

# Proceedings of the 6<sup>th</sup> FARAH-Day

Faculty of Veterinary Medicine  
(University of Liège - Belgium)

November 8, 2019

*One Health*

L'Animal et l'Homme, une même santé





# **Proceedings of the 6<sup>th</sup> FARAH-Day**

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

**November 8, 2019**

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

**Presses de la Faculté de Médecine vétérinaire de l'Université de Liège**  
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**COVER PICTURE CREDITS:**

**Sonia Parrilla-Hernandez**, Dpt of Functional Sciences, FARA

The love in science

### ***Welcome to the 6<sup>th</sup> FARAH Day***

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

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

The founding principles of the FARAH incorporate the notion of interaction between scientists of the Centre and, as such, the annual meeting of the scientific staff gives us the opportunity to share our knowledge. Also, it is now under the auspices of the FARAH that the annual meeting will be held with the same organizers (i.e. members of the Scientific Staff). This edition gathers about 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 6<sup>ème</sup> journée du FARAH***

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

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

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

**Laurent Gillet**, Président du FARAH

**Frédéric Farnir**, Vice-président

**Dominique Votion**, Secrétaire

# Organisers of the meeting

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**Véronique Defize**

**Jean-Michel Dusoulier**

**Marie-Eve Lechanteur**

**Michel Motkin**

**Anne Wlasowski**







# Program

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

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

**Prof. F. Bureau, Vice-Rector of research**

**09:15 Invited speaker**

***Dr Delrez Natacha, laureate of the international contest "Ma thèse en 180 secondes"***

*« Il était une fois l'anguille, le virus et la luciole »*

***Followed by:***

*Infection of European eel by Anguillid herpesvirus 1:*

*physical contacts between infected and naïve eels enhance viral transmission*

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

**Chair: B. Machiels & M.M. Garigliany**

*09:45 **Sophie Egyptien** - Optimizing drone raising in Belgium and semen cryopreservation techniques*

*09:55 **Xiang Du** - Development of a small animal model for adenovirus-vectored oral vaccination against Canine Distemper Virus*

*10:05 **Linde Gille** - Cocoa by-products as a possible cause of acute illness and death in fattening bulls*

*10:15 **Céline Maquet** - Ly6Chi monocytes are key orchestrators of gammaherpesvirus lifecycle*

*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)

**Chair: ML. Scippo & L. Gillet**

*11:15 **Benoit Renaud** - Diagnostic and prognostic value of hypoglycin A, methylenecyclopropyl acetic acid-carnitine and, acylcarnitines profile in horses with atypical myopathy*

11:25 **Shi Fang Li** - Identification of genetic variants affecting immune traits in Belgian White-Blue cattle

11:35 Questions

11:40 Short talks

**Mamadou Diallo** - The Zalpha domain containing protein ORF112 of Cyprinid herpesvirus 3 is associated with RNA nucleocytoplasmic transport

**Caroline Leonard** - Ear canal microbiota and mycobiota – effect of preventive use of a AIS topical in atopic dog without any sign of active otitis

**Hélène Casalta** - Outbreak of post-parturient hemoglobinuria associated with hypophosphatemia in a dairy herd

**Noémie El Agrebi** - Belgian case study on flumethrin residues in beeswax: Possible impact on honeybee and prediction of the maximum daily intake for consumers

**Yuan Gao** - A single nucleotide polymorphism of Cyprinid herpesvirus 3 ORF131 determines the formation of syncytia in cell culture

**Lucie Gillard** - Identification of the cellular protein interactome of Kaposi's sarcoma-associated herpesvirus tegument protein through a Mammalian Protein-Protein Interaction Trap approach

**Yunlong Hu** - Cyprinid herpesvirus 3 prevents the formation of stress granules induced by arsenite treatment

**Phuong Nguyen** – Foodborne zoonotic trematodes in Bithyniid snails (Gastropoda: Bithyniidae) in Northern and Central Vietnam

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

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

**Chair: V. Delcenserie & T. Marichal**

14:00 **Ludovic Martinelle** - Impact of feeding calves waste milk containing antibiotic residues on the rise of antimicrobial resistance in fecal *Escherichia coli*

14:10 **Clovis Wouters** - Untargeted metabolomics studies focusing on prognosis and resistance factors leading to the identification of new potential biomarkers in equine atypical myopathy

14:20 Questions

14:30 Short talks

**Salem Djebala** - Prophylactic antibiotic usage by Belgian veterinarians and breeders during elective caesarean section in Belgian Blue cattle

**Louisa Ludwig-Begall** - Replicative fitness recuperation of a recombinant murine norovirus – in vitro interplay of genetic shift and drift

**Achille Ouedraogo** - Bovine Papular Stomatitis and Pseudocowpox viruses found in ticks infesting cattle in West Africa

**Haiyan Zhang** – Genomic and biologic comparisons of anguillid herpesvirus 1 strains

**Olivier M. Zannou** - Cattle transhumance between Burkina Faso and Benin: corridors and health risk factors associated to ticks

**15:00 Invited speakers**

**Prof. Philippe Bossaert**

« Mesures de réduction d'antibiotiques dans la médecine vétérinaire:  
le bâton ou la carotte ? »

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

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

**Chair: S. Grulke & E. Thiry**

16:00 **Véronique Renault** - Pilot study assessing the possible benefits of a higher level of implementation of biosecurity measures on farm productivity and health status in Belgian cattle farms

16:10 **Owen Donohoe** - Transcriptomic comparison of zebrafish and carp larvae after inoculation with Cyprinid Herpesvirus-3

16:20 **Caroline Deblon** - Serological "aspecific" reaction against BoHV1 in Belgium: possible cross-reactivity of other herpesviruses?

16:30 **Annalisa Canale** - An attenuated recombinant vaccine against Cyprinid herpesvirus 3 induces intraepithelial acquired immunity and prevents transmission of a virulent strain

16:40 Questions

16:50 Short talks

**Jeremy Mortier** - *Dynamic contrast-enhanced computed tomography in dogs with nasal tumors: protocol development and perfusion analysis*

**Amira Preure** - *Helminth infection is associated with the accumulation of lung interstitial macrophages and increased susceptibility to gammaherpesvirus infection in C57BL/6 mice*

**17:00 – Summer school**

**17:05 - Closing session**

**Rectoral team**

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

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# **Oral presentations**

## Oral presentations

### **Infection of European eel by Anguillid herpesvirus 1: physical contacts between infected and naïve eels enhance viral transmission**

Delrez N.<sup>1</sup>, Haiyan Z.<sup>1</sup>, Denoël M.<sup>2</sup>, Mélard C.<sup>3</sup>, Liefbrig F.<sup>4</sup>, Suarez N.<sup>5</sup>, Davison A.<sup>5</sup>, Boutier M.<sup>1</sup> and Vanderplasschen A.<sup>1</sup>

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Over the last few decades, the number of European eel (*Anguilla anguilla*) reaching Europe has declined by 99%, justifying its current 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. Here, we aimed to investigate the pathogenesis of the AngHV-1 in his natural host using *in vivo* bioluminescent imaging system (IVIS). First, we produced a recombinant strain (hereafter called LucGFP strain) encoding a bicistronic reporter expression cassette inserted in the intergenic region between open reading frame (ORF) 32 and ORF33. This cassette driven by the EF1 promoter led to detectable expression of both firefly luciferase and copepod GFP in infected cell cultures. Next, AngHV-1 portal of entry was investigated in juvenile eels infected with the LucGFP strain. We used different modes of inoculation mimicking epidemiological conditions, than latter analysed fish by IVIS according to time post infection (p.i.). Ingestion of infectious material (oral contamination) led to no detectable infection. Immersion in water containing the virus (contamination by immersion) and intraperitoneal (IP) injection led to a strong bioluminescent signal by IVIS analysis from day 3 post infection. Among these groups, clear clinical signs appeared from day 6 p.i. and were characterized by lethargy, discolored skin patches, ulcers, erythema and in some cases, abnormal vertical swimming. First IVIS signals were detected on the skin – mainly on the head and tail-, while internal organs were still negative. Finally, naïve eels were contaminated by cohabitation with infected eels. This mode of inoculation led to rapid and efficient transmission of AngHV-1 through epidermal infection. Localization of foci of infection on the skin of contaminated eels strongly suggested the roles of physical interactions in the transmission process. Together, the data obtained in the present study demonstrate that the skin is the portal of entry of AngHV-1 in eels and unravel the importance of physical interactions between infected and naïve subjects in the epidemiology of the infection.

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### **Optimizing drone raising in Belgium and semen cryopreservation techniques.**

Egyptien S., Deleuze S.

*Obstetric and Reproduction of small animals, comparative veterinary medicine, FARAH, ULiège*

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Honeybee semen conservation is of worldwide scientific interest with increasing urgency to improve results in order to conserve biodiversity. Gamete cryopreservation from selected lines is of growing interest also. Indeed, selection of bees tolerant to varroa is one of the main actual objectives in honeybee research. Cryobanking of these gametes will be exploitable internationally by simple transport. In order to improve cryopreservation, different parameters are studied such as influence of drone age. To study this criterion, we need a method to raise large numbers of drones of known age without impairing the colony's stability. We also need objective characteristics of semen quality. One of these is semen viability studied by epifluorescence or flow cytometry using SYBR-14 and propidium iodide dyes. This report describes the development of this technique and first attempts of cryopreservation. It also details the first steps of validation of the fluorescent supravital staining by flow cytometry as a reference to test new dyes. We can conclude that the rearing method allows to raise large numbers of drones of known age and needs to be tested again during the next breeding season and that the flow cytometry technique needs to be further validated with comparison to epifluorescence microscopy.

### **Development of a small animal model for adenovirus-vectored oral vaccination against Canine Distemper Virus**

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Distemper is a fatal and highly contagious disease of young carnivores, which is due to infection by Canine Distemper Virus (CDV), a member of the genus Morbillivirus in the Paramyxoviridae family. Although CDV has initially been described as a pathogen of domestic dogs, it has increasingly become a threat for multiple wildlife carnivore species worldwide but also for several primates raising concerns about a potential zoonotic risk of CDV infection in humans. Conventional live modified vaccines are commercially available and are widely used in dogs. While these vaccines provide a rapid and robust immunity, the use of injectable vaccines is unfeasible for wildlife. Alternative vaccination strategies are therefore needed. In particular, oral immunization may offer several advantages over the traditional parentally administered vaccines and has previously revealed successful for wildlife populations. In this project, using Mouse Adenovirus Type 1 (MAV1), we developed a protocol of oral vaccination of wildlife species against CDV with adenovirus-vectored vaccines. Firstly, recombinant MAV-1 strains expressing various forms CDV glycoproteins H or F have been generated. Secondly, expression of the transgenes has been checked *in vitro*. Finally, we investigated the immune response against CDV generated by the oral administration of the different MAV-1 recombinant vaccines in mice. Altogether, the results obtained in this study should help us to better define oral vaccination strategies for the protection of wildlife species against CDV.

