LIÈGE université
Médecine Vétérinaire

LIÈGE
université

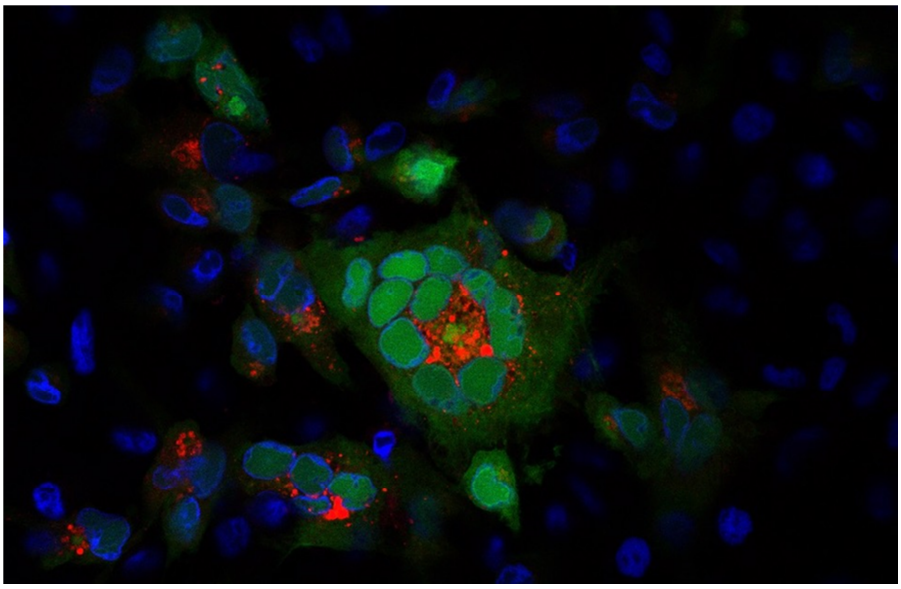
FARAH

# Proceedings of the 5th FARAH-Day

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

**October 26, 2018**

*One Health*
L'Animal et l'Homme, une même santé



# Proceedings of the 5th FARAH-Day

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

**October 26, 2018**

**Edited by** C. Bayrou, J.-F. Cabaraux, C. Delguste, C. Douny, C. Gatez, T. Jauniaux, L. Ludwig, J. Ponthier, A. Sartelet, D. Thiry, D.-M. Votion

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

**COVER PICTURE CREDITS:**

**Y. Gao**, Immunology-Vaccinology (B43b), Department of Infectious and Parasitic Diseases, FARAH

CyHV-3 syncytial viral plaque

## *Welcome to the 5th 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 80 abstracts dedicated to fundamental, clinical and or applied researches.

**Laurent Gillet**, President of the FARAH

**Frédéric Farnir**, Vice-president

**Dominique Votion**, Secretary

## *Bienvenue à la 5ème 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 centaine de travaux ayant trait à la recherche fondamentale, clinique et/ ou appliquée.

**Laurent Gilet**, Président du FARAH

**Frédéric Farnir**, Vice-président

**Dominique Votion**, Secrétaire

# Organisers of the meeting

## ORGANISING COMMITTEE

**Calixte Bayrou**
*Dpt of Morphology and Pathology*

**Jean-François Cabaraux**
*Dpt of Animal Production*

**Catherine Delguste**
*General Services*

**Caroline Douny**
*Dpt of Food Sciences*

**Carine Gatez**
*Dpt of Functional Sciences*

**Thierry Jauniaux**
*Dpt of Morphology and Pathology*

**Louisa Ludwig**
*Dpt of Infectious and Parasitic diseases*

**Jérôme Ponthier**
*Clinical Dpt of Companion Animals and Equids*

**Arnaud Sartelet**
*Dpt of Animal Production*

**Damien Thiry**
*Dpt of Infectious and Parasitic diseases*

**Dominique-M. Votion**
*Clinical Dpt of Companion Animals and Equids*

## SCIENTIFIC COMMITTEE

President

**Catherine Delguste**
*General Services*

Members

**Jean-François Cabaraux**
*Dpt of Animal Production*

**Caroline Douny**
*Dpt of Food Sciences*

**Thierry Jauniaux**
*Dpt of Morphology and Pathology*

**Jérôme Ponthier**
*Clinical Dpt of Companion Animals and Equids*

**Arnaud Sartelet**
*Dpt of Animal Production*

**Damien Thiry**
*Dpt of Infectious and Parasitic diseases*

**Dominique-M. Votion**
*Clinical Dpt of Companion Animals and Equids*

## TECHNICAL SUPPORT

**Véronique Defize**
**Jean-Michel Dusoulier**
**Nathalie Guillaume**
**Marie-Eve Lechanteur**
**Dino Marinutti**
**Michel Motkin**
**Frédéric Paquer**
**Anne Wlasowski**

LIÈGE université
Médecine Vétérinaire

LIÈGE université


Val d'Hony Verdifarm
A Henry Schein Company


VWR
We Enable Science

MSD
Animal Health

zoetis

eppendorf

# Program

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

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

Prof GILLET Laurent, President of FARAH

**09:15 Invited speaker**

**Winner of the "Etienne Baise award"**

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

**Chairmen: *Stéphanie CLAEYS and Frédéric FARNIR***

*09:45* ***GOFFIN E.***, *Characterization of oral murine adenovirus type 1 infection in mouse and evaluation of the protection induced against a respiratory homologous infection*

*09:55* ***WIMMER-SCHERR C.****, Atypical Myopathy: is there a relationship between serum hypoglycin A and MCPA-Carnitin levels and the composition of faecal microbiota?*

*10:05* ***FOURRIER M.***, *The carp olfactory system: a highway to the brain for Cyprinid herpesvirus 3*

*10:15* ***DIALLO M****., The Zalpha domain containing protein ORF112 encoded by Cyprinid herpesvirus 3: Is its Zalpha domain the only important part?*

*10:25 Questions*

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

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

**Chairmen: *Annick HAMAIDE and Philippe BOSSAERT***

*11:15* ***GAO Y****., Development of Cyprinid herpesvirus 3 as a recombinant vector vaccine*

*11:25* ***DAO MINH H****., Striped Catfish (Pangasianodon hypophthalmus) genetic selection under saline conditions in the Mekong Delta*

*11:35 Questions*

11:45 Short talks

***Borelli E****., Albumin, Urea and Cholesterol: 3 haematological parameters as markers of nutritional status and prognosis in hospitalised calf.*

***Delrez N****., Infection of European eel by Anguillid herpesvirus 1: from basic research to conservation programs*

***Doan T.Q.****, A realistic mixture of Persistent Organic Pollutants (POPs) reveals possible synergism to inhibit the transactivation activities of the rat Aryl hydrocarbon Receptor (rAhR) in vitro*

***El Agrebi N****., Perception of risk factors affecting bee colonies (Apis mellifera) health and mortality in Belgium*

***Gao Y****., A single nucleotide polymorphism of ORF131 encoded by Cyprinid herpesvirus 3 determines the formation of syncytia in cell culture*

***Guérin V.****, β-lactamase-encoding gene identification by microarrays in phenotypically resistant Escherichia coli from young calves in Wallonia, Belgium*

***Habets A****., Isolation of sero-pathotype specific bacteriophages against unconventional Shiga toxin-producing (STEC) and enteropathogenic (EPEC) Escherichia coli from diarrheic calves.*

***Hache-Carré de Lusancay A****., Effects of oral administration of omeprazole on equine faecal microbiota*

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

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

Chairmen: ***Johann DETILLEUX and Arnaud SARTELET***

*14:00* ***RENAULT V.****, Economic impact of contagious caprine pleuropneumonia and cost-efficiency analysis of the vaccination programs based on a one-year continuous monitoring of flocks in pastoral area*

*14:10* ***JACQUEL J****., Impact of selenium deficiency on the cardiac function of double-muscled calves*

*14:20* ***LOOS P****., A Gammaherpesvirus Affects Type 2 Innate Lymphoid Cells in the Context of HDM-Induced Asthma*

*14:30 Questions*

14:45 Short talks

***Humbel M.**, Preliminary results on the characterization of alternative horse housing systems in Belgium and France*

***Ludwig-Begall L**., Replicative fitness recuperation of a recombinant murine norovirus – investigating the interplay between genetic shift and genetic drift in an in vitro norovirus model*

**15:00 Invited speakers**

**DESMECHT Daniel & THIRY Etienne**

**Hot Topic: AFRICAN SWINE FEVER**

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

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

**Chairmen: *Antoine CLINQUART and Jérôme PONTHIER***

*16:00 **MAQUET C.**, Ly6Chi monocytes are key orchestrators of gammaherpesvirus lifecycle*

*16:10 **SAUVAGE A.**, Detection of intraocular Leptospira spp. by real-time polymerase chain reaction in horses with recurrent uveitis in Belgium*

*16:20 Questions*

16:30 Short talks

***Morvan L.,** Zebrafish as a model for Cyprinid Herpesvirus 3 infection and for investigation of ORF112 function*

***Myster F**., Development of a flow cytometry panel to characterize bovine peripheral blood leukocytes*

***Nguyen Thi Xuan P**., Habitat preference of freshwater snails as intermediate hosts of potential foodborne zoonotic trematodes in Thanh Hoa, Vietnam*

***Radermecker C.**, Particular neutrophils mediate environment-driven onset of airway allergy through NETs release*

***Razafimahefa R.**, Development of alternative methods to RT-qPCR to detect and quantify infectious norovirus particles in food*

***Vande Catsyne C.A.**, The lipid phosphatase SHIP2/INPPL1 controls chondrocyte hypertrophy and matrix mineralization in a murine model for opsismodysplasia*

**17:15 - Awards**

*Dr Dominique VOTION, Board member of FARAH*

**17:30 - Closing session**

**17:45 - Cocktail and poster session 4** (Room P, building B45)

**20:00 – Dinner and Dancing party** (Room P, building B45)

# Table of Contents

# Oral Presentations

# Short Talks

# Posters

## Veterinary public health

## Sustainable livestock production

## Comparative veterinary medicine

# Oral presentations

### Striped Catfish (Pangasianodon hypophthalmus) genetic selection under saline conditions in the Mekong Delta

Dao Minh Hai[1,2], Duong Thuy Yen[2], Nguyen Thanh Phuong[2], Pham Thanh Liem[2], Bui Minh Tam[2], Do Thi Thanh Huong[2], Bui Thi Bich Hang[2], Vo Nam Son[2], Mutien-Marie Garigliany[3], Patrick Kestemont[4], Nicolas Antoine-Moussiaux[1], Frédéric Farnir[1].
*[1.] FARAH/Sustainable Animal Production, Fac. of Vet. Med., ULiege; [2.]Colleges of Aquaculture and Fisheries, Can Tho University, Viet Nam; [3.]FARAH/Veterinary Public Health, Fac. of Vet. Med., ULiege; [4.]Research Unit in Environmental and Evolutionary Biology, NARILIS, Univ. of Namur*
***Corresponding author:*** *f.farnir@uliege.be*

Stripped catfish (*Pangasianodon hypophthalmus*), a commercially important species cultured in Mekong Delta region in Southern Vietnam, is facing a significant challenge due to salinity intrusion as a result of climatic changes. In this study, we attempt to carry out a genetic selection program targeting fish showing improved adaptation to salinity. 22000 catfish fry (870 families) was produced from crossing 29 females and 30 males from three different provinces in Mekong Delta to serve as genetic diversity resource fish for the study. The catfish fry (with weight between 4 and 5 g) has been put progressively in the targeted saline conditions (10 ppt of salinity). Subsequent sequential upward selection of around 50 % of the survival and healthy fast-growing fish at 3 stages of weight (100, 300 and 1000 g) was implemented. In parallel, another set of fish was raised under similar conditions (pond, salinity, density, feed) and underwent a random selection process to serve as negative control. After 3 selection stages (one year), the average weight of selected group and random group were 1.380 ± 175 g and 793 ±230 g, respectively. The DWG (daily weight gain) of selected group was higher than random group in all selection period. Further steps of this study will be the generation of the next generation of fish, the assessment of the heritabilities for growth traits and of the genetic diversity of the remaining fish, and, later, the identification of genes and QTL involved in salinity tolerance in this species.

---

### The Zalpha domain containing protein ORF112 encoded by Cyprinid herpesvirus 3: Is its Zalpha domain the only important part?

Mamadou Diallo[1], Léa Morvan[1], Yunlong Hu[1], Alain Vanderplasschen[1]
*[1]Immunology-Vaccinology, Department of Infectious and Parasitic Diseases (B43b), Fundamental and Applied Research for Animals & Health (FARAH), Faculty of Veterinary Medicine, University of Liège, Liège, Belgium*
***Corresponding author:*** *mdiallo@uliege.be*

Zalpha domains are 66 aa long domains, which bind to left handed dsDNA and dsRNA. Zα-domains have been identified in a limited number of cellular and viral proteins involved in innate immunity and its inhibition, respectively. The present study was devoted to the ORF112 protein encoded by Cyprinid herpesvirus 3 (CyHV-3). ORF112 is a 280 aa protein essential for viral replication in cell culture. The ORF112 protein consists of a long non-essential N-terminal glutamine rich domain with no recognizable homology and a short essential C-terminal region consisting of a single Zalpha domain. ORF112 is expressed in CyHV-3 infected cells both in the cytoplasm and the nucleus. It was also shown to localize in stress granules induced by arsenite treatment. Here, we investigated in the context of ectopic expression the role of the two regions of ORF112 in its subcellular localization. The major findings were: (i) ORF112 (aa 1-280) is expressed exclusively as a cytoplasmic protein due to its active nuclear export, while the expression of only its Zalpha domain (aa 189-280) leads to cytoplasmic and nuclear localization. (ii) ORF112 co-localizes with arsenite-induced stress granules. (iii) This co-localization was not affected by mutation affecting the Z-DNA/Z-RNA binding activity of ORF112 Zalpha domain. (iv) Expression of ORF112 N-terminal region (aa 1-189) co-localized with arsenite-induced stress granules, in contrast to the expression of ORF112 Zalpha domain (aa 189-280). Together, these results suggest that the N terminal glutamine rich domain of ORF112 contains a NES signal and is responsible for the localization of the full length protein in arsenite-induced stress granules.

# Oral presentations

## The carp olfactory system: a highway to the brain for Cyprinid herpesvirus 3

Fourrier M.[1], Nguyen L. [2], Boutier M.[1] and Vanderplasschen A.[-1]

[1.] *Immunology-Vaccinology (B43b), FARAH, ULg.*
[2.] *GIGA-Neurosciences, ULg.*
***Corresponding author:*** *mfourrier@uliege.be*

Cyprinid herpesvirus 3 (CyHV-3) causes a lethal disease in common and koi carp. This virus has inflicted substantial financial losses to the aquaculture sector. *In vivo* bioluminescent imaging (IVIS) of infected fish revealed replication in the olfactory rosette soon after inoculation by immersion in water containing the virus, and latter, into the central nervous system (CNS). Together these observations suggested that CyHV-3 could use the olfactory nervous system to invade the CNS. The goal of this study was to test this hypothesis. Using topical infection of the olfactory rosette, we were able to demonstrate that CyHV-3 infects neuronal cells present in the olfactory epithelium. IVIS analyses demonstrated that CyHV-3 spread in a time manner from the olfactory rosette to the olfactory bulb before migrating through the olfactory tract to reach the telencephalon. Experiments based on neurectomy of the olfactory tracts demonstrated that CyHV-3 also uses different routes to invade the CNS. Next, to investigate the neuroinvasion of the olfactory system by CyHV-3 using light-sheet microscopy, we produced a recombinant strain expressing EGFP as a constitutive reporter gene. Light-sheet microscopy analysis of carp entire CNS system allowed us to visualise at the level of individual cells and in a 3D manner, the neuroinvasion of CyHV-3.

---

## Development of Cyprinid herpesvirus 3 as a recombinant vector vaccine

Gao Y.[1], Boutier M.[1], Davison A. [2] and Vanderplasschen A.[1]

[1.]*Immunology-Vaccinology (B43b), Dep. of Infectious and Parasitic Diseases, Fac. of Vet. Med., FARAH, ULiège;* [2.]*University of Glasgow Centre for Virus Research, Glasgow, United Kingdom*
***Corresponding author****: yuan.gao@doct.uliege.be*

Cyprinid herpesvirus 3 (CyHV-3), is a member of the genus Cyprinivirus, family Alloherpesviridae, order Herpesvirales. CyHV-3 is responsible of a lethal disease in common and koi carp (Cyprinus carpio) which are both economically important species. In this study, we investigated the potential of a CyHV-3 attenuated vaccine strain developed by the host lab as a platform for the development of recombinant vaccines expressing antigen of other fish pathogens. First, we investigated whether the ORF56-57 double deletion encoded by the attenuated vaccine strain could provide a potential site for transgene insertion: (i) by analyzing the stability of the ORF56-57 deletion, and (ii) by inserting a luciferase expression cassette at the site of deletion. The results obtained showed a high stability of the ORF56-57 deletion and that the ORF56-57 deletion site is compatible with an efficient expression of luciferase as detected in vivo by IVIS analyses. Next, the glycoprotein G (gG) of Spring Viremia of Carp Virus (SVCV), a pathogenic rhabdovirus that also infects common carp, was selected as a candidate transgene. An expression cassette was inserted within the ORF56-57 deletion. Immunofluorescent staining of cells infected by the obtained recombinant virus revealed expression of glycoprotein G and the stability of the transgene. These results support the testing of the ability of this CyHV-3 vector vaccine expressing SVCV gG to induce an immune protection against both CyHV-3 and SVCV.

## Characterization of oral murine adenovirus type 1 infection in mouse and evaluation of the protection induced against a respiratory homologous infection

Goffin E.[1], Javaux J.[1], Bisteau M. [2], Destexhe E. [2], Spindler K.R. [3], Machiels B. [1] and Gillet L.[1]
*[1.] Immunology-Vaccinology, Dep. of infectious and parasitic diseases, Fac. of Vet. Med. – FARAH, ULiège; [2.] GlaxoSmithKline Biologicals, Rixensart, Belgium; [3.] Microbiology/Immunology, University of Michigan, Ann Arbor, Michigan, USA*
***Corresponding author:*** *emeline.goffin@uliege.be*

Replication-competent adenoviruses appear as promising vectors for the development of oral vaccines. However, as human adenoviruses replicate efficiently only in a highly restricted host range, researches on this topic have suffered from the lack of reliable animal models. In this study, we used *mouse adenovirus type 1* (MAV-1) to develop a small animal model for oral replicative adenovirus vaccines. We firstly characterized MAV-1 oral infection in mice. Although no clinical signs were observed following MAV-1 oral administration, viral DNA was detected in various organs all over the body and a specific and neutralizing humoral response was mounted against the virus. We then evaluated the protection induced by MAV-1 oral infection against a respiratory homologous challenge. Our observations showed that oral immunization prevents weight loss associated with intranasal MAV-1 infection and protects against lung inflammation and viral replication. Finally, we showed that at least 100 TCID50 of MAV-1 administered orally were sufficient to confer immune protection to mice. Altogether, these results show that MAV-1 offers a reliable model for oral vaccination based on replicative adenoviruses. This model provides a valuable tool to study replicative adenoviruses in their natural host and to investigate the potential of these viruses as vectors for oral vaccination.

---

## Impact of selenium deficiency on the cardiac function of double-muscled calves

Jacquel J.[1], Lecoq L.[2], Rollin F.[1], Amory H.[2].
*[1.]Clinical departement of production animals, Fac. of veterinary medicine, Liege, Belgium.*
*[2.]Clinical departement of small animals and equids, Faculty of veterinary medicine, Liege, Belgium.*
***Corresponding authors:*** *jujacquel@gmail.com, Laureline.Lecoq@uliege.be.*

Myocardial dysfunction was assessed in a population of Belgian Blue calves (BBB) suffering from vitamin E (VitE) and selenium (Se) deficiency. Twenty-four BBB calves aged 1 to 8 weeks were selected from 3 Walloon farms with a known selenium deficiency. Each calf was submitted to a clinical examination, a cardiac ultrasound examination and has been subjected to blood sampling. VitE, Se, troponin I levels and glutathione peroxidase activity were measured for each animal. Conventional ultrasound measurements (diameters of the left atrium, the aorta and the pulmonary artery, and left ventricular shortening fraction) as well as a two-dimensional speckle-tracking analysis were performed. Two groups of animals were formed based on the calculated average heart rate (AvHR):group 1 calves had an AvHR lower than 120 (Gr1,n=14) and group 2 calves had an AvHR greater than 120 (Gr2, n=10). Sonographic and biochemical results of both groups were subjected to statistical variance analysis using T-tests. Shortening fraction was significantly lower in group 2, which is consistent with the general impression of dyskinesia in this group during echocardiography. Global specke-tracking results also tended to point out a myocardial malfunction within group 2 although only the values of circumferential and radial A peaks were significantly different between the two groups. Blood Se level was significantly different from one group to the other with a deficiency more pronounced in group 2. The level of blood Se in BBB calves aged 1 to 8 weeks is thus correlated with cardiac function and heart rate could be used as a clinical screening parameter of cardiac dysfunction secondary to Se deficiency.

# Oral presentations

## A Gammaherpesvirus Affects Type 2 Innate Lymphoid Cells in the Context of HDM-Induced Asthma

Loos P., Maquet C., Javaux J., Machiels B.* and Gillet L.*
* co-last authors
*Immunology-Vaccinology, Department of infectious and parasitic diseases, Faculty of Veterinary medicine – FARAH, University of Liège, Belgium.*

***Corresponding author:*** *pauline.loos@uliege.be*

Persistent viruses, such as gammaherpesviruses, profoundly imprint the immune system of their hosts. Accordingly, infection with murid gammaherpesvirus 4 (MuHV-4) inhibits the development of airway allergy following the instillation of house dust mite extracts (HDM) in mice. Group 2 innate lymphoid cells (ILC2s) play a major role in the initiation, maintenance and memory of type 2 immune responses. This work aims to investigate the impact of MuHV-4 infection on pulmonary ILC2s. Our results showed that MuHV-4 infection modifies functional properties of ILC2s as early as 8 days post-infection. Specifically, the production of type 2 cytokines such as IL-5 and IL-13 by ILC2s was decreased after HDM-sensitization or allergic challenge. This reduced cytokine production was associated with the decreased expression of the canonical Th2 transcription factor GATA-3. Although not exhibiting any characteristic of plasticity towards an ILC1 phenotype, these ILC2s from MuHV-4 infected mice showed a lower expression of the PD-1 and KLRG1 activation markers. Accordingly, MuHV-4 infection impaired the recruitment of some described "inflammatory" ILC2s without affecting the multiplication of the "resident" ILC2s. Overall, these results show that MuHV-4 infection significantly and sustainably affects the lung ILC2s population and that this may have a determining role in the subsequent development of immune responses against respiratory allergens.

