T0) were mixed with water and chopped using an immersion blender to get a mixture (T1), which was left uncovered during 30 minutes (T2) and filtered through a sieve to obtain a fecal infusion (T3). Samples were taken at different steps of the procedure (Tn), stored at 4°C and rapidly analyzed. Additional T3 samples were frozen (-20°C) for 1 (T3_{1M}) and 6 months (T3_{6M}) until analysis. Bacterial 16S rDNA amplicon profiling associated to propidium-monoazide treatment (PMA_t) was performed in each sample to select living bacteria. After PMA_t, total flora (TF) was measured using quantitative PCR analysis. Bacterial community structure, composition and TF were assessed (significance $p < 0.05$).

No significant differences in ecological indices or abundance of the major bacterial genera were identified after freezing compared to initial fecal sample. Estimated alive TF (mean \pm SD) in T3_{1M} (9.73 ± 0.1 log gene copies/g) and T3_{6M} (10.39 ± 0.2 log gene copies/g) was not significantly lower than before freezing (10.29 ± 0.3 log gene copies/g). Results were similar for all 3 aliquots.

Results suggest that the processing method described in this study is replicable and the resultant fecal inoculum can be frozen up to 6 months for use in future FMT without affecting bacterial viability.

Chemical composition of cactus cladodes (*Opuntia ficus indica*) during the growing season in Northern Morocco

El Otmani S.¹, Chebli Y.¹, Chentouf M.¹, Hornick J.L.² and Cabaraux J.F.²

1. Regional Center of Agricultural Research - Tangier, National Institute of Agricultural Research, Avenue Ennasr, BP 415 Rabat Principale, 10090 Rabat, Morocco

2. Department of Veterinary Management of Animal Resources, FARAH, IVT, Faculty of Veterinary Medicine, University of Liège

Corresponding author: samira.elotmani@inra.ma

In Northern Morocco, forest rangelands are the major 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 livestock feeding by alternative feed resources incorporation. Cactus cladodes are an unconventional resource widely available in the Mediterranean area of Northern Morocco that could take place in ruminant diet. In order to introduce them, it is necessary to characterize their chemical composition. This work aims to determine the chemical composition of cactus cladodes during the growing period. Twelve samples of young cladodes were collected from three different places of Tangier during four periods (April to June) corresponding to the growing season (spring). The studied composition parameters were dry matter, ash, crude protein, ether extract, nitrogen-free extract, fibers (NDF and ADF), and condensed tannins. Results showed that ash, NDF, condensed tannins, and nitrogen-free extract were stable during the growing season ($P > 0.05$), despite they had a tendency to be variable ($P < 0.1$), except for NDF. However, dry matter, crude protein, ether extract and ADF contents varied during the growth ($P < 0.05$). Contrary to crude protein content, these affected parameters tended to increase significantly with ageing. In conclusion, cladodes have interesting chemical composition that allowed them to be incorporated in ruminant diet.

Gentamicin concentration in nasal lavage in healthy dogs after inhalation therapy

T. Biénès¹, A. Lyssens¹, H. Machiels¹, M-E. Hercot¹, A. Fastrès¹, A-C. Tutunaru¹, M. Deville², C. Charlier², F. Billen¹, C. Clercx¹

1. Dept of Clinical Sc., FARAH 2. Lab. of Clinical, Forensic, Industrial and Environmental Toxicology, CIRM, CHU

In human medicine, topical administration of antimicrobials is a common treatment of upper respiratory tract diseases and gentamicin inhalation has shown promising results for chronic rhinosinusitis management. Because of gentamicin's narrow therapeutic index, inhalation therapy is interesting to allow high local bioavailability in sinonasal cavities with less systemic impact. In canine upper respiratory tract disease, antimicrobials inhalation efficacy for treatment of rhinosinusitis has not been assessed. Nasal lavage (NAL) might be a useful diagnostic and therapeutic method, but a well standardized protocol has not been described. This study's aims were (1) to describe a NAL standardized procedure and total cell count in healthy beagle dogs;(2) to compare gentamicin concentrations in NAL-fluid (NALF) obtained after administration of nebulization protocols of different duration. Ten healthy beagles were used. A standardized NAL procedure was performed in each dog prior to gentamicin administration, as well as after one week of administration of two different nebulization protocols. Gentamicin (50mg/ml) was nebulized twice daily either for 10 minutes per session (± 95 mg) (10-min-protocol) or for 3 min per session (± 28.5 mg) (3-min-protocol), separated by a one-week wash out period. NAL was performed under anesthesia, within 30 min after last nebulization. Gentamicin determination was performed on NALF by particle enhanced turbidimetric inhibition immunoassay. Dogs were placed in ventral recumbency, the nasopharynx was manually obstructed and a 12 Fr fenestrated catheter was introduced in the first third of left nasal cavity for administration of 20 mL of isotonic saline and retrieval. NAL procedures yielded 5 to 16mL of NALF (mean \pm std deviation of 11.1 ± 2.55 mL) with poor cellularity (17.50 ± 19.70 cells/ μ L), mainly consisting in keratinized (51.1%) and epithelial cells (46.4%), with no or few neutrophils. NALF gentamicin concentration was higher in the 10-min-protocol compared with the 3-min-protocol (13.66 ± 8.28 mg/L and 6.28 ± 3.23 mg/L; $p=0.013$). In spite of an obvious dilution effect related to the NAL procedure, NALF gentamicin concentrations were correlated with the duration of inhalation and were high, compared to the minimum inhibitory concentration reported for aminoglycoside-sensitive bacteria (2-4mg/L). This study shows that,(1) the NAL method seems reproducible and yields samples that can be used for measurement of local drug concentration as a surrogate of intranasal drug concentration and (2) gentamicin nebulization appears as reproducible therapeutic procedure with interest for the management of bacterial rhinosinusitis in dogs, although the most adequate and efficacious nebulization protocol still needs to be determined.

Influence of gammaherpesvirus infections on the antibody repertoire of their host

Baiwir J.¹, Xiao X.¹, Lété C.¹, Machiels B.¹, Gillet L.¹

1. Immunology-vaccinology, Dept of infectious and parasitic diseases, Veterinary public health, FARAH, ULiège

Corresponding author: jerome.baiwir@uliege.be

Gammaherpesviruses (γ HVs) are ubiquitous viruses that have co-evolved with their hosts. Although these infections remain asymptomatic in most of the individuals, they can cause cancers, mainly lymphoproliferative disorders, in immunocompromised people. After primary infection, most of γ HVs undergo latent expansion in germinal center (GC) B cells and persists in memory cells. In this project, using next generation sequencing and Murid Herpesvirus 4 (MuHV-4), a mouse γ HV, we characterized the effect of a γ HV infection on the antibody repertoire of its host. Firstly, we showed that B cells of MuHV-4 infected mice display distinct VDJ recombination frequencies and isotypes switching routes compared to the ones of non-infected mice. Secondly, using a YFP expressing MuHV-4 strain, we compared the repertoire of MuHV-4 infected and non-infected B cells. Surprisingly, we observed that infected cells displayed a distinct repertoire than the one of their non-infected counterparts with a different speed of clonal expansion. These results suggest therefore that MuHV-4 infection is not random and establishes preferentially in some B cells. In the future, identifying the common determinants of these infectable B cell subsets could help us to better understand γ HVs lifecycle and the lymphoproliferative disorders that they induce. More generally, it could help us to better understand how our environment and especially some infections agents shape our immune responses.

Oral presentations

Congenital urethral sphincter mechanism incompetence in 11 bitches: a retrospective preliminary study

Porsmoguer C., Hamaide A., Noël S.

Department of Clinical Sciences (Companion Animals), FARAH, Faculty of Veterinary Medicine, ULiège, Belgium

Corresponding author: charles.porsmoguer@uliege.be

This study aims to describe effects of medical treatment and sterilization in bitches with congenital urethral sphincter mechanism incompetence (CUSMI).

Diagnosis of CUSMI was based on history (urinary incontinence since early age), exclusion of anatomical abnormalities by diagnostic imaging and urethral pressure profiles. Follow-up data were collected via standardized telephone owner's interview. Continence scores (1 to 5; with 1 fully incontinent and 5 fully continent) were assigned.

Eleven bitches were included (7 sterilized before first oestrus (BO), 4 after first oestrus (AO). Before treatment, CS was significantly lower in the BO group (mean=1.4), compared to the AO group (mean=2.5). All bitches received phenylpropanolamine (PPA) 1.5mg/kg SID after diagnosis. After PPA treatment, CS was not different between the two groups and only 3/11 bitches were fully continent. Nevertheless, CS after treatment (mean=3.8) was significantly higher than CS before treatment (mean=1.9). Improvement of incontinence was observed before or during oestrus in 3/4 dogs of the AO group (with 2 being fully continent). However, a significant worsening of the CS (mean decrease=1.4) was reported after sterilization in 5/11 dogs (2/7 from BO group and 3/4 from AO group).

At long-term follow-up, 5/10 dogs were fully continent after treatment modulation.

Bitches with CUSMI may be less responsive to medical treatment than bitches with acquired USMI. Even if improvement of incontinence can be observed before or during oestrus, worsening of clinical signs may occur after sterilization. Further investigation is needed on a larger number of dogs to confirm those preliminary results.

Understanding the growth of *Clostridioides difficile* *in vitro*

Martinez E.¹, Taminiau B.¹, Daube G.¹

1. Food Science Department - Microbiology, FARAH, ULiège

Corresponding author: emartinez@uliege.be

Clostridioides difficile is a Gram positive bacterium sporulant and required strict anaerobiosis. The intestinal carriage of *C. difficile* can be asymptomatic, but in cases of infection it is associated with different clinical signs of disease that can vary from mild diarrhea to pseudomembranous colitis or even death. Several growth curve assays were carried out to study the behavior of the spores and vegetative forms of a *C. difficile* strain 078 under different conditions: in nonspecific culture medium, in digestive culture medium, and with and without human feces. Then, *C. difficile* was grown in microbial fermenters with and without controlled conditions in the simulator of the human intestinal microbial microorganism (SHIME). When the pH was not controlled, no spore germination or growth was observed. However, *C. difficile* spore growth was observed and *C. difficile* toxins were produced when the pH was controlled within a range of 6.6-6.9. An entire dynamic infectious model of *C. difficile* infection was studied but no germination was observed. Then, expression of *C. difficile* virulence gene was studied in an *in vitro* insert plate model. A peak of expression of the virulence gene was observed at 24 hours. The next step of our study will use metatranscriptomics to identify the expression of certain genes that have an impact on colonization.

A persistent gammaherpesvirus infection reprograms the alveolar macrophage niche for the long-term by dampening type 2 properties of group 2 innate lymphoid cells

Pauline Loos¹, Jérôme Baiwir¹, Céline Maquet¹, Justine Javaux¹, Rémy Sandor¹, François Lallemand², Thomas Marichal³, Bénédicte Machiels^{1,*}, Laurent Gillet^{1,*}

1. Laboratory of Immunology and Vaccinology, Faculty of Veterinary Medicine, FARA, ULiège, Liège, 4000, Belgium 2. Centre Hospitalier Universitaire de Liège, Département de Physique Médicale, Service médical de radiothérapie, Belgium, Liège, 4000, Belgium 3. Laboratory of Immunophysiology, GIGA-Research and Faculty of Veterinary Medicine, ULiège, Belgium, Liège, 4000, Belgium.

* co-senior authors

Corresponding author: pauline.loos@uliege.be

Immunological dysregulation in asthma is associated with changes in microorganism exposure early in life. Gammaherpesviruses (γ HVs) are highly prevalent human viruses that establish lifelong infection and deeply shape host immunity. Using Murid herpesvirus 4 (MuHV-4), a mouse γ HV, we showed that, after infection, lung resident and bone marrow-derived group 2 innate lymphoid cells (ILC2s) display lasting reduced capacity to expand and to produce type 2 cytokines in response to house dust mites, contributing to asthma protection. Importantly, we uncovered that ILC2s represent essential niche cells imprinting the tissue-specific identity of monocyte-derived alveolar macrophages upon infection. In particular, MuHV-4 infection disrupts the physiological ILC2-epithelial cells circuit that programs monocytes-derived alveolar macrophages for type 2 functions without affecting their differentiation. These results reveal that persistent γ HV infection shapes the alveolar landscape much beyond the initial acute infection through long-term effect on ILC2 niche cells.

Social interactions between European eels mediate the transmission of Anguillid herpesvirus 1 through biting

Delrez N. ¹, Haiyan Z. ¹, Denoël M. ², Ovidio M. ³, Larbanoix L. ⁴, Davison A. ⁵, Donohoe O. ¹ and Vanderplasschen A. ¹

1. Immunology-Vaccinology, FARA, Faculty of Veterinary Medicine, University of Liège, Liège, Belgium

2. Laboratory of Fish and Amphibian Ethology, University of Liège, Liège, Belgium

3. Laboratory of Fish Demography and Hydroecology, University of Liège, Liège, Belgium

4. Center for Microscopy and Molecular Imaging, Gosselies, Belgium

5. MRC-University of Glasgow, United Kingdom

Corresponding author: natacha.delrez@uliege.be

Within the last 50 years, the European eel (*Anguilla anguilla*) has turned from one of the largest freshwater fishery resources in many areas of Europe to a critically endangered species. Among potential causes of the population decline, infection by Anguillid herpesvirus 1 (AngHV-1) is thought to play a key role. This virus is now widespread both in aquaculture facilities and in the wild population. How exactly this virus reached such high prevalence in a host population in decline remains an enigma. The aim of the present study was to investigate the pathogenesis of AngHV-1 in his natural host using *in vivo* bioluminescent imaging. First, we produced a recombinant strain (hereafter called LucGFP strain) encoding a luciferase-GFP reporter expression cassette. This recombinant allowed us to investigate the portal of entry and the transmission mode of AngHV-1. These experiments led to the following observations: (i) There is an increase of sensibility to the virus according to the developmental stage of the fish. While glass eels were not sensitive by the natural route tested, inoculation of yellow eel led to infection of all subjects expressing bioluminescent signal on the gills, periodontal mucosa and skin foci mainly distributed on the tail and the head. (ii) These observations suggested that the gills and the periodontal mucosa represent portals of entry of the virus into naïve subjects and that biting could contribute to transmission of the virus between subjects. (iii) Transmission modes were investigated using yellow eels. Notably, our results indicated two modes of viral transmission: indirectly through water acting as an abiotic vector, and directly through social interactions, more specifically through bites. Given the low stability of the virus in the water, a strong ability of the eel epidermal mucus to neutralize the virus and the reduction of eel population in the wild, transmission through biting is likely to explain how AngHV-1 reaches such a high prevalence among the eel population. To the best of our knowledge, this is the first description of this mode of viral transmission in an aquatic environment.