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### **Cocoa by-products as a possible cause of acute illness and death in fattening bulls**

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A farm fattening +- 1000 bovines a year contacted the Ruminant clinic in January 2019 due to a high number of animals dying unexpectedly (23 since September 2018). The affected bulls weighed between 300-500 kg. Symptoms consisted of stiffness, muscle tremors, depression, and tachycardia without fever. Antibiotic and other medicinal treatment seemed without effect. Death was usually observed within 48 hours of symptom onset. Necropsy did not yield a clear diagnosis. Selenium values had been determined on 5 animals on the suspicion of white muscle disease (WMD), they were low. Since then, (organic) selenium was supplemented without effect. Blood was taken of three symptomatic animals to determine LDH, CPK and Troponine I values. WMD was seen as the primary suspect. However, troponine I was not elevated, reducing the possibility of cardiomyopathy. CPK was elevated, but not as high as one would expect in a WMD case, easily explained by extended recumbency. Feed analysis showed the addition of 8.9% cocoa shell pellets till 17/10/2018 and 5% onwards. Serum of two symptomatic animals was tested for theobromine, one was positive. Analysis of the theobromine content in the feed showed it lay above the EFSA guideline of 300 mg/kg for growing cattle. Symptomatology matched that of case reports of theobromine intoxication in calves and cattle. Deaths could be related to a cumulative effect (T1/2 is 14-21 hours) and the high palatability, leading to increased selection by the animals.



## Oral presentations

### Ly6C<sup>hi</sup> monocytes are key orchestrators of gammaherpesvirus lifecycle

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Gammaherpesviruses (γHVs) are 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 persist in the host in a symbiotic relationship. Hence, these persistent infections usually do not cause major harm in healthy adult host. In some instances, the persisting herpesvirus infections might even provide some benefits to the host. In that context, our laboratory recently showed that a pulmonary infection with Murid gammaherpesvirus 4 (MuHV-4), a γHV infecting mice, induces the recruitment of Ly6C<sup>hi</sup> monocytes from the bone marrow to the site of primary infection. These recruited cells have been shown to regulate some heterologous responses against aeroallergens and block development of allergic asthma. While the recruitment of these Ly6C<sup>hi</sup> monocytes induced by MuHV-4 infection confer a benefit to the host in the context of allergic asthma, their direct role in MuHV-4 lifecycle remains unknown. The objective of this study is to decipher the importance of Ly6C<sup>hi</sup> MOs in MuHV-4 lifecycle. To that end, we investigated whether Ly6C<sup>hi</sup> MOs affect MuHV-4 replication and investigated the role of Ly6C<sup>hi</sup> MOs in the establishment of both innate and adaptive immune response to MuHV-4. By using complementary mouse models deficient for Ly6C<sup>hi</sup> MOs, we highlighted the importance of Ly6C<sup>hi</sup> MOs recruitment in the MuHV-4 lifecycle. Our results show that, at early time points after MuHV-4 infection, recruitment of Ly6C<sup>hi</sup> MOs is associated with reduced viral replication, clinical signs, and neutrophilic infiltration in lungs. Moreover, these recruited Ly6C<sup>hi</sup> MOs produced high levels of IL-10 suggesting regulatory properties and dampen the establishment of MuHV-4 specific adaptive immune response. These results highlight that Ly6C<sup>hi</sup> MOs are key orchestrators of immune response following γHV infection.

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### Diagnostic and prognostic value of hypoglycin A, methylenecyclopropyl acetic acid-carnitine and, acylcarnitines profile in horses with atypical myopathy

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Equine atypical myopathy (AM) is an acute environmental intoxication linked to hypoglycin A (HGA). Previous studies showed that blood of diseased animals exhibited HGA, methylenecyclopropyl acetic acid-carnitine (MCPA) and, a severely modified acylcarnitines profile (AC). This latest reflects the biochemical derangement induced and is thought to be useful to diagnose AM and to prognosticate the chances of survival. In contrast to clinically affected horses, HGA but not MCPA was detected in the blood of healthy co-grazing horses (Co-G). The aim of this study was to confirm the diagnostic and/or prognostic value of HGA, MCPA and AC in AM. A retrospective and exploratory blood analysis was conducted on European AM cases reported to the Atypical Myopathy Alert Group from autumn 2006 to 2018. Confirmed or highly probable AM cases (n=155) and their Co-G (n=75) were included in the study. Serum concentration of HGA, MCPA and AC were determined by previously validated methods. Both, HGA and MCPA concentrations were statistically higher in serum of AM cases than in Co-G (HGA = 4.12±0.38 vs. 1.60±0.21 μmol/l and MCPA = 332.50±71.06 vs. 15.85±5.22nmol/l, respectively) challenging their usefulness for diagnosis. Among AM cases, survivors had a calculated prognosis for survival (based on AC) of 19±3% vs. 51±6% in non-survivors. All Co-G had a prognosis above 95%. For the first time, MCPA was detected in apparently healthy Co-G. An early detection of HGA/MCPA in Co-G might be a promising step in prophylaxis whereas the survival prognosis may be used to balance the chances of success with cost management, human involvement and with animal suffering.

### Identification of genetic variants affecting immune traits in Belgian White-Blue cattle

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One of the main challenges of the agriculture is to increase its capacity to produce in order to respond to the growing needs worldwide and, at the same time, to reduce the use of synthetic inputs. This is particularly true for beef cattle. The Belgian White-Blue breed (BWB) is the most muscled breed in the world and generates a big interest to improve meat production in pure breeding but also in industrial crossbreeding. In order to speed up and to improve the identification of the best genitors, Genomic Selection (GS) has recently been initiated in BWB. GS aims at predicting the future phenotype of newborns by reading their genomes for genetic variants. Until now, the identification of these variants has mainly been focused on production features and has not really considered resistance to pathogens. However, recent studies in humans indicate that about 50% of the variability of immune responses is explained by genetic determinants. With the ResistOmics project, we aim at identifying genetic markers associated with a better resistance of BWB individuals against infectious diseases. To that purpose, we characterized the diversity and the functionality of peripheral blood leukocytes of 150 young BB-B bulls housed at the Center for Bovine Selection (AWE, Ciney, Belgium) at two different time points. Firstly, a flow cytometry analysis has been performed in order to distinguish and characterize the different peripheral blood leukocyte populations: neutrophils, B cells,  $\gamma\delta$  T cells, CD8/CD4 T cells, NK cells, monocytes and dendritic cells. Secondly, these leukocytes have been re-stimulated *ex vivo* by 16 different selected pathogen extracts or synthetic molecules in order to determine the level of secretion of different cytokines. In the future, association of these phenotypic traits with genomic and transcriptomic information should allow us to identify genetic variants responsible of immune traits in bovine in order to orientate selection. This approach should also reveal new fundamental mechanisms of the regulation of immune responses in cattle.

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### Impact of feeding calves waste milk containing antibiotic residues on the rise of antimicrobial resistance in fecal *Escherichia coli*

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In order to draw full benefits from milk it is still commonplace in Europe to feed waste milk containing antimicrobial residues to calves. The purpose of this study was to determine and quantify risks associated with this practice for the development of antimicrobial resistance in fecal *E. coli* of calves. During eight weeks, 36 Holstein calves and seven Belgian blue calves were fed waste milk. This milk was tested for antimicrobial residues using Extensio milk analysis platform. Feces from calves were collected weekly, then underwent bacteria isolation (n=130) and antibiograms (n=134). Control group consisted of 10 calves exclusively fed with milk powder. Most commonly found residues in waste milk were beta-lactams and aminoglycosides (39 and 37% of samples respectively). Thirteen percent were free from any residues. Bacteriological analysis revealed overall 98% of *E. coli* strains among which 12 EHEC, 41 CS31A, and 14 F17a. Twenty-six percent of antibiograms found *E. coli* strains sensitive to all tested antibiotics. Kanamycin resistance was the most common (66%) whereas no strain was reported resistant to colistin. Multivariable analysis showed no significant differences between waste milk fed calves and control group regarding mean number of resistances, resistance prevalence against critical antibiotics and multiresistance prevalence, as well as resistance prevalence against each individual antimicrobial. Age was a protective factor of resistances against some antibiotics. Isolation of EHEC strains could be associated with consumption of waste milk. Additional data collection is still ongoing to determine exactly how waste milk feeding affects antimicrobial resistance.

## Oral presentations

### Untargeted metabolomics studies focusing on prognosis and resistance factors leading to the identification of new potential biomarkers in equine atypical myopathy

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Atypical myopathy (AM) is a severe rhabdomyolysis affecting grazing horses. AM is caused by the ingestion of hypoglycin A, which is found within seeds and seedlings from sycamore maple. Once ingested, hypoglycin A is metabolized into a toxic compound that impairs lipid metabolism. The lethality rate is comprised between 61 to 74 %. In AM affected pastures, healthy cograzing horses (i.e. asymptomatic) account for 57.5%. The aim of the study was to identify blood discriminant biomarkers with prognosis and resistance relevance in AM. With this purpose, the first blood sample was analysed by comparing pairwise groups: i) ill vs. healthy cograzing horses ii) non-survivor vs. survivor horses. Serum or plasma heparin lithium samples were analysed in biological triplicate using an untargeted polar metabolomic approach with an ultra-performance liquid chromatography coupled with mass spectrometry. Metabolic data were evaluated using unsupervised and supervised analyses and machine learning algorithm. Thirty-six metabolites, taken independently, were all found to fully discriminate ill and healthy cograzing horses. Two signatures of five metabolites each were found to discriminate survivor and non-survivor horses. Two acylcarnitines (i.e. isobutyrylcarnitine and hexanoylcarnitine) were identified with a high confidence level, using an in-house library. Fourteen other metabolites were putatively identified, based on online libraries. Further investigations are required in order to validate signatures.

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### Pilot study assessing the possible benefits of a higher level of implementation of biosecurity measures on farm productivity and health status in Belgian cattle farms

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A proper level of biosecurity (BS) is necessary in order to prevent and control the introduction and spread of infectious diseases. Nevertheless, previous studies highlighted a low implementation level of biosecurity measures (BSM), especially in cattle farms. Among the reasons of non-implementation, the cost-benefits ratio and the utility of the BSM were the most frequently expressed by the farmers. In order to support a long-term behavior change, it is therefore necessary to gather more information and evidence on their cost-effectiveness and their importance or utility in terms of disease prevention and control. The objectives of this study were to determine whether the farm or farmers' profile correlated with the implementation level of BSM and if there was a positive correlation between the BSM implementation and the farm production and health parameters. A stratified and randomised survey was conducted in 100 Belgian farms to collect data on BSM implementation. The health status and production data of the surveyed farms were provided by the Regional Animal Health Services provided the farm. Different BS score and sub-scores were calculated for each farm based on the implementation level of different BSM grouped in 16 domains. The study highlighted a significant and negative correlation between the mortality rates in adult cattle (over 24 months) and young calves (0-7 days) and different BS compartment scores. It also demonstrated that the farms having a higher general BS score were more likely to have a BVD free status. These evidence-based findings are encouraging as they demonstrate the benefits of implementing BSM and could promote their adoption by farmers.