---

## Ly6C$^{hi}$ monocytes are key orchestrators of gammaherpesvirus lifecycle

Maquet C., Loos P., Javaux J., Gillet L.* and Machiels B.*

* co-last authors

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

**Corresponding author:** *c.maquet@uliege.be*

The majority of the human population will be infected with one or more herpesviruses during life. In particular, gammaherpesvirus (γHVs) represent highly prevalent human viruses as the best studied γHVs, Epstein-Barr virus and the Kaposi's Sarcoma-associated Herpesvirus, infect respectively some 90% and up to 40% of human populations. Through coevolution with their hosts, γHVs have developed numerous mechanisms to control the immune response and successfully survive in the host in a symbiotic relationship. In some instances, the persisting herpesvirus infections might even provide some benefits to the host against other infections. In that context, a pulmonary infection with Murid gammaherpesvirus 4 (MuHV-4), a γHV infecting mice, induces the recruitment of inflammatory monocytes (Ly6C$^{hi}$ MOs) from the bone marrow to the site of primary infection. While the recruitment of these Ly6C$^{hi}$ MOs induced by MuHV-4 infection confers a benefit to the host to protect against asthma, their role in MuHV-4 lifecycle remains unknown. By using complementary mouse models deficient for Ly6C$^{hi}$ MOs, we highlighted the importance of Ly6C$^{hi}$ MOs recruitment in the MuHV-4 lifecycle. Our results show that, at early time points after MuHV-4 infection, recruitment of Ly6C$^{hi}$ MOs is associated with reduced viral replication, clinical signs, and neutrophilic infiltration in lung and with enhanced NK and CD8 T cells responses. Moreover, these recruited Ly6C$^{hi}$ MOs produce high levels of IL-10 suggesting regulatory properties. This therefore suggests that Ly6C$^{hi}$ MOs are key orchestrators of immune response following γHV infection.

## Economic impact of contagious caprine pleuropneumonia and cost-efficiency analysis of the vaccination programs based on a one-year continuous monitoring of flocks in pastoral area

Renault V.[1], Hambe H.A.[2], Van Vlaenderen G.[3], Timmermans E.[4], Berkvens D.[5] and Saegerman C.[1.]
*[1] Res. Unit of Epidem. & Risk Analysis applied to Vet. Sci. (UREAR-ULg), FARAH, ULiège; [2] County coord., Accelerated Value Chain Progr.–Livestock Component (AVCD–LC), Intern. Livestock Res. Instit., Kenya; [3] Indep. consultant; [4] Techn. Coord., Vét. Sans Frontières-Belgium (VSF-B); [5] Instit. of Tropical Medecine, Antwerp, Belgium*
***Corresponding author**:* vrenault@uliege.be

In Kenya, contagious caprine pleuro-pneumonia is one of the most prevalent infectious diseases affecting the small ruminants in pastoral areas with adverse consequences on the livelihoods despite the organization of bi-annual vaccination campaigns. The cost efficiency of the vaccination program in a pastoral context has been difficult to assess due to a lack of reliable data. Indeed the dynamic of flock population, illiteracy level and limited outreach make it difficult for researchers to collect the information needed for such studies. Nevertheless such analysis are of prime importance in order to evaluate the efficiency of the control programs and justify the organization of national vaccination campaign either as part of a disease eradication program and/or households economy support program in order to protect the livestock, which represents the main livelihoods of the pastoral population. A continuous flock monitoring was performed for a period of 1 year in Turkana County to collect data on the flock dynamics and record the different causes of mortalities. A stochastic model was developed to evaluate the economic losses due to CCPP in a standard flock of 100 heads over a year and the cost of the vaccination in order to evaluate the cost-efficiency of the vaccination programs based on different efficiency scenarios (95%, 50% and 20% efficiency). The model shows that the vaccination program remains cost-efficient even with a 20% efficiency rate only (average cost ratio of 5.715 and S.D. of 3.914). Nevertheless, a higher efficiency rate is required to achieve long term results in terms of flock productivity and diseases control.

---

## Detection of intraocular *Leptospira* spp. by real-time polymerase chain reaction in horses with recurrent uveitis in Belgium

Sauvage A.[1], Monclin SJ.[1], Elansary M.[2], Hansen P.[3] & Grauwels MF.[1]
*[1.]Dep. of Clinical Sci., Companion & Equine An., Ophthalmology, FARAH, ULiège; [2.]Unit of Animal Genomics, GIGA-R & College of Vet. Med., FARAH, ULiège; [3.]Vet. Lab. Synlab/Collard, Liège, Belgium*
***Corresponding author***: asauvage@uliege.be

Equine Recurrent Uveitis (ERU) has been associated with *Leptospira* spp. infection. The objectives of the study were to establish the prevalence of intraocular *Leptospira* spp. in ERU affected and healthy eyes of horses examined at the Equine Clinic of the University of Liège by real-time PCR and to compare the results of the aqueous and vitreous humour of the same eye. Sixty-six eyes from 59 client-owned horses with a diagnosis of equine recurrent uveitis were studied from May 2015 to December 2017. Fifty healthy eyes from 28 euthanized horses for unrelated reasons examined during the same period were included in the control group. Intraocular fluids (aqueous and/or vitreous humours) from ERU-affected eyes were sampled and analysed by real-time PCR for *Leptospira* spp. Aqueous and vitreous humours from the control group were processed in the same way. Both groups were comparable regarding age, sex, eye sampled, humours sampled but not regarding breeds, with an over-representation of Warmbloods and Appaloosas in the ERU-group. The prevalence of *Leptospira* spp. was 30.3% (20/66 eyes) in the ERU-group. *Leptospira* spp. DNA was identified in 11 aqueous and 17 vitreous humours with 8 horses testing positive in the two humours, 9 horses testing positive for vitreous humour alone and 3 horses for aqueous humour alone. The phi-correlation between aqueous and vitreous humour *Leptospira*-PCR results is 0.47 suggesting a low association. All the control eyes were negative. Leptospirosis is a potential cause of ERU in Belgium. Testing both intraocular media is advised whenever possible.

# Oral presentations

## Atypical Myopathy: is there a relationship between serum hypoglycin A and MCPA-Carnitin levels and the composition of faecal microbiota?

Wimmer-Scherr C.[1], Votion D.[1], Boemer F.[2], Cerri S.[1], Lefère L.[3], Palmers K.[4], van Loon G.[3], Amory H.[1], Daube G.[5], Taminiau B.[5], Cesarini C.[1]

[1] *Equine Section, FARAH Center, Faculty of Veterinary Medicine, ULiège*
[2] *Biochemical Genetics Laboratory, CHU Liege, ULiège*
[3]*Department of Large Animal Internal Medicine, Faculty of Veterinary Medicine, Ghent University, Merelbeke, Belgium*
[4]*De Morette Equine Clinic, Asse, Belgium*
[5]*Department of Food Sciences, Microbiology, FARAH Center, ULiège*
***Corresponding author:*** ccesarini@uliege.be

Ingestion of Hypoglycin A (HGA) is associated to the development of atypical myopathy (AM) in horses. This toxin and its principal metabolite methylenecyclopopyl acetic acid-carnitine (MCPA-carnitine) are detected in the serum of horses showing clinical disease. However, some horses exposed to HGA do not develop AM, and thus far, the factors protecting those horses from developing clinical disease are yet to be identified. This study aims to assess the correlation between faecal microbiota and HGA and MCPA-carnitine levels in the serum of 19 horses with confirmed AM. Faecal and serum samples were obtained on admission to the referral hospital. Determination of MCPA-carnitine serum concentrations using tandem mass spectrometry and HGA quantification using a modified aTRAQ® assay helped to confirm the disease. Bacterial taxonomy profiling was obtained by V1V3 16S amplicon sequencing from faeces. Comparison between both groups was performed with a two-tailed Mann-Whitney test ($p<0.05$). Spearman rank correlation between bacterial taxa and HGA and MCPA-carnitine were performed with MOTHUR. A total of 190,000 sequences were analysed and clustered to 296 genus level operational taxonomic units. Serum levels of HGA were positively correlated with the relative abundance of the *Prevotellaceae* family (rs 0,64) and MCPA-carnitine levels were negatively correlated with the relative abundance of the *Lachnospiraceae* family (rs -0,66). Results of this pilot study suggest that *Lachnospiraceae* could play a preventative role in the development of clinical disease. However, further research is needed to determine the exact role of the intestinal microbiome in the development of AM.

# Short talks

## Albumin, Urea and Cholesterol: 3 haematological parameters as markers of nutritional status and prognosis in hospitalised calf

Borelli E.[1], Rao A.-S.[2], Théron L.[2], Casalta H.[1], Djebala S.[1], Léonard M.[1], Sartelet A.[1], Toutati K.[1], Bayrou C.[1,2]

[1.] *Bovine Clinic, Comparative veterinary medicine, FARAH, ULiège.*

[2.] *RumeXperts, ULiège.*

[3.] *Dpmt of Pathology, Veterinary public health, FARAH, ULiège.*

***Corresponding author:*** *calixte.bayrou@uliege.be*

Protein-energy malnutrition of calves is often undervalued in clinical practice, although its negative consequences for the treatment and the prognosis in animals. The aim of this study was to determine the association between biomarkers of malnutrition (such as cholesterol, albumin and urea) and mortality in hospitalized calves affected by various diseases (especially neonatal diarrhea and arthritis). A total of 42 calves were enrolled in the study and biochemical analyses were carried out to measure serum cholesterol, albumin and urea. Blood samples were collected on admission in the clinic, as well as before discharge or death, in order to assess their evolution during the hospitalization. Subsequently, the results were compared between the calves which survived (n = 22) and those which died (n = 20). The results showed significantly differences of urea (p=0.0321) and albumin (p=0.0222) between the two groups at the moment of admission. Calves which died had higher values of urea (68,6 mg/dL) and lower values of albumin (24,5 g/L) compared to those which survived (43,9 mg/dL and 27,6 g/L respectively). Urea/albumin ratio was calculated and the cut-off value of 2.3 presented a sensibility of 65 % and a specificity of 91 % in predicting mortality of hospitalized calves. Calves which survived showed a significant increase of serum cholesterol during hospitalization (+2,9 mg/dL daily) compared to those that died, in which cholesterol decreased (-3 mg/dL daily) (p=0.0135). According to the findings of this study, serum cholesterol, albumin and urea have proved to be valid indicators of severity of disease and prognosis in hospitalized calves.

---

## Infection of European eel by Anguillid herpesvirus 1: from basic research to conservation programs

Delrez, N. [1], Boutier M. [1], Lieffrig F. [2], Denoël M. [3], Mélard C. [4] and Vanderplasschen A.[1]

*1. Immunology-Vaccinology, Dept of Infectious and Parasitic Diseases, FARAH, ULiège; 2.CER Groupe, Aye, Belgium; 3. Lab. of Fish and Amphibian Ethology, Behavioral Biology Unit, Dept of Biology, Ecology and Evolution, ULiège; 4. CEFRA, ULiège*

***Corresponding author:*** *natacha.delrez@uliege.be*

The European eel (Anguilla anguilla) is a catadromous fish with a complex and fascinating life cycle. Over the last few decades, the number of eels reaching Europe has declined by 99%, justifying its classification as a critically endangered species. Among the multiple factors contributing to this decline, viral infection caused by Anguillid herpesvirus 1 (AngHV-1) is thought to play a key role. In the present project, we aim to study the infection of the European eel by AngHV-1 as a homologous host-virus model to address fundamental questions related to this virus. With that goal in mind, we produced and characterized a recombinant virus expressing the firefly luciferase and copepodGFP as reporter genes using a transfection-infection approach. This viral strain and In vivo bioluminescence Imaging was used to address various aspects of viral pathogenesis: (i) the portal of viral entry into the host, (ii) viral spreading within the infected host, (iii) the effect of water temperature on the outcome of the infection, and (iv) transmission of the virus between subjects. Our results showed that infection by immersion in water containing the virus induced a less pronounced infection compared to inoculation by intra peritoneal injection. Thus, the latter mode of inoculation was selected for the following experiments. AngHV-1 infection was found to develop between 20 and 28°C, with a positive relationship between water temperature and clinical signs and IVIS signal. Next, we investigated AngHV-1 transmission from IP infected fish to naïve cohabitants. Interestingly, viral transmission to cohabitant fish was efficient and they expressed high positive signal on the skin. In the present study we established an efficient and original model to study the pathogenesis of AngHV-1 in its natural host.

# Short talks

## A realistic mixture of Persistent Organic Pollutants (POPs) reveals possible synergism to inhibit the transactivation activities of the rat Aryl hydrocarbon Receptor (rAhR) in vitro

Doan TQ[1], Muller M[2], Berntsen HF[3], Zimmer KE[4], Verhaegen S[3], Ropstad E[3], Connolly L[5], Scippo ML[1]
*[1] Dept of Food Science, FARAH, ULiège; [2]GIGA-R, Lab. for Organogenesis and Regeneration, ULiège; [3]Dept of Production Animal Clinical Sciences, Section of Experimental Biomedicine, NMBU-Fac. of Vet. Med., Oslo, Norway; [4]Dept of Basic Sciences and Aquatic Medicine, Section of Biochemistry and Physiology, NMBU-Fac. of Vet. Science, Oslo, Norway; [5]Institute for Global Food Security, School of Biological Sciences, Queen's University Belfast, Northern Ireland, UK.*
***Corresponding author**: tqdoan@uliege.be*

While organisms are exposed to mixtures of persistent organic pollutants (POPs), few studies have addressed the mixture effect. This study aims to determine how a realistic mixture of POPs affects the transactivation of the rat Aryl hydrocarbon Receptor (rAhR) in vitro. Luciferase reporter Dioxin responsive rat hepatoma cell lines (DR-H4IIE) were used to screen both rAhR agonistic and antagonistic activities of 29 POPs: six perfluorinated (PFAA), seven brominated (Br), and 16 chlorinated (Cl) compounds. Only 5 (2 Cl and 3 Br) out of the 29 compounds presented rAhR agonistic activities while 16 (13 Cl and 3 Br) were rAhR antagonists. No effect was observed for PFAAs. To test possible interactions between these compounds, a mixture of these 29 POPs and six sub-mixtures (PFAA, Br, Cl, Cl + Br, Cl + PFAA and Br + PFAA), prepared based on the concentrations found in Scandinavian human blood, were tested for the same activities. Not surprisingly, the POP mixture also displayed a rAhR antagonistic activity (IC50 = 371 ± 52 times the blood level) with the lowest effective concentration found at 75-time blood level. This level could be plausibly reached in humans after a food contamination incident or in highly exposed sub-populations. Testing the sub-mixtures and calculating IC50 using an additive model showed that the Cl mixture is responsible for 80% of the antagonism of the POP mixture, but the antagonist effect of the Cl + PFAA was the same as the response of the POP mixture. This indicates that PFAAs are probably non-specific rAhR antagonists as they did not induce any antagonist response when tested alone, indicating a possible synergistic effect.

## Perception of risk factors affecting bee colonies (*Apis mellifera*) health and mortality in Belgium

El Agrebi N.[1], Danneels E.[2], de Graaf D., Saegerman C.[1]
*[1] Res. Unit of Epid. and Risk Analysis applied to vet. Sci. (UREAR-ULg), FARAH, ULiege, Belgium; [2] Lab. of Molecular Entomology and Bee Pathology, Dept of Biochem. and Microbiology, Univ. of Gent*
***Corresponding author**: nelagrebi@uliege.be*

Understanding beekeepers' perceptions of risk factors having an impact on bee health and mortality is essential to analyze the reasons for adopting or rejecting some management practices. To date, no study on how beekeepers perceive and manage these risks has been carried out. Adopting strategies that mitigate risk to health and bee mortality is an action involving behavioral changes. In order to better understand the factors that determine changes, as well as the decision-making and action process, in Belgian apiaries, a perception survey was designed and launched online, based on the Health Belief Model (HBM) commonly used in human medicine. This sociological survey concerns 355 randomly distributed beekeepers all over Belgium. A first descriptive analysis of the data shows that beekeepers tend in general to take little risk, their perception of climate change, *Varroa destructor* and management practices is acceptable. On the other hand, their perception of pesticides use in beekeeping and agriculture is confused. A Welch test comparing beekeepers' perceptions in function of mortality rates, indicates that beekeepers (N=213), with mortality rates <10% have a significantly better perception of risk factors for their colonies and apply more measures limiting these factors. Despite a real perception of risk, the constraints of investing time in the execution of these actions and the lack of feeling of the financial impact that the loss of a colony entails, are the main obstacles to the implementation of measures to limit the risk. The results of this study highlight the importance of taking socio-economic determinants into account in any strategy aimed at mitigating the risks associated with bee mortality.

## A single nucleotide polymorphism of ORF131 encoded by Cyprinid herpesvirus 3 determines the formation of syncytia in cell culture

Gao Y.[1], Boutier M.[1], Davison A. [2] and Vanderplasschen A.[1]

[1.] *Immunology-Vaccinology (B43b), Department of Infectious and Parasitic Diseases, Faculty of Veterinary Medicine, FARAH, ULiège*
[2.] *University of Glasgow Centre for Virus Research, Glasgow, United Kingdom*
***Corresponding author**: yuan.gao@doct.uliege.be*

Cyprinid herpesvirus 3 (CyHV-3), is a member of the genus Cyprinivirus, family Alloherpesviridae, order Herpesvirales. CyHV-3 is responsible of a lethal disease in common and koi carp (Cyprinus carpio) which are both economically important species. When comparing the replication in cell culture of seven CyHV-3 strains, we observed that three of them formed syncytial viral plaques (including the previously described FL strain), while the others did not. Syncytia formation by herpesviruses relies on cell fusion that occurs during viral infection. This phenomenon is driven by the expression of virion transmembrane proteins (VTPs) on the surface of infected cells. Consequently, we compared the sequences of the ORFs encoding VTPs for the seven CyHV-3 strains. This analysis revealed that the three strains forming syncytia share mutations in ORF27 and ORF131: a disruption of ORF27 inducing frameshifts and a single nucleotide polymorphism (SNP) in ORF131 causing one amino acid substitution. Using bacterial artificial chromosome (BAC) cloning and prokaryotic mutagenesis, we demonstrated that the single nucleotide polymorphism observed for ORF131 determines the formation of syncytia in cell cultures.

---

## β-lactamase-encoding gene identification by microarrays in phenotypically resistant *Escherichia coli* from young calves in Wallonia, Belgium

Guérin V.[1*], Thiry D.[1], Galleni M.[2], Saulmont M.[3], Mainil J.[1]

[1.] *Bacteriology, Department of Infectious and Parasitic Diseases, FARAH and Faculty of Veterinary Medicine, ULiège, Belgium*
[2.] *Biological Macromolecules, Department of Life Sciences, Centre for Protein Engineering (CIP), ULiège, Belgium*
[3.] *Regional Animal Health and Identification Association (ARSIA), Ciney, Belgium*
[*] ***Corresponding author:** vguerin@uliege.be*

Since 2016, a decrease of β-lactam resistance of Escherichia (E.) coli from calves is observed at ARSIA, maybe as a consequence of the regulation of the use of human critical antibiotics in livestock, like 3rd/4thG cephalosporins. The most frequent β-lactam resistance mechanism is the production of β-lactamase enzymes (BLA). Their classification is highly complex, but 4 groups can be proposed: classical BLA (BLAC), cephalosporinases (AmpC), extended spectrum BLA (ESBL) and carbapenemases (CPE). The aim of this study was to identify the BLA-encoding genes present in E. coli from calves with a β-lactam resistance phenotype at the disk diffusion assay (DDA). For 4 months, E. coli with β-lactam resistance profiles were collected at ARSIA from calves with enteritis or septicaemia. After initial growth on Gassner agar plates, 3 colonies from faeces or intestinal contents were tested to identify their virulotype and one positive isolate per calf was tested by DDA. When pure culture was obtained from organs one isolate was also tested by DDA. Of the collected isolates, 94 with different resistance profiles were chosen to be tested with the Check-MDR CT103XL® microarray. A concordance of 72% between the detected genes and the phenotypes was observed. In isolates with ESBL resistance profiles, only blaCTX-M genes were detected. In isolates with AmpC resistance profiles blaCMY II and blaDHA genes were identified. The blaTEM-WT genes were detected in isolates with BLAC resistance profiles and also in isolates harbouring ESBL/AmpC-encoding genes. In the future, PCR will be performed on the whole collection to identify the genes present and to follow their incidence for 3 years.

# Short talks

## Isolation of sero-pathotype specific bacteriophages against unconventional Shiga toxin-producing (STEC) and enteropathogenic (EPEC) Escherichia coli from diarrheic calves

Habets A.[1], Duprez J-N.[1], Iguchi A.[2], Saulmont M.[3], Thiry D.[1], Mainil J.[1]

[1]*Bacteriology, Parasitic and Infectious Disease, FARAH, ULiège.*
[2]*Department of Animal and Grassland Sciences, Faculty of Agriculture, University of Miyazaki, Miyazaki, Japan 889-2192.*
[3]*Regional Agency for Animal for Health and Identification, Ciney, Belgium.*
***Corresponding author:*** *audrey.habets@uliege.be*

*Escherichia coli* producing the Shiga toxins (STEC) and/or the attaching-effacing (AE) lesion (EPEC) cause enteritis and (bloody) diarrhoea in young calves and in humans. STEC and EPEC can belong 7 serogroups frequently identified worldwide: O26, O103, O111, O121, O145, O157 and O165. Beside these classical "gang of 7" serogroups, unconventional serogroups can be identified as previously demonstrated with the O80 EPEC. The use of active and specific bacteriophages as biocontrol agents seems to be a promising alternative against unconventional STEC/EPEC and have been employed for diagnostic. The first aim of this project was to identify those 6 unconventional serogroups among 76 STEC and EPEC isolated between 2008 and 2015 from diarrheic calves at ARSIA. Two triplex PCRs have been applied either for the O146_O182_O183 serogroups or for the O123/186_O156_O177 serogroups. The second objective of this project was to isolate specific bacteriophages against these unconventional serogroups from wastewater or farm slurry. So far, the first triplex PCR identified 4 O182-positive and 2 O183-positive STEC and EPEC. The second triplex PCR identified 10 O123-186-positive, 2 O156-positive and 13 O177-positive STEC and EPEC. A total of 4 potentially specific bacteriophages respectively active against O5, O123, O146, O186 were obtained. The further steps of this study will be to perform: (i) the PFGE profile comparison of the calf and healthy cattle PCR-positive STEC and EPEC between themselves and with human STEC and EPEC; (ii) the identification of still other unconventional serogroups among STEC and EPEC from diarrheic calves; (iii) the host range spectrum of the 4 potentially specific bacteriophages.