Oral presentations

Gentamicin concentrations in bronchoalveolar lavage and serum in healthy dogs after inhalation therapy

A. Lyssens¹, T. Biénès¹, A. Fastrès¹, H. Machiels¹, C. Moreau¹, A-C. Tutunaru¹, M. Deville², C. Charlier², C. Clercx¹, F. Billen¹

1. Dept of Clinical Sciences, FMV, FARA, ULiège 2. Laboratory of Clinical, Forensic, Industrial and Environmental Toxicology, CIRM, University Hospital of Liège, Belgium

Corresponding author: aurelie.lyssens@uliege.be

Inhalation therapy is frequently used in dogs while little is known about how multiple possible factors influence the delivery of drugs into the lower airways. Gentamicin nebulization has been shown to be efficacious for treating dogs with *Bordetella bronchiseptica* infection that are poorly responsive to conventional antimicrobial treatment using an empirical protocol. In companion animals, a minimum inhibitory concentration (MIC) of gentamicin of approximately 1-4mg/L has been described for *Bordetella bronchiseptica* isolates. The aim of this study was to determine the concentration of gentamicin in the lower airways and serum of dogs after nebulization with undiluted gentamicin, according to the duration of nebulization during spontaneous breathing. Ten healthy beagles were prospectively included. A standardized bronchoalveolar lavage (BAL) procedure was performed in each dog after 1 week of administration of each of 2 different gentamicin nebulization protocols separated by a one-week wash out period. The 2 protocols consisted in nebulization of gentamicin (50mg/mL) twice daily either for 10 minutes per session (± 95 mg) (10-min-protocol) or for 3 minutes per session (± 28.5 mg) (3-min-protocol). BAL fluid (BALF) was obtained under general anesthesia using a bronchoscope within 15 minutes after administration of the last nebulization. Three 20ml aliquots of isotonic saline were instilled through the endoscope channel, 2 into the right caudal lobe successively and 1 into the left caudal lobe. Blood was obtained within 5 minutes after BALF. BALF and serum gentamicin concentration were determined by particle enhanced turbidimetric inhibition immunoassay. Concentrations between protocols were compared using a paired t-test. The BAL procedure yielded 23 to 32mL of BALF (median volume of 27.85mL). BALF and serum gentamicin concentrations were higher after 10-min-protocol compared with 3-min-protocol (2.41 ± 0.87 mg/L; mean \pm SD; versus 1.25 ± 0.31 mg/L; $p < 0.0001$ and 1.05 ± 0.55 mg/L versus 0.34 ± 0.24 mg/L; $p = 0.001$ in BALF and serum respectively) while the BALF to serum ratio did not differ between the protocols (5.1 ± 4.9 versus 2.8 ± 1.6 ; $p = 0.214$). Since the proportion of epithelium lining fluid in BALF is about 2 to 3%, we can assume that both protocols provide the minimum level gentamicin concentrations required to kill *Bordetella bronchiseptica* in the lower airways of dogs, while the serum concentrations remain below the toxic ranges (> 2 mg/L). Although the most adequate and efficacious nebulization protocols still need to be determined, treating dogs, diagnosed with a *Bordetella bronchiseptica* infection and reluctant to oral antimicrobial therapy, with a 3-min-nebulization of undiluted gentamicin should be as efficacious as the 10-min-protocol so far described.

Post-mortem mesenchymal stem cells: cryopreservation strategies

Levoz M.¹, Dubois A.², Toppets V.¹, Piret J.¹, Grobet L.², Antoine N.¹, Shikh Alsook M.K.¹

1. Department of Morphology and Pathology, Histology, FARA, Uliège

2. Department of Morphology and Pathology, Embryology, FARA, Uliège

Corresponding author: marine.levoz@uliege.be

The repair of equine tendinopathies is presently a clinical challenge. These injuries often lead to an early end of the athletic career of the affected horses. This study aimed to further explore the use of ligament-derived mesenchymal stem cells isolated post-mortem and to establish a successful cryopreservation strategy for their administration in veterinary medicine. The use of cryoprotectants of natural origin such as glycerol instead of dimethylsulfoxide, the most commonly used cryoprotectant, was able (i) to preserve the cells of interest on the long term, (ii) to preserve their viability and to ensure cell proliferation after thawing, (iii) and to maintain the characteristics of mesenchymal stem cells. The stemness of these cells was confirmed by their capacity to differentiate into osteogenic, chondrogenic and adipogenic lineages and by their phenotyping using flow cytometry.

Study of the use of IDEXX PROCYTE for total and differential cellular counts of bronchoalveolar lavage fluid in healthy dogs

H. Machiels, A. Fastrès, T. Biénès, A. Lyssens, A. Schlachet, K. Phan, F. Billen, E. Roels, C. Clercx
Department of Clinical Sciences, Faculty of Veterinary Medicine, FARA, University of Liege, Belgium

Bronchoalveolar lavage fluid (BALF) analysis is routinely used in canine and feline respiratory medicine. BALF manual counting allows total and differential cellular count measurement (TCC and DCC, respectively), but is time-consuming and requires training and expertise. Accordingly, the aims of the present study were (1) to assess usefulness of the IDEXX PROCYTE analyzer in comparison with manual counting for in clinic-BALF TCC and DCC measurement and (2) to assess the effects of a pre-treatment with either a mucolytic agent or a filtration on BALF TCC and DCC, in healthy dogs. Ten healthy beagles were prospectively included. BALF was collected using three aliquots of 1mL/kg of saline solution. Manual TCC and DCC were calculated using a hemocytometer and a cytopspin preparation of naïve BALF, BALF treated with dithiothreitol 0.15% (DTT) solution and BALF filtered through a 70µm Cell Strainer. For DCC, a total of 200 cells were counted at high power field, by 2 independent operators. Automatic TCC and DCC were also calculated using the IDEXX PROCYTE analyzer on DTT-treated and filtered BALF. Results were compared using intraclass correlation coefficients (ICC) (interobserver agreement), Bland-Altman plot analysis and Wilcoxon signed rank test (Procyte versus manual counting), and Friedman test (DTT and filtering effects). A good to excellent agreement was found between observers for manual TCC and DCC in each BALF type (ICC: 0.9-0.77; $p < 0.02$). There was no significant difference in manual TCC between naïve, DTT-treated or filtered BALF ($p = 0.741$) and also no significant difference in manual DCC between the different treatments ($p = 0.628$). There was no significant difference in TCC between manual and PROCYTE counting neither after DTT ($p = 0.373$), nor after filtering ($p = 0.477$) and a good agreement was found between the 2 methods (ICC: 0,87-0,82; $p \leq 0,001$). However, PROCYTE did not provide pertinent DCC, since macrophages were not identified. Results of this study demonstrated that BALF TCC can be adequately measured using both manual and PROCYTE counting, and is not impacted by pre-treatment with DTT or filtering. On the contrary, IDEXX PROCYTE does not appear suitable for DCC measurement. Effects of DTT and filtration procedures on TCC and DCC measurements should be further analyzed in inflammatory samples from dogs with lower airway diseases.

Ly6C^{hi} monocytes balance regulatory and cytotoxic CD4 T cell responses to control virus induced-immunopathology.

Céline Maquet¹, Jérôme Baiwir¹, Pauline Loos¹, Justine Javaux¹, Rémy Sandor¹, Fabienne Perin², Matthias Mack², Didier Cataldo³, Laurent Gillet^{1,4,*}, Bénédicte Machiels^{1,4,*}.

1. Lab. of Immunology and Vaccinology, Faculty of Veterinary Medicine, FARA, ULiège, Liège, 4000, Belgium 2. Department of Nephrology, University Hospital Regensburg, Regensburg, Germany 3. Laboratory of Tumor and Development Biology, GIGA-Cancer and "Centre Hospitalier Universitaire de Liège (CHU)", Liège, 4000, Belgium.

Corresponding author: c.maquet@uliege.be

The gammaherpesviruses (γ HVs) Epstein-Barr virus and Kaposi's Sarcoma-associated herpesvirus are among the most prevalent human viruses. Establishing lifelong persistence, γ HVs have coevolved with their host conferring symbiotic relationship and appear to broadly influence the function of the immune system. Understanding the balance between pro-inflammatory and immunomodulatory pathways triggered by these viruses is of major importance since mechanisms underlying deleterious inflammation induced by highly pathogenic respiratory viruses, notably SARS-COV-2, are not well known yet. Here, we show that pulmonary infection with Murid herpesvirus 4 (MuHV-4), a mouse γ HV, drives the recruitment in the airways of Ly6C^{hi} monocytes (MOs) that control the host immune response. Indeed, the absence of those MOs elicited a severe virus-induced immunopathology associated with the systemic release of inflammatory mediators. Indeed, by using complementary mouse models deficient for MOs, we highlighted that absence of MOs was associated with an exacerbated disease revealed by an important weight loss and by a systemic release of various inflammatory mediators following infection. Mechanistically, MuHV-4 imprinted MOs recruit CD4 T cells in the airways and trigger immunosuppressive signalling pathways through the PD-L1/PD-1 axis, thereby dampening the deleterious activation of cytotoxic CD4 T cells. These results uncover a new role for MOs in modulating CD4 T cells functions and reveal key pathways that could be targeted to interfere with the detrimental immunopathological cascade during respiratory viral infections.

Short talks

Genetic and epidemiological analysis of BRSV strains that circulate in the Wallonia region of Belgium

A. Demil¹, H. Boshra², D. Cassart², L. Gillet³, J. Evrard⁴, F. Grégoire⁴, F. Rollin⁵, C. Bayrou⁵, M.M. Garigliany², M. Dourcy¹

1. Anatomy, Department of Morphology-Pathology, FARA, ULiège 2. Pathology, Department of Morphology-Pathology, FARA, ULiège 3. Immunology-Vaccinology Laboratory, Department of Infectious and Parasitic Diseases, FARA, ULiège 4. ARSIA (« Association Régionale de Santé et d'Identification Animales »), Ciney, Belgium 5. Bovine Health service, Clinical Department of production animals, FARA, ULiège

Corresponding author: mickael.dourcy@uliege.be

Bovine respiratory syncytial virus (BRSV) is a virus belonging to the genus *Orthopneumovirus*, within the *Pneumoviridae* family (ICTV, 2020). BRSV is a major cause of bovine respiratory disease complex (BRD) as well as regular winter outbreaks of respiratory disease in cattle and is therefore responsible for important economic losses on the farming industry. The BRSV contains 11 viral proteins, among which the attachment glycoprotein (G). Displaying the highest reported mutation rate among BRSV strains, the G gene is commonly used as target during phylogenetic analysis and BRSV is classified to date into ten different subgroups (I to X). Recent studies achieved in different countries (Italy, Croatia and more recently Brasil and Japan) reported the circulation of new, genetically unique BRSV strains (groups VII to X) with mutations within essential immunodominant region. In this study, the genetic diversity of circulating BRSV strains in Belgium, which is unknown since end of 1990's, was investigated. Interestingly, our phylogenetic analyses revealed emergence and circulation of BRSV strains belonging to subgroup VIII, as observed elsewhere in Europe. The spatiotemporal distribution of BRSV isolates has been studied in different countries and should continue, as establishment of molecular surveillance of BRSV in different geographical regions can improve the identification of outbreaks, resulting in the implementation of preventive measures aimed to control the disease. Correlation between BRSV isolates circulating in Belgium and epidemiologic features like age, cattle breed, weather and geographical distribution has also been analysed here.

Vitamin and antioxidant supplementation in the prevention and treatment of atypical myopathy - a pilot study to establish relevant tolerance intervals and assess the feasibility of supplementation

Nagy D.¹, François A.C.², Renaud B.², Kruse C.², Stern D.², Cheramy-Bien J.P.³, Pincemail J.³, Votion D.M.⁴

1. Equine Clinic, Comparative Veterinary Medicine, FARA, ULiège 2. Pharmacology and Toxicology, Department of Functional Sciences Comparative veterinary Medicine, FARA, ULiège 3. Department of Cardiovascular Surgery, CREDEC and Platform Nutrition Antioxydante et Santé, CHU and ULiège 4. Equine Pole, Comparative veterinary Medicine, FARA, ULiège

Corresponding Author: acfrancois@uliege.be

In horses suffering from atypical myopathy (AM), vitamin and antioxidant supplementation was associated with a higher survival rate. However, the suspected state of oxidative stress in these sycamore maple tree intoxicated horses has not been studied. In this study, in order to establish relevant tolerance intervals of blood oxidant/antioxidant biomarkers in leisure-horses and to evaluate the feasibility of oral supplementation as a potential preventative measure, 16 horses from two stables were sampled in November 2020 and again after 4 weeks of daily oral supplementation with a mixed commercial of vitamins E, B, L-lysine and selenium preparation (n=8) or 4 weeks without supplementation (n=8). The oxidant/antioxidant status of a hospitalised AM case was subsequently compared. Inter-stable differences in selenium and glutathione peroxidase, and supplement-related increases in selenium were found, while functional biomarkers of oxidative damage were unchanged by supplementation reflecting likely soils mineral deficiencies alongside adequate antioxidant compensation. Furthermore, known correlations between several biomarkers was confirmed. The AM case showed high levels of most antioxidants. These results support the possibility of establishing leisure-horse specific norms for circulating oxidant/antioxidant biomarkers, however larger samples or healthy co-grazers should serve as references. Furthermore, AM cases are not all necessarily under systemic oxidative stress at hospitalisation, but longitudinal follow-up of a wider panel of biomarkers in various tissues is needed to evaluate oxidative stress, and thus better guide vitamins and antioxidants use in therapy and prevention.

Short talks

***In vitro* evaluation of the aryl hydrocarbon receptor transactivity induced by human intestinal metabolites**

E. Goya-Jorge¹, I.E. Gonza¹, P. Bondue¹, C. Douny², M.L. Scippo², V. Delcenserie¹

1. Laboratory of Food Quality Management, Department of Food Sciences, FARAH - Veterinary Public Health, University of Liège, B43b, 4000 Liège, Belgium 2. Laboratory of Food Analysis, Department of Food Sciences, FARAH - Veterinary Public Health, University of Liège, B43b, 4000 Liège, Belgium

Corresponding author: egoya@uliege.be

The gastrointestinal tract is directly exposed to the environment and constitutes one of the first lines of defense against harmful antigens in the diet or the microbiota. Highly expressed in intestinal cells, the aryl hydrocarbon receptor (AhR) plays a crucial role in gut mucosal integrity and in intestinal immune function. Substances derived from food and the microbiota in the intestinal lumen can induce AhR transcriptional activity and thus maintain intestinal barrier homeostasis. We suggested the combination of a simulator of the human gut microbial ecosystem (SHIME) with cell-based AhR transactivity bioassays. Thereby, the effects of human gut microbiota metabolism on the AhR signaling pathways could be evaluated. Three AhR reporter gene cell lines (T47D, HepG2, HT29) from human mammary, hepatic, and intestinal tissues, respectively, were used to test mixtures of microbiota-derived metabolites that were generated in a 3-days experiment on a SHIME system. The SHIME samples induced an AhR agonist activation in the different cell lines, with slightly tissue-dependent effects. Microbiota produced metabolites, such as short-chain fatty acids (SCFA) and tryptophan catabolites were quantified chromatographically in the SHIME samples, and they might be causing the AhR agonist activity of the mixture. However, more detailed investigations are needed to identify the contribution of individual metabolites to AhR activation. This is an innovative suggestion to study *in vitro* the AhR modulation caused by the human intestinal metabolism.

A novel role for CXCR2-dependent neutrophil recruitment in the skin and lung in the anti-helminth type 2 immune response

Petrellis G.¹, Marichal T.^{2,3,4}, Dewals B.G.¹

1. Immunology-Vaccinology, Department of infectious and parasitic diseases, Faculty of Veterinary medicine – FARAH, University of Liège, Belgium 2. Laboratory of Immunophysiology, Grappe Interdisciplinaire de Génoprotéomique Appliquée (GIGA) Institute, Liege University, Liege, Belgium 3. Faculty of Veterinary Medicine, Liege University, Liege, Belgium 4. Walloon Excellence in Life Sciences and Biotechnology, Wallonia, Belgium

Hookworms, an important family of soil-transmitted helminths (STH), represent a source of great disability and economic losses in humans and livestock. Infected hosts mount a type 2 immune response to eliminate the hookworms and repair the damaged tissue. Here, we investigate the role of recruited neutrophils recruited via C-X-C Motif Chemokine Receptor 2 (CXCR2) in a mouse model of hookworm infection. BALB/c female mice treated orally with navarixin, a CXCR2 antagonist, were infected with *Nippostrongylus brasiliensis* (Nb) intradermally, mimicking the natural route of infection. Using flow cytometry we observed that navarixin treatment, effectively reduced the number of neutrophils in the skin, the airways and the lung parenchyma, without affecting other cellular populations. Histology analysis of skin sections revealed a role for CXCR2 recruited neutrophils in controlling the inflammation at the site of hookworm entry. Moreover, recruited neutrophils seem to exacerbate the lung haemorrhage between day 1 and day 2 of the infection. CXCR2 recruited neutrophils in the lungs are able to control the load of larvae in the lung and further the number of adult worms in the gut. Focusing on the metabolism of the worm in the host revealed no significant differences between CXCR2 treated and untreated groups, indicating that neutrophils have a mediating role in the protective anti-helminth immunity. Further investigation with intravital imaging and single cell RNA sequencing will shed light to the role of different subsets of neutrophils and their migratory behaviour in the context of anti-helminth type 2 immunity, will provide useful immunotherapeutic targets.