### Transcriptomic comparison of zebrafish and carp larvae after inoculation with Cyprinid Herpesvirus-3

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Herpesviruses have mainly evolved by co-speciation with their hosts, leading to narrow host ranges. As such, among herpesviruses, there is a high degree of complexity and diversity with regard to viral host interactions. Cyprinid herpesvirus-3 (CyHV-3) is the archetype of fish alloherpesviruses. It causes mortality only in common and koi carp. Recently, we compared the ability of CyHV-3 to infect carp and zebrafish larvae (both members of the Cyprinidae family). Using in vivo imaging, light-sheet microscopy and RNAseq we observed that zebrafish larvae are permissive (all 156 viral ORFs transcribed, replication within and spread among host cells) to CyHV-3 infection after inoculation by pericardial injection. However, zebrafish larvae displayed rapid clearance of the infection by 4 days post inoculation (dpi). In contrast, carp larvae exhibited more intense viral replication, morbidity and the onset of mortality by 2 dpi. RNAseq analysis performed on larvae indicated that carp exhibited delayed upregulation of several pathways compared to zebrafish in response to infection, including the NOD-like receptor signalling and cytokine receptor interaction pathways. Also, on both 1 and 2 dpi, the DNA replication pathway was upregulated in zebrafish, but downregulated in carp ( $p > 0.005$ ). The most prominent response to infection in zebrafish was the upregulation of the proteasome pathway (including immunoproteasome) ( $p > 0.005$ ), however, in carp, this pathway showed no significant change during the experiment. These key differences in pathway responses may play or reveal key roles in enabling zebrafish larvae to control CyHV-3 infection, and thus, they will be investigated further.

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### Serological “aspecific” reaction against BoHV1 in Belgium: possible cross-reactivity of other herpesviruses?

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Infectious bovine rhinotracheitis (IBR) is a disease in cattle caused by bovine herpesvirus 1 (BoHV1). In Belgium, since 2012, a mandatory official control program has been implemented, which assigns two different statuses to IBR-free herds: I3 for vaccinated herds or I4 for unvaccinated herds. Two serological ELISA tests are used in the control program, detecting antibodies raised against either glycoprotein B (expressed in both vaccine and field strains of the virus) or glycoprotein E (only for field strains). During the annual serological monitoring of I4 herds (ELISA gB negative, unvaccinated), unexpected positive IBR serological reactions (ELISA gB positive but gE negative) were reported. Some were confirmed by a positive reaction in ELISA test detecting total antibodies and/or seroneutralisation test against BoHV1 (« pseudovaccination »). But others, on the contrary, were not confirmed by other BoHV1-specific serological tests (true gB « aspecific » reactions). For some herds the positive reactions concerned only one animal (single reactor) and were transient, disappearing at the second serological control of the animal. But in other cases, the animal remained positive and needed to be culled to avoid losing the I4 herd status. Further, in other herds, aspecific reactions appeared repetitively and concerned a larger number of animals with, as a consequence, the loss of the I4 status downgraded to I3 status (gB positive/gE negative). Beside involuntary vaccination or circulation of recombinant or vaccine strains, the presence of others herpesviruses in herds was investigated to explain such aspecific reactions caused by possible cross-reactivity in IBR gB ELISA.

## Oral presentations

### **An attenuated recombinant vaccine against Cyprinid herpesvirus 3 induces intraepithelial acquired immunity and prevents transmission of a virulent strain**

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Cyprinid herpesvirus 3 (CyHV-3) is causing severe economic losses worldwide in common and koi carp industries. Recently, the laboratory of Immunology-Vaccinology developed a live attenuated recombinant vaccine compatible with mass vaccination of carp by immersion in water containing the virus. The vaccine was proved to induce an immune protection at the portal of viral entry which is the skin epidermis covering the body and the fins. Here, we further investigated the immune protection expressed by vaccinated subjects. First, we studied the role of the epidermal mucus in the protection induced by the vaccine. Neutralization assays performed with soluble mucus extracts of vaccinated subjects or carp that survived a wild-type infection revealed a neutralizing activity comparable to the one observed for naïve fish. Using bioluminescent in vivo imaging system (IVIS) and a virulent strain of CyHV-3 expressing luciferase, we investigated the effect of epidermal mucus removal just before inoculation of the virus. These experiments demonstrated that despite the removal of the epidermal mucus on immune subjects (either vaccinated or survivor of a wild-type infection) the challenging virus was not able to infect the skin. In contrast, this treatment enhanced the infection of naïve subjects. Using IVIS, we demonstrated that the intraepithelial protective immunity observed in vivo was also detectable ex vivo in fin explants. Next, we investigated the effect of vaccination on the ability of an immune fish exposed to a wild-type strain to contribute to its spread. Our results demonstrated that immune fish (either vaccinated or survivor of a wild-type infection) were not able in contrast to naïve fish to contribute to the spread of a wild-type strain.

**Short talks**

## Short talks

### **The Zalpha domain containing protein ORF112 of Cyprinid herpesvirus 3 is associated with RNA nucleocytoplasmic transport**

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Nucleocytoplasmic transport occurs through nuclear pore complexes and is mediated by saturable transport receptors that shuttle between nucleus and cytoplasm. In the nucleus of eukaryotic cells, RNA molecules are assembled into ribonucleoprotein (RNP) complexes that are then translocated into the cytoplasm. Several proteins harboring RNA binding domains (RBD) such as dsRBD, RRM and Zalpha exhibit nucleocytoplasmic shuttling. Zalpha domain binds to left handed dsRNA and dsDNA. The most studied Zalpha domain containing protein ADAR1 is able to shuttle between nucleoplasm and cytoplasm in addition to its RNA editing activity. The present study was devoted to ORF112 encoded by Cyprinid herpesvirus 3. Recently, we demonstrated that the Zalpha domain of CyHV-3 ORF112 is essential for viral replication in cell culture. In the present study, we report several observations supporting its association with RNP complexes and nucleo-cytoplasmic shuttling: (i) the insertion of two NES signal in the coding sequence of ORF112 did affect neither viral replication nor its shuttling activity; in contrast insertion of 2 NLS abolished the replication of the virus; (ii) we found that ORF112 localizes in cytoplasmic stress granules induced by arsenite treatment; (iii) ORF112 forms dynamic aggregates due to its intrinsically disordered (IDD) N-terminal glutamine rich Prion related domain (PrD). RNAs and RNA-binding proteins are recruited in ORF112 aggregates. Together, these data suggest a role of CyHV-3 ORF112 with RNA transport and/or functions.

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### **Ear canal microbiota and mycobiota – effect of preventive use of a AIS topical in atopic dog without any sign of active otitis.**

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The use of topical anti-inflammatory steroids (AIS) to control the inflammation of the external ear canal can represent an interesting alternative to maintain a balance of bacterial and fungal flora. Ten dogs with atopic dermatitis are selected. A treatment with an instillation of 0,3ml of Mometasone 0,1% (Elocom lotion®) is applied twice a week during four weeks in the right ear canal. The left ear is used as an untreated control. A clinical and a cytological exam of the external ear canals are performed at Day 0, Day 14 and D28 to determine a clinical index (IC: minimum 0 and maximum 12). Samples are taken at Day 0, Day 14 and Day 28 for the microbiota (identification of the specificities of the hypervariable segments V1-V3 of the 16s DNA amplicons with a taxonomic assignment carried out by the Mothur software using the SILVA database) and the mycobiota identification (identification of the variables of the ITS areas with a taxonomic assignment carried out by the Mothur software using the UNITE v7.2 fungal ITS database). The IC indexes of the right and left ears are slightly different at Day 0 and Day 28 but not significantly ( $p=0,302$ ). The indexes at Day 0 and Day 28 are significantly different ( $p=0,024$ ) for the treated ears but not for the untreated ears ( $p=0,647$ ). The microbiota analysis of the external ear canals are similar to observations previously reported. There is no significantly difference between the treated or untreated external ear canal microbiota (Kruskal-Wallis or Friedman Test for the nonparametric paired data) for the richness, evenness or diversity. The mycobiota appears very variable from one dog to another and often different from the right to the left. The most abundant family are malasseziaceae and cladosporiaceae. There is no significantly difference between the treated or untreated external ear canals mycobiota (Kruskal-Wallis or Friedman Test) for the richness, evenness or diversity.

### **Outbreak of post-parturient hemoglobinuria associated with hypophosphatemia in a dairy herd**

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Post-parturient hemoglobinuria is described with a low incidence in dairy herds. We herein describe a fatal post-parturient hemoglobinuria outbreak in a Belgian dairy herd of two hundred cows. Four cows in early lactation were referred at the at the Clinic for Ruminant of Liège for depression, decubitus, decreased feed consumption and milk production associated with anemia and hematuria. Blood analysis revealed severe regenerative anemia with hemolysis and neutrophilia. Investigation for bacterial infection and blood parasite gave negative results. Blood plasma analysis indicated hypophosphatemia; a suspicion of post-parturient hemoglobinuria was made. Hospitalized animals received blood transfusion, supportive intravenous fluids and organic phosphorus provided intravenously. A herd visit was performed to investigate the cause of hypophosphatemia. It appeared that phosphorus daily intake for lactating cows was half the minimum recommended, especially in the lactation peak for highly productive cows. This could be explained by a ration almost composed of pressed beet pulps, straw and protein concentrate without adequate mineralization. In order to correct this deficiency, it was recommended to add two kilos of bran to the daily ration of the cows, as well as 150g of dicalcium phosphate, and no additional case has been reported since then. Post-parturient hemoglobinuria affects high-lactating cows during the first month after calving. This herd outbreak was original because of the high number of clinical and subclinical cases, and because of the ration content that permitted to reach a very high production of milk even though the mineralization was totally inadequate.

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### **Belgian case study on flumethrin residues in beeswax: Possible impact on honeybee and prediction of the maximum daily intake for consumers**

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To assess the health risk posed by flumethrin residues in beeswax to honeybees and honey consumers, 124 wax samples randomly distributed in Belgium were analysed for flumethrin residues using liquid chromatography/tandem mass spectrometry analysis. The risk posed by flumethrin residues in beeswax to honeybee health was assessed through the calculation of a non-pondered and a pondered Hazard Quotient by the prevalence rate of flumethrin considering an oral or topical exposure. No statistical difference was found when comparing both the average flumethrin residues concentrations and contact and oral pondered hazard quotients between apiaries with lower and equal or higher than 10% of colony loss. Flumethrin residues estimated daily intake by Belgian consumers through honey and wax ingestion was estimated via a deterministic (worst-case scenario) and a probabilistic approach. The highest estimated exposure was less than 0.1% of the theoretical maximum daily intake for both approaches, meaning no risk for human health.



## Short talks

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

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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 of seven CyHV-3 strains in cell culture, we observed that three of them formed syncytia. 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. With the goal to understand the genetic determinism of the syncytia phenotype and to identify the VTP(s) responsible for CyHV-3 syncytia formation, we compared the sequences of the ORFs encoding VTPs for seven CyHV-3 strains. This analysis revealed that the three strains forming syncytia shared mutations in ORF27 and ORF131: 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 in ORF131 mediates the formation of syncytia in cell cultures. Furthermore, we demonstrated that the SNP responsible for syncytia formation is associated with a higher viral replication *in vitro* but a lower virulence *in vivo*.