---

## Effects of oral administration of omeprazole on equine faecal microbiota

Hache-Carré de Lusancay A.[1], Taminiau B.[2], Lecoq L.[1], Daube G.[2], Amory H.[1], Cesarini C.[1]
[1] *Equine Section, FARAH Center, Faculty of Veterinary Medicine, ULiège*
[2] *Department of Food Sciences, Microbiology, FARAH Center, ULiège*
***Corresponding author:*** *ccesarini@uliege.be*

Equine Gastric Ulcer Syndrome is a frequent disease in horses, and the proton pump inhibitor omeprazole is the drug most commonly used for its management. In human and small animal medicine, omeprazole administration has been associated to shifts in faecal microbiota and an increased incidence of *Clostridium difficile* enterocolitis, especially in prolonged treatments. The purpose of this study was to assess the effects of omeprazole administration on faecal microbiota in healthy adult horses. This pilot study was conducted on eight healthy horses from the university research herd, 100% stabled and fed haylage. Two faecal samples to be used as controls were obtained from each horse at a 7 day interval, previously to any treatment. Faecal samples were also taken before (day 0) and after (day 7) a 7-day treatment period with omeprazole at 4mg/kg PO q24h. Fecal samples were placed in a preservation medium kit (Stool DNA Stabilizer (PSP® Spin Stool DNA Plus Kit 00310, Invitek)) and were frozen at -20°C until analysis. Bacterial taxonomy profiling was obtained by V1V3 16S amplicon sequencing from faeces. Analysis of alpha and beta diversity was performed with MOTHUR. Most common bacterial phyla identified in faeces included *Firmicutes, Bacteroidetes, Verrucomicrobia* and *Fibrobacter*. No significant differences were found in composition and diversity of faecal microbiota between treatment and control samples nor before and after 7 days of omeprazole treatment. In conclusion, equine faecal microbiota is not significantly modified in healthy horses by a 7-day treatment with oral omeprazole (4mg/kg). Further work is required to assess the effect of longer treatment periods.

## Preliminary results on the characterization of alternative horse housing systems in Belgium and France

Humbel M.[1], Cabaraux J.F.[1], Vandenheede M.[1]
[1]*Department of veterinary management of animal resources, FARAH, Fac. of Vet. Medicine, Uliège*
***Corresponding author:*** *mhumbel@uliege.be*

Individual horse stabling raises health and welfare concerns. Alternative systems are emerging (called "active stable", "Paddock Paradise®", "equi-tracks"). They intend to provide group housing, feeding day long, outdoor living and design to enhance movement. Their specificities and eventual effects on horse's behaviour, health and welfare need further investigation.Belgian and French alternative's owners were recruited to answer a questionnaire regarding herd structure, available areas, management and horse health. Data were collected from 35 herds (2 to 49 horses), 85.7% were mix gender herd. Mean available area per horse was 614.8 ±728.8 m² (pasture excluded) with 9.8 ± 13.4 m² shelter area per horse.Forage distribution methods varied, with 51.9% adding a net on the forage or using individual net (45.7%) to slow down ingestion. 25.7% never received feed complement beside forage and 48% received homemade mix rather than commercial ones. Most herds (97.1%) had access to grass pasture during summer. To increase locomotion at least one forage dispenser was away from the shelter (51.5%), or water set apart (42.9%) or tracks were used (91.4%). 60% of herds had alternative foot care, shoeing being refused in most cases. 21.8% did not receive any chemical deworming. 18.6% of the horses received veterinary care over the last year for foot abscesses (5.3% of horses), wounds (2.7%), skin problems (2.4%), colics (2.2%). This study was a first step to characterise those "horse alternative housings" in Belgium and France. Results will highlight strengths and weaknesses of those alternatives, pointing improving strategies in terms of horse health and welfare.

---

## Replicative fitness recuperation of a recombinant murine norovirus – investigating the interplay between genetic shift and genetic drift in an *in vitro* norovirus model

Ludwig-Begall L.F.[1], de Oliveira-Filho E.F.[1,2], Lu J.[3], Hosmillo M.[3], Mathijs E.[1,4], Di Felice E.[1,5], Goodfellow I.[3], Thiry E.[1], Mauroy A.[1,6]
[1.]*Veterinary Virology and Animal Viral Diseases, FARAH, Uliège;* [2.]*Dept of Virology, Aggeu Magalhães Institute, FIOCRUZ, Recife, PE, Brazil;* [3.]*Division of Virology, Dept of Pathology, Univ. of Cambridge, Cambridge, UK;* [4.]*Dept of Virology-Molecular Platform, Vet. & Agrochemical Research Centre, Brussels, Belgium;* [5.]*Facoltà di Med. Vet., Univ. degli Studi di Teramo, Italy;* [6.]*Staff direction for risk assessment, Control Policy, Federal Agency for the Safety of the Food Chain, Brussels, Belgium*
***Corresponding author:*** *etienne.thiry@uliege.be*

Noroviruses are recognised as a major cause of viral gastroenteritis in humans. Molecular mechanisms driving norovirus evolution are the accumulation of point mutations and recombination. Recombination can create great changes in a viral genome, potentially eliciting a replicative fitness cost, which must be compensated via the adaptive capacity of a recombinant virus. In this study, the capability of replicative fitness adaptation and genetic characteristics of a previously *in vitro*-generated recombinant murine norovirus (WU20-CW1) were evaluated at the start and end of ten *in vitro* passages. Our data provide evidence of viral adaptation after a recombination event that induced fitness loss of an infectious recombinant. Replicative fitness regain of the recombinant was demonstrated by growth kinetics differences and increase of mean viral lysis plaque size after ten passages. Point mutations at consensus and sub-consensus population level were characterised via NGS and putatively associated to fitness changes. To investigate the effect of observed genomic changes in the context of both a parental CW1 wild-type virus backbone and a lab-generated inter-MNV chimeric plasmid, mutations were introduced via overlap mutagenic PCR into plasmids containing either cDNA construct under control of a truncated T7 polymerase promoter. A DNA-based reverse genetics system allowed recovery of infectious virus at similar titres for all parental constructs, indicating that no mutation was so deleterious as to impair virus rescue. The impact of separate and combined mutations in a recombinant vector is under evaluation via passaging and phenotype characterisation.

# Short talks

## Zebrafish as a model for Cyprinid Herpesvirus 3 infection and for investigation of ORF112 function

Morvan L.[1], Diallo M.[1], Hu Y.[1], Po-Tsang L. [1], Rakus K.[1] and Vanderplasschen A[1].

[1] *Department of Infectious and Parasitic Diseases, Immunology-Vaccinology, FARAH, ULiège.*
***Corresponding author**: lea.morvan@uliege.be*

The innate immune system relies on many molecules that act as nucleic acid sensors, detected based on their structure, subcellular localization or sequence. Zalpha domains bind to left-handed dsDNA (Z-DNA) or dsRNA (Z-RNA). The description of Zalpha domains in proteins of the host innate immune system but also in viral proteins suggests that even the conformation of the nucleic acid could be exploited by the innate immune system.

Carp and zebrafish encode PKZ, a paralogue of the dsRNA-dependent protein kinase (PKR) expressed by all vertebrates. While PKR possesses dsRNA binding domains, PKZ has Z-DNA/Z-RNA binding domains.

Recently, it was demonstrated *in vitro* that ORF112 of Cyprinid herpesvirus 3 (CyHV-3) encodes a Zalpha domain protein over-competing the binding of PKZ to Z-DNA. It was therefore hypothesized that ORF112 directly or indirectly inhibits PKZ and/or PKR activation.

While carp encode 4 versions of PKZ, the widely used zebrafish model only encodes one and represents a powerful tool to further investigate the potential interaction of ORF112 with PKZ. However, adult zebrafish are thought to not be permissive to CyHV-3 infection. Zebrafish were knocked out for PKZ and/or PKR. These fish may be used to test the hypothesis of ORF112 inhibiting the PKZ and PKR pathways as it is hypothesised that KO fish will allow infection.

Micro-injection of wildtype zebrafish larvae 3 days post-fertilisation showed signal for several days post-infection after which it decreased. The viral clearance observed in the larvae may be the result of an interferon response. Using morpholinos that knock out all interferons in zebrafish larvae would allow to observe whether the infection is more intense, hence answering the question of clearance being interferon-dependent or not.

---

## Development of a flow cytometry panel to characterize bovine peripheral blood leukocytes

Myster F.[1], Davis W.[2], Charlier C.[3] and Gillet L.[1*]

1. *Immunology-Vaccinology, Department of infectious and parasitic diseases, Faculty of Veterinary medicine – FARAH, University of Liège, Belgium.*
2. *WSU monoclonal antibody center, Washington state university, Pullman, USA*
3. *Unit of genomics animals, GIGA, Uliège*

**Corresponding author:** *fmyster@uliege.be*

In the cattle industry, the selection of the best genitors is essential to maintain the competitiveness of the different breeds. The RESISTOMICS project aims at identifying in Belgian Blue breed (BB-B) genetic variants that could improve the health of animals via genomic selection. To that purpose, a detailed picture of the immune system of different individuals from a population is required. However, studying bovine immune responses has been hampered by the lack of specific reagents. Thus, flow cytometry characterization of peripheral blood leukocytes is limited due to the scarcity of conjugated antibodies available for cattle. In this project, using a new way to conjugate monoclonal antibodies, we have designed 7 to 9 colors panels allowing us to characterize better circulating leukocytes. This allowed us to identify with unprecedented details the following populations: neutrophils, B cells, the different clusters in γδ T cells, CD8/CD4 T cells, natural killer cells, monocytes and dendritic cells as well as activated cells in these populations. In the near future, this approach will be applied to the analysis of the immune system of a homogenous population of young BB-B bulls housed at the Centre for Bovine Selection (AWE, Ciney, Belgium). The results obtained in this study should open new ways for cattle selection but also for the study of bovine immune system.

## Habitat preference of freshwater snails as intermediate hosts of potential foodborne zoonotic trematodes in Thanh Hoa, Vietnam

Nguyen T. X. P. [1,2], Bui T.D. [2], Hoang V.H.[2], Dinh T.K.H [2], Losson B.[1], Lempereur L.[1]
[1.] *Department of Infectious and Parasitic Diseases, Faculty of Veterinary Medicine, ULiège*
[2.] *Institute of Ecology and Biological Resources, Hanoi, Vietnam Academy of Science and Technology*
***Corresponding author:*** *NguyenThiXuanPhuong@student.uliege.be*

Foodborne zoonotic trematode infections (FZTi) are neglected tropical diseases in Southeast Asia, including Vietnam, caused by plantborne and fishborne trematodes. Their complicated life cycles involve freshwater snails as first intermediate hosts. A cross-sectional study was conducted in Ha Vinh and Ha Duong communes, Thanh Hoa Province in April, 2018 to investigate trematode diversity with emphasis on FZTi in different freshwater snail species from different habitats (river, canal, pond, and rice field). Among 25,576 examined snails, a total of fifteen snail species was found with significant differences between habitats. Of nine infected snail species, twelve different morphological types of eight cercariae groups were detected with an overall prevalence of 3.0% (amphistome, armatae, brevifurcate-pharyngeate, echinostome, monostome, megalurous, parapleurolophocercous and vivax). The highest overall infection was 57.7% in snails collected from rice fields while the lowest was 1.68% in ponds. Rivers were found to harbor the most diverse trematode fauna (9 types). *Melanoides tuberculata* was the most preferred intermediate host with high prevalence of infection (12.2%). Monostome emerged as the most common cercaria, contributing 49.9 % of all infections, followed by echinostome (27.7%). Some of cercaria groups found in this study might be potential FZTi such as small liver flukes (parapleurolophocercous) and intestinal flukes (amphistome, echinostome and parapleurolophocercous). *Fasciola* spp were not recorded in this study. However, molecular identification of cercariae is required to give an insight into trematode species composition, especially for FZTi.

---

## Particular neutrophils mediate environment-driven onset of airway allergy through NETs release

C. Radermecker[1], C. Sabatel[1], S.L. Johnston[2], M. Toussaint[2], F. Bureau[1], T. Marichal[1]

[1] GIGA-R, Cellular and Molecular Immunology, University of Liege, Liege, Belgium
[2]Airway Disease Infection Section, National Heart and Lung Disease Institute (NHLI), Imperial College London, London, UK
***Corresponding author:*** *c.radermecker@uliege.be*

Environmental changes are responsible for the dramatic rise in the prevalence of asthma worldwide. Decreased exposure to microbial products such as lipopolysaccharide (LPS) and respiratory viral infections represent two major risk factors for asthma, yet the mechanisms linking such conditions and host allergic susceptibility remain unclear. First, we set up two mouse models, a virus-induced asthma model and a model of asthma promoted by exposition to low LPS doses. In these models, only previously infected mice or mice exposed to low LPS doses displayed the characteristics of asthma following sensitization and challenge to house dust mite (HDM). Then, using single-cell RNA sequencing, we found that pro-allergic environments (low LPS doses and respiratory virus) induced the recruitment into the lungs of $CXCR4^{hi}CD49d^{high}LAMP\text{-}1^{high}$ neutrophils releasing neutrophil extracellular traps (NETs). The role of NETs in asthma onset was then demonstrated using three NETosis inhibitors in our models. Infected or low LPS doses exposed mice exhibited strong decrease of all asthma features when treated with NETs inhibitors compared to non-treated mice. Finally, to address how NETs promote the induction of a Th2 immune response, we analysed by flow cytometry the distinct subpopulations of lung dendritic cells (DCs) in our models. We observed, during the NETs release phase, a recruitment of monocytic-derived DCs responsible for allergic sensitization. This recruitment was abrogated when NETS were inhibited. In conclusion, our study reveals how unrelated environmental risk factors commonly shape immune responses, by recruiting particular neutrophils which release NETs, to promote asthma.

# Short talks

## Development of alternative methods to RT-qPCR to detect and quantify infectious norovirus particles in food

Razafimahefa R.[1], Ludwig-Begall L.[1], Mauroy A.[1,2], Thiry E.[1]
[1.] *Vet. Virology and Animal Viral Diseases, Department of Infectious and Parasitic Diseases, FARAH, ULiège;* [2.] *Staff Direction for Risk Assessment, Control Policy, Federal Agency for the Safety of the Food Chain, Brussels, Belgium*
***Corresponding author:*** *etienne.thiry@uliege.be*

Human noroviruses are a major viral cause for gastroenteritis outbreaks. Molluscs, which filter contaminated water and accumulate noroviruses in their digestive tissues, are a typical vector for human infection. Since *in vitro* culture of human noroviruses is not viable for routine analysis, the murine norovirus is used as surrogate. RT-qPCR, the established molecular method for detection of human noroviruses in food, does not allow the distinction of infectious and non-infectious viruses. Our study addresses this issue by using intercalating agent propidium monoazide (PMA)-treatment prior to RT-qPCR on murine noroviruses. PMA has been shown to enter only viruses with compromised capsids; subsequently viruses with intact capsids remain impermeable and thus yield detectable signals via RT-qPCR. We have shown PMAxx, a new version of PMA, to be efficient in partially differentiating infectious from non-infectious viruses in PBS. A further investigation to adapt this detection in complex matrices (mussel digestive tissues) is ongoing. The ISO-norm for virus extraction incorporates proteinase K treatment causing capsid damages and thus impairs the recovery of infectious viruses. We developed an alternative mechanical extraction that needs improvements for more suitable results. Another method relies on flow cytometry coupled to RT-qPCR (long range RT) to assess genome integrity. A preliminary sandwich ELISA assay presented promising results for further experiments to assess sensitive differentiation between treated (UV, heat) and untreated viral protein epitopes by flow cytometry. The final objective is to detect and quantify only infectious noroviruses in naturally contaminated mussels via these methods.

---

## The lipid phosphatase SHIP2/INPPL1 controls chondrocyte hypertrophy and matrix mineralization in a murine model for opsismodysplasia

Vande Catsyne C.[1], Molina-Ortiz P.[1], Sayyed S.[1], Moës B.[1], Azzi H.[1], Muller J.[2], Heusschen R.[2], Caers J.[2], Schurmans S[1].
[1.] *Laboratory of Functional Genetics, GIGA-Molecular Biology of Disease, ULiège, Belgium.*
[2.] *Laboratory of Hematology, GIGA-Inflammation, Infection & Immunity, ULiège, Belgium.*
***Corresponding author:*** *cavandecatsyne@ulg.ac.be*

Opsismodysplasia is a human skeletal dysplasia characterized by delayed bone maturation. Most patients have a facial dysmorphism with shortened long bones associated with mutations in the gene INPPL1 that codes for the SH2 domain containing inositol 5-phosphatase 2 (SHIP2). These symptoms are shared by $SHIP2^{\Delta/\Delta}$ mice of which this enzyme has been catalytically inactivated in all cell types. The goal of this study is to analyze in these deficient mice the endochondral ossification process as the mechanism by which the lack of SHIP2 affects this process in patients with opsismodysplasia is unknown. Histological analysis of $SHIP2^{\Delta/\Delta}$ mice confirmed shortening of long bones (femur and tibia) and the reduced size of the tibial growth plate caused by a decreased number of hypertrophic chondrocytes. Micro-CT analysis have shown that $SHIP2^{\Delta/\Delta}$ mice also expressed less mineralization in the primary spongiosa of femur. Following inactivation of SHIP2, *in-vitro* micromass culture of murine chondrocytes expressed less mineralization of the matrix, increased phosphorylation of FGFR3, MEK and ERK proteins and increased osteocalcin mRNA synthesis. To address the hypothesis that delayed mineralization could be due to increased osteocalcin hormone synthesis, we have transduced lentivirus in ATDC5 cells to knock-down mRNA osteocalcin with shRNA. We successfully managed to restore mineralization in absence of SHIP2 by reducing level of osteocalcin. Altogether, these results show that SHIP2 protein controls *in-vitro* formation of matrix mineralization and meet what we observed in *in-vivo* murine model. In the future, it could herald a treatment if our results are confirmed in human patients.

# Short talks

# Posters

# Veterinary Public Health

# Posters

## Schmallenberg virus over time: serological follow-up of the wild cervids population to characterize the endemicity in Wallonia

Bayrou C.[1], Volpe R.[2], Lesenfants C.[2], Coupeau D.[3], Muylkens B.[3], Desmecht D.[1] and Linden A.[2]

[1.] *Dpt of Morphology and Pathology, FARAH, ULg*
[2.] *Surveillance Network of Wildlife Diseases, Dpt. Of Infectious Diseases, FARAH, ULg.*
[3.] *Veterinary Integrated Research Unit, Faculty of Sciences, Namur Research Institute for Life Sciences (NARILIS), University of Namur (UNamur), 5000 Namur, Belgium.*

***Corresponding author:*** *calixte.bayrou@ulg.ac.be*

Since the end of 2011, the Schmallenberg virus (SBV) widely spread across Europe causing an epizootic outbreak of abortions and congenital hydranencephaly-arthrogryposis syndrome in domestic ruminants. In Wallonia, after only nine months of SBV circulation, the seropositivity in the bovine population reached 91% (Garigliany *et al.,* 2012). This very high level of seroconversion was the sign of the very efficient spreading of the virus among ruminants. After the epidemic phase, the question arose of an endemicity of the virus in Europe. We herein present the long-term follow-up of the seroprevalence of SBV in wild cervids in Wallonia from 2012 to 2017. Our data show the virus was widely circulating in 2016 by contrast with the other post-epidemic years. Those data support the assumption of a 5 years cycle of re-emergence as it was reported for the related Orthobunyaviruses in endemic countries.

---

## Detection of *mcr* genes conferring colistin resistance in *Escherichia coli* isolated from different animal species in Belgium between 2008 and 2011

Berrah A., Thiry D.*, Muylaert A., Duprez J.-N., Mainil J.

*Bacteriology, Department of Infectious and Parasitic Diseases, FARAH and Faculty of Veterinary Medicine, ULiège, Belgium*

***Corresponding author****: damien.thiry@uliege.be*

In November 2015, the first plasmid-located transferable mechanism of resistance to colistin was identified in a porcine isolate of *Escherichia (E.) coli* in China. During the following years several other *mcr* (after "mobilized colistin resistance") genes were described (*mcr-2* to *mcr-8*) in different bacterial species. Today the *mcr* genes have been identified worldwide in food-producing animals (cattle, pigs, poultry). But amazingly enough, some *mcr* genes have also been detected in enterobacteria isolated many years before 2015. The original purpose of this study was to test collections of *E. coli* isolated in Belgium between 2008 and 2011 from canaries, cats, cattle, cervids, dogs, ducks, hedgehogs, horses, humans, rabbits, hares, piglets, poultry, reptiles and vultures, for the presence of *mcr* genes. A total of ca. 4500 *E. coli* isolates were streaked on LB agar plates containing 1 µg/ml of colistin. The growing isolates were tested with a pentaplex PCR for the mcr-1 to mcr-5 genes. Of the 459 growing E. coli, 45 tested positive for the *mcr-1* gene (from 26 bovines, 3 cervids, 2 hedgehogs, 12 piglets and 2 reptiles), 28 for the *mcr-2* gene (from 5 bovines, 1 rabbit, 21 piglets and 1 poultry), 4 for the *mcr-4* gene (from 1 bovine and 3 piglets), and 6 for the *mcr-5* gene (from 6 piglets). Two porcine *E. coli* tested positive with more than one PCR. Our results confirm the presence of *mcr* gene(s) in *E. coli* isolated years before 2015, not only from domestic animals but also from wild mammals and birds and from reptiles. The pentaplex PCR-negative isolates will be further tested for the presence of the *mcr-6* to *mcr-8* genes and of chromosome-mediated colistin resistance mechanisms.