Virus-induced inhibition of superinfection as a means for accelerating fitness-based selection of cyprinid herpesvirus 3 single nucleotide variants *in vitro* and *in vivo*

Gao Y.¹, Bernard N.¹, He B.¹, Zhang H.¹, Desmecht S.², Vancsok C.¹, Boutier M.¹, Suárez N.³, Davison A.³, Donohoe O.^{1,4}, and [Vanderplasschen A.¹](mailto:a.vdplasschen@uliege.be)

1. Immunology-Vaccinology (B43b), Department of Infectious and Parasitic Diseases, Faculty of Veterinary Medicine, FARA, ULiège 2. Laboratory of Animal Genomics, GIGA-Medical Genomics, GIGA-Institute, University of Liège, Liège, Belgium 3. University of Glasgow Centre for Virus Research, Glasgow, United Kingdom 4. Bioscience Research Institute, Athlone Institute of Technology, Athlone, Co. Westmeath, Ireland

Corresponding author: a.vdplasschen@uliege.be

Cyprinid herpesvirus 3 (CyHV-3) is the archetype of alloherpesviruses and is advantageous to research because, unlike many herpesviruses, it can be studied in the laboratory by infection of the natural host (carp). Previous studies have reported a negative correlation among CyHV-3 strains between viral growth *in vitro* (in cell culture) and virulence *in vivo* (in fish). This suggests the existence of alleles conferring enhanced fitness *in vitro* but reduced fitness *in vivo*, and vice versa. Here, we identified syncytial plaque formation *in vitro* as a common trait of CyHV-3 strains adapted to cell culture. Comparison of the sequences of virion transmembrane protein genes in CyHV-3 strains, and the use of various recombinant viruses, demonstrated that this trait is linked to a single nucleotide polymorphism (SNP) in ORF131 that results in codon 183 encoding either an alanine (A) or a threonine (T) residue. In experiments involving infections with recombinant viruses differing only by this SNP, the 183A allele associated with syncytial plaque formation was the more fit *in vitro* but the less fit *in vivo*. In experiments involving co-infection with both viruses, the more fit allele contributed to the purifying selection of the less fit allele by inhibiting superinfection. This study illustrates how the fundamental biological properties of some viruses and their hosts may have a profound impact on the degree of diversity that arises within viral populations.

The effect of *In Ovo* administration of Rosemary and Artemisia Essential Oil on hatchability, hatch weight, weight loss and post hatch mortality rate on Japanese quail (*Courtnix Courtnix Japonica*)

A. Aberbour¹, M. Iguer-Ouada³, L. Touazi², J-L. Hornick¹, N. Moula^{1,4}

1. Dept of Vet. Manag. in Animal Resources, FMV, FARA, ULiège 2. Dept of Agroalimentary, Fac. of Nature and Life Sciences, Farhat Abbas Univ., Setif, Algeria. 3. Dept of Biol. Sciences of the Environ., Fac. of Nature and Life Sciences, Abderrahmane-Mira-Univ., Bejaia, Algeria. 4. Animal Facilities, ULiège

Corresponding author: nassim.moula@uliege.be

Herbs and Plants extracts used in poultry nutrition as feed additives show beneficial effects on gastrointestinal microflora, growth performances and quality of animal products. Particularly, *in Ovo* pathway is a biotechnology approach that contributes significantly to improve poultry production. The aim of this study was to determine *in Ovo* injection effects of Rosemary and Artemisia essential oils (EOs) on hatchability, chick weight at hatching and mortality rate after birth. A total of 120 fertile eggs were used. They were divided into three groups at the thirteenth day of incubation: control (eggs injected with 30µl of distilled water) and two treatments with eggs injected with 3µl/30µl (EOs/distilled water) of Rosemary essential oil or Artemisia essential oil. The results showed that the hatchability of the fertile eggs was lower in Rosemary and Artemisia essential oil treatment groups compared to the control group. Artemisia showed a significant effect ($p < 0.05$) on chick weight, compared to Rosemary and control group. Weight losses in the essential oil treatment groups were lower when compared to the control group, the Artemisia group presenting the lowest weight loss (31.07%) compared to the Rosemary and control groups (34.23 %; 37.16 %, respectively; $P < 0.05$). The control group was the most affected by chick losses (13 chick during the first week) compared to the Rosemary and Artemisia groups, with 2 and 0 chick losses, respectively. In conclusion, these preliminary results indicated that *In Ovo* administration of Artemisia and Rosemary essential oils enhanced chicks hatching weight and post-hatching vitality rate.

Short talks

Jurassic Virology as a means for studying the evolution of a glycoprotein encoded by Cyprinid Herpesvirus 3

Noah Bernard¹, Yuan Gao¹, Catherine Vancsok¹, Owen Donohoe^{1,2} and Alain Vanderplasschen¹

1. Immunology-Vaccinology, Dept of Infectious and Parasitic Diseases (B43b), FARAH ULiège 2. Bioscience Research Institute, Technological Univ. of the Shannon: Midlands Midwest, Athlone, Co Westmeath, Ireland
Corresponding author: noah.bernard@uliege.be

Viral glycoproteins expressed on virions and on the surface of infected cells play key roles in the biological cycle of viruses. They are involved in key biological processes such as virus entry into host cells (binding or fusion), egress from infected cells and modifications of host cells biological properties including their immunogenicity. Cyprinid Herpesvirus 3 (CyHV-3) is a member of the family *Alloherpesviridae*, genus *Cyprinivirus*. Since its emergence in the late 1990's, this virus causes a lethal disease in common and koi carp worldwide. One of the essential transmembrane glycoproteins encoded by CyHV-3 is encoded by ORF131. This gene consists of 3 exons highly conserved in the CyHV-3 species. Only the last exon is found in the closely related virus species Cyprinid Herpesvirus 1 and 2. Interestingly, we report here that the first two exons and the intron of CyHV-3 ORF131 gene are homologous (97% similarity) to sequences of the common carp genome strongly supporting the hypothesis that the 5' region of the ORF131 gene unique to CyHV-3 represents a recent acquisition from the ancestor of carp or a closely related species. By identifying sequences with the highest similarity and their localization in the carp genome, we hypothesized a multistep evolutionary scenario to explain the genesis of the actual sequence of CyHV-3 ORF131 sequence. Interestingly, this scenario implicates the existence of infectious intermediates with increasing fitness: the ancestral form (consisting only of the third exon), the intermediates C and CB forms (resulting from the two consecutive acquisitions of cellular sequences). To test this evolutionary model, the three ancestral forms were produced using prokaryotic and eukaryotic recombination technologies. This approach demonstrated that the predicted ancestral forms are able to replicate. Even more interesting, the comparison of actual CyHV-3 with the three predicted ancestral forms demonstrated a progressive increase of virulence *in vivo*. To the best of our knowledge, this is the first study to investigate the evolution of a viral gene through evidence-based reconstruction of extinct ancestral evolutionary intermediates, representing various stages of host genome incorporation. This may provide strong insights into the reasons for preferable selection of certain genotypes over others during the evolution of CyHV-3.

The impact of grey seals on the mortality of other marine mammal species in the North Sea between 2011 and 2020

Servaty M.¹, Garigliany M.¹, Haelters J.², Hasanaj A.¹, Jauniaux T.³, Karpouzopoulos J.⁴

1. Faculté de Médecine vétérinaire, Département de morphologie et pathologie (DMP), Pathologie générale et autopsies, FARAH: Santé publique vétérinaire 2. Royal Belgian Institute of Natural Sciences, Direction of Natural Environment 3. Faculté de Médecine vétérinaire, Département de morphologie et pathologie (DMP), Faculté des Sciences Département de Biologie, Ecologie et Evolution, FARAH: Santé publique vétérinaire 4. Coördination Mammalogique du Nord de la France (CMNF), 806 rue haute, 62850 Alembro

Corresponding author: m.servaty@student.uliege.be

This study focuses on grey seal predation on harbour porpoises and harbour seals. The predation is an emerging cause of death for both species. During autopsy, several samples were taken on suspicious bite. In the framework course of this study, new techniques of DNA detection were developed in order to assess the predation of grey seals on harbour porpoises and harbour seals more efficiently. Old samples were also analyzed with these techniques, resulting in a more reliable study. Our results show that the grey seal attacks are more frequent in the winter and at the beginning of spring. It focuses its attack on juveniles with good health. The lesions present on the preys were similar to the lesions described in other countries. Our new techniques in DNA detection show a more reliable study during samples (old and new) analysis. The increase in attacks by grey seals could result from the increase of the fishing activity near the coast during winter and consequently the decrease in food availability. The increase of grey seal population in the North Sea can also influence the number of attacks. This study highlights the need to have uniformed protocols among countries that must be applied during each stranding, the importance of avoiding contamination and also shows the importance of performing autopsies to determine the cause of death.

Assessment of SPP1 and FN1 in serum, bronchoalveolar lavage fluid and lung tissue samples from dogs affected with canine idiopathic pulmonary fibrosis

Fastrès A.¹, Roels E.¹, Antoine N.², Clercx C.¹.

1. *Department of clinical sciences, FARAHA, Uliège*

2. *Department of morphology and pathology, Histology, FARAHA, Uliège, Uliège*

Corresponding author: afastres@uliege.be

Canine idiopathic pulmonary fibrosis (CIPF) is a chronic disease affecting West Highland white terriers (WHWTs)^{1,2}. Osteopontin (SPP1) and fibronectin (FN1) are associated with pulmonary fibrosis in men³⁻⁶ and are overexpressed in bronchoalveolar lavage fluid (BALF) macrophage clusters in CIPF⁷.

The aim is to investigate whether these molecules are potential disease markers. SPP1 and FN1 serum and BALF concentrations were measured using canine ELISA kits in CIPF WHWTs (n=24), healthy aged-matched WHWTs (n=13) and healthy terriers (n=15). Proteins were also localized in lung tissue by immunohistochemistry.

SPP1 serum concentrations were higher in CIPF compared with healthy WHWTs and terriers, and in healthy WHWTs compared with terriers. There were negatively correlated with PaO₂ in WHWTs. Higher SPP1 BALF concentrations were found in CIPF and healthy WHWTs compared with terriers. Intense labelling was reported in all groups in ciliated epithelial cells, smooth muscular cells surrounding large vessels and some macrophages. Moreover, in all CIPF WHWTs, the pneumocytes II and the extra cellular matrix were labelled, while it was the case in only 57% of healthy WHWTs and not present in terriers.

FN1 serum concentrations were lower in CIPF and healthy WHWTs compared with terriers. No difference was found between groups in BALF. There was no evidence of differences in FN1 labelling. The results suggest that SPP1 is involved in CIPF pathogenesis and could predispose that breed to the disease. However, further studies are required to determine its interest as biomarker or potential therapeutic target.

Analysis of Synchronous and Asynchronous In Vitro Infections with Homologous Murine Norovirus Strains Reveals Time-Dependent Viral Interference Effects

Louisa F. Ludwig-Begall¹, Elisabetta Di Felice², Barbara Toffoli¹, Chiara Ceci³, Barbara Di Martino³, Fulvio Marsilio³, Axel Mauroy^{1,4} and Etienne Thiry^{1,*}

1. *Veterinary Virology and Animal Viral Diseases, FARAHA, Uliège*

2. *Department of diagnosis and surveillance of exotic disease, IZS, Teramo, Italy*

3. *Faculty of Veterinary Medicine, Università degli Studi di Teramo, Teramo, Italy*

4. *Staff direction for risk assessment, Control Policy, FASFC, Brussels, Belgium*

Corresponding author: etienne.thiry@uliege.be

Human noroviruses are recognised as a leading global cause of sporadic and epidemic viral gastroenteritis. Viral recombination is a key mechanism shaping the evolution and diversity of noroviruses. Single-cell coinfection by multiple viruses is the ultimate prerequisite to viral recombination. Under natural conditions, various environmental, host, and virus factors may influence the probability of synchronous coinfections and may determine the delay or even the absolute achievability of asynchronous cellular superinfections. Here, we determine the effect of a temporal separation of *in vitro* infections with the two homologous murine norovirus (human norovirus surrogate) strains MNV-1 WU20 and CW1 on the composition of nascent viral populations. WU20 and CW1 were either synchronously inoculated onto murine macrophage cell monolayers (coinfection) or asynchronously applied (superinfection with varying titres of CW1 at half-hour to 24-hour delays). Both quantification of genomic copy numbers and discriminative screening of plaque picked infectious progeny viruses 24 hours after initial co-or superinfection, demonstrated a time-dependent predominance of primary infecting WU20 in the majority of viral progenies. Our results indicate that a time interval from one to two hours onwards between two consecutive norovirus infections allows for the establishment of a barrier that reduces or prevents superinfection; this first demonstration of time-dependent viral interference for noroviruses has clear implications for norovirus epidemiology, risk assessment, and potentially treatment.

Short talks

Sensitivity and permissivity of zebrafish larvae to alloherpesviruses

Streiff C.¹, He B.¹, Manfroid I.², Morvan L.¹, Delrez N.¹, Zhang H.¹, Fourrier M.¹, Van Leeuw V.¹, Suárez N.M.³, Davison A.J.³, Diallo M.A.¹, Betoulle S.⁴, Donohoe O.^{1,5} and Vanderplasschen A.¹

1. Imm.-Vacc, Dept of Infect. and Parasitic Diseases, FARA, ULiège 2. Zebrafish Dev. and Disease Models lab., GIGA-Molec. Biol. of Diseases, ULiège 3. MRC-Univ. of Glasgow Centre for Virus Res., Glasgow, UK 4. UMR-I 02 Stress Environn. et BIOSurv. des milieux aqua.(SEBIO), UFR Sc. Exactes et Nat., Univ. de Reims Champagne-Ardenne, France 5. Bioscience Res. Instit., Technol. Univ. of the Shannon, Ireland

Corresponding author: cindy.streiff@uliege.be

The zebrafish (*Danio rerio*) is an experimental model which has been increasingly used in virology. It has proved to be an interesting model to study Herpes Simplex Virus Type 1 (HSV-1) but also diseases affecting reared fish such as the spring viraemia of carp (SVC). It has recently been shown that Cyprinid Herpesvirus 3 (CyHV-3) can replicate in a zebrafish cell line established from 1-day-old embryos (ZF4), this being followed by a rapid clearance of the virus. Our work aimed to investigate the potential of the zebrafish model to study the pathogenesis of three alloherpesviruses associated with diseases, namely the Cyprinid Herpesvirus 2 (CyHV-2, affecting goldfish and gibel carp), the CyHV-3 (affecting common and koi carp) and the Anguillid Herpesvirus 1 (AngHV-1, affecting eel species). With that goal in mind, ZF4 cells and zebrafish larvae have been infected by recombinant strains of each virus species expressing GFP and luciferase as reporter genes. Viral replication was then monitored by exploiting fluorescence microscopy, bioluminescence imaging (IVIS), time-lapse microscopy and real-time live-cell imaging (Incucyte). Results showed that only CyHV-2 and CyHV-3 were able to infect ZF4 cells and zebrafish larvae. However, both viruses were quickly cleared after infection, with live microscopy indicating the potential implication of programmed cell death response. This was confirmed by transcriptomic analysis of whole zebrafish larvae infected with CyHV-3. Using differential gene expression data mapped to KEGG and GO annotations, we identified differentially regulated pathways and gene sets. We then generated a network of these based on functional/gene overlap and overlaid the network with differential regulation data. Further examination of this network revealed that in addition to upregulation of many proteasome related processes, the largest and most connected cluster of up-regulated processes comprised of those related to the innate immune response and programmed/non-programmed cell death. Furthermore, within this primary cluster, necroptosis was the most significantly upregulated pathway. These results suggest that it is these specific defence mechanisms that play a key role in survival of zebrafish challenged with CyHV-3.