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### **Identification of the cellular protein interactome of Kaposi's sarcoma-associated herpesvirus tegument protein through a Mammalian Protein-Protein Interaction Trap approach.**

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Gammaherpesviruses ( $\gamma$ HVs) are widespread viruses that cause lifelong infections in many mammalian species and represent a significant cause of diseases. Thus, in humans, Kaposi's sarcoma-associated herpesvirus (KSHV) is associated with several cancers and is therefore a major subject of research. Among the different constituents of the  $\gamma$ HVs virions, tegument proteins play major roles in virus entry, morphogenesis and egress but also in early evasion of innate immune recognition. In order to identify new roles of KSHV tegument proteins, we performed a screening of the protein interaction between KSHV tegument proteins and a pool of 18,000 human proteins using a mammalian protein-protein interaction trap approach (MAPPIT). This analysis identified 347 potential interactions between KSHV tegument proteins and cellular partners. These results represent therefore a pool of promising information for a better understanding of KSHV lifecycle and for the development of new therapeutic strategies against this virus.

### Cyprinid herpesvirus 3 prevents the formation of stress granules induced by arsenite treatment

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Eukaryotic cells can express specific mechanisms that quickly block protein synthesis when facing various types of stress, including viral infections. Stress granules (SGs) are discrete cytoplasmic inclusions into which stalled translation initiation complexes are dynamically recruited in cells subjected to stresses. Incubation of cells with arsenite is a well known process to induce the formation of SGs. Recently, we observed that CyHV 3 prevents the formation of SGs induced by arsenite treatment in infected cells. This phenomenon was proved to be time-dependent. Resistance to arsenite treatment was observed after at least 12 hours of infection. We also observed that transfection of CCB cells with the entire genome of CyHV-3 cloned as a bacterial artificial chromosome (BAC) conferred protection to arsenite treatment applied 48 hours after transfection. With the goal in mind to identify CyHV-3 ORF(s) causing this phenotype, we produced a series of recombinant CyHV-3 BAC plasmids, each plasmid being deleted for approximately 10 consecutive ORFs. We found that all plasmids were able to inhibit SGs induced by arsenite with exception of the plasmid deleted for ORF100 to ORF111. These results suggested that one or several proteins encoded by ORF100 to ORF111 could be responsible for the resistance to arsenite. Next, we tested the ability of the proteins encoded by ORF100 to ORF111 expressed ectopically in CCB cells to prevent the formation of arsenite induced SGs. We observed that both ORF101 and ORF104 inhibited SG formation by approximately 50%. Finally, a BAC plasmid deleted for both ORF101 and ORF104 was produced and tested for its ability to inhibit arsenite-induced SGs. The double deleted plasmid did not confer any protection to transfected cells demonstrating that ORF101 and ORF104 are the proteins responsible for the resistance conferred by CyHV-3 against arsenite treatment. Further studies are required to unravel the mechanisms by which CyHV-3 prevents the formation of SGs and the biological relevance of this phenomenon.

### Foodborne zoonotic trematodes in Bithyniid snails (Gastropoda: Bithyniidae) in Northern and Central Vietnam

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Foodborne zoonotic trematode (FZT) infections are widely distributed and continue to be an important public health problem in Southeast Asia, especially in Vietnam. The life cycle of some important liver flukes and intestinal flukes involves bithyniid freshwater snails as the first intermediate hosts. A cross-sectional study was conducted in Yen Bai and Thanh Hoa Provinces in dry and rainy seasons to investigate diversity of FZT in bithyniid snails from different habitats. Among 26,463 examined bithyniid snails, a total of four species was found in which *Bithynia fuchsiana* and *Bithynia goniomphalus* were only present in Thanh Hoa while *Allocinma longicornis* and *Parafossarulus striatulus* were present in both provinces. Of four infected snail species, eight cercariae groups were detected. The highest overall infection in Yen Bai was recorded in rice field (35.1%, rainy season) while the highest one in Thanh Hoa was in canal (7.6%, rainy season). *Bithynia fuchsiana* was found to harbor the most diverse trematode fauna (7 types). As regards potential FZT, parapleurolophocercous emerged as the most common cercariae in Yen Bai while echinostome is the most common in Thanh Hoa. Remarkably, for the first time in Thac Ba Lake-Yen Bai, *Clonorchis sinensis*, the most prevalent and harmful human liver fluke in Asia, was found in *P. striatulus* based on molecular identification (ITS2 region). Other important intestinal fluke, *Echinochasmus japonicus*, was found in both four host snail species of the Bithyniidae present in Thanh Hoa. This finding has confirmed the presence of FZT in these areas and alerted scientists and managers to bring an urgent action for integrated control intervention.

## Short talks

### **Prophylactic antibiotic usage by Belgian veterinarians and breeders during elective caesarean section in Belgian Blue cattle**

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The Antimicrobial Consumption and Resistance in Animals (AMCRA) aims to quantify and reduce veterinary antibiotic (AB) use in Belgium and provides practical guidelines. A survey was sent to Wallonian veterinarians in order to assess the use of AB before, during and after bovine elective caesarean section (ECS), the most common surgery in Belgian rural practice.

Among 380 contacted veterinarians, 113 answered the survey. All respondents use AB during ECS. Most respondents use penicillin in standard situations, while a minority use second or third choice drugs (amoxicillin, oxytetracyclin or lincomycin-spectinomycin). The majority of veterinarians administer AB during or after surgery, while a minority administer AB preoperatively. Most veterinarians apply an AB treatment of 1 day only. The administration route (IM, IP, IU, between muscle layers) varies widely. Many veterinarians perform an IP injection of AB, which is not registered for cattle. The dosage of AB varies enormously and is often excessive, particularly when multiple injection routes are used, and is often in conflict with AMCRA guidelines.

Results show a striking lack of consistency in the AB therapy during ECS by rural veterinarians. Concretely, the use of second and third line products should be strongly discouraged. The use of unregistered application routes, such as IP injection, cannot be justified. Evidently, the injection of excessive or insufficient doses of antibiotics should be avoided at all cost. A major challenge lies in the education of veterinary students and the sensitization of veterinary practitioners in order to avoid or unlearn unnecessary habits concerning AB use.

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### **Replicative fitness recuperation of a recombinant murine norovirus – in vitro interplay of genetic shift and drift**

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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. Here, the capacity for 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 induced fitness loss of an infectious recombinant. Replicative fitness regain of the recombinant was demonstrated via growth kinetics differences and increase of mean viral lysis plaque size after serial passaging. 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. Subsequent DNA-based reverse genetics recovery of infectious virus at similar titres for parental and recombinant constructs indicated 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.

## Bovine Papular Stomatitis and Pseudocowpox viruses found in ticks infesting cattle in West Africa

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Tick-borne viruses is a research field that is still largely unexplored in the West African sub-region. Most of the work on tick-borne viruses in this part of the continent has been carried out as part of research activities in human health, and more rarely in animal health. Despite their harmful effects on ruminants, and in spite of their zoonotic nature, no study has yet reported the occurrence of virus in ticks in Burkina Faso to our knowledge. In this study, we used real time PCR assays to achieve a wide screening of viruses infecting ticks, collected on cattle in eastern Burkina Faso. A total of eight groups of viruses were investigated both by generic and specific RT-PCR, followed by sequencing by Next Generation Sequencing (NGS). A total of 665 ticks were distributed into 178 pools. Two genera have been identified such as parapoxvirus and flavivirus. Bovine Papular Stomatitis virus (BPSV) was evidenced in 18 pools of ticks with a absolute prevalence of 10.11%, while Pseudocowpox virus (PCPV) in 7 pools with 3.93%. Presence of Crimean Congo Hemorrhagic Fever and Rift Valley fever viruses were not confirmed. Although the cattle on which the ticks were collected were asymptomatic, the question of the vectorial competence of these tick species with regard to the transmission of BPSV and PCPV remains questionable. Further *in vitro* study will be needed.

## Genomic and biologic comparisons of Anguillid Herpesvirus 1 strains

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Anguillid herpesvirus 1 (AngHV-1), also known as herpesvirus anguillae (HVA) belongs to the *Cyprinivirus* genus of the *Alloherpesviridae* family. To date, the genome sequence of only two AngHV-1 isolates has been published and there is only a limited knowledge of the virulence and pathogenesis of this viral species. The goal of the present study was to address these lacunas. Here, we sequenced a further 7 strains of AngHV-1 isolated from different geographical origins. The sequences of the 9 genomes were then analysed. Phylogenetic analyses revealed a low genomic variability among strains, although two main genetic lineages could be identified. Recombination detection analyses indicated putative occurrence of 5 recombination events during evolution. They also suggested the existence of a third, yet unidentified lineage. Analysis of disrupted genes identified those that are not essential for viral growth *in vitro*. *In vitro* biologic comparison of AngHV 1 strains revealed important differences of viral growth (up to 10 times). To further compare the strains *in vivo*, we produced for 4 strains representative of the viral species recombinants expressing a fusion reporter protein consisting of luciferase and copepod green fluorescent protein (LucGFP). Analysis of AngHV-1 LucGFP strains showed that: (i) the intergenic insertion of the reporter cassette did affect neither the expression of the flanking ORF32 and ORF33 nor the replication of the virus *in vitro*; (ii) the recombinant strains expressed luciferase and GFP. Using the LucGFP recombinants and *In vivo* Imaging (IVIS), we tested the sensitivity of glass eels to AngHV-1 infection. We demonstrated that glass eels are resistant to AngHV-1 infection after inoculation through natural routes (oral contamination or immersion in water containing the virus) but sensitive when inoculated by intraperitoneal injection.

## Short talks

### **Cattle transhumance between Burkina Faso and Benin: corridors and health risk factors associated to ticks**

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Livestock plays a key role in the macro economy of West Africa and provides livelihoods for millions people. The main strategy in cattle rearing in West Africa is pastoralism, including transhumance, a seasonal migration of cattle with its herders. This adaptive strategy aims to optimize livestock access to water and pastures. This strategy can favour pathogens and vectors spread. This study aimed to highlight, firstly the corridors and grazing areas used by Burkina Faso (BF) transhumant cattle herds, secondly the characteristics of departure and arrival areas of transhumance and thirdly, the risk factors related to the introduction and the spread of the invasive tick *Rhipicephalus (Boophilus) microplus* (Bm) in free areas. For that purpose, GPS devices were given to 27 herders to monitoring a transhumance season of BF cattle herds. The analysis of the 14966 spots emitted by the GPS devices with the QGIS software permits to know accurately the four main different corridors and the five main grazing areas used by cattle herds during the transhumance. Statistical analysis of vegetation index (NDVI) and temperature data, revealed significant differences between departure and arrival areas. Colder temperatures were recorded in areas where Bm ticks were found. Additionally, the NDVI is better whereas the rainfall is not significantly different. The invasiveness and adaptability of Bm and the frequent stays of transhumant herds in infested areas suggest its potential introduction and establishment in free areas soon. Moreover, frequent intrusions of the domestic cattle in the wildlife reserves is another risk of introduction of Bm in wildlife and other pathogens sharing.