## Effect of plant extracts as dietary components on striped catfish fillets quality during iced storage

Dao N.L.A.[1,2], Degand G.[2], Brose F.[2], Douny C.[2], Phu T.M.[1], Quetin-Leclercq J.[3], Hue B.T.B.[4], Bach L.T.[4], Nhu T.Q.[1], Hang B.T.B.[1], Huong D.T.T.[1], Phuong N.T.[1], Kestemont P.[5], Scippo M-L.[2]

*[1] College of Aquaculture & Fisheries, Can Tho Univ., Vietnam; [2]Dept of Food Sciences, Fac. of Vet. Med., FARAH, ULiège ; [3]Louvain Drug Research Instit., Pharmacognosy research group, UCL; [4]College of Natural Sciences, Can Tho Univ., Vietnam; [5] Dept of biology, Fac. of Sc., Univ. of Namur.*

***Corresponding author:*** *nladao@ctu.edu.vn*

The aim of this study was to examine the effect of dietary supplementation with herbal extracts on the changes of striped catfish flesh during iced storage for 16 days. Five plant extracts were selected for experimental diets. Each extract was tested at two concentrations: *Euphorbia hirta*, *Mimosa pudica* and *Azadiracha indica* were added to the diet at concentrations of 0.4% and 2% (w/w), while 0.2% and 1% (w/w) of *Phyllanthus amarus* and *Psidium guajava* L. were used. Fish were fed daily at 3-5% of body weight for 2.5 months. After that, the fish flesh was collected, stored under ice and investigated for the changes of total viable counts (TVC), peroxide value (PV) and sensory properties during 16 days of storage. Results showed that fillets coming from fish fed with diet containing 1% of *P. amarus* or *P. guajava* displayed the lowest TVC and pH values during the early storage period. *P. amarus* and *E. hirta* were effective in retarding the lipid oxidation in striped catfish flesh, and *E. hirta* treatment allowed to retain good sensory characteristics. These results obviously showed that *P. amarus* at concentration of 1% could be a potential prospect for its both natural antioxidant and antimicrobial properties, and that it could be used in aquaculture to improve the quality of striped catfish fillet during post-harvest stage under iced storage.

---

## Abortion due to Salmonella spp. in southern Belgium, the last decade

Delooz Laurent[1,2], Saulmont Marc[1], Wesley Mattheus[3], Florence Crombé[3], Saegerman Claude[2]

*(1) Dépt Epidémiologie et Encadrement Sanitaire, Association Régionale de Santé et d'Identification Animales, Ciney (2) Research Unit of Epidemiology and Risk Analysis applied to veterinary science (UREAR-ULiège), FARAH, Fac. of Vet. Med., ULiège (3) Dépt des Maladies infectieuses humaines-Service scientifique des Maladies bactériennes humaines, Sciensano, Bruxelles*

***Corresponding author:*** laurent.delooz@arsia.be

Salmonellosis is a global disease of animals, with potential major economic impact and important zoonotic capacities. This disease is caused by Salmonella spp. and lead to abortion in cattle. In southern Belgium, seven different serovar of Salmonella enterica subsp. enterica were diagnosed this last decade. Since 2009 in Belgium, a national surveillance programme based on the reporting of abortions and analyses on their products reached several objectives including official surveillance of bovine brucellosis but also the monitoring of other bovine abortive diseases including systematically salmonellosis. The diagnosis is performed on a sample of abomasal fluid using a culture on blood agar and a consecutive serotyping of Salmonella from positive culture.
From 2009 until august 2018, 36,969 aborted fetuses were analysed in southern Belgium. The study revealed that 1.83% (95% CI: 1.69-1.97) of the abortions are caused by salmonellosis and that S. enterica serovar Dublin was the most frequent isolated serovar then Typhimurium, Enteritidis, Livingstone, Choleraesuis, Braenderup and Montevideo (in decreasing order of frequency). These five last serovars are not currently included in Salmonella vaccines distributed in Belgium. Despite significant differences in prevalence according to the year and the fact that Salmonella infects cattle throughout the year, significant less numerous positive results are observed in February, March and April (OR = 0.021 [95% CI: 0.017-0.027]; $p < 0.001$) in comparison with other months. The exact origin of the disease seasonality remains unknown but the grazing and the favorable climatic conditions seem to be the most likely explicative hypothesis.

# Posters

## CHROMagar™ Mastitis medium: assessment of the new formulation and comparison with the previous one

Didesse E.[1], Duprez JN.[2], Mainil J.[2], Thiry D.[2]
[1.] *HEPL André Vésale, Bachelor's degree "Medical Laboratory Technologist"*
[2.] *Department of Infectious and Parasitic Diseases, Bacteriology, FARAH, ULiège*
**Corresponding author:** jean-noel.duprez@uliege.be

CHROMagar™ Company has developed several chromogenic culture media specific either for bacteria or for pathologies. Among them, in 2016, this firm has developed two media for the research of the main bacterial pathogens in the context of bovine mastitis: the CHROMagar™ Mastitis medium (Gram-positive and Gram-negative). A previous study highlighted several problems with this formulation (selectivity of Gram-negative medium, difficulty of differentiation of some genus, growth of common contaminants). This study aims to assess a new formulation composed of 3 media: Gram negative, Staphylococcus and Streptococcus and to compare them with the old formula and the usual media. Sensitivity and specificity were calculated from pure strains while ease of handling was evaluated on milk samples. Moreover, the ease of the medium preparation from powders has also been compared. The new formulations solved the Gram-negative medium selectivity problem, improved the detection of the main Gram-positive bacteria by allowing them to grow more easily and by eliminating some common contaminants. Nevertheless, a new problem of Staphylococcus differentiation appeared, some pathogen agents were no longer detected and the media preparations were more difficult.

---

## Analysis of volatile short-chain fatty acids in different biological samples with SPME-GC-MS

Douny C., Verachtert P., Brose F., Scippo M-L.

*Department of Food Science, Faculty of Veterinary Medicine, FARAH, University of Liège, Liège*
**Corresponding author:** cdouny@uliege.be

Volatile short-chain fatty acids (SCFA) belong to a group of aliphatic monocarboxylic acids with low molecular weight and short chain length (C2–C6). The nature and concentration of these organic compounds are of interest because they are natural products from the degradation of organic matter constituting key intermediate metabolites in many biological processes (Raposo et al, 2015).
Therefore, a GC-MS method has been developed to evaluate the concentration of seven short-chain fatty acids in biological samples: acetic acid (C2), propionic acid (C3), butyric acid (C4), isobutyric acid (iC4), valeric acid (C5), isovaleric acid (iC5) and hexanoic acid (C6).
SCFA were extracted using solid phase micro-extraction (SPME) with a DVB/CAR/PDMS fibre (Supelco), separated on a Focus GC gas chromatographer (Thermo Fisher Scientific) using a Supelcowax-10 column (30 m × 0.25 mm, 0.2 µm) (Supelco) and analyzed with an ion trap PolarisQ mass spectrometer (Thermo Fisher Scientific). The peaks were identified by comparing their mass spectrum and retention times with those of the corresponding standards.
Until now, the developed method was applied to quantify SCFA in gastrointestinal juice coming for the SHIME, ruminal juice and bovine blood.
Performances of the analytical method were evaluated using two Quality Control (QC) samples containing SCFA at different levels. Those QC were analyzed 10 times each, on different days. The calculated coefficients of variation obtained under reproducibility conditions varied between 5.7-15.3% and 3.5-6.3% for the 7 SCFA in QC1 and QC2, respectively. The trueness obtained varied between 105.0-122.4% and 97.9-105.2% for the 7 SCFA in QC1 and QC2, respectively.

References: Raposo et al. (2015) Journal of Chromatography A, 1413, 94–106.

## Adaptation and assessment of the L.O.U.I.S test for a fast detection of *Salmonella sp.* and *Yersinia sp*. in veterinary medicine

Duprez JN., Mainil J., Linden A.

*Department of Infectious and Parasitic Diseases, Bacteriology, FARAH, ULiège.*
***Corresponding author:*** jean-noel.duprez@uliege.be

With the announced disappearance of the API biochemical gallery, the problem of simple and relatively fast bacteria identification will arise. In 2004, Wilson G. developed an economic test allowing a rapid detection of *Salmonella* and *Shigella* species in human medicine: Lysine, Ornithine, Urease, Indol Screenig test (L.O.U.I.S test). It announces a sensitivity of 100% and a sensitivity of 94%. This study proposes to evaluate this test for the detection of *Salmonella* and also for the research of *Yersinia* in veterinary medicine. Indeed, for the research of these genus the medium usually used in our laboratory (BPL modified medium and Yersinia CIN) gives many false positive colonies causing a significant loss of time and money. A total of 50 bacterial strains currently isolated during *Salmonella* / *Yersinia* research in animals and 18 control strains were tested. A score was given for each result to evaluate the sensitivity and specificity of the LOUIS test in veterinary medicine. We obtained respectively 100% and 83.3% for *Salmonella* and 82.7% and 96.3% for *Yersinia*.

---

## Identification of unconventional O serotypes of enteropathogenic (EPEC) *Escherichia coli* from intestinal contents of healthy cattle at slaughterhouse

Ikeda R.[1], Habets A.[1*], Duprez J.-N.[1], Iguchi A.[2], Korsak N.[3], Thiry D.[1], Mainil J.[1]
[1] *Bacteriology, Parasitic and Infectious Disease Department, FVM, FARAH, ULiège.*
[2] *Depart. of Animal and Grassland Sciences, Fac. of Agriculture, Univ. of Miyazaki, Miyazaki, Japan.*
[3] *Food Inspection, Department of Food Science, FMV, FARAH, ULiège*
***Corresponding author:*** *Audrey.habets@uliege.be*

*Escherichia coli* producing the attaching-effacing (AE) lesion are called enteropathogenic *E. coli* (EPEC) and are responsible for diarrhoea in animals and humans. EPEC are subdivided into typical (t) EPEC producing the "Bundle Forming Pili" type 4 fimbriae and isolated from humans, and atypical (a) EPEC not producing the BFP and isolated from humans and animals, including diarrheic young calves and healthy adult cattle. tEPEC and several aEPEC belong to specific serotypes, but different aEPEC can also belong to classical Shigatoxigenic *E. coli* (STEC) O serotypes (O26, O103, O111, O121, O145, O157, O165) and derive from STEC after loss of genes encoding the Shiga toxins. In 2014 several hundreds of EPEC from healthy cattle at two slaughterhouses in Wallonia, but the prevalence of the classical STEC O serotypes was low and all were negative for the recently described O80 EPEC serotype. The aim of this study was to identify 6 other unconventional O serotypes (O123/186, O146, O156, O177, O182, O183) recently identified by PCR in different bovine aEPEC and STEC by one of us, amongst 312 EPEC isolated at slaughterhouse that previously tested negative, applying two triplex PCRs either for the O146_O182_O183 or for the O123/186_O156_O177 serotypes. So far the first triplex PCR identified 1 O146-positive and 8 O182-positive EPEC. The second triplex PCR identified 30 O156. The further steps of this study are: (i) the identification of still other unconventional serogroups among EPEC (ii) the identification of their H antigen-encoding genes; (iii) the comparison of these bovine EPEC between themselves, with EPEC from diarrheic calves and with STEC from cattle and humans belonging to the same serotypes.

# Posters

## First description of a genital hypoplasia in a striped dolphin (Stenella coeruleoalba) stranded in Northern France

Jauniaux T.[1], Dabin W.[2], Franssen M.[1], Gregoire T.[1], Karpouzopoulos J.[3], Sarlet M.[1], Garigliany M.M.[1]
*[1] Department of Pathology, FARAH, ULiege; [2] Observatoire PELAGIS, UMS 3462, Univ. de La Rochelle-CNRS, La Rochelle, France ; [3] Coordination Mammalogique du Nord de la France (CMNF), Alembon*
***Corresponding author:*** *t.jauniaux@ulg.ac.be*

Gonad atrophy has been described in case of hermaphrodism. In cetacean, two cases of hermaphrodism have been reported: a beluga whale *Delphinapterus leucas* from Canada and a common dolphin *Delphinus delphis* from UK. The two cases were considered as true hermaphrodism as two testicles and two separate ovaries were observed for the beluga while one ovotestis and a contralateral ovary were reported for the dolphin. In July 2017, an adult striped dolphin (*Stenella coeruleoalba*) stranded in northern France, displaying swimming disorders compatible with a neurological syndrome. Externally, the phenotype was a female with a relatively caudal genital slit separated of the anus by 2 mammary gland slits. The dolphin weighted 63 kg and was severely emaciated. At the necropsy, no mammary gland tissue was reported under the slit while a micro-penis (18 mm) was present in the genital slit. Macroscopically, gonad could not be identified, only a 2cm on 0.5cm organ was present unilaterally on the left abdominal wall at the place of gonad. Samples have been collected for histology, microbiology and genetic. Under the microscope, the most significant observation was a severe non-suppurative meningo-encephalitis. The organ on the left abdominal wall was a testis that exhibited complete hypoplasia without spermatogenic activity. The sex was genetically identified as a male. The animal was negative for Morbillivirus and Brucella. The origin of the penis and testis hypoplasia and the absence of contralateral gonad could not be determined and are considered as being congenital. For terrestrial mammals, such congenital defects could be associated intra-uterine pathogens infection.

---

## In vitro and in vivo susceptibility of methicillin resistant Staphylococci isolated from cow mastitis to lytic bacteriophages

Ngassam Tchamba C.[1], Duprez J.N.[1], Fergestad M.[2], De Visscher A.[3], L'Abee-Lund T. [2], De Vliegher S. [3], Wasteson Y. [2], Blanchard Y.[4], Lavigne R.[5], Mainil J.[1], Thiry D.[1]
*[1]Dept of Infectious and Parasitic Diseases, Bacteriology, FARAH, ULiège; [2]Dept of Food Safety and Infection Biology, Fac. of Vet. Med., Norwegian Univ. of Life Sciences, Norway; [3]M-team and Mastitis and Milk Quality Research Unit, Dept of Reproduction, Obstetrics and Herd Health,, Fac. of Vet. Med., Ghent Univ.; [4]Viral Genetics and Bio-security Unit, ANSES, Ploufragan-Plouzané lab., France; [5]Lab. of Gene Technology, Dept of Biosystems, KU Leuven, Heverlee, Belgium*
**Corresponding author**: *cngassam@student.uliege.be*

Methicillin Resistant Staphylococci (MRS) represent a potential hazard for public health via the inter-*Staphylococcus* transferability of the mobile "Staphylococcal Chromosome Cassette" (SCC) carrying the *mec* genes encoding the resistance. The aims of this study were to characterize MRS strains originating from Belgium and Norway, and to assess their susceptibility to lytic bacteriophages. Between May and December 2016, 273 staphylococci were isolated from milk samples of cows with mastitis in Belgium and Norway. After phenotypic characterization, the isolates were tested by disc diffusion assay for the cefoxitin and submitted to several PCRs targeting the *mec* A, *mec* C genes and SCC-mec types. The lytic activity of 3 bacteriophages (Romulus, Remus and DSMZ_105264) was then assessed *in vitro* (lysotyping) and *in vivo* (Galleria melonella larvae model) on a selection of strains including MRS and Methicilin Sensitive Staphylococci (MSS) at equal rate. A total of 10 isolates were detected as MRS including 5 *Staphylococcus aureus* (SA) and 5 *Staphylococcus non-aureus* (SNA). The lysotyping of 20 strains including the 10 MRS and 10 MSS (5 SA and 5 SNA) showed that 4 of them were lysed by Romulus, Remus including 3 MSSA from Belgium and 1 MSSA from Norway. No correlation were observed between the methicillin resistance and the lytic activity of the phages. The in vivo tests are now being carried out to assess the efficacy of these phages in Galleria mellonella larvae.

## Potential resident bacterial microbiota in udder tissues of culled cows sampled in abattoir

Pirard B.[1], Crèvecoeur S.[1], Taminiau B.[1], Daube G.[1]
[1] *Food Science Department - Microbiology, FARAH, ULiège*
***Corresponding author:*** barbara.pirard@uliege.be

Aseptic milking samples and microbiological analyses are used in routine for bovine mastitis diagnosis. In practice, it's difficult to sample mammary gland tissues (MGTs) out of risks for cows health or milk production. We thus design a study based on samples taken in abattoir. It aimed to identify, quantify, compare the mammary secretions (MSs) and MGTs microbiota of macroscopically healthy dairy bovine mammary glands, by microbiological analyses and amplicon sequencing. We harvested 13 couples of MSs and MGTs samples, originated from the same quarter of reformed cows. Aseptic milking has been done just before culling and MGTs had been taken of the carcasses on slaughter line. Total and specific microbiological counting and metagenetic analysis were performed. Results showed one main bacterial genus, *Corynebacterium*, generally found in the milk in higher proportions than in tissues. When it dominates clearly other populations in MSs, it can be found in the same quarter tissues. In case of identification of pathogenic bacteria in MSs, the same pathogens were detected in tissues from the same quarter but in very different proportions: higher for *Streptococcus uberis*, lower for *Staphylococcus spp* or *Enterococcus faecium*. Data show also that species evenness and beta diversity are greater in MGTs than in MSs. In contrast, species richness is higher in MSs samples. These results show a potential resident microflora in MGTs of culled cows in abattoirs but those must be completed and confirmed on a larger number of samples. Hypothesis about nature of such a resident flora will also have to be confirmed on producing cows before studying bacterial-host interactions.

---

## Genomic comparison of Anguillid herpesvirus 1 strains

Haiyan Zhang[1], Natacha Delrez[1], Yuan Gao[1], Nicolás M. Suarez[2], Andrew J. Davison[2], Maxime Boutier[1], Alain Vanderplasschen[1*]

[1] *Immunology-Vaccinology, Department of Infectious and Parasitic Diseases (B43b), Fundamental and Applied Research for Animals & Health (FARAH), Faculty of Veterinary Medicine, University of Liège, Liège, Belgium*
[2] *MRC-University of Glasgow Centre for Virus Research, Glasgow, United Kingdom*
***Corresponding author:*** a.vdplasschen@uliege.be

Anguillid herpesvirus 1 (AngHV-1) is a large and complex double-stranded DNA virus that belongs to the *cyprinivirus* genus of the *Alloherpesviridae* family. AngHV-1 is the etiologic agent of a lethal disease of both European and Japanese eels causing great economic loses to the aquaculture sector. To date, the genome sequence of only two AngHV-1 isolates have been published. In this study, we have sequenced a further 7 strains from different geographical sources and performed genomic analyses. The major findings were: (i) phylogenetic analyses revealed that the 7 strains sequenced in the present study and the two previously sequenced strains distributed in two genetic lineages; (ii) recombination detection analyses indicated the existence of 7 recombination events and suggested the existence of a third, as yet unidentified lineage; (iii) gene disruption analysis identified genes that are not essential to viral growth *in vitro*. This study illustrates the importance of multi-strain sequencing as a source of information to study virus evolution and gene function.

# Posters

## Cyprinid herpesvirus 3 and Anguillid herpesvirus 1 inhibit arsenite-induced stress granules

Yunlong Hu[1], Mamadou Diallo[1], Léa Morvan[1], Alain Vanderplasschen[1]

[1] *Immunology-Vaccinology, Department of Infectious and Parasitic Diseases, FARAH, Faculty of Veterinary Medicine, University of Liège*

***Corresponding author:*** huyunlong@doct.uliege.be

Different types of stress can induce phosphorylation of eIF2α by activation of five different eIF2α kinases: unfolded proteins activate PERK, oxidative stress activates HRI, amino acid deprivation activates GCN2, dsRNA activates PKR, and Z-RNA and Z-DNA activate PKZ. Phosphorylation of eIF2α leads to inhibition of protein synthesis and to the accumulation of stalled translation initiation complexes into dynamic cytoplasmic stress granules (SG). Incubation of cells with arsenite is a well know process to induce the expression of SG. Arsenite activates PKR and HRI kinases. Mammalian herpesviruses have been shown to expression proteins able to inhibit the formation of arsenite-induced SG as a consequence of the roles of these proteins in the inhibition of the cell autonomous immune system. Here, we investigated whether this property is conserved in alloherpesviruses infecting fish: Cyprinid herpesvirus 3 (CyHV-3) infecting common carp and Anguillid herpesvirus 1 (AngHV-1) infecting European eels. We observed that both virus are able to inhibit the formation of SG induced by arsenite treatment as long as the viral infection initiates at least 12 h before the stress of the cells. The similarity of the data and the phylogeny existing between the two viruses suggest a conserved mechanism of inhibition. Our aim is to identify and characterize the viral protein responsible of this effect among the proteins conserved between CyHV-3 and AngHV-1.

# Posters

# Sustainable livestock production

## Contribution to the study of the prevalence of digestive parasites in goat farms of the region of Laghouat

Becheur M.[1], Tennah S.[2], Hafsi F[2]., Ferhat F.[1], Medjelled N.[1], Laouadi M.[1,2], Ghalmi F.[2]

[1] *Department of Agronomy, University Amar Telidji,* BP 37G road of Ghardaïa 03000, Laghouat, *Algeria*
[2] *Laboratory of research Management of Local Animal Resources (GRAL), National Veterinary College of Algiers,* road Issad Abbes, El Alia, Algiers**,** *Algeria*
***Corresponding author:*** *fghlmi@yahoo.fr*

The objective of this work is to the study of the prevalence of digestive parasites on goats in the region of Laghouat and determined the effect of the age, sex, breed and type of breeding. Our study was realized over a period of two months (February and March 2018) on a sample of 97 animals. We collected 97 faecal samples from 11 farms. The methods used for the coprological examination were flotation and sedimentation. On the 97 goats used, 12 were infested with a general prevalence of 12.37%. This also made it possible to identify several parasitic species, namely: one species of protozoa (Ei*meria spp* with a rate of 9.28%), three species of nematodes (N*ematodirus spp* with a rate of 4.12%, *Trichuris spp* with 2.06% and Co*operia spp* with 1.03%), and one species of trematode (*Fasciola hepatica* with a rate of 1.03%). The age, sex, breed, and type of breeding had no significant effect on prevalence recorded. It would be interesting to deepen this study by increasing the sample and adding autumn and winter to evaluate the effect of seasons.

---

## Phenotypic and morpho-biometric characterization of the Algerian dromedary populations in southern Algeria

Bouzid H.[1], Tennah S.[1], Azzag N.[1], Ghalmi F.[1], Kafidi N.[1], Moula N.[1,2]

[1] *Laboratory of research Management of Local Animal Resources (GRAL), National Veterinary College of Algiers, road Issad Abbes, El Alia, Algiers, Algeria*
[2] *Fundamental and Applied Research for Animals & Health (FARAH), University of Liege*

***Corresponding author:*** tensaf2004@yahoo.fr

Aim of this study was to characterize the Algerian dromedary in the steppes and sub-Saharan area for their phenotypic and morpho-biometric characters. The characterization study was focused on the observations on external appearance of the dromedary. The observations were expressed in percentage for the qualitative characters and in average for the quantitative characters. In total, 17 characters were studied (4 qualitative and 13 quantitative). From January through September 2017, 177 adult camels (164 females and 13 males) were studied. The camels were distributed in five study regions (Laghouat, M'sila, Djelfa, Biskra and El Oued). The results of this study showed a high frequency of brown color in female camels. Neck and chest circumference, and size of the hump by sex were significantly (<0.05) different among the wilayas of M'sila and Laghouat. Principal Component Analysis (PCA) and Agglomerative Hierarchical Clustering (AHC) for quantitative measurement of the traits categorized the males and females in 3 different groups for both. The respective variance between these 3 groups represents 53.07% and 78.22% of the total variance. The results of this study can be a basis for the description and standardization of dromedary populations, in the region of steppes and sub-Saharan in Algeria.