Anguillid herpesvirus 1 ORF35 is dispensable for viral replication in cell culture but essential for efficient infection of European eels (*Anguilla Anguilla*)

H. Zhang¹, N. Delrez¹, Y. Gao¹, N.M. Suarez², A.J. Davison², O. Donohoe^{1,3}, A. Vanderplasschen¹

1. Dept of Infectious and Parasitic Diseases, Immunology-Vaccinology, FARA, ULiège 2. MRC-Univ. of Glasgow Centre for Virus Research, Glasgow, UK 3. Bioscience Research Institute, Technological Univ. of the Shannon: Midlands Midwest, Athlone, Co Westmeath, Ireland

Corresponding author: haiyan.zhang@doct.uliege.be

Anguillid herpesvirus 1 (AngHV-1) is a member of the *Cyprinivirus* genus of the *Alloherpesviridae* family. It is the etiologic agent of a lethal disease that affects at least two freshwater eel species and is the cause of economic loss within the eel aquaculture sector. AngHV-1 is also thought to contribute to the drastic decline of eels in the wild. To date, no vaccines are available against AngHV-1. Recently, we produced a live attenuated recombinant vaccine against the phylogenetically related virus Cyprinid herpesvirus 3 (CyHV-3) causing a lethal disease in common and koi carp. This CyHV-3 recombinant attenuated vaccine encodes a deletion of CyHV-3 ORF57, which is an orthologue of AngHV-1 ORF35. In the present study, we investigated the effect of AngHV-1 ORF35 deletion on AngHV-1 replication in cell culture and its virulence in its natural host the European eel (*Anguilla Anguilla*). With that goal in mind, we produced recombinant strains deleted for ORF35 using two parental strains, 1) the UK strain and 2) a recombinant derived from the former strain encoding a Luciferase reporter cassette inserted into a non-coding intergenic region. Analyses of ORF35 deleted recombinants demonstrated that the deletion of ORF35 does not affect AngHV-1 replication in cell culture. However, plaque size assays revealed that deleted recombinants produce smaller plaques. Interestingly, *in vivo* bioluminescent analyses (IVIS) revealed that ORF35 is essential for efficient viral replication *in vivo* after inoculation using different modes such as immersion in water containing the virus, intraperitoneal injection or intra-dermic injection. The importance of AngHV-1 ORF35 for efficient replication *in vivo* was also supported by *ex vivo* infection of fin explants. Experiments to unravel the roles of ORF35 *in vivo* and to test the potential of AngHV-1 ORF35 deleted recombinant as a vaccine candidate are under progress.

Antimicrobial resistance of *Escherichia coli* isolated from freshwaters, hospital effluents and bathing water in Belgium

L. Crettels^{1,2,*}, L. Champon^{1,*}, N. Burlion¹, R. Breyer¹, J. Mainil², P. Servais³, J. Korfer¹, D. Thiry²

1. Dept of Microbiology, ISSeP, Scientific Institute of Public Service, Liège 2. Bacteriology, Dept of Infectious and Parasitic Diseases, FMV, FARA, ULiège 3. Ecology of aquatic systems, Univ. Libre de Bruxelles, Brussels

*the 2 authors have equally contributed to the work

Corresponding author: l.crettels@issep.be

Antimicrobial resistance is recognized by the WHO as one of the greatest threats to global health. The presence of enteric bacteria in aquatic systems, which may carry antimicrobial resistance, represents a vector for its spread in the environment. The purposes of this work were for the first aim to evaluate the level of antimicrobial-resistant *E. coli* isolates in freshwaters and hospital effluents. The samples were collected from 24 locations along the Ourthe, Vesdre, Amblève and Meuse rivers and effluents of hospitals. For the second aim, *E. coli* were isolated from 9 locations of bathing water including rivers and lakes during 6 successive months in 2021. A total of 938 *E. coli* strains were isolated for the first aim while 925 strains were isolated for the second one on Chromogenic Selective Tryptone Bile X-glucuronide (TBX) and TBX supplemented with amoxicillin (TBX+AMX) media. Disk-diffusion assays were performed following the EUCAST's recommendations to assess the antimicrobial resistance against selected antibiotics. For freshwaters and wastewaters, 32.7% of strains were at least resistant to one antibiotic and 24.6% were multiple antimicrobial resistant strains on TBX. For freshwaters, wastewaters and bathing-waters, the highest resistance rates were found for ampicillin (AMP), amoxicillin/clavulanic acid (AMC) and sulfamethoxazole/trimethoprim (SXT). The lowest resistance rates were observed for carbapenems, which are last resort antibiotics. For each sampling point, antimicrobial-resistant *E. coli* were isolated, which clearly demonstrates the extent of the phenomenon in the environment and the importance of studying it in a One health context.

Does tourist pressure affect behaviour and feeding strategies of a commensal group of Barbary macaques, *Macaca sylvanus*, in Gouraya National Park (Bejaia, North-East Algeria)?

M. Boumenir¹, F. Brotcorne², M. Iguer-Ouada³, J-L. Hornick¹, N. Moula^{1,4}

1. Dept of Veterinary Management in Animal Resources, FMV, FARA Center, ULiège, Belgium 2. Research Unit SPHERES, ULiège, Belgium 3. Dept of Biological Sciences of the Environment, Faculty of Nature and Life Sciences, Abderrahmane-Mira-University, Bejaia, Algeria 4. Animal Facilities, ULiège, Belgium

Corresponding author: nassim.moula@uliege.be

Barbary macaque, *Macaca sylvanus*, "Ivekki" in Berber language, endemic to North Africa is an endangered species of primates undergoing strong anthropic pressure. The objectives of this study were to investigate the impact of tourist pressure on behavioural profile and feeding strategies of a group of Barbary macaques in Gouraya National Park (Bejaia, North-East Algeria). Using 10-min focal animal sampling, 51 hours of behavioural observations have been collected on 12 identified adult individuals (6 males and 6 females) over 03 months covering the touristic and non-touristic season. We measured the influence of tourist pressure by using three distinct factors: season (touristic and non-touristic season), tourist intensity (as assessed by daily number of cars present in the study site) and human presence (number of persons present at <15 m macaques at the start of the focal sample). The findings showed that moving and feeding time decreased during the tourist season, whereas resting and grooming time increased. When tourist intensity increased, similar results were obtained. On the other hand, with higher human presence, agonistic interactions amongst the macaques, which are linked to food competition, were more frequently observed. Finally, during the tourist season, consumption of natural foods (fruits, seeds, leaves, etc.) decreased significantly in favor of anthropogenic food (Pasta, nuts etc.). To conclude, the results show that both behavioural profile and diet composition of commensal Barbary macaques were significantly influenced by tourist pressure, suggesting an ecological and behavioural flexibility of the group, with implications in terms of energy expenditure.

Short talks

Staphylococcal enterotoxins (SEs) genes screening of *Staphylococcus aureus* strains isolated from Walloon artisanal farm cheeses and assessment of their ability to produce SEs *in vitro*

Pirard Barbara¹, Fatihi Abdelhak², Lefebvre Donatien^{2,3}, Nia Yacine², Hennekinne Jacques Antoine², Daube Georges¹, Clinquart Antoine¹

1. Lab. of Food Microbiology & Lab. of Food Technology, FARA, ULiège, Belgium 2. Lab. for Food Safety, French Agency for Food, Environmental and Occupational Health & Safety (ANSES), Univ. Paris-Est, 94700 Maisons-Alfort, France 3. Technologies pour la Santé (DMTS), SPI, Univ. Paris-Saclay, CEA, INRAE, 91191 Gif-sur-Yvette, France

Corresponding author: barbara.pirard@uliege.be

Cheese can be associated with Staphylococcal Foodborne Poisoning Outbreaks (SFPO) caused by *Staphylococcus aureus* (*S. aureus*), which is able to produce staphylococcal enterotoxins (SEs) in food. Up to now, more than 26 SEs have been identified but the most reported ones are the five "classical" (Sea to See), while newly described SEs have also been demonstrated to cause SFPO (Chieffi et al., 2020). In this study, cheeses from 32 operators producing at farm level were analysed at the beginning and at the end of their shelf life in order to isolate, identify and assess the toxigenic capacity of *S. aureus* strains. A total of 49 *S. aureus* was isolated on Baird Parker agar and identified by sequencing of the 16s RNA gene. They were screened by real-time PCR for the presence of 11 SEs genes (*sea, seb, sec, sed, see, seg, sei, sej, seh, sep, ser*). Toxin production capacity of these isolates was tested and quantified by ELISA sandwich immunoassays according to the methods used by the EURL for Coagulase Positive Staphylococci. SEs genes detected by PCR were *sea, sec, seg, sei, sep*. The most frequently observed were *seg* and *sei* genes (27/49 strains or 55%), which are part of the enterotoxin gene cluster (*egc*) and production of *Sei* was confirmed for all these strains by ELISA. These results show that, *egc* SEs genes are present in cheeses from Wallonia terroir. However, no SFPO was reported since 2016 in Belgium (Sciensano, 2019). Further studies should then be conducted in order to better circumscribe public health risk linked to the production of *egc* SEs in Walloon cheeses but also to enhance the ability to detect them in food products in routine.

Posters

Veterinary Public Health

1. Genomes of Anguillid Herpesvirus 1 Strains Reveal Evolutionary Disparities and Low Genetic Diversity in the Genus *Cyprinivirus*

Donohoe O.^{1,2}, Delrez N.¹, Zhang H.¹, Davison A.³ and Vanderplasschen A.¹

1. Immunology-Vaccinology (B43b), FARA, Faculty of Veterinary Medicine, ULiège

2. Bioscience Research Institute, Technological University of the Shannon: Midlands Midwest, Athlone, Co Westmeath, Ireland, Athlone, Co. Westmeath, Ireland

3. University of Glasgow Centre for Virus Research, Glasgow, United Kingdom

Corresponding author: owen.donohoe@uliege.be

Anguillid herpesvirus 1 (AngHV-1) is a pathogen of eels and a member of the genus *Cyprinivirus* in the family *Alloherpesviridae*. We have compared the biological and genomic features of different AngHV-1 strains, focusing on their growth kinetics *in vitro* and genetic content, diversity, and recombination. Comparisons based on three core genes conserved among alloherpesviruses revealed that AngHV-1 exhibits a slower rate of change and less positive selection than other cypriniviruses. We propose that this may be linked to major differences in host species and corresponding epidemiological circumstances. Efforts to derive evolutionary rate estimates for cypriniviruses under various theoretical models were ultimately unrewarding. We highlight the potential value of future collaborative efforts towards generating short-term evolutionary rate estimates based on known sequence sampling dates. Finally, we revealed that there is significantly less genetic diversity in core gene sequences within cyprinivirus species clades compared to species in the family *Herpesviridae*. This suggests that cyprinivirus species may have undergone much more vigorous purifying selection post species clade divergence. We discuss whether this may be linked to biological and anthropogenic factors or to sampling bias, and we propose that the comparison of short-term evolutionary rates between species may provide further insights into these differences.

2. Seasonal variation of aflatoxin M1 in raw milk from Benin with an emphasis on risk assessment of human exposure

Alphonse Dossou^{1,2}, Caroline Douny¹, Yann Madode², Paulin Azokpota², Souaïbou Farougou³, Samiha Boutaleb¹, Marie-Louise Scippo¹, Antoine Clinquart¹

1. Dept of Food Science, Fac. of Veterinary Medicine, FARA, Vet. Public Health & Sustainable Livestock Production, ULiège, Liège, Belgium 2. School of Nutrition, Food Sciences and Technology, Laboratory of food sciences, Faculty of Agronomic Sciences, University of Abomey-Calavi, Cotonou, Benin 3. Communicable Diseases Research Unit, Polytechnic School of Abomey Calavi, University of Abomey-Calavi, Cotonou, Benin

Corresponding author: wadossou@uliege.be

Aflatoxin M1 (AFM1) is a natural toxin found in milk produced in hot and humid regions such as Benin. It may be associated with liver damage and some types of cancer. The main objective of this work is to determine the seasonal variation of AFM1 in raw milk from Benin with an emphasis on risk assessment of human exposure. For this purpose, 24 samples of cow's milk from six farms were collected during the rainy and the dry season of 2020. AFM1 levels were determined by ELISA. AFM1 was found at quantifiable levels ($> 0,005 \mu\text{g}/\text{kg}$) in all samples collected during the dry season and only in 3 samples collected during the rainy season. However, no significant difference was observed in the AFM1 contamination regarding the season (mean values using the upper bound approach of 0.02 ± 0.03 and $0.06 \pm 0.06 \mu\text{g}/\text{kg}$ wet weight for rainy and dry season respectively). A deterministic approach was used to calculate the AFM1 exposure corresponding to the worse case (Percentile 95) daily consumption of 192 g of milk. For an adult of 60 kg body weight (bw), the exposure would be $6.4 \cdot 10^{-5}$ and $1.6 \cdot 10^{-4} \mu\text{g}/\text{kg}$ bw/day for the rainy and dry season, respectively. According to EFSA, the risk linked to the intake of AFM1 from food should be assessed using the approach of the margin of exposure (MOE), based on benchmark doses (BMDL) determined for animal liver carcinogenicity of AFB1 and AFM1. The BMDL10 for AFB1 is $0.4 \mu\text{g}/\text{kg}$ bw/day and a potency factor of 0.1 has to be taken into account for AFM1 (EFSA, 2020). The MOE (ratio between the BMDL10 and the exposure) were 625000 and 25000 for AFM1 ingestion through ingestion of milk collected during the rainy and dry season, respectively. In conclusion, this preliminary and limited risk assessment show MOE higher than 10.000, which indicate an absence of concern about the AFM1 intake from raw milk consumption in Benin.

Posters

3. Study of the importance of tegument proteins, and in particular of pORF63, in the biology of gammaherpesvirus infection.

Gillard L.¹, Maquet C.¹, Myster F.¹, Javaux J.¹, Lemmens I.², Tavernier J.², Machiels B.¹, Gillet L.¹

1. Immunology-Vaccinology, Department of infectious and parasitic diseases, Faculty of Veterinary medicine – FARAH, University of Liège, Belgium.

2. Department of Medical Protein Research, VIB, University of Ghent, Belgium

Corresponding author : Lucie.Gillard@uliege.be

Gammaherpesviruses (γ HVs) are widespread viruses that cause lifelong infections in many mammalian species and represent a significant cause of diseases. Thus, in humans, Kaposi's sarcoma-associated herpesvirus (KSHV) is associated with several cancers and is therefore a major subject of research. Among the different constituents of the γ HVs virions, tegument proteins play major roles in viral cycle. In particular, a potential role in immune evasion has been proposed for the tegument protein encoded by KSHV open reading frame 63 (ORF63). However, KSHV study is limited by the absence of established animal model and by the lack of permissive cell lines. In this work, we used Murid herpesvirus 4, a phylogenetically related virus, to decipher the roles of ORF63 in the gammaherpesvirus lifecycle. Surprisingly, our study revealed the existence of two distinct MuHV4 pORF63 isoforms. While we previously showed that the long pORF63 isoform was essential for the migration of viral capsids toward the nucleus during entry, a role for the short ORF63 protein had never been addressed so far. In order to investigate the specific function during infection, we generated two different knock-out mutants; one expressing only the short form (STOP) and one coding for none isoform (double-STOP). A comparative analysis of these mutants revealed that the absence of the short protein was associated with a dramatic growth deficit in vitro. The purpose of this work is to decipher the precise function of pORF63 isoforms during infection. Such research could allow a better understanding of KSHV lifecycle and promote the development of new therapeutic strategies against this virus.

4. The role of A4 and A10 genes in the pathogenesis of a lethal bovine

lymphoproliferative disease induced by alcelaphine gammaherpesvirus 1

Gong Meijiao¹, Myster Françoise¹, Javaux Justine¹, Van Campe Willem², Roels Stefan², Mostin L.², Kerkhofs P.², Vanderplasschen A.¹, Dewals B.G.¹

1. Immunology-Vaccinology, Department of infectious and parasitic diseases, Faculty of Veterinary medicine – FARAH, University of Liège, Belgium.