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### **Dynamic contrast-enhanced computed tomography in dogs with nasal tumours: protocol development and perfusion analysis**

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Nasal tumours are common neoplasms in dogs and are associated with high morbidity and mortality rate. Megavoltage radiotherapy is the treatment of choice yet is associated with high cost and logistical constraints for the owners and significant side effects for the patients. In humans, perfusion parameters derived from dynamic contrast-enhanced computed tomography are correlated with response to chemoradiation and help in the therapeutic decision. The aims of the study are to set up a quick and easy perfusion computed tomography protocol that could be routinely used in clinical practice, to assess it on a small population of dogs with nasal tumours and to determine the intra-observer agreement of the perfusion parameters. Six dogs were included in the study and comprised 4 carcinomas and 2 sarcomas. Perfusion computed tomography was easily incorporated within the conventional computed tomography protocol and was quick and successful in all dogs. Tumoral blood flow, blood volume and mean transit time were calculated for each dog twice and intraclass correlation coefficients were calculated. The 2 sarcomas had the lowest tumoral blood flow, suggesting that sarcomas are less perfused.

### **Helminth infection is associated with the accumulation of lung interstitial macrophages and increased susceptibility to gammaherpesvirus infection in C57BL/6 mice**

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Parasitic helminths can imprint innate cells such as lung macrophages for accelerated clearance in a secondary infection. However, it is unknown how helminth-induced macrophage changes in the lung affect the response to concurrent bystander infection with viruses. Here, we have examined the lung macrophage responses after *Schistosoma mansoni* egg-induced inflammation or *Nippostrongylus brasiliensis* infection and observed a severe expansion of CD11b+ interstitial macrophages while siglec-F+ alveolar macrophages were reduced. Interestingly, expansion of interstitial macrophages was more pronounced in C57BL/6 compared to BALB/c mice and we observed a strong upregulation of the M(IL-4) alternative activation marker YM1 in interstitial macrophages of C57BL/6 that was less pronounced in BALB/c mice. We then examined the susceptibility of both mouse strains to murid gammaherpesvirus 4 (MuHV-4) infection and observed that helminth exposure rendered C57BL/6 mice highly susceptible to MuHV-4 acute infection whereas BALB/c mice controlled viral infection earlier as previously published by our group through the expansion of “virtual” memory CD8+ T cells (TVM) (Rolot et al., 2018. Nat Commun. 2018 Oct 30;9(1):4516). We further confirmed the role of IL-4-induced TVM by helminth in the CD8-mediated control of MuHV-4 in both C57BL/6 and BALB/c strains, suggesting that the increased early susceptibility of C57BL/6 mice to MuHV-4 does not affect the induction of enhanced effector CD8+ T cell responses. Finally, we observed increased proportions of MuHV-4-infected macrophages 4 days after viral infection when C57BL/6 mice were exposed to helminths, suggesting that helminth infection modifies the lung macrophage niche to become more permissive to MuHV-4 infection.

# Posters

# **Veterinary Public Health**



## Posters

### 1. Presence of Maed-Visna infection in western Algeria sheep flocks

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Maedi-Visna is a worldwide insidious disease that has a significant economic impact in sheep flocks. In Algeria, few data exist about MVV presence in sheep floc, described studies are limited to some regions of the country. The aim of this study is to detect anti-MVV antibodies in sheep population in western Algeria sheep flocks and to estimate the influence of some parameters on the infection appearance (age, sexe, herd size, presence of goat and flock type). A total of 184 samples were randomly selected from three Algerian western regions (Oued el Fodda, Gdyel, Sig) then analyzed by ELISA, of which 6% were seropositive, including 2% in Oued el Fodda, 8% in Gdyel and 18% in Sig, the herd seroprevalence was estimated at 33%. In addition, it was found that both of age and flock type had a significant effect on the increased risk of virus infection.

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### 2. Impact of 3'-sialyllactose and *Bifidobacterium crudilactis* on infant microbiota and *Escherichia coli* O157:H7 virulence modulation, using the SHIME® model

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In a previous work, cell free spent media (CFSM) obtained from culture of *Bifidobacterium crudilactis*, a bifidobacteria of bovine origin, and a major bovine milk oligosaccharide (BMO), the 3'-sialyllactose (3'SL), modulated virulence gene expression of *Escherichia coli* O157:H7. The aim of this study was to evaluate this effect directly on microbiota. The gastrointestinal model SHIME® was inoculated with feces from a young child and four treatments were successively administrated: 3'SL, *B. crudilactis*, 3'SL and *B. crudilactis* simultaneously and CFSM from 3'SL and *B. crudilactis* culture. Collected samples have been analysed for SCFA concentrations using HPLC, and microbiota composition using pyrosequencing. In addition, impact of SHIME® samples have been assessed on *E. coli* O157:H7 virulence genes expression. The results showed that SCFA levels were stable during the experiments with mainly production of acetate, propionate and butyrate. Metagenetic analysis showed a microbial diversity in transverse (TC) and descending colon (DC) close to feces, dominated by *Bacteroides*, *Prevotella* and *Fusobacterium*, while the ascending colon (AC) showed a microbial diversity dominated by *Veillonella*. Probiotic treatment with *B. crudilactis* seemed to increase proportions of bacteria beneficial to host health (*Prevotella*, *Lactobacillus*, *Lachnospiraceae*, *Prevotella*, *Bacteroides*, *Akkermansia*). Also, SHIME® fractions tended to down-regulate virulence gene expression of *E. coli* O157:H7 (*ler*, *fliC*, *luxS*, *stx1* and *qseA*). Interesting effects have therefore been highlighted after this first run. However, those trends have to be validated with the further replicates on the SHIME® system.

### 3. Detection and eradication of *Helicobacter* spp. infection in laboratory mouse colony

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Endemic infection with *Helicobacter* spp. is frequently detected in research mice colonies. So far, several species have been isolated including *H. hepaticus*, *H. bilis*, *H. muridarum*, *H. typhlonicus* and *H. rodentium* as the most prevalent ones. The transmission of this pathogen is through fecal-oral route, followed by persistent long-term shedding in infected individuals. In immunocompetent mice the infection is subclinical, however, emerging evidence show that it can have significant health implications leading to typhlocolitis, intestinal and hepatic tumors and mammary gland neoplasia. Moreover, the infection alters reproduction successes with increase in fetal resorption, decline in neonatal weight and decreased number of weaned pups detected in both experimentally and naturally infected mice. In addition to the impact on animal health and welfare, the infection might have confounding effects on obtained research results; therefore, regular screening and eradication are key for obtaining a pathogen free colony. In order to eliminate a naturally acquired *Helicobacter* infection in our animal facility, we have attempted an eight-week long treatment protocol with amoxicillin, clarithromycin, metronidazole and bismuth, administered in drinking water. This treatment route is more economical, time-efficient and noninvasive compared to other alternatives for eradication such as treatment with medicated diet, oral gavage or cross fostering. To determine the results of the administrated therapy we have performed qPCR of DNA extracted from fecal pellets.

### 4. Rat Aryl Hydrocarbon Receptor (rAHR) and human Estrogen Receptor (hER) agonistic activity of blubber sampled from pre-moult and post-moult North Sea grey seals (*Halichoerus Grypus*)

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We investigated the *in vitro* activity of blubber extracts from North Sea Grey seals (*Halichoerus grypus*) on rat aryl hydrocarbon receptor (rAhR) and human estrogen receptor (hER), before and after moulting by Chemical Activated Luciferase gene eXpression assays. Thirty blubber biopsies were taken from 15 yearling seals (7 females and 8 males) from the Isle of May and Moray Firth, UK before and after moulting, during March to August 2018. For rAhR agonistic activity, the response was below the assay limit of quantification, except for the extracts from a female seal, showing 24 and 17 pg TCDD eq./g at pre-moult and post-moult stages, respectively. No AhR antagonistic activity was recorded. However, when cells were co-exposed to EC50 TCDD and the extracts together, pre-moult samples from 4 of the 8 male seals, and the female described above produced rAhR agonistic activity. It seems thus that pre-moult extracts act additively or synergically with TCDD and contain more compounds able to activate rAhR than post-moult samples. Samples from 10 of the 15 seals produced a response in hER agonistic activity assay. For two male seals, only pre-moult extracts induced a response, corresponding to 8 and 57 pg E2 eq./g, respectively. However, no clear difference in the ER activity of extracts from pre-moult and post-moult blubber was observed, opposite to that observed for the rAhR agonistic activity. No antagonistic activity was recorded in hER assays, but, as expected, a clear additive effect was observed for the sample from the male seal with the highest E2 eq. content.

## Posters

### 5. Physico-chemical and technological assessment of curdled milk and Wagashi Gassire cheese from traditional processes in northern Benin

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In Benin, fresh cow milk is processed into various products such as curdled milk (CM), Wagashi Gassiré (WG) cheese, butter etc. Among all of these products, the WG remains the most consumed, in rural as well as in urban areas, and significantly contributes to proteins intake of the population. WG is a soft cheese obtained by hot coagulation of pasteurized fresh milk using the latex of *Calotropis procera*. CM is obtained by natural fermentation of fresh cow milk. The traditional processing and preservation methods of CM and WG and the hygiene practices are often inappropriate and lead to variability in safety and sensory quality. This study is part of a R&D project funded by ARES-CCD aiming at improving the traditional processing and preservation methods of CM and WG by participatory action research in partnership with the actors of the milk value chain in Benin. To achieve that goal, several samples will be collected and submitted to physicochemical analysis for residues of veterinary drugs and pesticides detection/quantification. Additional analysis of chemical contaminants including polycyclic aromatic hydrocarbons will be done on heat-processed WG. The expected results of this study are namely the development and validation (viability, purity and shelf life) of culture starters that could easily be used by curdled milk processors, the determination of optimal scales for heat treatment and the concentration of the enzyme extracted from *Calotropis procera* suitable for good coagulation. At the end of this study, the traditional processing and preservation methods of CM and WG will have been improved.

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### 6. Preservation of avian genetic resources: elaborating strategies to support an underestimated global emergency

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Poultry is the largest animal protein source for human populations (meat and eggs). Recent sanitary events with zoonotic issues (highly pathogenic influenza A virus infections) reminded us how avian populations are worryingly sensitive to infectious threats, with potentially high societal, economic as well as ecological consequences. Yet, avian genetics resources preservation carries out unsatisfactory methods in terms of costs and efficiency. Indeed, live animals are sometimes maintained in scattered breeding units, with potential genetic bottleneck and drift issues. On the other hand, gametes and embryos cryopreservation suffer either of low and highly variable efficiency (sperm) or of intrinsically unfavorable structure and physiology (ova and embryos). We propose a couple of technological approaches to contribute to avian genome preservation: (i) cryopreservation of whole embryonic blastodiscs, followed by their reimplantation in surrogate decellularized eggs and (ii) *in testis* transdifferentiation of mesenchymal stem cells into spermatozoa. Although the former necessitates joint cryopreservation and avian embryology high-end skills, it could allow recovering the whole preserved genome at once in newborns. The latter approach could safeguard the genome of sterile, debilitated or even dead animals, and could also take advantage of inter-strains or interspecies intra-gonadic transplantation when carrier availability or integrity is a concern. If successful, these proof-of-concepts could pave the way to novel avian genetic resources preservation paradigms and provide with new technological options for avian genetic engineering.