# Posters

## Camel breeding system and management of animal genetic resources in the steppes and sub-Saharan area of Algeria

Bouzid H.[1], Tennah S.[1], Kafidi N.[1], Antoine-Moussiaux N.[1,2]

[1] *Laboratory of research Management of Local Animal Resources (GRAL), National Veterinary School of Algiers, road Issad Abbes, El Alia, Algiers, Algeria*
[2] *Fundamental and Applied Research for Animals & Health (FARAH), University of Liege, Boulevard de Colonster, 20, building B43, 4000 Liege, Belgium*

***Corresponding author:*** *nantoine@uliege.be*

The objective of this work is to identify breeding systems in the study area and to determine the management practices of the camel genetic resources in order to make the dromedary farming systems sustainable. Direct interviews were conducted with 37 breeders in the steppe and sub-Saharan zone of Algeria. The survey included open and closed questions. The data were subjected to a general descriptive analysis. Multiple correspondence Analysis (MCA) and agglomerative Hierarchical clustering (AHC) were conducted on variable selections to differentially diagnose the context of genetic resources management. About herd mobility, three quarters of the farms were sedentary (73%), 19% practiced seasonal transhumance and only 8% were nomads. Livestock was the main activity of 54% of respondents, herds associating mainly dromedary at goats. The results show a global management of camel herds in southern Algeria highly submitted to the tradition. The sale of milk was a cultural impossibility for 52% of breeders. The purpose of breeding was for a long time and still remains a matter of social positioning and a form of capitalization. This mode of management influences the structure and composition of the herd. Support for breeders wishing to engage in commercial breeding should be considered, including breed improvement intend. The strong tradition will probably be an asset in a concomitant strategy for the conservation of the genetic diversity, which remains to be characterized.

---

## Effect of cow dung and manure of laying hens on growth and reproduction of *Eudrilus eugeniae*

Byambas P.[1,3], Lemtiri A.[2], Francis F.[2], Begone Ndong T.[3], Hornick JL.[1]

[1] *University of Liège, Faculty of Veterinary Medecine, Veterinary Animal Resource Management, Avenue Cureghem 6 bât. 43. B-4000 Liège, Belgium*
[2] *University of Liège-Gembloux Agro-BioTech. Functional and Evolutionary Entomology. Passage des Déportés, 2. B-5030 Gembloux, Belgium*
[3] *National Center of Technology and Scientific researches (CENAREST), Forestry and Agricultural Research Institute (IRAF), Trois quartiers, BP: 2246 Libreville, Gabon*

***Corresponding authors:*** jlhornick@ulg.ac.be ; manherbee@yahoo.fr

The objective of this study was to compare the effects of cow dung and manure of laying hens, associated to one of three carbonish substrates, on weight and population of *E. eugeniae*. Each substrate was sown with twenty (20) adult worms, 0.7 to 1 g weight. The weight of worms was measured at the beginning and at the end of breeding. The number was collected weekly until 60 days of breeding. The average number of worms increased by 2.38 (60±6.78 in cow dung, 40±6.81 in manure of laying hens and 43±8.23 in control). The average weight of worms was 27.19±1.21, 24.41±1.20, and 22.41±1.44 g, respectively. These results suggest that cow dung is better than manure of laying hens for vermiculture of *E. eugeniae*.

## Delphi method study about the need for stakeholder involvement in an indigenous cattle-breeding program in developing countries

Y. Camara [1,2], B. Govoeyi [2], N. Moula [2], M. M. Sissokho [1], N. Antoine-Moussiaux [2*]

[1] *Institut Sénégalais des Recherches Agricoles, Kolda, Senegal*
[2] *FARAH, Sustainable Animal Production, ULiège,*

***Corresponding author:*** *nantoine@uliege.ac.be*

Stakeholder involvement is key in breeding programs' success. The identification of stakeholders, their categories, respective role and weight in the overall process, therefore constitutes a crucial aspect of animal breeding. This study mobilises the Delphi method to collect expert opinions on these elements of breeding scheme design. In the 1st and 2nd rounds, 17 and 12 experts answered our questionnaires respectively. In the 1st round, 18 stakeholders were cited; the most frequent are State, research and farmers (65%, 57%, and 47%). Statistical analysis reveals a disparity in assigned scores (1 to 4.5) and a moderate consensus on the importance of stakeholders (Kendall W=0.4, P<0.01). In the 2nd round, a strong agreement on the participation of breeders, State and research (92%, 75% and 67% respectively) and a low to moderate consensus on that of the breeders' cooperatives, the private sector and NGOs (33%, 33%, and 25%) are revealed. A divergence on the importance of State actions and breeders (75% and 67% of interquartile responses) also appears. Between the 2 rounds, experts views converge on stakeholder involvement (Wilcoxon test W=31, P<0.05) but differ on their importance (Wilcoxon test W=38, P<0.05). The qualitative and factorial correspondence analyses gave three types of actors playing major roles: Group 1 (State, NGOs and funding institutions) has the roles of financing, subsidizing and capacity building; Group 2 (farmers and their organizations) is in charge of the breeding program management, the genetic progress production and the breed conservation; Group3 (Research) provides a scientific support (genetic and economic evaluation, technologies development).

---

## Spatio-temporal behavior of goats in forest pasture of northern Morocco

Chebli Y.[1,2], Chentouf M.[2] J. L. Hornick [1] and Cabaraux J.F.[1]

[1] *Department of veterinary management of animal resources, FARAH, IVT, Faculty of Veterinary Medicine, ULiege*
[2] *INRA, Regional Agricultural Research Centre of Tangier, 78 Av. Sidi Mohamed Ben Abdellah, Tangier, Morocco*
***Corresponding author:*** *ychebli@doct.uliege.be*

Grazing is associated with significantly different physical activities compared to confined goats. These activities are rarely studied despite their importance in the management and use of pastoral resources. The purpose of this study was to characterize the spatio-temporal movement and the distance travelled by goats in forest pasture of northern Morocco. To accomplish this work, eight goats were equipped with GPS collars, for 3 days during the spring and summer. The grazing area and distance travelled were estimated higher in summer compared to spring. This can be explained by low forage supply in pasture during drought season. The results obtained will be very useful to the enrichment of our database in order to develop a model for the rational management of silvopastoral resources in northern Morocco.

# Posters

## Diet composition of goats in forest pasture of northern Morocco

Chebli Y.[1,2], Chentouf M.[2] J. L. Hornick [1] and Cabaraux J.F.[1]

[1] *Department of veterinary management of animal resources, FARAH, IVT, Faculty of Veterinary Medicine, ULiege*
[2] *INRA, Regional Agricultural Research Centre of Tangier, 78 Av. Sidi Mohamed Ben Abdellah, Tangier, Morocco*
***Corresponding author:*** *ychebli@doct.uliege.be*

In forest pasture, diet selection of goats is affected by the interaction of several factors such as goat characteristics, climate and existing pastoral species. Understanding the diet composition of grazing goat will help to improve their productivity. The aim of this study was thus to determine the forage selection and the diet composition of goats grazing in a silvopastoral area in northern Morocco. Direct observation was used as a technique to evaluate grazing behavior of goats, during three consecutive days for three seasons (autumn, spring and summer). The goats spent more grazing time in autumn and summer. During this period, they performed the highest number of total bites. Intake rate and bite weight of palatable species depended significantly to the pastoral species and the season. The shrub was the most important part of the diet of goats. Goat remains an adapted animal to the seasonality of forest vegetation in northern Morocco.

---

## Lifetime of 5 non-commercial boluses evaluated in dairy cows by X Rays and plasmatic Trace Elements concentrations

Cheleux G.[1], Rollin F.[1], Wajda-Dubos J-P.[2], Chery L.[2], Liège P.[3,] Dubreucq P.[1], Busoni V.[1], Guyot H.[1]

[1] *Department of Production Animals FARAH, ULiège.*
[2] *Vétalis Technologies, Châteaubernard, France.*
[3] *Anistème Biosciences, Saint-Christol, France.*
***Corresponding author:*** *gcheleux@uliege.be*

Introduction: Boluses are commonly used to supplement cattle in trace elements (TE) but very few data can be found to confirm their behaviour and lifetime (LT).
Aim of the study: To check experimentally the presumed LT (20 to 180 d) of 5 non-commercial boluses, combining radiographic examinations and plasmatic TE assays.
Materials & methods: 5 experimental boluses were administrated each to 3 non-productive adult Holstein Friesian cows. Reticulum radiographs (RR) and plasmatic TE investigations were performed regarding a specific schedule depending on bolus specifications. Descriptive statistics include means ± SD, max. concentrations (Cmax), and max. concentration time (Tmax) of plasmatic TE. When possible, half-life time (T1/2) was calculated.
Results: RR showed that boluses were not always visible and could regularly move between reticulum and rumen. Thereby, a positive result confirms the presence of the bolus while a negative one does not exclude it. A burst of plasmatic TE concentrations within 7 d after administration has also been observed, followed by a decrease. Plasmatic TE assays showed that T1/2 tend to increase with bolus LT for selenium and cobalt.
Discussion: This burst could be the consequence of a most important TE release from the beginning and/or could be due to a progressive decrease of absorption of TE after 7 d related to homeostatic regulations, storage in the specific target organs and/or excretion in urine.
Conclusion: This original study is a premiere demonstrating that physical presence of a bolus is not always related to its biochemical activity. Further investigations are needed for a better characterization.

## Genetic counseling in horse

M. Dequenne[1], J. Detilleux[1]

[1] *University of Liège, Faculty of Veterinary Medicine, FARAH, ULiège*
***Corresponding author:*** *mdequenne@uliege.be*

Genetic disorders are frequent in dogs and horses. Evaluation of the risk of occurrence of such disease is important to reduce their transmission across generation. Evaluation of such risk is based upon information on genetic and non-genetic factors, and upon the utility value of the animal. The evaluation is not always straightforward which makes it difficult for horse care providers to advice clearly and intelligibly their clients. Bayesian networks (BN) are especially suited for such situation thanks to their transparency, flexibility to predict events by combining different sources of information and handling uncertainty. As an example, we propose a BN to evaluate the risk of an offspring suffering from Polysaccharide Storage Myopathy (PSSM1) when mating two horses phenotypically healthy. Clinical signs include inconvenient muscular dysfunction that prevents the horse to work properly. The responsible mutation is quite common in particular breeds. The transmission is autosomal dominant with incomplete penetrance. In the proposed BN, knowledge may be on the diet, test result and disease status of the parents. We derived other necessary information from current knowledge of PSSM1 mode of inheritance and sampled prior probabilities from Beta distributions when necessary. Arbitrary utilities (0 to 100) were given to the mating in line with the offspring expected genotype. All computations were done on Netica 5.12. Results showed the offspring of the mating has a probability of 4.6% and 27.3% to show PSSM1 symptoms if the diet is adequate or not. His/her probability to be homozygous recessive is 10.5% and 62.0% and the expected utility of the mating increases from 64.5 to 78.7 if one or both parents are healthy. With this example, one realize the method is useful to discuss objectively issues in genetic counseling. It is also fast to solve, easy to implement and it allows new information to be included while discussing with decision partners.

---

## Admixture mapping revisited: using stratification as an allied rather than an enemy

Dor A.[1], Frédéric Farnir[1].

[1] *FARAH/Sustainable Animal Production, Faculty of Veterinary Medicine, University of Liege (B43), 4000-Liege, Belgium*
***Corresponding author***: *f.farnir@uliege.be*

Genomic association studies aim at detecting associations between phenotypes (e.g diseases) and genomic regions. In that context, stratification - the simultaneous presence of several sub-populations in the sample - can be a confounding factor. Indeed, stratification tends to increase the number of false positive results: the association statistics will increase not only in the candidate region but also in other genomic regions unlinked to the studied phenotype. Many methods aim at controlling for stratification. Our idea is to try to take advantage of it. This idea is as follows: let's assume that a sampled population is made of 2 sub-populations from which we randomly sample cases and controls. The proportions of cases and controls sampled from each sub-population will usually differ. We demonstrate that the statistical test value calculated for each marker under the (null) hypothesis of absence of association in this context can become highly significant, leading to spurious association detections. This problem occurs when the allelic frequency of the tested allele differs between sub-populations and, simultaneously, when the sampled proportions of cases and controls in a sub-population differ. To exploit this result, we propose three procedures: the first one to control the false positive rate when the sub-population structure is known, the second one to increase the mapping accuracy using controlled mixture of sub-populations, and the third one to correct for stratification when the underlying structure of the sample is unknown.

# Posters

## Chemical composition of spiny cactus cladodes (*Opuntia ficus indica f. amyclae*) under age and collection period effect in Northern Morocco

El Otmani S.[1,2], Ayadi M.[2], Chentouf M.[2], Hornick J.L.[1] and Cabaraux J.F.[1]

[1] *Department of veterinary management of animal resources, FARAH, IVT, Faculty of Veterinary Medicine, Uliege*
[2] *INRA, Regional Center of Agricultural Research- Tangier, 78 Av. Sidi Mohamed Ben Abdellah, 90010, Tangier, Morocco*
***Corresponding author:*** selotmani@doct.uliege.be

In Northern Morocco, goat herd is the most dominant. His feeding is based on forest rangelands and characterized by strong seasonal variability responsible of the low productivity. Feed improvement is necessary. Cactus cladodes represent an available resource that can take their place in goat feeding. In order to introduce them, it is necessary to characterize their chemical composition. This work aims to determine the chemical composition of cactus cladodes according to age and collection period. Twenty four samples of young and old cactus were collected in three places in Tangier in four periods (April to June). In the laboratory, measurement parameters of cladodes were determined and samples were dried in oven at 60°C and subsequently ground and sieved to 1 mm diameter. The studied composition parameters were dry matter, ash, crude protein, ether extract, fibers and phenolic compounds. The chemical composition of these samples was determined by the methods of AOAC (1997). From results, age affected morphological measurements with superiority of mature cladodes ($P<0.001$). Length and width increased significantly with time ($P<0.001$). However, thickness and weight were similar in all periods. Nutritional quality of cladodes decreased significantly with ageing. Ash and fibers were lower and protein, moisture, fat and phenolic compounds were higher in young cladodes. Collection period affected moisture, protein, crude fiber, ADF and condensed tannins. Except ADF, these affected parameters decreased by time. In conclusion, cladodes have interesting chemical composition especially young cladodes that allowed them to be incorporated in ruminant feed diet.

---

## Phenotypic assessment of milk production in cattle rearing in the east of Algeria

Kerbache I.[1], Tennah S.[1,2], Azzag N.[1], Kafidi N.[1,2,3] , Ghalmi F.[1]

[1] *Laboratory of Local Animal Resources Management, Superior National Veterinary School of Algiers, Algiers, Algeria.*
[2] *Division of Genetics and Biostatistics, Department of Animal Production, Faculty of Veterinary Medicine, University of Liege, Liege, Belgium.*
[3] *Canadian Food Inspection Agency, Ottawa, Canada.*
***Corresponding author:*** tensaf2004@yahoo.fr

The aims of this study are to improve knowledge of dairy cattle breeding in the eastern part of Algeria and to characterize the effects of some key factors -related to livestock farming- affecting milk production and quality. A "follow-up-intervention" approach was adopted on six ranches over a period of ten months, illustrating the diversity of dairy cattle rearing situations in four cities in eastern Algeria: Mila, Setif, Constantine, and Jijel. In parallel, individual milk samples from 100 cows were collected monthly for production measuring and physicochemical quality analyzers. An analysis of the chemical composition of 23 food samples distributed daily in each farm was also carried out. Our results show that the average production per cow and per day was ranged from 19.48 to 25.41 kg, percent fat content from 3.11 to 4.25% and percent protein content from 2.69 to 3.37%. The herd effect is shown to be significant for the milk yield, the percent fat and the percent protein ($p< 0.001$). An effect significant of the breed ($P< 0.001$), the age at first calving ($P< 0. 01$), the number of lactation ($P< 0.05$) and season of calving ($P< 0.05$), were shown for all measurements.

## Contribution to study the prevalence of *Cryptosporidium spp* on goat farms in the Laghouat area

Laouadi M.[1,2], Tennah S.[2], Hafsi F[2]., Chettih I.[1], Naoum I.[1], Becheur M.[1], Ghalmi F[2].

*1 Department of Agronomy, University Amar Telidji, BP 37G road of Ghardaïa 03000, Laghouat, Algeria*
*2 Laboratory of research Management of Local Animal Resources (GRAL), National Veterinary College of Algiers, road Issad Abbes, El Alia, Algiers, Algeria*

***Corresponding author:*** *tensaf2004@yahoo.fr*

The objective of the present work was the search of *Cryptosporidium spp* on goat farms in Laghouat region, as well as the study of the relationship between the prevalence of this parasite with some factors, which are sex, age, type of breeding, breed and treatment. Our study was carried out on 97 animals, from 11 farms, on a period of 2 months (February and March 2018). For this purpose, 97 faecal samples were taken and the modified Ziehl - Nielsen method was used. Of the 97 goats studied, 25 were infested with *Cryptosporidium spp*, giving a general prevalence of 25.77%. Statistical analysis of the influence of the factors of variation on the general prevalence revealed no significant effects ($p<0.05$) about sex, age and treatment. On the other hand, the effect of the type of breeding and the breed were significant ($p<0.01$). Finally, the parasite prevalence recorded, despite moderate, must be taken seriously to avoid their detrimental effects on the health and zootechnical performance of the animals studied.

---

## Full Moon, or any other lunar phase, is not associated with a higher birth rate in cattle

Martinelle L.[1], L. Delooz [2,3], S. Graff[4], J.-L. Deville[5] and E. Moyse[6]

*[1] Experimental Station CARE-FEPEX, FARAH, ULiege*
*[2] Department of Biostatistics and Bioinformatics applied to Veterinary Sciences, Sustainable livestock production, FARAH, ULiege*
*[3] ARSIA asbl, Ciney, Belgium*
*[4] Pneumology Department, CHU Liege, Liege, Belgium*
*[5] CHR Verviers East Belgium, Verviers, Belgium*
*[6] Epidemiology and Risk Analysis Applied to Veterinary Sciences, Veterinary Public Health, FARAH, ULiege*
***Corresponding author:*** *lmartinelle@uliege.be*

There has been a persistent belief regarding the putative influence of the lunar cycle on birth rate in human as well as in cattle. Any parameter allowing to better anticipate and control the calving is of great interest to improve herd management.
In this retrospective study we examined a total of 476 043 calving occurring in 2015 in the Walloon region of Belgium to evaluate the influence of the lunar cycle on the distribution of cattle deliveries. Analyses were done using a linear model with the day of the week, the month, sex and type of cattle (dairy n=144005, beef n=280972 or mixed n=51066) as fixed effects.
Further analysis using linear regression on residual values showed that the month and the day of the week can explain 95 % of the births ($R^2_{adj}$ =0.949). A mixed model was used to test the effect of the moon (4 phases or 8 phases) on residuals. A clear "Tuesday effect" regarding an increased birth rate for dairy and mixed cattle was reported. This is most likely caused by calf fattening management in Belgium: male calves are mostly grouped by livestock dealers starting on Tuesdays. Indeed, livestock dealers do not buy calves before the age of two weeks. Therefore there is a bias in birth declarations in order to sell calves sooner.
When further testing according to a 4 or 8 phases lunar cycle there were no effect of any particular phase on birth frequency ($P$=0.07 and $P$=0.58, respectively, $R^2_{LR}$ = 0.003).
The results of the current study do not support the hypothesis of a relationship between lunar cycle and the frequency of calving in the Walloon Region of Belgium. Lunar phases are of no help to predict calving.

# Posters

## Feeding on larvae of black soldier flies doesn't substantially modify chicken caecal microbiota

N. Moula, J.-F. Cabaraux, E. Dawans, B. Taminiau, J. Detilleux
*University of Liège, Faculty of Veterinary Medicine, FARAH, ULiège*
***Corresponding author:*** *nassim.moula@uliege.be*

Although insects have been proposed to feed poultry, few studies have assessed their impact on microbiota even though it has an important role in chicken performance. Here, we aimed at characterizing the change in caecal microbiota of chicken fed larvae of black soldier fly (BSF) raised on horse manure. We collected larvae at the pre-pupal stage and stored them directly at - 20°C. In parallel, we raised 40 one-day-old male Ross chicks and individually fed them a commercial feed with either 0% or 8% of de-frosted BSF larvae. We collected bacterial DNA from samples of larvae, their growing substrate, and from the caecal content of the chickens for microbiota characterization with the software Mothur. We used analyses of variance to determine whether percent abundances of caecal operational taxonomic units (OTUs) were different in chicken receiving one or the other diet, after adjusting for the effect of replication. Around 20% of the genus *Dysgonomonas* were retrieved in de-frosted BSF larvae and in the growing substrate after their passage. This may be one of the mechanisms used by the larvae to transform manure because these bacteria have a fermentative metabolism producing acids and no gas. A total of 37 families and 5,275 species were detected in chicken caecal microbiota. *Firmicutes* were the most abundant phylum (90.83%) followed by the *Bacteroidetes* (6.93%). The majority of sequences within the *Firmicutes* phylum belonged to the families *Ruminococcaceae* (33.01%) and *Lachnospiraceae* (46.17%). No significant differences were found between experimental and control groups in the mean relative abundances of bacterial OTUs. However, relative abundances of both *Rhodobacteraceae* and *Bacillaceae* were very low (<0.5%) and significantly lower in the ceaca of birds receiving BSF than control diets.