2. Siensano, Brussels, Belgium

Corresponding author: meijiao.gong@uliege.be

Alcelaphine gammaherpesvirus 1 (AIHV-1) is carried asymptotically by wildebeests. Upon transmission to various ruminant species including cattle, this virus is responsible for malignant catarrhal fever (MCF), a deadly lymphoproliferative disease caused by latently infected CD8 T cells. However, the mechanisms involved in the pathogenesis of MCF remain poorly understood. Interestingly, RNAseq analysis was carried out on purified CD8+ T cells from uninfected or MCF-developing calves. Besides information on the host gene regulation during MCF, the RNAseq data could provide information about the viral expressed regions across the AIHV-1 genome. AIHV-1 specific genes A4 and A10 were observed to be expressed in CD8+ T cells. Both gene encode putative homologs of the STP and Tip oncoproteins expressed by related Saimiriine gammaherpesvirus 2, which also infects T lymphocytes. Thus, A4 and/or A10 might be involved in reprogramming infected T cells, resulting in their uncontrolled activation. To investigate the role of A4 and A10 in the pathogenesis of MCF, recombinant viruses impaired for A4 or A10 expression were produced using the AIHV-1 BAC clone. We observed that the lack of A4 or A10 did not affect the viral fitness in vitro. Using the rabbit experimental model to induce MCF, we observed that A4 was not essential for MCF induction but demonstrated that A10 expression in vivo was essential to induce CD8+ T cell expansion, MCF lesions and hyperthermia in the infected animals. In parallel, an A10-FLAG recombinant virus was constructed to further identify interacting proteins. Future work will aim to determine the role of the ITAM and SH3 motifs in the cytoplasmic domain of A10 through generation of mutant viruses that will be further used in rabbit infection experiments. Based on these results and future perspectives, we suggest that A10 can constitutively trigger T cell activation pathways upon infection of CD8+ T cells and lead to MCF.

5. Impact of food additives on intestinal microbiota

I. Gonza^{*1}, C. Douny², V. Mohring³, M.L. Scippo², E. Louis⁴ and V. Delcenserie¹

1. ULiège – Food Quality Management, Food Science Dept, FARAHA 2. ULiège – Food Analysis, Food Science Dept, FARAHA 3. UCL – Master in Biomedical Sciences 4. ULiège and CHU of Liège – Department of Gastroenterology

Corresponding author: iegonza@uliege.be

Accumulating evidence demonstrates a contribution of dietary emulsifiers in the increase of prevalence of diseases associated with intestinal inflammation, such as inflammatory bowel disease. Two emulsifiers in particular, polysorbate 80 and carboxymethylcellulose, profoundly impact intestinal microbiota leading to gut inflammation. The impact of other food additives on the intestinal microbiota composition and function is less known. Some mucolytic bacteria such as *Mucispirillum schaedleri*, *Ruminococcus* and *Anaeroplasm* has been related directly with fibrosis induction while other bacteria such as *Oscillospira* and *Coprococcus* were negatively related. The aim of this work is to determine how food additives influences intestinal microbiota towards a dysbiosis, an inflammation and the subsequent formation of fibrosis. Six food additives (polysorbate 80, maltodextrin, titanium dioxide, sodium nitrite, sucralose and kappa - carrageenan) were tested in *in vitro* batch cultures model of intestinal microbiota for 72 hours. The tested concentrations were based on the acceptable daily intake (ADI) established by the Expert Committee on Food Additives (JECFA) or on the estimated daily exposure calculated by the European Food Safety Authority (EFSA) assuming in both cases an average weight of 70 kg. A mix of fecal samples from 5 healthy donors was used in the *in vitro* batch models. Changes in microbiota were assessed every 24h using qPCR targeting bacterial groups involved in short-chain fatty acid (SCFA) production or in inflammation. In addition, SCFA production was assessed using GC/MS. The main changes observed were for polysorbate 80 who after 72h of fermentation dramatically decreased butyrate and propionate. This was confirmed by qPCR analysis with a decrease of *Clostridium* cluster XIVa (butyrate producing bacteria) and *Bacteroides/Prevotella* spp. (propionate producing bacteria). Titanium dioxide increased propionic acid after 24h of fermentation with promotion of Bacteroidetes and the inhibition of *Bifidobacterium*, *Streptococcus* spp., *Enterococcus* spp. and *Clostridium* clusters IV and XIVa. After 72h, maltodextrin, induced an increase of acetic acid, propionate, butyrate and total SCFA. In addition, maltodextrin promoted the growth of *Bifidobacterium*, *Clostridium* clusters IV and XIVa.

6. Illumination of Cyprinid Herpesvirus 2 infectious cycle in goldfish (*Carassius auratus*) using *in vivo* bioluminescent imaging

Bo He.¹, O Donohoe^{1,2}, Alain Vanderplasschen.^{1*}

1. Dept of Infectious and Parasitic Diseases (B43b), Immunology-Vaccinology, FARAHA, Uliège 2. Bioscience Research Institute, Technological University of the Shannon: Midlands Midwest, Athlone, Co Westmeath, Ireland

Corresponding author: bo.he@doct.uliege.be

During the past two decades, as a consequence of growth in international trade, Cyprinid herpesvirus 2 (CyHV-2) reached a worldwide distribution. Its high economic impact on the aquaculture sector has subsequently stimulated an increase in fundamental and applied researches on this virus. However, fundamental knowledge on CyHV-2 pathogenesis such as its portal of entry into the host are still unknown. In the present study, we aimed to use bioluminescent *in vivo* imaging to investigate this further. With this goal in mind, we produced a recombinant strain expressing luciferase and copepod GFP reporter genes (LucGFP strain). We compared the recombinant to the parental strain in terms of genome sequence, expression of genes flanking the reporter gene insertion site, replication in cell culture and virulence *in vivo* after inoculation by IP injection. All of these tests indicated no significant difference between the recombinant and parental strains. Using this recombinant strain, the CyHV-2 portal of entry was investigated using two modes of inoculation mimicking different epidemiological scenarios: 1) immersion in water containing virus and 2) cohabitation with subjects initially infected intraperitoneal injection (IP). Despite the fact that subjects inoculated with the LucGFP strain by IP exhibited high virulence comparable to the parental strain, subjects inoculated by immersion and cohabitation both exhibited abortive infections. Surprisingly, the IVIS data demonstrated that the LucGFP strain inoculated through the two modes of infection tested led to an abortive infection (cohabitation mode or controlled immersion mode). The data also indicated that for both modes of inoculation, the intestine, may act as portal of entry and represents the first step towards a greater fundamental understanding of CyHV-2 pathogenesis.

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Notably, this data demonstrates that despite their phylogenetic relationship *Cyprinivirus* species use very different strategies to enter their respective hosts.

7. *Escherichia coli* O80 isolated from healthy cattle at slaughterhouse

R. Ikedas^{1,a}, K. Nakamura², N. Korsak³, J.-N. Duprez¹, T. Hayashi², D. Thiry¹, J.G. Mainil¹

1. Bacteriology, Department of Infectious Disease and 3. Food inspection, Department of Food Science, Faculty of Veterinary Medicine, Centre for Fundamental and Applied Research for Animals and Health (FARAH), University of Liège (ULiège), Belgium

2. Department of Bacteriology, Faculty of Medical Sciences, Kyushu University, Fukuoka, Japan

Since 2014, Shiga toxin (Stx)-producing *Escherichia coli* (STEC) of the previously rare serotype O80:H2 have been isolated from humans suffering diarrhoea and haemolytic uremic syndrome. In addition to Stx, STEC O80:H2 produce the attaching effacing (AE) lesion and harbour a pS88 plasmid carrying genes responsible for systemic infection (Ingelbeen B et al., 2018). STEC and enteropathogenic *E. coli* (EPEC) O80:H2 harbouring the pS88 plasmid have also been isolated from young calves with diarrhoea (Habets et al., 2021). Conversely, they have not been isolated from healthy cattle (Thiry et al., 2018). The aim of this work was to isolate and characterize *E. coli* O80:H2 from healthy cattle at slaughterhouse in Belgium. Faecal samples were collected in 2020 at slaughterhouse and incubated overnight in coliform enrichment broths that were tested with a PCR for the O80 antigen. PCR-positive broths were inoculated onto five semi-selective agar plates. Up to 5 colonies were picked up from each plate and tested twice with the O80 PCR. PCR for the H2 antigen, *eae* and *stx* genes were further performed on the O80 PCR-confirmed colonies. Finally, whole genome sequencing (WGS) was carried out on 3 O80-positive colonies to identify their full serotypes and virulotypes. A total of 450 colonies were picked up from the 35/149 enriched faecal samples positive with the O80 PCR. Of them, 5 colonies from 3 samples were confirmed as positive with the O80 PCR. Conversely none tested positive with the PCR for the H2 antigen, *eae* and *stx* genes. The WGS showed that 3 colonies are *E. coli* O80:H45 and negative for H2, *eae* and *stx*. In conclusion, no *E. coli* O80:H2 was isolated from healthy cattle in this slaughterhouse in 2020.

8. Virulence genes expression in *Clostridioides difficile* in a insert plate model

Martinez E.¹, Doumont L.¹, Taminiau B.¹, Daube G.¹

1. Food Science Department - Microbiology, FARAH, ULiège

Corresponding author: emartinez@uliege.be

Clostridioides difficile is a Gram positive bacterium sporulant and requires strict anaerobiosis. The intestinal carriage of *C. difficile* can be asymptomatic, but in cases of infection it is associated with different clinical signs of disease that can vary from mild diarrhea to pseudomembranous colitis or even death. Expression of *C. difficile* virulence gene was studied in an *in vitro* insert plate model. The objective of this study was to identify the timing of expression of virulence gene of *C. difficile*. In compartment "in", a nutritive matrix, salt biles and a quantity of *C. difficile* spores were placed. In compartment "out", a nutritive matrix and salt biles were placed. Several plates were made to study a plate without feces, two with a variation of the concentration of spores, two with a variation of the concentration of feces concentration and four with a variation of the microflora. Total DNA and RNA were extracted and RT-qPCR targeting *mldA* (involved in cell division), *gluD* (housekeeping genes), *tcdB* and *tcdA* (toxins genes) This method of culture allows the growth of *C. difficile* under different growth conditions. The plate without feces is the benchmark. The expression of genes varies depending on the growth conditions and some donors (D2 and D4) will promote the production of toxins. Among the donors, donors 4 gives us a pattern that is closer to the expression of the virulence genes of the insert without feces. The next step of our study will use metatranscriptomics to identify the expression of certain genes that have an impact on colonization.

9. The nucleocytoplasmic shuttling of Cyprinid herpesvirus 3 ORF112 is important for efficient replication in cell culture

Sébastien Pirotte¹, Mamadou Diallo¹, Yunlong Hu¹, Alain Vanderplasschen¹

1. Immunology-Vaccinology, FARAHA, Faculty of Veterinary Medicine, University of Liège, Liège, Belgium

Corresponding author: spirotte@student.uliege.be

The innate immune system relies on a large number of nucleic acid sensors. Nucleic acids can be detected based on their structure, subcellular localization, sequence or even their conformation. Zalpha (Zα) domains bind to left-handed dsDNA (Z-DNA) or left-handed dsRNA (Z-RNA). Three cellular proteins contain Zα domains: ADAR1, ZBP1, and PKZ. Zα domains have also been described in two phylogenetic groups of viruses: the E3L protein of chordopoxviruses and ORF112 encoded by some of the cypriniviruses including Cyprinid herpesvirus 3 (CyHV-3). Many of these proteins are capable of shuttling. Shuttling refers to the ability of a protein to travel between the nucleus and the cytoplasm through the nuclear pore complex. Shuttling is frequently associated with the presence of localization signals in the primary sequence proteins. NLS (nuclear localization signal) and NES (nuclear export signal) stimulate localization in the nucleus and the cytoplasm, respectively. We have recently made some major findings related to the Zα domain of CyHV-3 ORF112: (i) ORF112 Zalpha domain is essential and sufficient for viral replication in cell culture. (ii) Within infected cells, ORF112 aggregates in RNA condensates. (iii) The localization of these condensates evolves during the infection. Bioinformatic analyses predicted the presence of NLS and NES in the N-terminal part of ORF112. To investigate the importance of ORF112 shuttling in the replication cycle of CyHV-3, we constructed two recombinant viruses encoding CyHV-3 Zalpha domain of ORF112 fused at its N-terminal end to two NLS or two NES and we compared their growth to a control virus encoding the Zalpha domain only. Here, we report that an impairment in the shuttling activity of ORF112 impacts the growth of CyHV-3 *in vitro*.

10. Typology of strandings, causes of cetacean mortality and study on *Brucella spp.* in Northern France from 1995 to 2020

Polaert R., Jauniaux T., Karpouzopoulos J., Garigliany M., Sarlet M., Hasanaj A., Dabin W.

Laboratory of Morphology and Pathology, Veterinary Public Health, FARAHA, ULiège

Corresponding author : raphaelle.polaert@student.uliege.be

In the current context where strandings are increasing every year, we highlighted the main lesions and causes of mortality of cetaceans stranded along the French northern coastline between 1995 and 2020, the harbour porpoise (*Phocoena phocoena*) being used as a reference. Necropsies were performed and organ sampled following a standard protocol. The autopsy data thus collected were compiled with the database of the Pelagis Observatory of La Rochelle. The most frequent post-mortem findings included net marks, subcutaneous and muscle bruising, emaciation, respiratory and gastric parasitism, acute pneumonia, lung congestion and edema. The two main causes of mortality were bycatch in fishing nets (41%) and infectious processes (40%), followed by emaciation (10%) and predation by grey seals (*Halichoerus grypus*) (5%). The annual distribution of strandings is very heterogeneous. Bycatch was mostly observed in March and April and its incidence has increased. Infectious processes, mostly acute pneumonia associated with severe parasitic infestation, appear all year round. New causes of mortality have been identified, notably emaciation alone and predation by grey seals. Overall, there has been an increase in strandings over the last two decades, which can be explained by the large-scale movement of harbour porpoise population from the northern part to the southern bay of the North Sea. This increase may also be strongly influenced by other factors such as the increase in bycatch, the lack of food causing emaciation of the species and the

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emerging attacks of grey seals. The study also revealed high incidence (12.5%) of brucellosis among cetaceans.

11. Identification of *Corynebacterium ulcerans* isolated from European hedgehogs (*Erinaceus europaeus*) in Wallonia and characterization of its zoonotic potential

A. Van Goethem¹, G. Gilliaux¹, J. Paternostre¹, C. Lesenfants¹, A. Mertens¹, A. Linden¹

1. *Departement of Infectious and Parasitic diseases, Wildlife service, FARAH, ULiège*

Corresponding author : avangoethem@uliege.be

Corynebacterium ulcerans is an emerging zoonotic pathogen that has been isolated from hedgehogs. The objectives of this study were (1) to determine the prevalence of this bacterium in two cohorts of hedgehogs and (2) to clarify its zoonotic potential. 218 hedgehogs were necropsied and classified into two cohorts according to the absence (cohort A, control group) or presence (cohort B) of specific skin lesions. Molecular and conventional analyses were performed on samples from both cohorts. The necropsies revealed a high proportion (64.7%) of individuals with ulcerative skin lesions. The frequency of detection of tox-bearing *Corynebacteria* of the diphtheriae complex was much higher in cohort B (80%) than in the control cohort (11.7%) and the invasive nature of these *Corynebacteria* is demonstrated. Mass spectrometry analysis confirmed the presence of *Corynebacterium ulcerans* in several samples and 100% of these strains produced diphtheria toxin *in vitro*. Without being able to establish a causal link, our results suggest that this *corynebacterium* is an opportunistic pathogen taking advantage of skin trauma to induce, alone or in synergy, specific skin lesions in hedgehogs. Moreover, the zoonotic risk is real. The fact that toxigenic *Corynebacterium ulcerans* strains were detected in the skin lesions of 75% of the animals (60 out of 80) that were treated/manipulated in the centres should encourage caution. Recommendations are proposed at the end of this work.