## 7. Assessment of CHROMagar™ COL-APSE for the Detection and Pre-Identification of Colistin-Resistant Bacteria in Veterinary Medicine

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Colistin (polymyxin E) resistance is increasingly reported in human, animal, or farm, food, and the environment and can be induced either by chromosomal mutations or after acquisition of the plasmid-located *mcr* genes. To identify and more easily control this rapidly spreading threat, effective diagnostic tools have become urgent. The recommended reference method (both EUCAST and CLSI) for assessing the susceptibility of bacteria to polymyxins is the dilution method in liquid medium (microdilution). The method of diffusion in solid medium (disks or strips) is not recommended because of the high molecular weight of this antibiotic, which prevents a good diffusion in the agar and renders the results unreliable. The dilution technique in a liquid medium nevertheless has drawbacks since it is not optimized for the routine diagnosis and since the cationic nature of the antibiotic increases the adsorption of these agents on the plastic surfaces leading to inaccurate minimal inhibitory concentration (MIC) determination. Chromagar™ has developed a selective and chromogenic medium (COL\_APSE®) that makes more reliable the detection of resistant bacteria and a first orientation of their identity. Nevertheless, up-to-date, it has been validated in human medicine only. This study aims to assess the sensitivity and specificity of this agar medium for animal bacteria (cattle, cervine, hedgehog, birds, cat, dog, pig, reptile, rabbit, chicken, duck, guinea fowl) by comparing their growth with the results of the recommended method and of the antibiogram by the disk diffusion assay. Moreover, the presence of the *mcr*-1 to *mcr*-5 genes will be searched for by specific PCRs in all isolates with a MIC > 2µg.l<sup>-1</sup>.

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## 8. Role of pORF63-HSP90 interaction in the gammaherpesvirus lifecycle

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The Kaposi's sarcoma herpesvirus (KSHV) is a relatively prevalent gammaherpesvirus (γHVs) in certain regions of the world, especially in Africa. The infection can lead to 3 lymphoproliferative diseases (Kaposi's sarcoma, primary effusion lymphoma and Castelman's disease) for which no standard treatment currently exists. The γHVs have developed different mechanisms to benefit from host proteins. Like all the γHVs, the KSHV holds a tegument layer, made of 9 different proteins. By their external position these proteins are the most likely to interact firstly with the host proteins during the viral life cycle. For this reason, we performed a screening of the protein interaction between KSHV tegument proteins and a pool of around 18 000 human preys by a MAPPIT (mammalian protein-protein interaction trap) analysis. This highlighted a strong interaction between the KSHV protein ORF63 (pORF63) and the heat shock protein 90 (HSP90). This protein acts like a chaperone and is known to play a pivotal role in many viral life cycles. The KSHV doesn't grow well in vitro and doesn't infect laboratory animals. This is why models are usually used to study his behavior. The main one is the Murine Gammaherpesvirus (MuHV-4). The MuHV-4 pORF63 codes for two proteins, a long and a short one. By performing viral mutants, we showed that the deleted pORF63 virions present a growth deficiency. This undergrowth could be due to a lack of use of the HSP90 by the depleted virions.

## Posters

### 9. Triplex PCR to detect CTX-M-1, CTX-M-2 and CTX-M-9 extended-spectrum-β-lactamase-encoding genes in bovine *Escherichia coli* isolates

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Extended-spectrum-BLA (ESBL) confer a resistance to some antibiotics classified as critical in human medicine, like 3rd/4th generation cephalosporins. Though the use of critical antibiotics in livestock is regulated since 2016, it remains important to follow the evolution of ESBL resistance, especially in enterobacteria. The aim of the study was therefore to identify the ESBL-encoding genes in *Escherichia (E.) coli* from young calves in Wallonia with an ESBL phenotype at the disk diffusion assay. During 2 calving seasons (A: 2017-2018 and B: 2018-2019), 152 (A) and 161 (B) *E. coli* with ESBL resistance profiles were collected at ARSIA from calves with enteritis or septicaemia. Of them, 50 were tested with microarrays and only bla<sub>CTX-M</sub> genes, coding for cefotaximases, were detected. Based on these results, all 313 *E. coli* of the collection were tested with PCR for the different genes coding for the CTX-M-1, CTX-M-2 and CTX-M-9 ESBL groups: 103 (A) and 84 (B) were positive for the CTX-M-1 group; 26 (A) and 24 (B) isolates, for the CTX-M-2 group; 25 (A) and 37 (B) isolates, for the CTX-M-9 group; and 4 (A) and 5 (B) isolates were negative. In 4 (A) and 1 (B) isolates, genes coding for CTX-M-1 and CTX-M-2 groups were simultaneously detected. In conclusion, the bla<sub>CTX-M</sub> genes are the most prevalent ESBL-encoding genes in our collection and of them, the genes coding for the CTX-M-1 group are the most prevalent, as described in the literature. The PCR-negative *E. coli* will be further tested with PCR for the other bla<sub>CTX-M</sub> genes, if any. Moreover, a third collect of isolates is already planned during the next calving season to follow the prevalence of ESBL *E. coli*.

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### 10. Identification of Shigatoxigenic (STEC) and enteropathogenic (EPEC) *Escherichia coli* O80 in young calves with diarrhea.

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*Escherichia coli* producing the Shiga toxins (STEC) and/or the attaching-effacing (AE) lesion (EPEC) cause enteritis and (bloody) diarrhea in young calves and in humans. STEC and EPEC can belong to 7 serogroups frequently identified worldwide: O26, O103, O111, O121, O145, O157 and O165. Beside these classical "gang of 7", unconventional serogroups can be identified as previously demonstrated with the zoonotic O80 EPEC detected for the first time in diarrheic calves in Belgium between 2008 and 2015. The purposes of this project were (i) to identify 7 unconventional serogroups among 279 STEC and EPEC isolated between 2008 and 2017 from diarrheic calves at ARSIA (ii) to identify the virulence genes and the MLST, and to confirm the serotypes of a selection of these strains by whole genome sequencing. Two triplex PCRs have been applied either for the O146\_O182\_O183 serogroups or for the O123/186\_O156\_O177 serogroups and one uniplex PCR for the O80 serogroup. So far, the first triplex PCR identified 4 O182-positive and 2 O183 positive. The second triplex PCR identified 12 O123/186-positive, 3 O156-positive and 29 O177-positive STEC and EPEC. The uniplex PCR identified 20 O80-positive EPEC and STEC. The MLST 29, 300, 342 and 765 were detected. The variants *eeae*<sub>ζ</sub>, *stx1a* and *stx1c* were predominantly found. In addition to the *eeae*, *stx1* and *stx2* genes, many other virulence genes were found. The further steps of this study will be to search after the O80 serogroup among STEC and EPEC isolated from healthy cattle and to answer the following question: are these calf EPEC true EPEC, STEC derivatives that lost *stx* genes or STEC precursors that could acquire *stx* genes in the future?

## 11. Fatty acids composition of ready-to-eat grilled pork consumed in Benin

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Pork is widely consumed all over the world under different processed forms including grilled pork. This study aimed to determine the fatty acids profile of traditional grilled pork processed in Benin and to estimate the daily intake of fatty acids from grilled pork consumption. For this, 300 consumers having grilled pork at least once per month were selected and 24 samples of grilled pork were collected to determine the profile of 23 fatty acids on GC/MS. Results shows oleic acid was the most abundant mono unsaturated fatty acids with a range between 24.4% and 48.8% of total fatty acids while linoleic acid was the most abundant omega 6-PUFA (Polyunsaturated fatty acid) with a range between 0.5% and 3.6% of total fatty acids. The daily intake (expressed in g/day) calculated with the average content of fatty acids (expressed in g/100g of product) for different level of consumption (minimum, median, 97.5th percentile and maximum) of PUFA ranged between 0.02- 2.3 (for men) and 0.005-1.5 (for women) while PUFA recommended intake ranged between 14-28 for men and 11-22 for women (SHC, 2016). The daily intake of omega 6 fatty acids from grilled pork ranged between 0.01-0.8 for men and 0.002-0.5 for women. The consumer having high level of grilled pork consumption (342.9 g/day) covers 7% (for men) and 6% (for women) of adult recommended intake (g/day) of omega 6 fatty acids which are 11-22 for men and 8.8-18 for women (SHC, 2016). The daily intake of omega 3 through grilled pork consumption ranged between 0.01-1.5 for men and 0.003-0.9 for women with a dietary reference value of 2.8-5.6 for men and 2.2-4.4 for women. Further studies should be undertaken on oxidation of grilled pork due to presence of PUFA.

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## 12. Influence of reduced levels of sodium nitrite on the growth and toxinogenesis of *Clostridium botulinum* in two meat products

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Sodium nitrite (NaNO<sub>2</sub>) is used in meat products manufacturing for its antimicrobial effect on *Clostridium botulinum*, responsible of botulism by production of neurotoxins. Its use can nevertheless be associated with nitrosamines production, which are considered as carcinogenic substances. There is therefore a high demand to reduce the nitrite incorporation rate in meat products. The objective of this study was to compare the growth and toxinogenesis of psychrotrophic *C. botulinum* Group II (non-proteolytic) type B in cooked ham and in frankfurters in function of various NaNO<sub>2</sub> incorporation rates (0, 30, 60 and 80 mg/kg) in order to evaluate the risk associated with this pathogen in two common cooked meat products. Ground pork and a preparation made for frankfurters were mixed with NaNO<sub>2</sub> (0, 30, 60 or 80 mg/kg), 1.35% or 1.80% of salt. The meat mixtures were then inoculated or not (negative control) with a cocktail of spores of three *C. botulinum* Group II (non-proteolytic) type B strains i.e. BL7, 300.05 and 815.12 at 4 log CFU/g and vacuum packed in portions of 50 g. Thermal treatment was applied in conditions simulating industrial process. The products were then stored 2 weeks at 4 °C + 1h at 20 °C + around 4 weeks at 8 °C. In conditions applied during the present experiment, reduction of NaNO<sub>2</sub> incorporation at 30 mg/kg allowed to prevent the growth of *C. botulinum* in both models during around 6 weeks. In contrast, total removal of nitrite didn't prevent growth and toxin production in the two meat models tested. This observation is in accordance with the results of a previous study reporting neurotoxin production in cooked ham without nitrite incorporation.

## Posters

### 13. Successful isolation of mesenchymal stem cells from human foreskin tissue

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Human mesenchymal stem cells (MSCs) hold great promise as novel innovative treatments for several debilitating and incurable conditions. The beneficial effects of these cells rely on their differentiation capacity and the secretion of chemokines, cytokines, and growth factors. Allogenic MSCs are immune-privileged and can therefore be used to repair injured tissues. Human foreskin could be a valuable tissue source of MSCs. They can be obtained via non-invasive tissue harvesting procedures. In this study, human foreskin tissue was surgically harvested from 2 children and 2 adult at the University Hospital of Liège in compliance with the recommendations of the ethics committee. MSCs were successfully isolated via enzymatic digestion. Isolated cells were characterized by plastic-adherent ability, fibroblast-like morphology and high proliferative capacity. Cell viability test using Trypan blue indicated that the viability was usually greater than 98 %. The *in vitro* trilineage differentiation assay revealed that these cells are able to differentiate into mesodermal adipogenic, osteogenic, and chondrogenic lineages. Flow cytometry analysis showed that they expressed CD90, CD105, and CD73, but are negative for CD45. Taken together, our research confirmed that MSCs isolated from human foreskin tissue meet the criteria defined by the International Society for Cell Therapy (ISCT). These cells offer an exciting alternative for the development of human tissue engineering strategies and cell-based therapies.