---

## Effects of *Artemisia herba alba* and Olive leaf (Olea europaea) powders on growth performance, blood biochemical parameters and carcass yields of broiler chicks

Ait-Kaki A.[1], Diaw M.T.[2], Geda F.[3], Moula N.[3]
*[1]Fac. of Sci., Mhamed Bougara Univ. of Boumerdes, Algeria; [2]Dep. of Animal Production, University of Thiès, Senegal; [3]Animal Production Department, FARAH, Uliège*
***Corresponding author:*** *Nassim.Moula@uliege.be*

Like the other Mediterranean countries, Algeria has a diverse variety of potential plants that can be used in poultry production. The objectives of this study were to evaluate the effects of supplementing *Artemisia herba alba* (white wormwood) and olive leaf (*Olea europaea*) powders in diets of chicks on growth performance, blood biochemical parameters and carcass yields.
The study was conducted from April to May 2017 in the region of Chemini, Algeria. Sixty, one-day-old male chicks (Ross-308) were set up in a completely randomized design and divided into 3 groups of 10 chicks each in 2 replications. We fed group 1 chicks with a standard commercial diet (SCD); group 2 received the same SCD with 2% replaced by powder of *Artemisia herba alba* and group 3 the same SCD with 2% replaced by powder of *Olea europaea*. The chicks were housed indoor and fed *ad-libitum*. Blood samples were collected from the wing vein on 5 chicks from each group and plasma was analyzed for glucose, triglycerides, urea, total proteins and cholesterol contents. The chicks were starved for approximately 12 h when slaughtered at 42 days old. Growth performance parameters were recorded as follows: feed intake daily, body weights every two weeks, and carcass yields at the end of 42 days of age. The parameters of growth performance, blood biochemistry and carcass yields were subjected to one-way analysis of variance to identify the variations in effects between the dietary treatments and the effects were considered significant at $P<0.05$. Results showed that inclusion of *Olea europaea* significantly increased mean body weight at 42 days old (2117.42±26.38 g, 2230.10±26.38 g and 2336.66±27.88 g in groups 1, 2 and 3) and decreased glycemia (2.24±0.06, 2.05±0.06 and 1.90±0.06 g/L in groups 1, 2 and 3) and cholesterolemia (1.13±0.05, 1.03±0.05 and 0.95±0.05 g/L in groups 1, 2 and 3). There was no significant difference for the feed conversion ratio (1.79, 1.87 and 1.81 in groups 1, 2 and 3), carcass yield that varied from 67.34 to 68.74%, triglyceride (0.68 to 0.73 g/L), urea (0.03 to 0.04 g/L), and total proteins (26.02 to 27.11 g/L) blood concentrations.
The incorporation of Artemisia and olive leaf powders into the broiler diet improved body weight at slaughter with moderate changes in blood biochemical parameters.

## Effects of *Urtica Dioica* flour supplementation on performance, egg production and quality, and selected blood biochemical parameters in quails

Sadoudi A.[1], Touazi L.[2], Moula N.[1], Geda F[1].

[1]Animal Production Department, FARAH, ULiège
[2]Department of Agronomy, University Ferhat Abas, Sétif, Algeria

***Corresponding author:*** *Nassim.Moula@uliege.be*

The objective of this study was to evaluate effects stinging nettle (*Urtica Dioica*) flour supplementation on growth performance, egg production and quality, and selected serum biochemical parameters. A total of 144, 10-week-old Japanese quails (*Coturnix coturnix japonica*) were uniformly divided into three groups consisted of 12 quails in each of four replications. The control group received a basic diet containing 0% stinging nettle (SN) and two other groups received 3 or 6% SN flour for 12 weeks. Live weight, feed intake and egg production were recorded and feed conversion efficiency was calculated. Egg was evaluated for indoor and outdoor quality and the cholesterol content of the egg yolk. At the end of the experiment, blood samples were taken and serum samples were analyzed for serum Ca, Mg, inorganic phosphorus (Pi), triglycerides and total cholesterol. The results of this experiment indicated that the supplementation the diets of quails with 6% SN meal significantly reduced ($P<0.01$) egg yolk cholesterol and serum cholesterol and triglyceride levels with no negative effect on performance. In conclusion, the present study demonstrated that the supplementation of diets of quails with stinging nettle has potential to reduce cholesterol levels in egg yolk and serum and triglyceride level in the serum with no negative effect on performance.

---

## Biodiversity and phenotypic characterization of local chickens in the wilaya of Bejaia, Algeria

Saidani M.[2], Tennah S.[2], Moula N.[1,2]

*[1] Fundamental and Applied Research for Animals & Health (FARAH), University of Liege, Boulevard de Colonster, 20, building B43, 4000 Liege, Belgium*
*[2] Laboratory of research Management of Local Animal Resources (GRAL), National Veterinary College of Algiers, road Issad Abbes, El Alia, Algiers, Algeria*
***Corresponding author:*** nassim.moula@uliege.be

Algeria, the largest country in Africa, is characterized by its pedoclimatic diversity. The diversity of species and breeds of domestic animals is associated with different regions of the country. Like other species, chicken is present everywhere in Algeria with an exceptional phenotypic diversity. The objective of this study was the morphobiometric characterization of the local chickens in the communes of Bejaia province. The work was carried out from February through September 2017 and involved 40 households raising the local hens. A total of 199 adult chickens including 156 hens and 43 roosters were characterized morpho biometrically. The local chickens in Bejaia were characterized by a large phenotypic diversity; the color of the feathers showed 22 phenotypes including red (19.6%), black (14.57%), white (14.07%), golden (9.05%), tan (6.03%), partridge (5.53%), coppery black (3.52%), salmon (3.52%), wheat (3.52%) and gray (3.02%). The distribution of feathers, although having 7 phenotypes, was dominated by the normal distribution (62.31%). The yellow color of the legs was found to be the most represented phenotype (72.36%). The average body weight of the roosters (2337.44 ± 82.75 g) was found to be significantly greater ($P<0.001$) than the hens (1710.01 ± 43.44 g).
The results obtained from this study emphasize the importance of better utilization of the local populations. Therefore, further study (especially genetic characterization) is required to improve and conserve the local breeds that will ultimately promote their long-term economic role in their native regions.

# Posters

## Lactation curves of Algerian local rabbits does according to dietary energy content

Saidj D.[1,2], AinBaziz H.[2], Hornick J.L.[3] and Moula N.[3]

[1]*Veterinary Sciences Institute, Saad Dahleb University, B.P. 270, Route de Soomâa, 09000. Blida, Algeria.*
[2]*Research Laboratory "Animal health and Production" High National Veterinary School, Algiers, Algeria.*
[3]*Fundamental and Applied Research for Animals & Health (FARAH), University of Liege, Boulevard de Colonster, 20, building B43, 4000 Liege, Belgium*

***Corresponding author:*** nassim.moula@ulg.ac.be

Maternal milk, exclusive feed of the pups during their first days of life, must be of good quality and sufficient quantity. For this, the feeding of their mothers must meet all these needs.
In this context, a total of 53 nulliparous Algerian local rabbit does, 2744g±83 live weight, were allocated individually to three groups that received isoproteic diets (15% PD) differing in their digestible energy (2300, 2450 and 2600 kcal DE/kg for diets A, B and C respectively) during the two first lactations. Diets were supplied *ad libitum* during experimentation.
The use of high energetic diet did not show effects on does weight, feed intake and milk production whatever the parity with milk production mean of 2718g during the three first weeks of lactations. No significant differences were found in born or live litter size and birth weight (P>0.05).
Total milk production does not vary according to the energy content of the diet (2677g for A group *vs*. 2766g for B group *vs*. 2711g for C group ; P>0.05) However, peak lactation occurs at different times (18$^{th}$, 20$^{th}$ et 20$^{th}$ days *PP* at first lactation and 19$^{th}$, 20$^{th}$ et 18$^{th}$ days $^{PP}$ at second lactation for A, B and C groups respectively), with a significant effect of parity; the rabbit produces more milk at the 2$^{nd}$ lactation (2614g±54 *vs*. 2822g±67; p=0.02).
In our experimental conditions, the change in energy content had no effect on milk production, but could probably vary the chemical composition of the milk, which has not yet been achieved.

---

## Characterization of sheep farming in urban areas in Senegal

Thior Y. El H.[1,2,3], Hornick J. L.[1], Missohou A.[2], Moula N.[1], Cabaraux J.F.[1]

[1.] *Department of veterinary management of animal resources, FARAH, IVT, Faculty of Veterinary Medicine, Uliege*
[2.] *Inter-States School of Veterinary Science and Medicine, Dakar, Senegal*
[3.] *Ministry of Livestock and Animal Production, Dakar, Senegal*
***Corresponding author:*** *thioryakhya@yahoo.fr*

In order to characterize sheep farming in urban areas in Senegal (Dakar and Thies), a cross-sectional study was conducted between May 2016 and September 2017. Farmers are mainly male probably due to the high cost of maintaining sheep in urban area. Sheep farming was considered as a main activity by only 4% of them. Professional breeders had larger flocks and sold or slaughtered higher number of animals per year. Herds were of breeding type and females were predominant. Several sheep breeds were observed. However, the Ladoum breed created by Senegalese farmers and the Touabire breed were more represented with respectively 40% and 60%. The distribution of fodder, concentrates and minerals was systematic. Groundnut hay was the most used fodder and its price varied from 0.16 to 0.63 €/Kg, depending on the period of the year. Fodder and concentrate were therefore generally distributed in a rationed way. Kitchen wastes were usually distributed to decrease the cost of feed. Reproduction was controlled in different ways. According to farmers, the age at which young males and females started breeding was about 9 months old and was significantly higher among experienced breeders. Urban sheep farmers dewormed systematically their animals, on average 4 times a year, with Ivermectine and Albendazole. Vaccination was significantly more practiced by breeders belonging to breeder associations. The available area per sheep in sheepfolds was larger in Dakar than in Thies and in professional farms. Overall, urban sheep farming is carried out in an intensified way but is still constrained by missfeeding, inappropriate medication and non-optimal management of reproduction.

## Feasibility study for the establishment of a marine mammal stranding network in West and Central Africa: the example of Senegal

Wund S.[1], Jauniaux T.[1], Kadja M.C.[2], Kaboret Y.Y.[2], Antoine-Moussiaux N.[1]

*[1]Fundamental and Applied Research for Animals & Health (FARAH), Faculty of Veterinary Medicine, University of Liege, Liege.*
*[2]Interstate School of Veterinary Science and Medicine of Dakar, Dakar-Fann, Senegal.*
***Corresponding author**:* sarah.wund@gmail.com

The purpose of this work was to study the feasibility of the project of establishing aquatic mammals stranding surveillance networks in the countries of West and Central Africa, taking Senegal as an example. The feasibility study was conducted directly in the field and gave encouraging results. The monitoring and management of marine mammals strandings brings numerous benefits on different fronts: to prevent dead animals to be in contact with the population in an uncontrolled way while risks to human health exist; to use the evidence collected as bio-health indicators for the environment and draw conclusions about the risks associated with the consumption of fish products; to know the prevalence of potentially zoonotic diseases carried by aquatic mammals; to improve scientific knowledge of these animals to contribute to conservation and protection measures; and finally, to save stranded live animals when it's still possible. This approach is fully in line with the "One Health" concept which advocates the existence of a single health including that of humans, animals and environment. This explains the central role that veterinarians must play in such networks. The establishment of stranding surveillance networks allows monitoring to be regular and relevant. They have already proven it in many countries but unfortunately they are still rare in Africa. In order to really improve the monitoring and conservation of these animals in the region the ideal would be to established networks in all coastal countries and make links between them. This would allow, among other things, the results obtained to be representative of the overall situation of the region.

# Comparative veterinary medicine

## The fluidity of equine sperm membrane monitored by electron paramagnetic resonance (EPR) spectroscopy

Blommaert D.[1], Franck T.[2], Lejeune J.P.[1], Donnay I.[3], Serteyn D.[1,2,4], Mouithys-Mickalad A.[2]

*[1]LINALUX-MLS, Centre Européen du cheval, Vielsalm, Belgium. [2]Center for Oxygen R&D (CORD), (ciRM and FARAH), ULiège, Belgium. [3]Institut des Sciences de la Vie, Université Catholique de Louvain, Louvain, Belgium. [4]Equine Pole, Fundamental and Applied Research for Animals and Health (FARAH), Faculty of veterinary Medicine, Liège, Belgium.*
***Corresponding author**: didierblommaert@hotmail.com*

Background: The sperm quality might be affected during freezing causing loss of motility and cell viability decrease. Ochsendorf et al. studied the fluidity of sperm plasma membranes in fertile and infertile men by EPR spectroscopy.[1] We have previously shown that the use of INRA 96 extender enriched with hydroxypropyl beta cyclodextrin-cholesterol complex (CDC) and 1% glycerol (INRA96-CDC-G) compared to INRA-freeze (IF) medium, significantly improved the *in vitro* parameters of post thawing semen quality.[2]
Objective: this study aimed: i) to describe an EPR spectroscopy process to study the membrane fluidity of equine spermatozoa (SPZ) using 5-DSA and 16-DSA as probes on 5 stallions having a good fertility and a good quality with post-thawing ii) to compare the values obtained with two different extenders used in our Center.
Materials & Methods: Frozen semen in IF or INRA96-CDC-G was thawed at 37°C, centrifuged (600xg, 10min, 37°C) and SPZ were washed once with HBSS+glucose (HBSS/g) (pH 7.4). After washing, the resulting pellets was resuspended in HBSS/g to obtain $5x10^7$ cells in 95 µL + 5 µl of the probe (5-DSA or 16-DSA, 0.5 mM). Cells were then placed in EPR tube for analysis.
Results & Conclusion: Our results showed good EPR characteristic signals for each probe. Both media (IF and INRA96-CDC-G) had similar parameters: 5-dsa probe IF (2A//= 58.9±1.8G; I-hf=4.6±1.7) and INRA-CDC-G (2A//=55.6±4.8G; I-hf=5.9±1.3), likewise, for 16-dsa (IF: 24.37±5.82 G and INRA-CDC-G: 26.87±3.17 G). Altogether, our results indicate that EPR technique can be applied to evaluate the membrane fluidity of equine sperm cells and suggest that INRA96-CDC-G does not modify the membrane fluidity compared to IF medium.

---

## Proteins secreted by equine muscle-derived mesenchymal stem cells: preliminary results

Dechêne L.[1,2], Dieu M.[3], Demazy C.[2,3], Niesten A.[1], Ceusters J.[1], Franck T.[1] Renard P. [2,3] & Serteyn D.[1]

*[1] Center for oxygen R&D, Faculty of veterinary medicine, FARAH, ULiège*
*[2] URBC, Biology department, University of Namur, Belgium*
*[3] Mass Spectrometry facility of Unamur (MaSUN), Biology dep., University of Namur, Belgium*
***Corresponding author:** ldechene@uliege.be*

Horses are outstanding athletes suffering from similar injury as humans. Therefore, it is considered as the best model to study cells therapy for human musculoskeletal diseases. Equine mesenchymal stem cells (MSCs) are usually harvested from bone marrow or adipose tissue, two invasive techniques with a number of cells not always sufficient for cell therapy. To circumvent this, our team has developed a minimally invasive technique to harvest MSCs from muscle tissue (WO2015091210). Based on a muscular microbiopsy, this sampling method is easy to perform and yields a high number of cells. The therapeutic potential of MSCs is due to their capacity of homing, differentiation, and the secretion of paracrine factors. The immunomodulatory capacity of MSCs is mostly attributed by the secretion of bioactive factor. Harnessing the properties of MSCs secretome, defined as all proteins present in MSCs supernatant, potentially represents a future "stem cell-free" therapy for disease with excessive inflammatory response.
This study aims to analyse the proteins secreted by equine muscle-derived MSCs (emMSCs) by mass spectrometry and identify key actors involved in the immunomodulatory effect of MSCs. Secreted proteins were identified and compared with a sample of serum-free medium (without cell contact). The detected proteins were mainly involved in biological cell processes such as cell communication and adhesion. In addition, several proteins with anti-inflammatory and/or anti-oxidant properties were detected.

# Posters

## Skin Biopsies From Humpback Whales (*Megaptera Novaeangliae*) Collected In Mozambique : Immunohistochemical Investigation Of Cytochrome P450 1A1

Dessard F.[1], Gallego P.[2], Mazzariol S.[3], Jauniaux T[1].

[1] *Pathology, Faculty of veterinary medicine, Liège, Belgium*
[2] *Marine Biology, Faculty of Sciences, Liège, Belgium*
[3] *Pathology, Faculty of veterinary medicine, Padova, Italy*
***Corresponding author:*** *fanny.dessard@gmail.com*

The humpback whales studied in this work feed during the austral summer in the cold waters of Antarctica, a region far away from the sources of emissions of Persistent Organic Pollutants (POPs). Their status as mysticetes places them at a low level of the food web, limiting their contamination by these pollutants via food, since they accumulate over the rungs. These humpback whales would therefore be little contaminated. However, during the fast accompanying their annual migration, they face an energetic challenge resulting in huge lipid mobilization. The concomitant recirculation of lipidic POPs results in an increased risk of occurrence of their adverse effects. In this study, the detection of CYP1A1 in skin biopsies using immunohistochemical (IHC) techniques was used to investigate the effects of POPs contamination of humpback whales. The IHC staining method has been established to obtain semi-quantitative data. Our results demonstrate a heterogeneous distribution of CYP1A1 induction levels among individuals, with 42.5% of the samples expressing the enzyme at an low level. However, linear trend curves indicate that increasing the number of samples would result in a majority of induction at a low level. This corresponds to the expected results when we take into account the place in the trophic chain of these animals and their place of feeding. Cells expressing CYP1A1 are endothelial cells of arterioles and capillaries, smooth muscle cells of vascular walls and fibroblasts.

---

## Effect of constant rate infusion of fentanyl on physiological variables during isoflurane anaesthesia in horses

Vanaga J.[1,2], Dupont J.[1], Gougnard A.[1,3] Detilleux J.[4], Serteyn D[1]., Sandersen C.[1]

[1] *Veterinary Anaesthesia, FARAH – Comparative Veterinary Medicine, University of Liège, Liege, Liege, Belgium*
[2] *Equine Clinic, Faculty of Veterinary Medicine, Latvia University of Life Science, Jelgava, Latvia*
[3] *Equine Clinic, National Veterinary School Toulouse, Toulouse, France*
[4] *Quantitative Genetics, FARAH - Sustainable Animal Production, University of Liège, Liege, Liege, Belgium*
***Corresponding author:*** charlotte.sandersen@uliege.be

The aim of the study was to investigate the influence of a fentanyl constant rate infusion (CRI) on parasympathetic tone activity (PTA) derived from heart rate variability and other anaesthetic variables in isoflurane anaesthetised horses.
Twenty-one horses undergoing general anaesthesia for various indications were included in this study. Ten of them were randomly assigned to the fentanyl group and received fentanyl 0.572 µg $kg^{-1}$ IV followed by a CRI of 0.1 µg $kg^{-1}$ $minute^{-1}$ during isoflurane anaesthesia, while the control group (n = 11) did not receive fentanyl. A commercially available PTA monitor was used to derive PTA data from ECG signals. Data between groups were analysed with univariate and t-test analysis, the significance was set $p < 0.05$. Results are expressed as mean ± standard deviation.
There were no significant differences between fentanyl and control group in weight (mean 455.6 ± 137.1 vs 513.2 ± 43.2 kg), anaesthesia duration (136.0 ± 52.2 vs 138.2 ± 47.2 minute) heart rate (40.59 ± 7.1 vs 42.4 ± 11), mean arterial pressure (91.64 ± 16.58 vs 82.75 ± 18.33 mmHg), mean dobutamine requirement (0.5059 ± 0.4438 vs 0.5721 ± 0.3415 µg $kg^{-1}$ $min^{-1}$), tidal volume (12.05 ± 1.58 vs 11.07 ± 1.14 mL $kg^{-1}$), and peak inspiratory pressure (23.65 ± 4.45 vs 22.62 ± 7.16 $cmH_2O$). A significant difference was observed in PTA values, which were 71.31 ± 11.77 in the fentanyl group and 58.19 ± 8.01 in the control group.
In conclusion, fentanyl CRI during isoflurane anaesthesia led to higher PTA values, which may suggest higher levels of antinociception. The potential effect on minimal alveolar concentration of isoflurane still needs to be investigated.

## Pyocolpos in a spayed queen with imperforate hymen: a case report

Egyptien S., N. Shimizu, N. Anne-Archard, F. Billen, S. Noël, S. Deleuze

*Clinical department of veterinary sciences, University of Liège, Liège, Belgium*

***Corresponding author:*** *segyptien@uliege.be*

A 1-y-old sterilized queen was presented for dysuria. Abdominal palpation elicited pain and revealed a firm, well-circumscribed mass dorsal to the bladder. Ultrasonography confirmed a caudal fluid filled abdominal structure extending into the pelvic cavity, displacing the colon dorsally and the urethra ventrally. Retrograde vagino-urethrography showed contrast in the vestibule, urethra and urinary bladder. Imperforate membrane at the vestibulo-vaginal junction with secondary vaginal distension was highly suspected. During surgery, a distended by purulent content vagina was observed, sub-total vaginectomy was performed. Bacterial culture showed *Enterobacter cloacae*. Definitive diagnosis of imperforate hymen is usually achieved by vaginoscopy. Retrograde vagino-urethrography can be used in some cases. This is the first report of imperforate hymen in the queen and also is the first case of pyocolpos. Origin of infection remains unclear. Contamination during neutering or via partial perforation of the hymen may be suspected. *Enterobacter cloacae* is a Gram - opportunistic pathogen of the urogenital tract of humans and animals. It is involved in multidrug-resistance spreading but its prevalence and clinical impact in veterinary medicine is unknown. In conclusion, this first report of persistent hymen in the queen highlights vagino-urethrography usefulness for diagnosing imperforate hymen in small patients, as well as the inclusion of congenital abnormality in the differential diagnosis of dysuria and the feasibility of sub-total vaginectomy by abdominal approach. Finally, it raises the question of *Enterobacter cloacae's* implication in nosocomial infection in veterinary medicine.

---

## The integration of a hybrid teaching in veterinary radiology and its effects on fifth year students

Etienne A.-L.[1], Delfosse C.[2], Busoni V.[1]

[1] *Diagnostic Imaging Section, Comparative veterinary medicine, FARAH, ULiège*

[2] *IFRES (Institut de formation et de recherche en enseignement supérieur), ULiège, Belgium*

***Corresponding author***: AL.Etienne@uliege.be

The integration of a hybrid teaching in veterinary radiology was born following the plethora of students and the disappearance of the clinics for 5th year students. It aims to improve the understanding and mastery of radiology key concepts while decreasing the passivity of students, facilitating their involvement, stimulating their motivation and promoting their deep learning. The aim of this study was to determine whether this hybrid device has positive effects on involvement, motivation and quality of learning of students during their week of radiology seminar. This study was conducted at the University of Liège on a sample of 57 students. The hybrid device consisted of a panel of online resources, online tests, theoretical reminders, face-to-face feedback and an immersion in clinics. Students' perceptions of the global and motivational assessment of the device and their learning were collected via three questionnaires. The online institutional platform has provided objective data on student participation and performance. A non-parametric Wilcoxon test was performed to compare pre- and post-device test scores. 98% of students completed tests. They perceived a high value of the hybrid device. They significantly improved pre and post device test scores ($p < 0.00001$). This device promoted methods of deep learning among half of the students. 75% of students used their course notes, 25% used the online reminder module, and none used the book available online. This device has positive effects on students'involvement and motivation. The use of the various available resources, the responsibility of the students and a solicitation of a deep learning approach remain areas for improvement. This study reveals some encouraging data and enlightens on regulatory tracks.