12. Of masks and methylene blue - the use of methylene blue photochemical treatment to decontaminate surgical masks contaminated with a tenacious small non-enveloped norovirus

Wielick C.¹, Fries A.¹, Dams L.¹, Razafimahefa R. M.¹, Heyne B.², Harcourt B.³, Lendvay T.⁴, Chu M.⁵, Willeart J. F.⁵, de Jaeger S.⁵, Haubruge E.⁵, Thiry E.¹, Ludwig-Begall L. F.¹

1. *Vet. Virology and Animal Viral Diseases, Dept of Infectious and Parasitic Diseases, FARAH Research Centre, Fac. of Vet. Medicine, ULiège* 2. *Dept of Chemistry, Univ. of Calgary, 2500 University Drive Northwest, Calgary, Alberta, Canada* 3. *Viral Special Pathogens Branch, Division of High Consequence Pathogens and Pathology, Centers for Disease Control and Prevention, Atlanta, USA* 4. *Dept of Urology, Univ. of Washington School of Medicine, Seattle Children's Hospital, Seattle, Washington, USA* 5. *Dept of Epidemiology, Colorado School of Public Health, Aurora, Colorado, USA* 6. *TERRA Research Centre, Gembloux Agro-Bio Tech, ULiège, Gembloux*

Corresponding author : Etienne.thiry@uliege.be

In the context of the ongoing severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) pandemic, the supply of personal protective equipment has been under severe strain. To address this issue, re-use of personal protective equipment, specifically that of medical face coverings, has been recommended. The reuse of these typically single-use only items necessitates procedures to inactivate contaminating human respiratory and oral pathogens. We previously demonstrated decontamination of surgical masks and respirators contaminated with infectious SARS-CoV-2 and various animal coronaviruses via low concentration- and short exposure methylene blue photochemical treatment (10 µM methylene blue, 30 minutes of 10.474 W/m² red light exposure). Here, we describe the adaptation of this protocol to the decontamination of a more resistant, non-enveloped oral virus and demonstrate efficient photodynamic inactivation of murine norovirus, a human norovirus surrogate; methylene blue photochemical treatment (100 µM methylene blue, 30 minutes of 10.474 W/cm² red light exposure) of murine norovirus-contaminated masks reduced infectious viral titres by over four orders of magnitude on surgical mask surfaces. Inactivation of a norovirus, the most resistant of the respiratory and oral human viruses, can predict the inactivation

of any less resistant viral mask contaminant. The inexpensive and universally accessible photochemical decontamination protocol developed here thus solidifies the position of methylene blue photochemical decontamination as an important tool in the package of practical pandemic preparedness.

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13. Siglec-2: a specific surface marker of IL-4-dependent virtual memory CD8⁺ T cells in the periphery

Bin Yang¹, Arnaud Lavergne², Benjamin G. Dewals¹

¹Immunology-Vaccinology, Department of infectious and parasitic diseases, Faculty of Veterinary medicine – FARAHA, University of Liège, Belgium.

²GIGA-Genomics Core Facility, University of Liège, Belgium.

Corresponding author: byang@uliege.be

Helminth infection can modulate the immune response to concurrent infections. Recent work from the laboratory has demonstrated that IL-4 during helminth infection could expand virtual memory CD8⁺ T cells (T_{VM}). T_{VM} cells are foreign antigen (Ag)-inexperienced displaying a memory-like phenotype. Helminth induced expansion of T_{VM} cells led to a subsequent raised Ag-specific CD8⁺ T cell activation that enhanced control of viral infection. In the present study, we have further investigated how IL-4 regulates T_{VM} cell expansion via single-cell RNA sequencing of peripheral CD8⁺ T cells. We have compared the heterogeneity of cellular gene signatures of CD8⁺ T cells after IL-4-complex treatment or infection with the helminth *Heligmosomoides polygyrus*. Using mice conditionally deficient for IL-4Rα in peripheral CD8⁺ T lymphocytes, we could identify a cluster of cells that specifically responded to IL-4 and upregulated signature genes of T_{VM} cells. Among upregulated genes in the T_{VM} cell cluster, we identified Cd22 as being upregulated specifically by IL-4. Cd22 gene encodes a protein named sialic acid-binding immunoglobulin-type lectin 2 (siglec-2) and was until recently thought to be restricted to some B lymphocyte subsets. CD22 protein expression could also be detected by flow cytometry and was restricted to IL-4-responsive T_{VM} cells, suggesting that this inhibitory receptor could be involved in the regulation of T_{VM} functions.

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14. The influence of the *in Ovo* administration of vitamin E, Ascorbic acid and their association on hatchability, hatch weight and egg weight loss of Japanese quail (*Courtnix Courtnix Japonica*).

A. Aberbour¹, M. Iguer-Ouada³, L. Touazi², J-L. Hornick¹, N. Moula^{1,4}

1. Dept of Vet. Management in Animal Resources, Fac. of Veterinary Medicine, FARAH Center, ULiege, Belgium

2. Department of Agroalimentary, Fac. of Nature and Life Sciences, Farhat Abbas University, Setif, Algeria

3. Dept of Biological Sciences of the Environment, Faculty of Nature and Life Sciences, Abderrahmane-Mira-University, Bejaia, Algeria

4. Animal Facilities, University of Liège, Belgium

Corresponding author: nassim.moula@uliege.be

In Ovo supplementation of poultry embryos has shown diverse advantages in poultry production by enhancing particularly embryonic development during incubation and posthatch performances. Prenatal vitamins C and E supply (*in Ovo* feeding) is indicated to improve hatchability, chick weight and growth performances by enhancing antioxidant status. However, Vitamin E potency is dependent on recycling efficiency. In this respect, Vitamin C is known to recycle Vitamin E by transforming its oxydized form to a reduced one, extending consequently Vitamin E action.

In this study Vitamins E and C alone and their association were injected *In Ovo* to investigate their impact on hatchability, hatching chick weight and post-hatching weight loss in Japanese quail. At the thirteenth day of incubation, fertile quail eggs (n=100) were distributed into five groups: control (20 µl of sterilized saline solution 0.9%) and four treatments with eggs injected with 20µl of vitamins solution: Vitamin E acetate (3mg solubilized in micro emulsion of Polyethylene glycol in saline solution, T1), Vitamin E α-tocopherol (3mg /egg solubilized in micro emulsions of Polyethylene glycol in saline solution, T2), Vitamin C (1mg, T3) and association of both Vitamin E α-tocopherol (3mg/egg) and Vitamin C (1mg, T4). The results showed that the vitamins association group showed the highest hatchability percentage compared to the other treatments. The highest value of weight hatch was found in Vitamin C compared to the other groups. In term of weight loss, the lowest value was recorded in Vitamin C treatment (28.6%).

In conclusion, based on the current preliminary results, it appears that vitamin C enhances hatching weight; whereas the association of vitamin E and Vitamin C enhances hatchability rate. By exploring other optimal concentrations, the effect of this association could be more significant.

15. Effects of replacing alfalfa hay with *Sulla flexuosa* hay in the diet of Moroccan Beni Arouss goats on milk production and quality: preliminary results

Boukrouh S.^{1,2}, Moula N.¹, Noutfia A.², El Otmani S.², Avril C.³, Hornick J.L.¹, Chentouf M.², Cabaraux J.F.¹

1. Department of veterinary management of animal resources, FARAH, IVT, Faculty of Veterinary Medicine, ULiège

2. INRA, Regional Agricultural Research Centre, Tangier, Morocco

3. HEPH Condorcet, agronomy category, Ath, Belgium.

Corresponding author: s.boukrouh@uliege.be

Sulla flexuosa (*Hedysarum flexuosum*) is a legume rich in condensed tannins and widely distributed in Northern Morocco. This study aimed to evaluate the effects of incorporating *Sulla flexuosa* hay as a replacement for alfalfa hay on goat milk production and quality. Thirty local Beni Arouss goats were divided into three experimental groups according to their physiological state and production level. The control group received a diet based on alfalfa hay (70%) as fodder; the test groups received either 35% of *Sulla flexuosa* hay and 35% of alfalfa hay or 70% of *Sulla flexuosa* hay. Over two months, milk production was weekly recorded and sampled to analyze the milk quality. No significant differences were observed between the control and test groups for fat (2.13 %), proteins (3.53%), lactose (4.54%), solids non-fat (9%), and mineral matter (0.83%) contents. Likewise, the milk acidity (pH and Dornic acidity) was not affected by the incorporation of *Sulla*. However, the lactation period significantly affected protein, lactose, mineral matter, and solids non-fat contents. *Sulla flexuosa* hay, which is easier to grow in Northern Morocco than Alfalfa, can be used as a substitute for Alfalfa in the diet of milked goats.

16. Influence of the diet on the composition of the earthworm *Eudrilus eugeniae*

Patrick Byambas^{1,2} Caroline Douny³ Nassim Moula¹ Marie-Louise Scippo³ Jean-Luc Hornick¹

1. Department of Fundamental and Applied Research for Animals and Health, Faculty of Veterinary Medicine, University of Liège, Quartier Vallée 2, av. de Cureghem 10 Bât. B43, 4000 Liège, Belgium.

2. Department of Zootechny, Institute of Agronomic and Forestry Research (IRAF), National Center of Technology and Scientific and Technological Research (CENAREST), Libreville, Gabon.

3. Laboratory of Food Analysis, Department of Food Sciences, Faculty of Veterinary Medicine, Fundamental and Applied Research for Animals and Health (FARAH), Veterinary Public Health, University of Liège, B-4000 Liège, Belgium.

Corresponding author: byambaspatrick@gmail.com

The earthworm *Eudrilus eugeniae* is a well-known source of protein in animal feeding but there is lack of information on its fatty acid profile. This study aimed to determine its nutrient components and fatty acid profile. Earthworms were bred in substrate containing organic matter; peanut powder was added in the experimental group diet. The nutrient components (protein, ash, and minerals) of the adult worms were determined according to the Kjeldahl method and by atomic absorption spectrophotometer. The fatty acid profile was determined by gas chromatography/mass spectrometry. The dry-matter protein content significantly increased in worms fed peanut powder ($p < 0.05$) as did the minerals measured.

The main fatty acid families had similar proportions in the groups. In the saturated fatty acids, C12:0 and C18:0 were present in higher proportions in both groups, whereas in monounsaturated fatty acids, C18:1 ω 9 prevailed. Significant differences ($p < 0.05$) in polyunsaturated fatty acids were observed between the two groups.

An increase in the proportion of ω 6 and ω 3 was observed in the experimental group compared to the control. This study showed that peanut powder significantly influenced nutrient components of *E. eugeniae*. The fatty acid profile of this species was similar to that of most animals. Earthworms' diet can be altered to improve the nutritional value of *E. eugeniae*.

17. Forest and pasture cover in Western Rif of Northern Morocco

Chebli Y.¹, El Otmani S.¹, Chentouf M.¹, J. L. Hornick² and Cabaraux J.F.²

1. INRA, Regional Agricultural Research Centre, Tangier, Morocco

2. Department of veterinary management of animal resources, FARAH, IVT, Faculty of Veterinary Medicine, ULiège

Corresponding author: youssef.chebli@inra.ma

Northern Morocco harbours the large-scale oak forests in the country, surrounded by an agricultural mosaic. The mountainous topography and the existing forest vegetation explain the strong predominance of grazing goats in these woodlands. 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 pasture. Mapping has become a central element of current natural resource management strategies to implement appropriate development policies. The objective of this study was to produce a land cover map of Chefchaouen in order to determine the forest and pastureland cover based on remote sensing data. This study allowed us to identify four types of pasturelands (forest, pasture mixed with forest, pasture, and pasture mixed with cropland). According to the classification results, forest, pasture, and cropland cover an area of 39, 3.9, and 3.1%, respectively. The largest cover land was recorded by pasture 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 pastures, because they have never been studied before. Overall, this research makes a contribution to know the current land cover types in Western Rif for future interventions in order to develop and manage forest and pasture areas.

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18. Silvopastoral system in northern Morocco: Importance, strategic functions and recent changes

Chebli Y.¹, El Otmani S.¹, Chentouf M.¹, J. L. Hornick ² and Cabaraux J.F.²

1. INRA, Regional Agricultural Research Centre, Tangier, Morocco

2. Department of veterinary management of animal resources, FARAH, IVT, Faculty of Veterinary Medicine, ULiege

Corresponding author: youssef.chebli@inra.ma

In the Mediterranean basin, the silvopastoral system has always been part of the regional economy. Silvopastoral areas contribute significantly to the development of the ecological and socio-economic factors of territorial sustainability. However, little attention has been paid to reviewing their importance, historical evolution, socio-economic conditions, and the climate change impact, mainly the southern Mediterranean region of northern Morocco. This review casts light on research on the silvopastoral system in northern Morocco. Sixty-nine peer-reviewed publications were selected for this systematic review. The findings revealed that silvopastoral areas play a very important role in animal feeding in Morocco. Animals are managed traditionally on these wood-lands where they graze throughout the year. The silvopastoral system has undergone several changes over these last decades. These changes have had a direct impact on these ecosystems causing the decline of biodiversity, forest degradation, and the increase of land use at the expense of silvopastoral areas. Consequently, the sustainability of the silvopastoral systems would undoubtedly be negatively affected. The review finishes with some crucial strategies and propositions that could be more encouraged to support sustainable management actions of silvopastoral resources. In the conclusion, we outline the need to assess the realistic socio-economic and ecological benefits of silvopastoral systems to promote their sustainable development.

19. Bacteriological analysis of black soldier fly frass

Detilleux, J. ¹, Duprez, J-N. ², Dawans, E. ¹, Mainil, J.²

1. Elevage sélectif, Sustainable livestock production, FARAH, University of Liège, Liège, Belgium

2. Bactériologie, Veterinary public health naire, FARAH, University of Liège, Liège, Belgium

Corresponding author: jdetilleux@uliege.be

The equine sector has increased during the last decades and currently about 3 million hobby and sports horses reside in the EU member states. This increase is associated with large amount of horse manure whose components may pollute field and streams. Black soldier fly larvae (BSFL) have the potential to reduce the organic matter and the load of pathogenic bacteria of chicken, pig and cattle manure. However, their effect on horse manure is still unknown. Here, BSFL initially provided by INAGRO® were raised on horse feces in a ratio of 1 to 3, 3 to 5 and >5 BSFL per gr of feces. All bins were placed in the same environment for a period of one month (3 replications). At the end, a sample of the feces was suspended in physiological water, plated on different selective agar plates and incubated overnight at 37°C in aerobic atmosphere. Numbers of colonies of *Escherichia coli*, coliforms, *Salmonella enterica*, mannitol-positive and mannitol-negative staphylococci, *Streptococcus* sp., *Enterococcus* sp. and *Bacillus subtilis* were recorded. A zero-inflated Poisson regression was used to estimate whether bacterial numbers were different according to initial load, ratio BSFL:feces, replication and bacterial species. Horse manure was found to be easily processed by the larvae. Differences across replications and initial loads were not significantly different from null. Numbers of *E. coli* and coliforms were significantly lower and mannitol-negative staphylococci could not be recovered in bins with more than 3 BSFL per gr of feces than without BSFL. These findings confirm the antibacterial activity of BSFL and their potential role in waste management.

20. Effect of Black Soldier Fly larvae on horse fecal emission of methane

Detilleux J., Dawans E., Cabaraux J.F.

Department of Veterinary Management of Animal Resources, FARAH Centre, Faculty of Veterinary Medicine, University of Liege, Belgium

Corresponding author: jdettleux@uliege.be

Decomposition of manure is associated with the emission of greenhouse gases (GHG), primarily in the form of carbon dioxide, methane (CH₄) and nitrous oxide. During their growth on manure, Black Soldier Fly Larvae (BSFL) may have the potential to reduce CH₄ emission because they can recycle large amounts of carbon into insect proteins and oils. This study evaluated whether the presence of BSFL in horse feces modifies direct emission of CH₄. To do so, feces sampled from one particular horse were incubated in three opened dynamic chambers. One chamber contained 500 gr of feces and the two others contained 500 gr of feces with 100 BSFL of mixed ages. Hourly CH₄ emissions were measured with a Guardian® gas analyzer during 17 consecutive days. The experiment was replicated twice. The mean amount of CH₄ measured in the chamber containing only feces was not significantly different from that measured in the ambient air. Inversely, the mean quantity measured in chambers with BSFL was higher, between 13 and 27 ppm by volume, than the quantity measured in the chamber containing only feces. These results suggest total direct CH₄ emissions from horse feces are affected by the presence of BSFL. In a review on CH₄ production in pig manure, it was shown that lack of oxygen, high temperature, high level of degradable organic matter, high moisture content, low redox potential, neutral pH and C/N ratio between 15 and 30 promote CH₄ production. Further analyses are currently undergoing to determine whether the presence or absence of BSFL has modified the physico-chemical nature of our horse feces and consequently the quantity of CH₄ emissions.