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### 14. The effect of mycotoxin detoxifiers in the gastrointestinal tract of a child: the baby SHIME

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In Africa, mycotoxins play a crucial role resulting in adverse health effects in both animals and humans (Zain, 2011). More specifically, in Kenya, the ingestion of high concentrations of aflatoxin B1 (AFB1) and fumonisin B1 (FB1) in certain regions is well known. There have been several outbreaks of aflatoxicosis resulting in a large number of casualties (Probst et al., 2007). In Europe, mycotoxin detoxifiers are frequently applied to animal feed to prevent and reduce acute and chronic mycotoxin toxicity (Dell'Orto et al., 2015). The main objective of this study is to test the efficacy of two mycotoxin detoxifiers in a human gastrointestinal tract.

Two European Food Safety Authority (EFSA) approved mycotoxin detoxifier feed additives were tested in a controlled experiment simulating the gastrointestinal tract of a child using the SHIME model. Concentrations of AFB1 (81.6 µg kg<sup>-1</sup>) and FB1 (2000 µg kg<sup>-1</sup>) mimicking African circumstances were tested in the system. After two weeks of stabilisation of the model, both mycotoxins without detoxifiers were added to the model for a one week period, followed by a week including the detoxifiers. Samples were analysed for mycotoxin concentration using LC-MS/MS, for short chain fatty acid concentration using GC-MS, and a metagenetic analysis was performed.

After a first trial, the data provides an indication on the effect of these detoxifiers when provided to humans. Two repetitions of the experiment are in progress. Based on the results of this *in vitro* trial, the decision will be made to further test these products *in vivo*.

### 15. *In vitro* and *in vivo* assessment of lytic bacteriophages against *Staphylococcus aureus* causing bovine mastitis

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The use of antimicrobials against *Staphylococcus aureus* (*S.aureus*) causing bovine mastitis can lead to the emergence of Livestock-Associated Methicillin Resistant *Staphylococcus aureus* (LA-MRSA) which represent a potential hazard for public health. The use of bacteriophages as an alternative to antimicrobials has been identified as a potential solution to tackle this problem. The aim of this study was to assess the efficacy of four lytic bacteriophages on a collection of *S. aureus*, by *in vitro* and *in vivo* assays (*Galleria mellonella* larvae model and mouse model). Between May and December 2016, 10 *S. aureus* isolates comprising five MRSA and five methicillin sensitive (MSSA) *isolates* were isolated from milk samples of cattle with mastitis in Belgium and Norway. After phenotypic and genetic characterization, the isolates were assessed *in vitro* for their susceptibility to four lytic bacteriophages (Romulus, Remus, ISP and DSM105264) and then *in vivo* in *G. mellonella* larvae and in a murine mastitis model. The *in vitro* assay demonstrated the lytic activity of these four bacteriophages on nine *S. aureus* isolates, while the *in vivo* assay showed that the larvae survival rate was below 50% after four days post inoculation and that the recovery of the mouse mastitis was incomplete after 48h post inoculation. However, a statistically significant difference was observed in the results between infected-PBS treated and infected-phage treated groups in the *G. mellonella* and the murine models suggesting an effect of the phage therapy on mastitis caused by *S. aureus*.

### 16. Development of microsphere-based assay technique combined with RT-qPCR to detect rapidly and quantify infectious norovirus particles in food

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Human noroviruses are a major viral cause for gastroenteritis outbreaks. Bivalve 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 relying on murine norovirus capsid detection by flow cytometry coupled to RT-qPCR. A magnetic microsphere-based assay coupled to immune enzyme assay was designed. The approach is to use two different antibodies for capture (clone 5C4) and detection (clone 2D3). We examined the efficacy of the antibodies by a sandwich ELISA assay. A first detection assay of murine norovirus by flow cytometry was successful, further experiments to assess sensitive differentiation between treated (UV, heat) and untreated viral protein epitopes from simple matrix (PBS) and complex matrix (mussels) are ongoing. The final objective is to detect rapidly and specifically only infectious noroviruses in naturally contaminated mussels.



## Posters

### 17. The use of selected plant extracts as antioxidant in feed preservation at ambient temperature

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The aim of this study is to investigate the effect of plant extracts added to fish feed during its preservation at ambient temperature. Five plant extracts were selected for fish feed storage experiment. Each extract was tested at two concentrations: *Euphorbia hirta*, *Mimosa pudica* and *Azadirachta indica* were added to the fish 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. Negative control (without antioxidant or plant extract), positive control (without plant extract but with 200 mg/kg of butylhydroxytoluene (BHT)) and plant extracts added diets were stored at room temperature (30 – 35°C) in bags similar to those used by feed companies. The sampling was done after 0, 2, 4, 6 and 8 weeks of storage for analysis of moisture, PV and TBARS. Lipid level in diet was determined at the beginning of the experiment. After 8 weeks of storage, the moisture of each batch increased during preservation, ranged from 6.02% to 13.61%. Feed batches added with *P. amarus* (0.2%) and *M. pudica* (2%) showed the highest (13.61%) and lowest (10.46%) moisture level, respectively. The lipid level of feed fluctuated from 7.31% to 8.65%. The highest value and the lowest value were found in feed batches added with *E. hirta* (2%) and *P. guajava* (0.2%). Moreover, adding plant extracts into feed seems to reduce lipid oxidation as shown from PV and TBARS values, except when feed was added with *A. indica* (0.4%). When looking to secondary oxidation products (TBARS), addition of *M. pudica* (0.4%) and *P. guajava* (1%) showed the best antioxidant properties. In conclusion, *M. pudica* extract would be the most appropriate natural preservative for use as a feed preservative showing the lower PV and TBARS in feed in which it was included, compared to feed added BHT, after 8 weeks of storage.

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### 18. Infection of zebrafish by Cyprinid Herpesvirus 3: a model to study host-pathogen-environment interactions

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Farmed and wild fish can easily encounter infectious pathogens as they are constantly in close interaction with their surroundings. Infectious diseases represent a significant health problem by impacting the productivity of fish farming industries and causing economic losses. Cyprinid Herpesvirus 3 is the causative agent of a deadly and highly contagious disease in koi carp and common carp. While it has recently been observed that this herpesvirus can also replicate in the zebrafish, this teleost species can clear the infection very rapidly. The severity of a disease can depend on the interactions of many variables such as the virulence of the pathogen, the susceptible of the host and the environment in which they encounter each other. The purpose of this study was to investigate whether two environmental contaminants, paracetamol and diclofenac, can alter the zebrafish immune system and thus affect the replication of the virus in this host. *In vivo* experiments have been carried out using recombinant viruses by means of fluorescence microscopy and bioluminescence *in vivo* imaging. Our results showed a significant increase of viral replication 12 hours post-infection in CCB cells exposed to either paracetamol (100 and 1000 µM) or diclofenac (10 µM) compared to that of the cells not exposed to drugs. Moreover, infected zebrafish exposed to paracetamol (100 µM) tends to show an infection peak delayed by a day compared to the other groups.

### 19. Safety and efficacy of mycotoxin detoxifiers as intervention strategies to reduce chicken exposure to mycotoxins and carry over to chicken products

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Mycotoxins are low-molecular weight and non-volatile chemical compounds naturally produced by toxigenic fungal metabolisms. Food safety is currently a global problem that require much attention to counteract possible food and feed contamination by these toxigenic fungi. High and low mycotoxins contamination levels both have effect on animal health and also to human due to secondary exposure through consumption of eggs, liver and meat derived from chickens fed mycotoxins contaminated feeds. Since contamination of food and feed is inevitable in the field, during harvesting, transportation or during storage and processing, mitigation strategies to reduce or eradicate negative mycotoxins effects have been explored. These include, among other methods, the use of mycotoxin binding agents that function when the mycotoxin is already present in the diets and are being ingested by animals. The binders can thus act as a safety measure mostly in situations where regular testing of feeds is not practical or where the accuracy of the testing is not assured. To evaluate the safety and efficacy of the mycotoxin binding agents, layers and broiler chickens will be fed corn based diets with either a control (with undetectable mycotoxins), aflatoxin low concentration (15 ppb) or/and fumonisins concentrated (20 ppm) or aflatoxin high concentration (500 ppb) or/and fumonisins concentrated (20 ppm). In selected experimental groups these diets will be supplemented with bentonite (aflatoxin binder) and fumonisin esterase (fumonisin detoxifier). There will be a total of 20 treatment groups with each treatment group consisting of 20 birds housed in 4 cages as per the guidelines of the ethical committee of ILRI. Mortality rate, body weight gain, feed conversion ratio, laying capacity, blood chemistry and hematology, immune response to vaccination, and histopathology of liver and spleen will be used to evaluate the animal health and productivity following the different treatments. The safety of the food (eggs, meat, liver, and plasma) from the chicken fed aflatoxin contaminated feeds will be assessed by analyzing the aflatoxins and its metabolites residues using ultra-performance liquid chromatography-tandem mass spectrometry (UPLC-MS/MS) methods. The first results will be presented.

# **Sustainable livestock production**

## 20. Effect of prickly pear oilcake (*Opuntia Ficus-indica. L*) as substitute of diet on animal performance and carcass characteristics of broiler chicken

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The objective of this study was to determine the effects of incorporating a meal of prickly pear seeds "*Opuntia Ficus Indica. L*" (local by-product of oil extraction from seeds), in the feed ration on growth performance and slaughter products of broilers. The 45-days trial included 150 one-day-old, unsexed chicks of the "Arbor Acres" strain. Five batches of 30 chicks (3x10 chicks per group), were randomly assigned to 5 diets, depending on the substitution rates of 0, 10, 20, 20, 30 and 40% (R0, R10, R20, R30 and R40) of the ration by the prickly pear cake. Compared to that of the control group, the final live weights and ADG were significantly lower in the R30 and R40 groups, with a decrease in food intake and consumption index. The Lot R10 showed an improvement in growth performance with a final live weight of 2823 g, a ADG of 61.9 g/day and a consumption index of 1.87. The mortality rate was 5% during the breeding period. For slaughter products, weight of thighs, breast, wings, gizzard, liver and heart, as well as weight of legs and head, decreased linearly and quadratically with the incorporation rate ( $p < 0.001$ ). On the other hand, the carcass yield was significantly improved with R10. These results indicate that growth of chicken is stable up to 20% when prickly pear cakes are incorporated. Substitution rates of 30 and 40% lead to a significant decrease in growth.

## 21. Zootechnical and economic prospects of the earthworm *Eudrilus eugeniae* in poultry farming in Gabon

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*Eudrilus eugeniae* is a nutritious source for omnivores, especially poultry. This study proposed to introduce this earthworm into the diet of the chicken and to measure the effects on zootechnical and economic performances. The experiment lasted 28 days and was performed with 20 chicks, Hubbard strain, divided into 2 homogeneous batches. The control group received one conventional food and the other received the same food supplemented with 3% fresh worm.