# Posters

## Comparison between ultrasonographic and magnetic resonance imaging findings in the podotrochlear apparatus of horses with foot pain and without significant radiographic findings

Evrard L.[1], Joostens Z.[1], Busoni V.[1]

[1] *Clinical Department of Small Animals and Equidae, Diagnostic Imaging Section, FARAH, ULiège*
***Corresponding author**: levrard@uliege.be*

Ultrasound (US) and magnetic resonance imaging (MRI) findings in horses with foot pain have been described in the literature; however, direct comparison between MRI and US findings has only been sporadically reported. This study aimed to compare MRI and US findings in the podotrochlear apparatus in horses with foot pain and no significant radiographic findings. Feet from horses with front foot lameness and no radiographic diagnosis were prospectively included to undergo MRI and US examination. The same experienced operator, blinded to the MRI results, performed the US examinations. Lesions of the podotrochlear apparatus were independently recorded with both modalities and findings compared. Twenty-three feet responded to the inclusion criteria. Abnormalities of the podotrochlear bursa were seen in 19 feet by MRI and in 13 feet by US. Suprasesamoidean deep digital flexor tendon (DDFT) lesions were detected in 20 feet with MRI and 21 feet with US, while 11/17 sesamoidean and infrasesamoidean DDFT abnormalities detected at MRI were identified at US. Bone marrow lesions were seen in 15 feet at MRI. Erosions of the palmar compact bone were identified in 15 feet by transcuneal US and in 12 feet at MRI. US was able to identify certain lesions of the podotrochlear apparatus detected by MRI in horses with no radiographic changes. With a complete radiographic examination, US should therefore be part of the routine imaging work-up for foot pain, either because MRI is not financially affordable for the owner or simply because its ability to detect lesions and to guide injections makes this modality a useful and cheap complement to the MRI examination.

---

## Like curcumin, the soluble curcumin derivative NDS27 inhibits ROS production by neutrophils and acts as substrate and reversible inhibitor of myeloperoxidase

Franck T.[1,2], Aldib I.[3], Boudjeltia K.Z.[4], Furtmüller P.G.[5], Obinger C.[5], Philippe Neven[6], Martine Prévost[7], Jalal Soubhye[3], Pierre Van Antwerpen[3], Ange Mouithys Mickalad[2], Didier Serteyn[1,2]
*[1]Dept of Clinical Sci., Equine clinic, FARAH, ULiège; [2]Centre of Oxygen, Res. and Devt-CIRM, Inst. of Chem., ULiège; [3]Lab. of Pharm. Chem. & Anal. Platform of the Fac. of Pharmacy, ULB; [4]Lab. of Exp.Med., Univ. of Brussels, U 222, ISPPC, CHU A.Vésale, Montigny-le-Tilleul; [5]Dept of Chem., Div. of Biochem., BOKU–Univ. of Natural Resources and Life Sci., Vienna; [6]Lab. of Med.Chem.-CIRM, Fac. of Pharmacy, CHU Liège; [7]Struct. and Function of Biological Membranes, Fac. of Sci., ULB.*
***Corresponding author**: t.franck@uliege.be*

A water-soluble curcumin lysinate incorporated into hydroxypropyl-β-cyclodextrin (NDS27) has been developed and shown anti-inflammatory properties but no comparative study has been made in parallel with its parent molecule, curcumin on the neutrophil ROS production and myeloperoxidase (MPO) activity. The effect of NDS27, its excipients (hydroxypropyl-β-cyclodextrin and lysine), curcumin lysinate and curcumin were compared on the PMNs ROS production using a chemiluminescence assay and on the peroxidase activity of MPO. The action mechanism of curcumin and NDS27 on the MPO activity was refined by stopped flow and docking analyses. It is shown that curcumin and NDS27 exhibit similar inhibition activities on ROS released by PMNs. We demonstrate that both curcumin and NDS27 are reversible inhibitors of MPO by acting as excellent electron donors for Compound I ($\sim 10^7$ $M^{-1}$ $s^{-1}$) but not for Compound II ($\sim 10^3$ $M^{-1}$ $s^{-1}$), thereby trapping the enzyme in the Compound II state. Curcumin is shown to bind to the heme cavity by β-stacking and formation of hydrogen bonds involving substituents from both aromatic rings. Despite its excipients, NDS27 acts similarly to curcumin as reversible inhibitor of MPO halogenation and peroxidase activity. Docking calculations show that hydroxypropyl-β-cyclodextrin is too bulky to enter MPO channel leading to the binding site suggesting a full release of curcumin from the cyclodextrin thereby allowing its full access to the active site of MPO. In conclusion, the hydroxypropyl-β-cyclodextrin of NDS27 enhances curcumin solubilization without affecting its antioxidant capacity and inhibitory activity on MPO.

## Update of diagnostic algorithm used to categorize reported cases to the Atypical Myopathy Alert Group

François A.-C.[1], Renaud B.[2], Weber M.[1], Marcillaud-Pitel C.[3], Gustin P.[1], Votion D.-M.[2]
*[1].Dept of Funct. Sci., FMV, Pharmacology and Toxicol.,FARAH,ULiège; [2.]Equine Pole,FARAH,Fac. of vet.Med.,ULiège; [3.]Réseau d'EpidémioSurveillance en Pathologie Equine (RESPE), Caen*
***Corresponding author:*** acfrancois@uliege.be

Introduction: Equine atypical myopathy (AM) is an intoxication following ingestion of hypoglycin A (HGA) contained in seeds and seedlings from *Acer pseudoplatanus*. A system of case declaration named atypical myopathy alert group (AMAG) was set up in 2004 and opened to European cases in 2006. In order to use these data, cases have to be allocated according to their probability of being true cases of AM. With the discovery of the cause of AM, new diagnostic methods have emerged. The purpose of this study is to propose an algorithm (1) to classify cases communicated to AMAG, (2) to help practitioers to improve their detection of AM and (3), to help them to diagnose AM.
Methods: We updated a previous algorithm used to categorize cases as being confirmed (CC), cases with a high probability (HP), low probability (LP) of having AM, cases with other diagnoses (OD) than AM and doubtful cases (DC)[1]. In this previous algorithm, classification of cases was based on history, clinical signs, biochemistry and histology. Histology can give false-positive and false-negative results. In our updated algorithm, we added (1) HGA detection in blood (prove the exposure to the toxin), (2) detection of MCPA, its toxic metabolite and (3), a particular profile of acylcarnitines in blood that signs the pathophysiological process of AM.
Results: The new algorithm kept the CC, HP, LP and OD groups but added the probable (P) cases category and suppressed the DC group. The history and clinical signs observed allowed a provisional ranking between LP, P and HP of having AM. The blood tests allow to confirm cases or to invalid AM. Conclusion: This new algorithm is currently being tested with the 2600 cases reported to AMAG for validation.

---

## Neutrophils/Lymphocytes Ratio (NLR) as a marker of inflammation associated with obesity in dogs

Gómez Fernández-Blanco C.[1], Leterrier M.[2], Diez M.[2], Peeters D.[1]

*[1.] Department of clinical sciences of small animals, Faculty of veterinary medicine, FARAH, ULiège*
*[2.] Department of veterinary management of animal resources, Small animals Nutrition, Faculty of veterinary medicine, FARAH, ULiège*
***Corresponding author:*** mdiez@uliege.be

The term metabolic syndrome (MS) refers to every kind of pathophysiological changes associated with obesity in humans. Obese dogs show some characteristics of MS, including subclinical inflammation, and also present comorbidities associated with obesity. In humans, as well as in dogs, the Neutrophils/Lymphocytes Ratio (NLR) has been used as a marker of inflammation. An increase of this NLR has been associated with a poor prognosis for some diseases. This study aims to evaluate the ability of NLR to identify an inflammatory condition in obese dogs. Using the Body Condition Score (BCS) system on a 9-point scale, a group of 15 lean dogs (group L, BCS=5), and a group of 23 obese dogs (group OB, BCS>6) were recruited to conduct this study. R free statistical software was used to investigate differences in NLR between the two groups, as well as to look for an association of NLR with BCS and weight. Group OB presented a higher NLR than group L (3.63 versus 2.72, p-value=0.03). NLR was correlated with the BCS (p-value=0.01, r=0.41) and weight (p-value=0.04, r=0.34) of dogs. These results reinforce the evidence of an inflammatory state in obese dogs similar to the MS described in humans, and allow us to propose NLR as a marker of inflammation and a risk factor for the development of obesity associated diseases in dogs.

# Posters

## What Happens When Equine Muscle-Derived Stem Cells Are Incubated With Synthetic Bone Scaffold?

Graide H.[1,2], Ceusters J.[1,2], Niesten A.[1], Dupont J.[3], Dechêne L.[1], Serteyn D.[1,2,3]

*[1.] Center for Oxygen, Research and Developpment (CORD), University of Liège, Institute of Chemistry, Belgium; [2.] RevaTis SA, Rue de la Science, 8 6900 Aye, Belgium; [3.] Department of Clinical Sciences, Equine Surgery, University of Liège, Faculty of Veterinary, Medicine B41, Belgium*

***Corresponding author:*** *helene.graide@doct.uliege.be*

A novel sampling method based on microbiopsy of skeletal muscle allows collecting a high number of autologous mesenchymal stem cells (WO2015091210) (Ceusters et al., 2017). These types of cells have shown potential for a plethora of regenerative medicine applications. Bone regeneration is one of the central clinical issues in rehabilitation medicine. Significant growth opportunities exist for synthetic bone scaffolds in association with stem cells as alternatives to biological bone grafts. Currently, 3D printing offer to create personalized synthetic bone matrix to improve cellular development and formation of new bone-like tissue.
To evaluate the ability of equine muscle-derived mesenchymal stem cells (mdMSC) to colonize synthetic bone scaffold, and subsequently to study cell changes.
Equine mdMSC have been achieved from a muscular microbiopsy by explant culture. Three-dimensional printing hydroxyapatite matrix seeded with mdMSC has been incubating at 37°C during 15 days. Adherent cells have been harvested, counted, colored with alizarin red or differentiated into adipocytes, osteocytes and chondrocytes.
After 2 weeks, equine mdMSC have been able to colonize the synthetic bone matrix, on the surface, but also in depth. This matrix has allowed reaching approximately 800 mdMSC per $mm^2$. Stem cells in contact with matrix have lost their trilineage differentiation ability and they have increased their calcium deposits.
Easily-accessible mdMSC combined with 3D printing synthetic bone matrix could have great potential in tissue engineering. These first results require additional repetitions.

---

## Bilateral laryngeal paralysis secondary to traumatic nerve damage in two dogs

Hamon M., Picavet PP., Etienne A-L., Guieu LV., Billen F., Claeys S., Noel S.

*Department of Clinical Sciences (Companion Animals and Equidae), College of Veterinary Medicine, FARAH, ULiège.*

***Corresponding author:*** *martin.hamon@uliège.be*

Cervical injuries are common in dogs and may be a disaster given the number of vital structures present in the region. Traumatic damage to the vagus or recurrent laryngeal nerve is identified as a possible cause of acquired laryngeal paralysis. However, its occurrence remains rare. Two dogs (a French Bulldog and a miniature Pinscher) were presented in emergency for cervical bite wounds. Bilateral laryngeal paralysis, associated with several tracheal tears, was diagnosed by respiratory tract endoscopy in both dogs. The French bulldog needed surgical intervention (temporary tracheostomy and left cricoarytenoid cartilage lateralization). A complete right caudal laryngeal nerve section was observed during surgery. The Pinscher has been treated conservatively with only anti-inflammatory drugs, skin wound dressing management and monitoring. The two dogs recovered uneventfully. The Pinscher was controlled endoscopically 6 months later and total recovery of laryngeal motion was observed. Cervical bite wounds can lead to laryngeal paralysis, which may be transient or require surgical treatment. Endoscopy is recommended in case of cervical traumas, especially for bite wounds. Associated with the clinical examination, this procedure is a key point in the management of these cases.

## Long-term outcome of the transobturator vaginal tape inside out (TVT-O) for the treatment of urethral sphincter mechanism incompetence in female dogs

Hamon M., Hamaide A., Noel S., Claeys S.

*Department of Clinical Sciences (Companion Animals and Equidae), College of Veterinary Medicine, FARAH, ULiège.*

***Corresponding author:*** *martin.hamon@uliège.be*

Urethral sphincter mechanism incompetence (USMI) is the most common cause of acquired urinary incontinence in female dogs. Surgery is recommended if the animal does not respond to medical treatment or becomes refractory, if side effects develop or if owners are reluctant to administer long-term medication. Stress urinary incontinence in women is mainly treated surgically by vaginal placement of suburethral slings, including the transobturator vaginal tape inside out. This technique was reported in female dogs in 2010 and short-term results were promising.
The objective of the present study was to evaluate the long-term outcome of the TVT-O in female dogs.
TVT-O tape was inserted in 12 incontinent bitches diagnosed with USMI. Follow-up information was evaluated by a telephone questionnaire and a continence score was attributed.
One year after surgery, 7 of 12 dogs were completely continent (58%). Two dogs were removed from the long-term analysis (1 dead and 1 lost). At a median follow-up time of 85 months (range 28 to 95 months), 4 /10 dogs were completely continent without medical treatment. Incontinence recurred in 6 dogs at a median time of 2 months after surgery (range 1 to 20 months). Among those, 4/6 regained continence and 2/6 had sporadic episodes of incontinence, with additional medical treatment. No postoperative complications were encountered.
TVT-O alone was successful in maintaining long term continence in 40% of the dogs. Additional postoperative medical treatment was effective in restoring continence in another 40 % of dogs.
TVT-O provides an alternative treatment of USMI in female dogs, that is safe and less invasive than standard surgical techniques.

---

## Radiographic projection influences the interpretation of proximal metatarsal radiographs

Joostens Z.[1], Garigliany M.M.[2], de la Rebière - de Pouyade G.[3], Audigié F.[4], Busoni V.[1].

*[1,2,3] Université de Liège, Faculty of Veterinary Medicine, 4000 Liège, Belgium. [1] Department of Medical Imaging, [2] Department of Pathology, [3] Department of Equine Surgery.*
*[4] CIRALE-IPC, UMR Biomécanique et Pathologie Locomotrice du Cheval, Ecole Nationale Vétérinaire d'Alfort, 14430 Goustranville, France.*
***Corresponding author:*** *zjoostens@uliège.be*

Radiography is a routinely used on-site imaging modality when bone pathology is suspected, but its sensitivity and specificity for diagnosing proximal suspensory enthesopathy are considered low. The present study aimed to assess the influence of x-ray beam incident angle in detecting radiographic signs of proximal suspensory enthesopathy and to evaluate inter-rater agreement by means of a blinded internet-based reading of normal and abnormal proximal metatarsal radiographs. In total 12 respondents submitted 948 responses. A 4 point Likert score was used to classify the proximal enthesis as definitely normal, probably normal, probably abnormal or definitely abnormal on radiographs of normal and abnormal hindlimbs taken at different angles. Correct interpretation and inter-rater agreement appeared to be significantly associated with radiographic incidence angle. Results show that a true lateromedial view of the proximal third metatarsus, obtained as a plantaro80-85°lateral-dorsomedial oblique of the hock, substantially reduces perceptual and interpretational errors and decreases the proportion of false positive and negative diagnoses of proximal suspensory enthesiopathy. Nevertheless overall sensitivity, specificity and inter-rater agreement remain only fair, even in the plantarolateral-dorsomedial plane.

# Posters

## Enhancing activity with a weight-loss program on obese dogs: a monitoring with accelerometers revealed differences between lean and obese dogs' activity, and before and during a weight-loss program

Leterrier M.[1], Gómez Fernández-Blanco C.[2], Moula N.[3], Salas A.[4], Jeusette I.[4], Peeters D.[2], Diez M.[1]

[1] *Department of veterinary management of animal ressources, Small animals Nutrition, Faculty of veterinary medicine, FARAH, ULiège*
[2] *Department of clinical science of small animals, Faculty of veterinary medicine, FARAH, ULiège*
[3] *Department of veterinary management of animal ressources, Biostatistics, Faculty of veterinary medicine, FARAH, ULiège*
[4] *Affinity Petcare, Barcelona, Spain*
***Corresponding author:*** *mdiez@uliege.be*

Obesity is spreading worldwide, and pets are frequently diagnosed overweight, even obese, by veterinarians in private practices. Canine obesity reaches 34% in Belgium. Two groups of privately owned Retriever dogs (Labrador and Golden) were enrolled: 15 Lean dogs (Group L) and 23 Overweight or Obese dogs (Group O). Dogs from Group L were submitted to a one-month diet with low energetic kibbles, as well as dogs from Group O until they reached their ideal weight. All dogs were fitted out an accelerometer in order to register their activities during at least 5 days. The device was attached to a dedicated collar, and could stand every kind of weather, so it could be wear at all times. The study shows no differences of activity between the two groups (Group O: Mean±ES, Group L: Mean±ES; p=0.09). Those are partial results as the study is still going on (all dogs enrolled did not have reach their target weight yet). The study failed to show a clear difference of activity between lean and obese Retrievers, but in the long term, it is expected to show that after weight loss, spontaneous activity is increased in dogs. To our knowledge, this is the first study of this range conducted on privately owned dogs.

---

## Guttural pouch mycosis due to *Trycophyton equinum* in a horse

Martin L.[1], A. Salciccia[1], C. Cesarini[1], C. Wimmer-Scherr[1], L. Lecoq[1]
[1] *Equine Section, FARAH Center, Faculty of Veterinary Medicine, ULiège*
***Corresponding author:*** *laureline.lecoq@uliege.be*

Guttural pouch mycosis (GPM), a disease mainly caused by Aspergillus spp., is a rare condition that can lead to fatal epistaxis and dysphagia. Arterial coil embolization is an effective method to prevent hemorrhage and resolve the mycotic plaques in most of the cases.
A 7-year-old gelding presented for chronic bilateral purulent nasal discharge. Endoscopic examination revealed bilateral GPM. *Tricophyton equinum* was cultured from the diphtheritic plaques. Coil embolization of both carotid arteries and the maxillary artery was performed on the left side followed 3 weeks later by embolization of the right internal carotid artery. Infusion of both guttural pouches with a solution of enilconazole and oxygen therapy were also initiated. The horse was discharged after 1 month with partial reduction of the size of the plaques. Two days after discharge, the horse presented with severe epistaxis caused by acute bleeding coming from the left guttural pouch. The common carotid artery was sutured, and the bleeding stopped. The following day, the horse developed neurological signs. He bled a second time 2 days after presentation at which point it was decided to euthanize it. Post-mortem CT-scan of the head and necropsy confirmed that coils were still in place, obturating completely the carotid and maxillary arteries. Despite all the treatment the mycotic lesions progressed to the point of lysing the septum and both ostium of the pouches. Origin of the bleeding was not identified but could have been caused by neovascularization or aberrant vascularisation. This is the first time *Trycophyton equinum*, is isolated from GPM. The unusual presentation as well as the course of the disease are uncommon for guttural pouch mycosis.

## Is laryngeal ultrasonography an operator-dependent method or a fast and reliably teachable diagnostic tool?

Mignini B.[1], Tosi I.[2], Evrard L.[3], Art T.[2], Busoni V[3].

*[1] Département clinique des animaux de compagnie et des équidés, Service d'Anesthesiologie générale et pathologie chirurgicale des grands animaux, Médecine vétérinaire comparée, FARAH, ULiège*
*[2] Département clinique des animaux de compagnie et des équidés, Service Physiologie neuro-musculaire et de l'effort, Médecine sportive des animaux, Médecine vétérinaire comparée, FARAH, ULiège*
*[3] Département clinique des animaux de compagnie et des équidés, Service d'Imagérie Médicale, Médecine vétérinaire comparée, FARAH, ULiège*
***Corresponding author:*** *bmignini@uliege.be*

The use of ultrasonographic laryngeal measurements in the horse has been suggested as a useful modality to assess diagnostic or prognostic indicators, and used to assist in planning surgical treatment of the larynx. The aim of this study was to assess the ability of operators of different degrees of experience in diagnostic imaging to obtain ultrasonographic images of the larynx of diagnostic quality and enabling to obtain reliable measurements.
Thirteen operators of 3 different levels of experience in equine ultrasound performed laryngeal echography on 3 healthy horses after having visualized a short tutorial video. Each operator was invited to obtain 3 different ultrasonographic images. Basihyoid depth, laryngeal diameter and distance thyroid cartilage – cricoid cartilage were respectively measured by an independent observer, on the 3 images obtained by each operator. A board-certified radiologist assessed the quality of the 39 images blindly using a 3-point scale.
Statistical analysis was used to assess reliability of measurements, as well as the correlation between image quality and operators' experience.
Results show a significant correlation between image quality and operator's experience; a good intra-group, inter-group and overall reliability of measures was found. Although image quality is operator-dependent, the results of the study suggest good transferability of a basic laryngeal ultrasound protocol for measurement acquisition after a short video training.

---

## Functions of the inositide-5-phosphatase Inpp5K in the B cells

Moës. B, Bai Q., Molina-Ortiz P., Vande Castyne C.-A., Azziz H. and Schurmans S.

*Laboratory of functional genetics, GIGA-R, University of Liege, Belgium.*

***Corresponding author****: B.moes@doct.uliege.be*

Inpp5K (Inositol polyphosphate 5-phosphatase K) hydrolyses, both PtdIns(4,5)P2 and PtdIns(3,4,5)P3, two phosphoinositides important for b cells differentiation and activation. Therefore; the aim of my project is to decipher the exact function of Inpp5K in b cells using a mouse model in which Inpp5K is specifically deleted in the hematopoietic system. Our results have showed that deletion of Inpp5K in the hematopoietic system induces strong architectural changes in the secondary lymphoid organs and a severe aggamaglobulinemia and lymphocytopenia. Indeed, Facs analysis showed that total number of B lymphocyte ($B220^{+}$) in blood and spleen of our KO mice are significantly decreased. We have also found that this decrease can be explain by a partial blockage of b cells differentiation at the stage between PreProB and ProB precursor. The analysis of IL7 pathways, an essential pathway for ProB cells survival and proliferation, has showed that absence of Inpp5K induces some anomalies in Pax5 and EBF1 expression but induces also an increase of AKT activation in ProB $Inpp5K^{-/-}$ cells. These results suggest that Inpp5K could be implicated in the regulation of IL7 pathways. More precisely, this regulation could occur by the control of the activation of AKT.