21. Chemical composition of some agricultural by-products in Northern Morocco

El Otmani S.¹, Chebli Y.¹, Chentouf M.¹, Hornick J.L.² and Cabaraux J.F.²

1. Regional Center of Agricultural Research - Tangier, National Institute of Agricultural Research, Avenue Ennasr, BP 415 Rabat Principale, 10090 Rabat, Morocco

2. Department of Veterinary Management of Animal Resources, FARAH, IVT, Faculty of Veterinary Medicine, University of Liège

Corresponding author: samira.elotmani@inra.ma

In Northern Morocco, goat feeding is based on forest rangelands characterized by seasonal variability and low availability. The substitution of conventional resources by agricultural by-products widely available could be used in ruminant diet. However, it is necessary to know their nutritional quality. This work aims to evaluate the composition of some agricultural by-products mostly available in the study area. Three samples of red fruits (strawberry and raspberry) and legumes (faba bean, chickpea, and bean) by-products were collected. The studied parameters were dry matter, ash, fibers (NDF, ADF, and ADL), and secondary compounds (total phenols, total tannins, and condensed tannins). The results showed that all parameters significantly differed according to the studied by-products ($P < 0.01$). Strawberry residues had the higher moisture content compared to the other by-products that were similar. Ash was lower in chickpea residues and higher in raspberry and bean by-products. The lower content of fibers (NDF, ADF, and ADL) was found in raspberry residues. Strawberry residues had the highest concentration of the three secondary compounds, while the lowest contents were observed in faba bean by-products. In conclusion, the use of agricultural by-products seems to be a solution to diversify and reduce feeding costs, and other nutritional parameters should be determined.

Posters

22. Thyroid ultrasound as a new tool to assess thyroid metabolism in cattle: preliminary results

J. Eppe^{1*}, E. Raguét¹, P. Petrossians², P. Bossaert¹, F. Rollin¹, H. Guyot¹

1. Clinical department of ruminants, FARAH, ULiège, Quartier Vallée 2, Avenue de Cureghem 7A-7D, Liège, 4000, Belgium

2. Department of Endocrinology, CHU of Liege, ULiège, Chemin de l'Hôpital 1, Liège, 4000, Belgium

Corresponding author: justine.eppe@uliege.be

Thyroid troubles are poorly documented in cattle. Ultrasonography is a tool used in other species to evaluate thyroid pathologies. The objective of this work is to describe the normal ultrasonographic appearance of the thyroid gland in cattle and to validate the reliability of its measurements.

In this study, 11 calves and 12 adult cows were enrolled at the University Veterinary Clinic of Liege. A thyroid ultrasonography was realized on each animal using a linear (calves) or convex (cow) probe on two views (transverse and longitudinal), measuring the length (l), width (w) and height (h) of each lobe and the height of the isthmus. Finally, the animals were euthanized and their thyroids were removed for weighing, measuring and histological examination. Volume was measured by liquid displacement, and calculated using two formulas: $\pi/6 * l * w * h$; $\pi/4 * l * w * h$.

The appearance of the thyroid was iso- or hyperechoic to the muscles and homogeneous. The capsule also appeared hyperechoic. Then, although the thyroid measurements are mostly significantly different ($p > 0.05$) from those in *post-mortem*, the volume measured did not differ significantly ($p > 0.1$) from the volume calculated. Ultrasound did not detect histological abnormalities such as follicular hypo- or hyperplasia. In one cow, a follicular cyst 0.4 cm in diameter was observed.

Further studies are needed to validate the ultrasound examination on a larger number of non-debilitated animals. Intra or inter-observer variability could also be measured. Additional work is needed to address other aspects of thyroid metabolism, such as the use of ultrasound Doppler and hormonal blood tests.

23. Tree and shrub fodders in sheep nutrition: forage preference for some species in Benin (West Africa)

Gnikplékpo E.L.R.^{1,3}, Accalogoun J.¹, Koura B.I.², Houinato M.R.B.¹, Hornick J-L.³, Cabaraux J-F.³

1. School of Sciences and Technics of Animal production, Faculty of Agronomic Sciences, University of Abomey-Calavi, Abomey-Calavi, Benin

2. School of Management and Exploitation of Livestock Systems, National University of Agriculture, Ketou, Benin

3. Department of Veterinary Management of Animal Resources, FARAH Centre, Faculty of Veterinary Medicine, University of Liege, Belgium

Corresponding author: Euvrard.Gninkplekpo@uliege.be

In tropical arid and semi-arid areas, tree and shrub fodders (TSF) largely contribute to sheep nutrition. Despite the important nutritional characteristics of TSF, the preference of most of these plants by small ruminants in these regions is still little known and could be helpful to develop strategies for the effective TSF utilization. Therefore, our study aimed to assess the forage preference of sheep for six tropical woody plants, namely *Annona senegalensis* (AS), *Blighia sapida* (BS), *Cassia siebebiana* (CS), *Mangifera indica* (MI), *Khaya senegalensis* (KS), and *Gmelina arborea* (GA). The trial was implemented at the Agricultural Research Centre (ARC-Ina, Benin) on eight adult Djallonke sheep (four rams and four ewes) of average live weight 18.22 ± 0.78 kg. The eight ones were housed in individual wooden pens and received Panicum hay and corn bran daily ration. During the cafeteria trial, sheep were offered 50 g dry matter (g DM) of each species in the morning for 30 min. For each plant, the ingested quantities, the consumption time, and the visit number were recorded. A GLM procedure with species and sex as fixed effects and their interaction was used for the statistical analyses. Significant differences ($p < 0.05$) were observed for both effects. The MI was the most ingested plant (47.8 ± 0.3 g DM; 46.7 ± 2.1 g DM), followed by BS (46.5 ± 3.4 g DM; 39.7 ± 10.5 g DM), GA (39.4 ± 3.6 g DM; 32.2 ± 6.4 g DM), KS (37.7 ± 5.5 g DM; 30.6 ± 6.1 g DM), AS (31.6 ± 8.1 g DM; 31.5 ± 4.9 g DM), and CS (21.9 ± 4.0 g DM; 20.2 ± 4.8 g DM), in males and females, respectively. This study is the first step in searching for efficient management of locally available TSF plants for sheep.

24. Characterisation and diversity of small ruminant herding systems in Benin (West Africa)

Zanou M.A.M.¹, Aoudji A.K.N.¹, Dossa L.H.², Antoine-Moussiaux M.³, Houinato M.R.B.²

1. *School of Rural Economics and Sociology, Faculty of Agronomic Sciences, University of Abomey-Calavi, Abomey-Calavi, Benin*

2. *School of Sciences and Technics of Animal production, Faculty of Agronomic Sciences, University of Abomey-Calavi, Abomey-Calavi, Benin*

3. *Department of Veterinary Management of Animal Resources, FARAH Centre, Faculty of Veterinary Medicine, University of Liege, Belgium*

Corresponding author: zanou.murielle@gmail.com

Bred in heterogeneous systems in many developing countries, including Benin, small ruminants (sheep and goat) are important for economies of these countries. Capturing the heterogeneity of these systems is a useful first step in analysing farm performance and rural livelihoods. In order to characterise small ruminant herding systems in Benin, a survey has been conducted with 802 farmers selected among five of eight agro-ecological zones of Benin. Four systems (S1, S2, S3 and S4) have been identified and differed in terms of animal genetic, breeding practices, feeding management, number of flock heads, labor used. Djallonke breed was predominant in S1, S2 and S3, while Sahelian breed dominated S4 (84.54%). Crossbreed of Djallonke and Sahelian breeds was present in the flocks of 16.52% of S3 farmers. The majority of breeders in the four systems used semi-claustration, but in S3, transhumance and rambling were observed (21.74%). Family labor was most used for breeding animals. However, in S3 and S4, respectively, 26.09% and 38.14% of herders also used occasional labor. Phytotherapy was most used in S4. Farmers of S3 were more active in breeding and 74.78% had more than ten heads of sheep or goat. They also bred many other animal species such as cattle, poultry, etc. Nearly half of S2 breeders left their flock freely grazing without supplementation, while animals were supplemented with woody fodder in S1, by-products of food processing in S3 and crop residues in S4. Constraints to small ruminant breeding varied according to the herding system. Limited access to credit was a major problem in S1 and S4, while social problems and animal theft strongly influenced breeding in S2 and S3.

Comparative veterinary medicine

25. Removal of two vaginal varicose veins by diode laser surgery in a 2-year-old female intact Bleu de Ham rabbit

Flore Brutinel 1*, Sophie Egyptien 1, Natacha Thilliez 2, Jérôme Abadie 2, Didier Marlier 1 and Stefan Deleuze 1

1. Department of Clinical Sciences, Companion Animals, Faculty of Veterinary Medicine, Fundamental and Applied Research for Animals & Health (FARAH), University of Liège, 4000 Liège, Belgium

2. Department of Biology, Pathology and Food Sciences, Oniris, Laboniris, 44300 Nantes, France.

Genital vascular abnormalities described in pet rabbits are almost exclusively endometrial venous aneurysms. This is the first detailed report of a vaginal localization of varicose veins in a doe and their treatment by laser surgery. A 2-year-old doe was presented with a vaginal mass protruding from the vulva associated with vaginitis that prevented reproduction. Six months after mass removal, she was sterilised and no sign of recurrence was observed. The animal was euthanized 6 months later because of recurrence. At histological examination, both the primary and the recurring lesions were diagnosed as voluminous varicose veins. Immunohistochemistry failed to identify progesterone receptors on the lesions. Recurrence after ovariohysterectomy and the absence of progesterone receptors in this case suggest that sex hormones are not involved in the etiopathogenesis of vaginal varicose veins in rabbits while they are strongly suspected in vulvar varicose veins in pregnant women. Although largely recommended in mares with similar lesions to limit bleeding during surgery, use of surgical diode laser has not yet been reported in does with vascular disorders.

In a recent study, the uterus of 1,928 pet rabbits that underwent ovariohysterectomy because of potential uterine disease or for disease-prevention purposes were submitted for histopathology. Endometrial venous aneurysms (VA) were identified on 3.8% of the rabbits with uterine lesions[2]. They are likely to be found in rabbits between 2 to 3 years. In this report, clinical signs, surgical removal and the histopathological findings of two vaginal varicose veins in a production rabbit are described. The resemblance of these lesions to those previously described in the endometrium is discussed, as well as a possible hormonal etiology.

26. Optimization of the Amplification of Equine Muscle-Derived Mesenchymal Stem Cells in a Bioreactor

Duysens J.^{1,2}, Graide H.^{1,2}, Franck T.¹, Niesten A.¹, Ceusters J.^{1,2}, Serteyn D.^{1,2}

1. Centre de l'Oxygène, Recherche et Développement (CORD), FARAH, University of Liège, Belgium

2. RevaTis SA, Rue de la Science, 8, 6900 Aye, Belgium

Adult mesenchymal stem cells play a crucial role in the homeostasis, renewal and repair of damaged tissue. The muscle derived Mesenchymal Stem Cells (mdMSCs) demonstrate potent anti-inflammatory and immunomodulatory effects similar to those derived from bone marrow. Systemic administration by intravenous injection appears to be safe, effective and is applied in several ongoing clinical trials in human medicine at an average dose of 1 to 2 million / kg body weight. If similar doses are needed in equine pathology, we will be forced to produce batches of over 500 million autologous MSCs.

Therefore, to reach this amount of muscle-derived mesenchymal stem cells (mdMSCs), an automated cell culture bioreactor (Quantum, Terumo BCT) composed of polyether sulfone hollow fibers was used. This feature greatly increases the area (2.1 m²) for cell multiplication, in comparison with classical T-flask (0.0175m²).

After different assays, the maximum expansion generated 220 million cells, it is more than twenty times than the initial seeding in nine days. The best returns are observed with the Bull's eye method and an optimal seeding between 10 to 30 million of stem cells. Moreover, harvested cells from the bioreactor keep their properties: fibroblastic shape, adherence to treated culture flask, trilineage differentiation ability (chondrocytes, osteocytes and adipocytes) and expression of CD-44 and CD-90 with a positive rate above 93% while CD-45 and MHCII are expressed with a positive rate below 0.5%. Finally, to reach our goal of 600 million cells, some parameters must be optimized such as the composition of the medium and the number of passages in this device.

Posters

27. Use of Computed Tomographic angiography for pulmonary hypertension assessment in a cohort of West Highland white terrier with or without canine idiopathic pulmonary fibrosis

Eugénie Soliveres^{1*}, Kathleen Mc Entee², Thierry Couvreur³, Aline Fastrès¹, Elodie Roels¹, Anne-Christine Merveille¹, Alexandru-Cosmin Tutunaru¹, Cécile Clercx¹, Géraldine Bolen¹

1. Department of Clinical Sciences, Companion Animals, Faculty of Veterinary Medicine, Fundamental and Applied Research for Animals & Health (FARAH), University of Liège, 4000 Liège, Belgium

2. Laboratory of Physiology and Pharmacology, Faculty of Medicine, Université Libre de Bruxelles, 1070 Brussels, Belgium

3. Department of Radiology, Christian Hospital Center Liège, Belgium

Corresponding author: esoliveres@uliege.be

Pre-capillary pulmonary hypertension (PH) is a comorbidity commonly reported in West Highland white terriers (WHWTs) affected with canine idiopathic pulmonary fibrosis (CIPF). Thoracic Computed Tomography (CT) is used in human patients with lower airways diseases and helps to identify comorbidities that could increase mortality rate such as PH. Indeed, diameter of the pulmonary trunk (PT), pulmonary trunk-to-aorta ratio (PT/Ao) and right ventricle-to-left ventricle ratio (RV/LV) are CT Angiography (CTA) parameters commonly used to assess the presence of PH. Pulmonary vein-to-right pulmonary artery ratio (PV/PA) is a new echocardiographic parameter used to diagnose PH in dogs.

The aims of this study were to evaluate the use of different CTA parameters to diagnose PH and the correlation of RV/LV ratio measurements between different CTA planes.

47 WHWT were prospectively enrolled, 22 affected with CIPF and 25 presumed healthy control dogs. CTA and echocardiography were performed and dogs were considered to have PH if PV/PA measured on echocardiography was < 0.7 .

WHWTs affected with CIPF had higher PT/Ao compared to control group. In WHWTs affected with CIPF, PT was larger in dogs with PH (15.38 mm) compared to dogs without PH (12.98 mm, $p = 0.003$). A cut-off value of 13.85 mm predicted PH in WHWTs affected with CIPF with a sensitivity of 90% and a specificity of 87% (AUC = 0.93). High correlations were observed between the different CTA planes of RV/LV.

The diameter of the PT measured by CTA may be used to assess PH in WHWTs with CIPF.

28. Thoracic ultrasonography for assessment of pulmonary fibrosis in West Highland white terriers with or without canine idiopathic pulmonary fibrosis

Eugénie Soliveres^{1*}, [Emilie Pierrot](mailto:emilie.pierrot@ulb.be)¹, Aline Fastrès¹, Elodie Roels¹, Cécile Clercx¹, Géraldine Bolen¹

1. Department of Clinical Sciences, Companion Animals, Faculty of Veterinary Medicine, Fundamental and Applied Research for Animals & Health (FARAH), University of Liège, 4000 Liège, Belgium

Corresponding author: esoliveres@uliege.be

Canine idiopathic pulmonary fibrosis (CIPF) is a pulmonary pathology reported in West Highland white terrier (WHWT). Thoracic computed tomography (CT) is commonly used to help in the diagnosis of CIPF. However, in dyspneic patients, sedation or anesthesia are not recommended and CT may not be available. B-mode ultrasonography (US) is used in human medicine to diagnose interstitial lung disease and especially idiopathic pulmonary fibrosis. In veterinary medicine, thoracic B-mode US has been described to be helpful in the diagnosis of various pulmonary diseases, including pulmonary cardiogenic oedema or aspiration pneumonia.

The aim of this study was to evaluate the usefulness of B-mode US in the diagnosis of CIPF.

Twenty-seven WHWT were prospectively enrolled, 16 affected with CIPF and 11 presumed healthy control dogs. Thoracic B-mode point-of-care US was performed. Presence of irregular pleural surface, ring down artifact and other lesions such as peripheral nodules were assessed and scored for each location. An overall cumulative score was calculated by adding the individual scores together (0 to 56).

WHWTs affected with CIPF (median=18) had statistically higher overall score compared to control group (median=10) ($p=0.023$). In CIPF group, the higher scored lesions were ring down artefact (median = 9) followed by irregular pleural surface (median = 6.5). Score for ring down artefact was statistically higher in CIPF group (median = 9) compared to control group (median = 4) ($p=0.024$). No preferential location for the lesions was observed.

B-mode US could be a useful tool to differentiate WHWT with or without CIPF.

29. Comparison Between Ultrasonographic and Standing Magnetic Resonance Imaging Findings in the Podotrochlear Apparatus of Horses With Foot Pain

Evrard L.¹, Joostens Z.², Vandersmissen M.¹, Audigié F.³, Busoni V.¹

1. Department of clinical sciences of companion animals and equids, Equine division, Diagnostic imaging section, FARAH, ULiège, Liège, Belgium

2. Medical imaging department, Equitom Equine clinic, Lummen, Belgium

3. Centre d'Imagerie et de Recherche sur les Affections Locomotrices Equines, Ecole nationale vétérinaire d'Alfort, Goustranville, France

Corresponding author: levrard@uliege.be

This prospective study aimed to blindly compare the ultrasonographic and standing magnetic resonance imaging (sMRI) findings in deep digital flexor tendon (DDFT), navicular bone, and navicular bursa in horses with foot pain, positive digital analgesia, and without definitive radiographic diagnosis. Ultrasonography detected more DDFT abnormalities (32/34 feet vs. 27/34 with sMRI) but identified less palmar navicular abnormalities (23/34 feet vs. 30/34 with sMRI). In suprasesamoidean DDFT lesions, which were mainly dorsally located, changes in echogenicity did not correspond to a particular pattern of sMRI signal change. Transcuneal ultrasonography did not allow assessment of morphology and extent of distal DDFT lesions, and sporadically discriminated the affected lobe compared to sMRI. Defects of the palmar compact bone were identified with both modalities except a parasagittal defect, which was only seen at sMRI.

30. A retrospective survey to assess owners' opinion on the impact of weight loss on quality of life in obese dogs.

Jergeay V.¹, Vandenneede M.¹, Jeusette I.², Diez M.¹

1. Department of Veterinary Management of Animal Resources, FARAH, ULiège, Belgium,

2. Affinity-Petcare, Barcelona, Spain.

Corresponding author: vjergeay@uliege.be

Quality of life (QoL) and its assessment has become a central topic in veterinary practice: obesity seems to decrease QoL, but weight loss (WL) has been reported to improve it. The aim of this study was to evaluate the impact of obesity and WL on QoL in dogs, after a 6 months WL program. This WL was performed on 28 privately-owned obese dogs (details previously presented). Afterwards, a survey was sent to the owners of the 23 dogs having completed the protocol. The survey assessed the owners' perception of the impact of being overweight, and also changes observed since WL. This survey was based on Likert scales, scores given by the owners, and scales filled by the owners. A Wilcoxon signed rank test with continuity correction and a Spearman correlation were performed on the data. Among the 15 responses received, respectively 8%, 33%, 80% and 92% of the owners found that obesity had a bad impact on their dog's mood, sleep, walking comfort and activity. In the same way, 13%, 27%, 60%, and 67% of the owners noticed an evolution in their dog's sleep, mood, walking comfort, and activity, after WL. Also 53% estimated that the dog was less breathless at the end of WL. Finally, the score given by the owners, for their dog's joy of life, and general health increased by 4% ($p=0.4$), and 15% ($p=0.05$) between before and after WL, but no correlation was found between these results and the WL percentage. Nevertheless, owners declared to be very happy to have made their dog lose weight (mean contentment score: 9.9/10). They considered their dog was healthier, especially regarding its motion capability. However, interpretation in terms of dog's QoL should need further research.

Posters

31. Exploring equine atypical myopathy through *in vitro* toxicity tests

Kruse C-J.¹, Renaud B.¹, François A-C.¹, Art T.¹, Mouithys-Mickalad A.², Niesten A.², Lemieux H.³, Votion D-M.⁴

1. Dept of functional sciences, Faculty of veterinary medicine, University of Liège (ULiège), Belgium 2. Center for Oxygen Research and Development (CORD), Faculty of sciences, University of Liège (ULiège), Belgium 3. Faculty Saint-Jean and Department of Medicine, University of Alberta, 8406-91 Street, Edmonton, AB T6C 4G9, Canada 4. Fundamental and Applied Research for Animals and Health (FARAH), Faculty of veterinary medicine, University of Liège (ULiège), Belgium

Corresponding author: caroline.kruse@uliege.be

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 fatty acid β -oxidation cycle. This inhibition results in a general decrease in mitochondrial respiration, as determined by high-resolution respirometry (HRR) previously applied to muscle samples taken from cases of AM. The severe impairment of mitochondrial bioenergetics may explain the high rate of mortality observed: about 74% of horses with AM die, most within the first two days of signs of poisoning. To this day, the mechanism of toxicity is not completely elucidated.

With the purpose of improving our understanding of the pathological process and to assess therapeutic candidates, we performed *in vitro* assays using equine skeletal myoblasts cultured from muscle biopsies and subjected them to toxins involved in AM. Through HRR, toxicity and viability assays, we have established that equine skeletal myoblasts do respond to the toxic metabolite MCPA. Indeed, a severe depression in mitochondrial respiration as well as cytotoxic responses were recorded after MCPA addition, rendering cell culture and *in vitro* assays interesting perspectives for further pathophysiological explorations of the condition.

32. The middle ear microbiota in healthy dogs is similar to that of the external ear canal

Léonard C.¹, Fontaine J.¹, Picavet P.¹, Taminiau B.², Daube G.², Claeys S.¹

1. Department for Clinical Sciences B67, Faculty of Veterinary Medicine, University of Liège, 4000 Liège, Belgium
2. Fundamental and Applied Research for Animal & Health (FARAH), Food Science Department, Faculty of Veterinary Medicine B43b, University of Liège, 4000 Liège, Belgium

Corresponding author: Caroline.leonard@uliege.be

Background - Otitis media can be a consequence of chronic otitis externa and could represent a perpetuating factor. Sparse information is available concerning the normal microbiota of middle ear. Aim - To compare the tympanic bulla (TB) microbiota with the external ear canal (EEC) microbiota in healthy dogs.

Material and methods - Six healthy experimental Beagle dogs were selected based on the absence of otitis externa, negative cytology and bacterial aerobic culture from the TB. Samples from the EEC and TB were collected directly after death using a total ear canal ablation and lateral bulla osteotomy. The hypervariable segment V1-V3 of the 16S rDNA was amplified and sequenced with a MiSeq Illumina sequence carried out by the Mothur software using the SILVA database. EEC DNA extracts of healthy client owned dogs from the study of Ngo *et al.* was used for the comparison.

Results - In Beagles, a significant difference ($P = 0.009$) for Chao1 richness index between the right and left EEC, but no significant differences between EEC and TB microbiota for Chao1 richness index ($P = 0.6544$), Simpson evenness index ($P = 0.4328$) and the reciprocal Simpson alpha diversity ($P = 0.4313$) was noted (Kruskal-Wallis test). A significant clustering ($P < 0,0001$) was observed between EEC of Beagles and client owned dogs (AMOVA).

Conclusions - The microbiota profile is similar in the EEC and TB in Beagles. The microbiota of the EEC of Beagles differs significantly from the one of healthy client owned dogs, suggesting involvement of environmental factors.

33. B-mode ultrasonographic abnormalities and power Doppler signal in suspensory ligament branches of non-lame working Quarter Horses

Rabba S.^{1,2}, Petrucci V.³, Petrizzi L.⁴, Giommi D.W.⁵, Busoni V.¹

1. Faculty of Veterinary Medicine, Department of Clinical Sciences of Companion Animals and Equids, Diagnostic Imaging Section, ULiège, Liège, Belgium 2. Istituto Veterinario di Novara, Department of Diagnostic Imaging, Granozzo con Monticello (NO), Italy 3. Equine practitioner, Frosinone, Italy 4. University of Teramo, Veterinary Teaching Hospital, Teramo, Italy 5. Clinica Veterinaria Maggiore, Maggiore (NO), Italy

Corresponding author: silviarabba@virgilio.it

Subclinical ultrasonographic B-mode abnormalities of the equine suspensory ligament (SL) branches have been reported and, in these cases, power Doppler (PD) may potentially help to distinguish non-active from active SL lesions, as suggested by the human medicine.

This study aimed to investigate the prevalence of subclinical ultrasonographic B-mode abnormalities and presence of PD signal in SL branches in a group of non-lame working Quarter Horses and to compare B-mode findings with PD findings in Quarter Horses in two different trainings (barrel racing and team roping).

A series of Quarter Horses in full training underwent a routine ultrasonographic examination (B-mode and PD examinations) of their SL branches (forelimbs and hindlimbs) for screening purpose. Two groups of these horses were used: a group of barrel racing horses (group B) and a group of team roping horses (group R). Inclusion criteria were absence of lameness, no evidence of a clinical SL branch injury and no known history of SL branch injury.

Twenty-one Quarter Horses, 14 barrel racing (group B) and 7 team roping horses (group R), were included in the study. B-mode lesions were seen more frequently in forelimbs than in hindlimbs. PD signal was not detected in all SL branches that were abnormal in B-mode. Horses of group R showed more B-mode abnormalities and more PD signal in branches abnormal in B-mode.

A combination of B-mode and PD ultrasonographic abnormalities can be found in normally working Quarter Horses. Results suggests that discipline may influence prevalence of subclinical ultrasonographic abnormalities.

34. Presence of neutrophil myeloperoxidase in lamellar tissue of horses with laminitis induced using a hyperinsulinemic model

N. Storms¹, C.E. Medina Torres², T. Franck³, A. Sole Guitart⁴, G. de la Rebière¹, D. Serteyn^{1,3}

¹ Department of Equids, Faculty of veterinary medicine, FARA, ULiège.

² Pferdeklinik Leichlingen GmbH, Leichlingen, Germany.

³ Center for Oxygen Research and Development, FARA, ULiège, Belgium.

⁴ School of Veterinary Science, The University of Queensland, Gatton, Australia.

Corresponding author: nazare.storms@uliege.be

Laminitis is a pathology of the equine digit resulting in failure of the dermo-epidermal interface. Inflammation is likely a central player in its pathophysiology. Neutrophil activation and the presence of myeloperoxidase have been observed in the black walnut heartwood extract model, used to investigate sepsis-related laminitis. However, less is known about the role of neutrophil activation in the prolonged euglycemic hyperinsulinemic clamp (pEHC) model. This model is used to investigate the pathophysiology of laminitis related to endocrine disturbances like hyperinsulinemia encountered in pituitary pars intermedia dysfunction and equine metabolic syndrome.

Five horses were included in this study and randomly assigned to a control group (n = 2) and a pEHC group (n = 3). Histological sections of lamellar tissue from all horses were obtained and immunohistochemically stained for myeloperoxidase and counterstained with hematoxylin-eosin. The sections were examined for histopathological evidence of laminitis and myeloperoxidase.

Histopathological changes that characterize insulin-induced laminitis and increased presence of myeloperoxidase, especially in the dermal lamellae, were observed in histologic sections of pEHC-treated horses compared to control horses.

Neutrophil activation with myeloperoxidase release may play a role in the pathophysiology of endocrinopathic laminitis. The implications of this finding warrant further investigation.

Posters

35. Does skill lab training for intravenous cannulation improve performance and satisfaction in veterinary students?

Alexandru Tutunaru^{1*}, Johann Detilleux², France Beaufays¹, Stefan Deleuze¹, Charlotte Sandersen¹

1. Clinical Dept of Companion Animals, FARAHA comparative vet. medicine, Fac. of Vet. Medicine, ULiège, Liège, Belgium 2. Dept of Animal Production, FARAHA public vet. health, Fac. of Vet. Medicine, ULiège, Liège, Belgium

Corresponding author: charlotte.sandersen@uliege.be

Background : The present study aims to demonstrate an improvement of intravenous cannulation skills after skill lab implementation for final year veterinary students.

Materials and methods

Previous experience, confidence to place an intravenous (iv) cannula and the success rate of iv cannula placement were evaluated in final-year veterinary students by using a questionnaire. First, the students enrolled in the study filled a questionnaire containing questions to determine previous experience and their personal opinion on the usefulness of mannequin experience, theoretical courses and their confidence to perform the cannulation successfully. Next, all students practiced iv cannulation during clinical rotation and their performance was recorded by auto-evaluation. Results were analysed by Chi-square tests where appropriate.

Results

Sixty-one students enrolled in the "non-experienced" group (NonExp), 38 students enrolled in the "clinical experience" (ClinExp) group, 35 students enrolled in the "skill lab group" (SkillLab), and 12 students in the group with experience in both, clinical and skill lab mannequin (ClinSkil). Previous experience was significantly different among groups with 77 % of the NonExp students have placed less than 5 catheters in dogs and 74% of the ClinExp group have placed more than 5 catheters before starting their clinical rotation. Students of all groups felt equally confident to place an iv cannula successfully. In the SkillLab group, 43 % of the students felt that mannequin training helped them placing an iv cannula, while 34% thought that mannequin experience did not help them and 23% had no opinion on this question. Success rate of iv cannula placement during clinical rotation was not significantly different among groups but tended to be higher in students that felt more confident.

Conclusion

The preliminary data presented here fails to demonstrate a difference between skill lab and clinical experience in iv cannula placement in final year veterinary students.

36. Plasma nucleosomes concentration in endurance horses: a new marker of inflammation induced by strenuous exercise?

Van Cauter R.^{1,2}, Herzog M.³, Caudron I.¹, Lejeune J-PH.^{1,2}, Franck T.^{1,4}, Michel G.³, Serteyn D.^{1,2,3}

1. Centre Européen du Cheval, Mont-le-Soie, Vielsalm

2. Département des sciences cliniques des équidés, Chirurgie et Orthopédie, FARAHA, Université de Liège

3. Volition, CA, USA

4. Centre de l'Oxygène, R&D (CORD), FARAHA, Université de Liège

Nucleosomes are made up of DNA wrapped around a core of histone octamer so that DNA can be condensed as chromatin into chromosomes. Diseases such as cancer or inflammation lead to cell death where chromatin is fragmented and released as mononucleosomes in the blood.

Additionally, activated neutrophils release extracellular neutrophil traps (NETs): a network of extracellular fibers made up of DNA, histones, and enzymes like elastase and myeloperoxidase (MPO). Recent work has shown that the concentration of H3.1 nucleosomes in the blood is a good marker for the measurement of NETs release in the bloodstream. The Nu.Q™ H3.1 test is an ELISA (enzyme-linked immunosorbent assay) directed on the central histone protein histone H3.1.

Our previous studies have shown in endurance horses that a severe inflammatory reaction occurs after 120 and 160 km and is related to a significantly higher plasma concentration of MPO.

The objectives of this study were to determine the changes in the plasma concentration of nucleosomes (H3.1) before and after endurance competition and their correlation with the number of red blood cells, the number of white blood cells, the level of total proteins, granulocyte levels and creatine kinase (CK) concentration.

Samples were taken from 25 endurance horses before and one hour after competition in two different races ranging from 70 km to 160 km.

We observed a significant increase in H3.1 nucleosomes and plasma CK concentration after running and a significant correlation between these two parameters.

These preliminary results highlight for the first time the possible role of the release of NETs during intense exercise in endurance horses.

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