# Posters

## A case of uterine hematoma in a mare after an endometrial biopsy

Parrilla-Hernández S.[1], Reignier F.[2], Barrière P.[2], Blard T.[2], Ponthier J.[3], Deleuze S[3]

[1] *Physiology of Reproduction, Comparative veterinary medicine, FARAH, Uliège*
[2] *INRA, UE1297 PAO, Nouzilly, France*
[3] *Equine Clinic of Reproduction, Comparative veterinary medicine, FARAH, Uliège*
***Corresponding author:*** *sparrilla@uliege.be*

Endometrial biopsy is an important, safe and painless procedure to assess equine uterine health. While minor bleeding at the site of sampling is a common condition, uterine hematoma is a rare complication. An endometrial biopsy was routinely performed on an unsedated 17 year-old welsh pony mare in estrus during a research protocol. From the next day on and during the following 7 days, a large amount of sanguineous fluid was observed in the uterine cavity during the daily ultrasound examination performed according with the protocol. No fever or any other systemic symptoms were noticed. Two weeks later, the ultrasound examination revealed an organised hyperechogenic mass compatible with a hematoma within the endometrial lumen. The mare was regularly controlled during the rest of the breeding season and no abnormalities in her cyclicity were observed. The size of the hematoma only began to decrease from the 3rd month after the biopsy onward and it disappeared completely 2 months later. This was the only complication following a biopsy in this study protocol that included a total of 70 biopsies on 49 mares and the very first case the authors got to see while biopsies have been common practice in their clinical and research activities for years. To our knowledge, this is the first time that the appearance of this condition is documented. The hematoma took 5 months to disappear at the ultrasound examination. During this time the mare could not be bred. Therefore, while endometrial biopsy should still be regarded as a safe procedure for the diagnosis of infertility in mares, the potential risk of a hematoma with its consequences for the breeding season has to be considered.

---

## Parotid purulent sialocoele treated by grass seeds extirpation

Picavet P.P. [1], Grauwels M. [1], Etienne A.-L. [1], Hamaide A. [1], S. Claeys[1]

[1.] *Department of Companion Animal Clinical Sciences, School of Veterinary Medicine, FARAH, University of Liège, Belgium*

***Corresponding author:*** *pierre.picavet@uliege.be*

Foreign body migration is a common syndrome in dogs, and clinical outcomes have a wide range of results. Very few reports are published on foreign bodies in the parotid gland or duct. Parotidectomy is usually required but is associated with a high complication rate. An 11-month-old female neutered French bulldog was initially presented to the referring veterinarian with a recurrent swelling under its right ear. Conservative and surgical treatments failed. On clinical examination, a non-painful subcutaneous swelling was present at the level of the right parotid gland. Iatrogenic facial nerve paralysis and secondary corneal ulcer were diagnosed. Regional ultrasonography was performed showing a fluid cavity that could be of salivary origin. Computed tomography with sialography was performed. Two vegetal foreign bodies protruding from the aperture of the parotid duct were removed. Medical treatment was installed as the cause of obstruction was identified and removed.
Oral amoxicillin-clavulanate was prescribed based on bacterial culture as well as antibiotic eyedrops and lacrymomimetics. At recheck 10 days later, the swelling had resolved and the corneal ulcer had healed. Two months after treatment, the dog remains asymptomatic except for permanent facial paralysis. Thus, a permanent partial temporal tarsorraphy was performed. This is the first report of a purulent parotid sialocœle treated by grass seed extirpation.

## The Atypical Myopathy Alert Group's case report process: strengths and weaknesses

Renaud B.[1], François A.-C.[2], Weber M.[2], Marcillaud-Pitel C.[3], Gustin P.[2], Votion D.-M.[1]

*[1.] Equine Pole, Fundamental and Applied Research for Animals & Health (FARAH), Faculty of veterinary Medicine, ULiège*
*[2]. Departement of Functional Sciences, Faculty of Veterinary Medicine, Pharmacology and Toxicology, FARAH, ULiège*
*[3.] Réseau d'EpidémioSurveillance en Pathologie Equine (RESPE), Caen, France*
***Corresponding author:*** benoit.renaud@uliege.be

Introduction: In 2004, the Faculty of veterinary Medicine of Liège University has initiated an epidemiosurveillance network for atypical myopathy (AM) called "atypical myopathy alert group" (AMAG) that records European cases since 2006. This European survey is based on the online reporting of cases through the horses' owners and veterinarians. As first lines of work, AMAG defined the clinical, pathological and epidemiological features of the disease based on 600 cases reported from 2006 to 2009[1]. Today, more than 2,600 cases, suspected to be suffering from AM, are registered in the AMAG's database. The objectives of the work are to assess the data incomes evolution and to identify the strengths and weaknesses of the network.
Methods: The online registered data collected from 2006 up to 2017 were analyzed following two guidelines: (1) the evolution of the reported cases since 2006, (2) the profile comparison between the new series of data (2010 to 2017) and the previous one (2006 to 2009) described by Van Galen et *al*. 2012. Results: The number of reported cases progressively increases over the years. About 2,002 new cases have been reported since 2009 and these cases emerge from a wider range of countries, including now Austria, Czech Republic, Spain and Portugal. On the same period, an extra 10 % of the cases have been declared outside the autumn peak incidence. Discussion: The spontaneous reporting is inevitably biased and thus, the true number of cases and real spatial incidence of the disease are unknown. The increased reporting may result from sensitization of the equine field to AM or from a true increase of AM cases.

---

## Potential new sources of hypoglycin A poisoning for equids kept at pasture in spring: a field pilot study

Renaud B.[1], François A.-C.[2], Habyarimana J.A.[2], Scippo M.-L.[3], Richard E.[4], Marcillaud-Pitel C.[5], Erpicum M.[6], Gustin P.[2], Votion D.-M.[1]

*[1.]Equine Pole, FARAH, Fac. of Vet. Med., ULiège; [2.]Dept of functional Sci., FARAH, Fac. of vet. Med., ULiège; [3.]Dept of Food Sci., Lab. of Food Analysis, FARAH, Fac. of Vet. Med., ULiège; [4.] BIOTARGEN / LABÉO Frank Duncombe, 14053 Caen cedex 4, France ; [5.] Réseau d'EpidémioSurveillance en Pathologie Equine (RESPE), Caen – France ; [6.] Department of Geography, ULiège*
***Corresponding author:*** benoit.renaud@uliege.be

Introduction: Equine atypical myopathy (AM) in Europe results from the ingestion of hypoglycin A (HGA) contains in samaras and seedlings of the sycamore maple tree (*Acer pseudoplatanus*). The disease is seasonal with outbreaks in autumn when samaras twirl to the ground and in spring because of sycamore seedlings. Up to now, measurement of HGA content in plant materials has been made at a given moment in time with no follow up of HGA concentration conducted over a season. The first objective of this study was to measure the concentration of HGA in samaras of sycamore trees fallen on the ground and to compare those results to the ones obtained in 2016. The subsequent objective was to examine other possible sources of HGA intoxication.
Methods: HGA was tracked in samaras of sycamore maple collected from 3 pastures already sampled in spring and summer 2016. As negative controls, samaras of field maple (*Acer campestre*), were also collected. Additional samples have been collected (1) inflorescences of sycamore maples, (2) rainwater in contact with sycamore inflorescences on a rainy day, (3) aqueous maceration of sycamore inflorescences and (4), stocked hay from 2016 in which HGA was detected. Results: HGA was detected in all plant material from sycamore but not field maples. Water in contact with sycamore seedlings tested positive for HGA but not in aqueous maceration of sycamore inflorescences. It was also found that HGA is still present in hay saplings from the previous years. Conclusion: Results of this field study highlight the interest of monitoring the toxicity at the pasture level in order to better define the grazing schedule in risky grasslands.

# Posters

## On-field Electro-ejaculation of wild Prezwalski horses: results and perspectives

G. Rigaux[1], S. Parrilla-Hernandez[2], S. Egyptien[2], C. Gatez[2], C. Carrasco Leroy[1], S. Deleuze[2], J. Ponthier[2]
[1]*Domaine des Grottes de Han, Han/Lesse, Belgium &*
[2]*Equine Reproduction, FARAH Comparative Veterinary Medicine, University of Liège, Belgium*
***Corresponding author:*** *Jerome.Ponthier@uliege.be*

Electro-ejaculation could enable semen exchange between wild equids conservation centres. 3 Prezwalski horses were darted (acepromazine, detomidine, butorphanol, etorphine) before IV anaesthesia was given (Guaiacolate, ketamine, xylazine) and oxygen was provided by tube. After bladder emptying and 2 minutes of prostatic massage, electro-ejaculator's probe was inserted in the rectum and stimulation sequence was: Cycle 1: 10 stimulations 0.5V, 10 stimulations 1.5V, 15 stimulations 2.5V, 3 minutes rest; Cycle 2: 10 stimulations 0.5V, 10 stimulations 1.5V, 15 stimulations 3.5V, 3 minutes rest; Cycle 3: 10 stimulations 1.5V, 10 stimulations 2.5V, 10 stimulations 3.5V, 15 stimulations 4.5V, 3 minutes rest; Cycle 4: 10 stimulations 0.5V, 10 stimulations 2.5V, 10 stimulations 3.5V, 15 stimulations 4.5V. Bladder was catheterised to detect spermatozoa. Raw semen motility was evaluated on field at 37°C. Samples were diluted (1/5) with INRA96 and sent to the lab within 4 hours. Procedure duration was 1 hour and no complication was observed during or after. For each horse, a total of 4, 2, 1,5 and 0,4 $x10^9$spz with respectively 56, 30 and 25% of progressive motility was obtained. Numbers of collected spermatozoa were higher on cycle 2 or 3 and decreased after. Spermatozoa number in urine was low. On-field semen motility was lower than extended semen motility analysed in lab. Our procedure using darting, intra-venous maintenance and an endotracheal tube reduced risks and was effective for electro-ejaculation of wild equids. Sufficient spermatozoa numbers were collected after 3 cycles of stimulations and dilution of raw semen in extender improved motility, enabling shipping and use for AI programs.

---

## Towards a better understanding of lung interstitial macrophages identity

J. Schyns[1,2], D. Pirottin[1,2], F. Bureau[1,2,3], T. Marichal[1,2,3]

[1]*Laboratory of Cellular and Molecular Immunology, GIGA-Research, University of Liège, Belgium*
[2]*Faculty of Veterinary Medicine, University of Liège, Belgium*
[3]*Walloon Excellence in Life Sciences and Biotechnology (WELBIO), Wallonia, Belgium*
***Corresponding author:*** *joey.schyns@uliege.be*

Lung interstitial macrophages (IM), constituting the non-alveolar lung macrophage compartment, have been shown to exhibit tolerogenic properties at steady-state by inhibiting the ability of dendritic cells to induce allergic type 2 responses against inhaled aeroallergens. Recently, several reports have provided experimental evidence that IM represent a heterogeneous population in the steady-state lung, and encompass at least two subpopulations, each of them likely carrying their own identity, e.g., phenotype, localization, differentiation program, and function. In order to assess the heterogeneity of IM in an unbiased way, droplet –based single-cell RNA sequencing experiments were performed on the IM pool, revealing three subpopulations of IM at the steady-state. Differentially expressed genes among the subpopulations allowed us to find specific surface markers that identify these phenotypically and functionally distinct subpopulations by flow cytometry, a crucial step to assess the identity of IM, and how such identity is imprinted by the local environment to fulfill the functional needs of the lung mucosa.

## Triamcinolone acetonide after intra-articular injection in knee in sheep

Tian J.*[1], Legrand N.*[2], Douny C.[3], Scippo ML.[3], Cambier C.[2], Fillet M.[4], Chiap P.[5], Dubrowski T.[5], Vandeweerd JM.[6], Gustin P.[2]

*[1]Fac. of Med., Shanghai Jiao Tong Univ., China; [2] Unit of Pharmacology; [3] Unit of Food analysis, FARAH, ULiège.; [4] CIRM-Dpt of Pharmacy, Fac. of Med. (ULiège); [5] Lab. of Toxicology CHU (ULiège); [6] Integrated Vet. Res. Unit (IVRU), Namur Research Inst. for Life Sciences (NARILIS), Univ. of Namur.*

***Corresponding author**: nlegrand@uliege.ac.be*

Intra-articular injections of glucocorticoids aim to control pain and inflammation caused by osteoarthritis. There is a lack of evidence and pharmacokinetics (PK) studies to support the empirical currently used dosage regimens. This study aimed 1) to determine the PK of triamcinolone hexacetonide (TH) and triamcinolone acetonide, its active metabolite (TA), in synovial fluid after intra-articular administration of a suspension of TH at 40mg and 10mg in sheep and 2) to compare the profiles of TA after injection of suspensions of TA or TH, both at 40mg. Twelve sheep were randomly allocated to three groups receiving respectively 40 mg TA (n=4), 40 mg TH (n=4) or 10 mg TH (n=4) in the left knee. Synovial fluids were sampled from day 1 up to day 21. The concentrations of TA and TH were measured by ultra-performance liquid chromatography mass spectrometry. TA concentrations measured after one day were higher in the group TA-40 mg (537762.3ng/ml) compared to those recorded in the group TH-40mg (22743.4 ng/ml), On day 21, the corresponding values were 2.5 and 33.5 ng/ml due to a significant higher value of T1/2β of TA in group TH-40 mg (6.0 versus 1.9 days). The differences between the mean values of AUC and T1/2β of TA were not significantly different between the groups TH-10 and -40 mg but T1/2β of TH was significantly higher in the group TH-40mg. In conclusion, TH injection maintains TA concentrations for a longer period of time than TA administration. Due to a possible saturation of esterases, the PK profiles associated to the high and low doses of TH were rather close suggesting that a dose of 10mg could provide an optimal benefit-risk.

***: these authors contributed equally to this work**

---

## Don't forget about ultrasound! Usefulness of ultrasonography in evaluating the equine head and throat regions

Vandersmissen M.1, Evrard L. 1, Joostens Z. 1, Busoni V.1

*1.Department of Companion Animals and Equine Clinical Science, Diagnostic Imaging Unit, FARAH, ULiège.*

***Corresponding author:** M.vandersmissen@uliege.be*

Ultrasonography is a non-invasive and affordable imaging modality used in equine practice. Its complementarity to radiography is widely demonstrated.
This presentation aims to illustrate the application of ultrasonography in the imaging of the equine head and throat through a retrospective clinical series.
Material and Methods
Medical records of 46 horses having undergone head and/or throat ultrasonography were reviewed. Horses having undergone ocular ultrasonography and horses affected by strangles were excluded.
In all horses, ultrasonography was used as a complement to radiography. 19 horses underwent ultrasonography to explore suspected bone or soft tissue infection. In these cases, ultrasonography was useful to confirm or demonstrate bone involvement, the presence of a sequestrum and areas of bone lysis. In preoperative assessment it also aimed to assess the morphology of fistulous tracts and abscesses. Power Doppler was useful to support the hypothesis of infection. 13 horses were examined for suspected neoplasia. In these cases, ultrasonography was used to assess tumor extent and vascularization, presence and extent of bone lysis and lymph nodes involvement, and to guide fine needle aspirations. In the remaining cases, ultrasonography was used to investigate miscellaneous conditions (focal swellings, lymph nodes, temporo-mandibular joints and laryngeal morphology).
This retrospective review indicates the usefulness of ultrasonography as an adjunct to radiography to complete the initial imaging investigation of the equine head and throat regions. Because of its low-invasiveness and wide availability, its use should be promoted.

# Posters

## MRI STIR hypersignal in the equine distal phalanx: retrospective study of prevalence and distribution in 96 horses

Vandersmissen M.[1], Evrard L. [1], Busoni V.[1]

[1.]*Dept of Companion Animals and Equine Clinical Science, Diagnostic Imaging Unit, FARAH, ULiège.*
***Corresponding author:*** *M.vandersmissen@uliege.be*

Increased Short-Tau Inversion Recovery (STIR) signal is commonly seen in the dorsal aspect of the distal phalanx (P3) and most of time considered asymptomatic. This study aimed to establish the prevalence of a STIR hypersignal in P3 and describe the distribution patterns in horses presented for MRI examination. Forefeet MRI examinations of horses either coming for MRI of the foot or having had MRI of the foot for comparison or as a complement to another region were reviewed. Presence and distribution of STIR hypersignal in P3 were assessed.
129 MRI examinations of 96 horses met the inclusion criteria (96 lame feet and 33 non-lame feet). STIR hypersignal was present at the dorsal aspect of P3 in all MRI examinations. The signal distribution could be classified in 4 different patterns: 1. thin dorsal line (47 feet), 2. small area restricted to the dorso-distal half of P3 (60 feet), 3. large area extending widely in P3 (18 feet), 4: generalized hypersignal involving the entire P3 (4 feet). Linear dorsal distribution and focal distribution limited to the dorso-distal P3 had a similar prevalence in lame and non-lame feet (35%-39% and 45%-52%), slightly higher in sound feet. Larger areas of STIR hypersignal were mainly seen in lame feet (16% and 4% for type 3 and 4 distribution against 9% and 0% in non-lame feet). Focal STIR hypersignal is routinely found in P3 and its high prevalence (sound and lame feet) suggests an adaptive origin. Larger areas of STIR hypersignal extending more proximal or deeper into P3 seem in contrary more prevalent in lame feet and more likely represent the result of a pathologic process.

---

## The electron paramagnetic resonance test of equine atypical myopathy induced mitochondrial dysfunction: a preliminary study

Zhou Y.[1], Votion D.-M.[2], Gustin P.[3], Mouithys-Mickalad A.[4]
*[1.]Fac. of Med., Shanghai Jiao Tong University, China; [2.]Equine Pole, FARAH, Fac. of vet. Med., ULiège; [3.]Dept of Functional Sciences, Fac. of Vet. Med., Pharmacology and Toxicology, ULiège; [4.]Centre de l'Oxygène, Recherche et Développement (CORD), CiRM, FARAH, ULiège, Belgium*

***Corresponding author:*** *A. amouithys@uliege.be*

Introduction: In Equine atypical myopathy (AM), mitochondrial oxidative phosphorylation capacity is severely altered as compared to healthy horses, which has been observed in muscle microbiopsies taken from AM-affected horses. In a cultured equine skeletal muscle cell model model, this mitochondrial dysfunction can also be reproduced by 1mM of MCPA (the toxin of AM). The reactive oxygen species (ROS) production is another important evaluation standard of mitochondrial function which can be monitored by the electron paramagnetic resonance (EPR) combined with spin trapping technique. The objective of this study was 1) to test if there is increased ROS production in MCPA-induced mitochondrial dysfunction in cultured cells, 2) to compare the effect of MCPA with rotenone and, 3) to check the effect of digitonin and ADP on MCPA ROS production. Methods: By using EPR-spin trapping, the ROS production of 2 million of cultured equine skeletal muscle cells was evaluated with α-(4-Pyridyl N-oxide)-N-tert-butylnitrone (POBN, as spin trap) 50 mM, MCPA 5 mM, reagents (malate 4 mM, glutamate 10 mM, digitonin (10 mg/1 ml DMSO, 0.8 µl/106 cells), ADP 25 mM). For rotenone assay, MCPA was replaced by rotenone 1µM. Results: Both 5 mM MCPA and 1µM rotenone produced a strong EPR signal caused by ROS production, indicating that MCPA could trigger the ROS production like rotenone. The use of digitonin and ADP could increase the ROS production initiated by MCPA. Conclusions: The MCPA-induced mitochondrial dysfunction is associated with ROS production. This preliminary study would benefit further researches on the mechanism of the MCPA toxic effect and provide a new method for the study of therapeutic agents.

## *In vitro* testing of equine atypical myopathy induced mitochondrial dysfunction

Zhou Y.[1], Gustin P.[2], Mouithys-Mickalad A.[3], Marcillaud-Pitel C.[4], Lemieux H.[5], Votion D.-M.[6]

*[1.]Fac. of Med., Shanghai Jiao Tong University, China; [2.]Dept of Functional Sciences, Fac. of Vet. Med., Pharmacology and Toxicology, ULiège ; [3.]Centre de l'Oxygène, Recherche et Développement (CORD), CiRM, FARAH, ULiège, Belgium ; [4.]Réseau d'EpidémioSurveillance en Pathologie Equine (RESPE), Caen, France ; [5.]Faculty Saint-Jean, University of Alberta, Edmonton, Alberta, Canada ; [6.]Equine Pole, FARAH, Fac. of vet. Med., ULiège*

***Corresponding author:*** *dominique.votion@uliege.be*

Introduction: Equine atypical myopathy (AM) is a highly fatal environmental intoxication without a specific cure. The condition is induced by the ingestion of seeds and/ or seedlings of *Acer pseudoplatanus* (sycamore maple tree). The key pathophysiological mechanism is MCPA (toxic metabolite of hypoglycin A) induced multiple acyl-CoA dehydrogenases deficiency. The objectives of this study were 1) to establish a cultured equine skeletal muscle cell model reproducing the AM-induced mitochondrial dysfunction with MCPA, 2) to test the effect of MCPA on fatty acids and, 3) to quantify the effect of MCPA on mitochondrial respiration. Methods: High-resolution respirometry was used to determine, with a dose-dependent test, the MCPA concentration that reproduces the muscle mitochondrial dysfunction seen in muscle microbiopsy samples taken from AM-affected horses. Protocols were extended to measure the effect of MCPA on acetylcarnitine, octanoylcarnitine and palmitoylcarnitine metabolism. Then, the effect of MCPA on NADH-linked, succinate-linked and/or electron transferring flavoprotein complex was determined using a dosage of MCPA that reproduces a 50% decrease in maximal OXPHOS. Results: MCPA 1 mM successfully reproduced the AM-induced mitochondrial dysfunction. With this concentration, MCPA significantly decreased the mitochondrial respiratory capacity linked to the different substrates tested. Conclusions: MCPA had an effect not only on β-oxidation but also on the entire mitochondrial function leading to question regarding the target of the toxin. This preliminary study would benefit further researches on the mechanism of the MCPA toxic effect and the studies on AM treatments

**Presses de la Faculté de Médecine vétérinaire de l'Université de Liège**

4000 Liège (Belgique)

D/2018/0480/9
ISBN 978-2-87543-128-8