The results reveal that the animals' growth and the average daily gain of the two groups were not significantly different ( $P > 0.05$ ) but the consumption index and the amount of feed consumed were significantly different ( $P < 0.05$ ). The average weight of the experimental batch ( $447.64 \pm 6.59$  g) was very close to that of the other group ( $446.28 \pm 6.61$ ). The 10 chicks of the control group consumed more food, for a value of 1.27 € (834 fcfa) against 1.25 € (821 fcfa) for the experimental group. The vermiculture device is relatively inexpensive, it is 108 € (70 592 fcfa) / year/m<sup>2</sup> for an estimated income of 1015 € (666 040 fcfa) / year/m<sup>2</sup>. These results suggest that the earthworm can be a good nutritional supplement for chicken feed and can generate valuable financial resources for the farmer.

## Posters

### 22. Seasonal variations in diet selection of goats in Rif mountain of Morocco

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Grazing goats' management in silvopastoral areas first requires the characterization of their feeding behavior in this heterogeneous landscape. This study was conducted in a forest pasture in Northern Morocco, to characterize the seasonal variation of goats' diet selection. Direct observation was used as a technique to evaluate the grazing behavior of goats for three seasons (autumn, spring, and summer). *Arbutus unedo*, *Cistus spp.*, *Erica arborea*, *Lavendula stoeches*, *Mentha pulegium*, *Pistacia lentiscus*, and *Quercus spp.* are the most palatable plant species selected by goats. Due to the low forage production of forest pasture during autumn and summer, goats spent more grazing time. Furthermore, goats recorded the highest bites number during these last same periods. Bite weight and ingestion rate of the most palatable species selected by goats depended significantly to the season and the vegetation type. Goats appeared to be adapted to season-to-season changes in forage production. In general, goats tended to selected shrubs independently to the season. Results of this study confirmed the adaptive feeding strategy of goats to the seasonality of forest vegetation.

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### 23. Seasonal variation of forage production of silvopastoral areas in the North-West of Morocco

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Extensive livestock farming plays a very important socio-economic role for livestock farmers in the mountainous region of North-Western Morocco. Silvopastoral resources are the main source of feed for goats. Recently, these resources are experiencing a strong tendency toward degradation. To ensure their sustainability evaluation of these resources are a necessity. This study aims to evaluate pastoral production and to investigate their use. Two representative forest rangelands were selected to conduct this study. To control spatial heterogeneity of vegetation, we used the stratification method based on *Quercus suber* density. To estimate seasonal forage production in each pasture, we used quadrats method. The interviews with goat herders were conducted during autumn and summer seasons. More than ninety species were counted in each rangeland. *Arbutus unedo*, *Cistus spp.*, *Erica arborea*, *Lavendula stoeches*, and *Quercus spp.* compose the main of forage production. For biomass, significant differences were noted according to the season and to the sampled pastures, with higher forage production recorded during spring and the lower during summer. Furthermore, we observed the appearance of degraded pastures dominated by unpalatable species. Grazing activity is practiced during all seasons, except in the winter where goat herders resort to the delimiting. Due to climate changes and pressure observed in the studied forest pasture, we expected that the availability and quality of silvopastoral areas continue to decrease over the next decades. The moderate use of forest pastures should be developed to ensure their sustainability.

#### 24. Study of the “destructured meat” defect in the Walloon pork value chain: effect of the *Ryr1* genotype

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The “destructured meat” (DM) defect affects the sliced cooked ham production industry. Since the 90’s, the incidence has increased due to the high production rates and the demand of consumers for products with less added ingredients. DM appears to be pale and soft, but it has to be distinguished from “PSE” meat. This defect results in significant losses in the mechanical slicing of cooked hams. The purpose of this work, part of a project studying the DM defect in the Walloon pork value chain, is to evaluate the possible link between the genotype for the halothane sensitivity (*Ryr1* gene) and this defect. The quality of carcass, meat and cooked ham has been evaluated in pigs issued from four farms preselected on basis of the mean ultimate pH (pHu) (2 farms with low pHu and 2 farms with high pHu), on a total of 506 pigs for the quality of the meat and the carcass, on 238 pigs for the quality of the cooked ham. More attention has been paid to one farm in which an equilibrated “NN”/“Nn” ratio was observed. Globally, the results obtained in the 4 farms on the pHu 5,30-5,49 and 5,80-5,99 classes, and on all animals of one of these farms show a genotype effect. The “Nn” genotype was associated with more favourable carcass quality indicators (carcass lean meat content, ham weight) and, conversely, with more unfavourable fresh meat quality indicators (pH1, pHu, drip loss, destructure score, color) than “NN”. By contrast, the *Ryr1* genotype has not been related to the incidence of the observed defects on cooked ham (“ointment”, holes, slice cohesion). Further statistical analysis is needed for studying the relation between measured parameters and DM defect.

#### 25. Non-Steroid Anti-Inflammatory Drugs utilization during bovine elective caesarean section in Wallonia

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In Belgium, more than 90% of Belgian Blue breed calves are born by caesarean section (CS). This surgery generates pain, usually well managed by the local administration of anesthetic. However, postoperative pain is rarely taken into account.

Non-Steroidal Anti-Inflammatory Drugs (NSAIDs) combine anti-inflammatory, antipyretic and analgesic action, suggesting that it improve cow comfort after surgical intervention.

A survey of nine questions about NSAIDs administration during the realization of elective CS was conducted among Wallonian rural veterinarians. The questions were either multiple choice questions, simple choice questions or short open-ended questions, with the possibility to add comments after each response.

A considerable number of veterinarians answered the survey (152). Only 4% of them use NSAIDs during CS. The most commonly used drug is meloxicam. The most preferred route of administration is intramuscular and the timing of administration is most often after the surgery. The reasons why veterinarians mention not to use NSAIDs during CS are the high price and the disagreement of breeders; additionally, many vets are not convinced of the beneficial effect for the cow’s well-being. A minority of veterinarians believe that their use reduces the rate of postoperative complications. However, a majority think that using them would reduce postoperative pain. Most veterinarians would be willing to use them if it improves the welfare of cows after CS and/or if NSAIDs becomes more affordable.

## Posters

### 26. Effect of olive cake and cactus cladodes incorporation on goat milk production and quality

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In Northern Morocco, the feeding of goat herds is based on forest rangelands characterized by seasonal variability, causing the low productivity of these herds. The overuse of rangeland may cause forest degradation and the extinction of some palatable species. The olive cake (OC) and cactus cladodes (CC) are two widely available alternative feed resources that could be incorporated in goat diet to satisfy their needs and reduce the rangeland overuse. This work aims to evaluate the effect of OC and CC on goat milk production and quality. Forty-four multiparous Beni Arouss goats were divided into four groups. The control group received a conventional feed and the test groups received respectively 20% OC, 30% CC, and 15% OC and 20%CC (OC+CC). During 3 months of lactation, a manual milking was performed to evaluate milk production and to collect samples for quality analysis. According to results, milk production, acidity, and composition (fat, proteins, lactose, solids non-fat, total solids and ash) were the same in all the groups. The incorporation of 20% OC increased C18:1n9cis, mono-unsaturated (MUFA) and n-9 fatty acids, and decreased C18:1n9trans (P<0.05). However, 30%CC administration increased C15, C18:1n9cis and C21, and decreased C4, C18:1n9trans, C18:2n6t, C20 and poly-unsaturated fatty acids (PUFA) (P<0.05). The OC+CC incorporation reduced C4, C18:1n9t, C18:2n6t, C22:6n3 and PUFA, and increased C18:1n9c and MUFA/PUFA. Fatty acids chain (SCFA, MCFA, and LCFA), saturated (SFA), desirable (DFA),  $\omega$ -3 and  $\omega$ -6 fatty acids,  $\omega$ -6/ $\omega$ -3, UFA/SFA, and PUFA/SFA ratios were similar to Control group. Thus, olive cake and cactus cladodes could take place in goat diet without a negative effect on milk production and quality.

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### 27. Effect of olive cake and cactus cladodes incorporation on goat kids carcass and meat quality

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In Northern Morocco, goat is the most dominant herd among ruminants. Its feeding is essentially based on forest resources that are responsible for its low productivity and may cause rangelands degradation. Olive cake (OC) and cactus cladodes (CC) are two alternative feed resources that could take place in goat feeding. This work aims to determine the effect of their introduction in the diet on carcass characteristics and meat quality of Beni Arouss goat kids. Forty-eight animals were divided into 4 homogeneous groups. The first control group received a conventional ration used by farmers, and the 3 other groups received a diet containing respectively 35% OC, 30% CC, and 15% OC and 15% CC (OC+CC). After 3 months of fattening, goat kids were slaughtered. Measurements were made to characterize carcass quality, and *longissimus* muscle was taken to determine meat quality. The incorporation of OC, CC or OC+CC was without an effect on carcass characteristics (carcass yield, color at back and saddle) except redness and yellowness at tail outline. The diet had no effect on pH, color, moisture and water retention of meat. However, higher level of protein and lower levels of fat and ash were observed with CC group and lower level of protein with OC group. The OC and CC introduction decreased palmitoleic acid (C16:1), and eicosatrienoic acid (C20:3n3) increased with 30% CC. The fatty acids groups and ratios were similar in all groups. In conclusion, 35% OC and 30% CC could be introduced into goat kids diet without any negative effect on carcass and meat quality.

## 28. Management of an infected non-penetrating thoracic wound in a Belgian Blue calf using honey and a tie-over bandage

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Wound management in cattle is challenging for several reasons, especially the low number of drugs authorised to treat livestock and the challenge to keep a wound clean.

Herein, we describe the wound management of a 105 days old, Belgian Blue, female calf referred in the clinic of the University of Liège for thoracic wounds, noticed two days ago.

The clinical examination highlighted high temperature and slight expiratory dyspnea. The thoracic area of the left side showed two large deep non-penetrating wounds. The first was 13x8cm large and the second was 7x3cm. They were necrotic, purulent and communicate between each other.

After abundant rinsing using 10 liters of diluted chlorhexidine at 0.05% to eliminate necrotic debris, a honey-based dressing was attached on the skin with eight non resorbable stiches. The systemic treatment consisted in the administration of meloxicam (MELOXIDYL®; 0.5 mg/kg).

The bandage change and wounds rinsing were performed twice daily during the first four days. On the fifth day, since the wounds were significantly less productive and a granulation tissue was lining their surface, a daily flush was decided. On the seventh day, a consequent wounds retraction was observed (diameter reduced by 1cm), subsequently, the cleaning was spaced out to every other day. On the eleventh day, the epithelial tissue was fully covering the wounds area, hence honey was replaced by Isobetadin gel® 10%. The total healing was achieved after 25 days of care.

In conclusion, honey is an authorized, cheap and effective product to treat cattle skin injury, and a tie-over bandage provides an efficient protection of the wound.

## 29. Socio-economic study of cattle breeding in Eastern Algeria

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The objective of this study is to establish a technical and economic reference framework for dairy cattle breeding in Eastern Algeria in order to improve the knowledge and level of technicality of farmers and to clarify the problems existing within the workshops concerning the structure and functioning, in particular technical and economic management.

A technical and economic follow-up was adopted in the form of a questionnaire in six farms over a ten-month period, illustrating the diversity of dairy cattle breeding situations in four Wilayas in Eastern Algeria: Mila, Sétif, Constantine and Jijel. The survey of 66 farms, comprising 1,477 dairy cows, shows that the majority of livestock farmers surveyed in this region are married (84.85%), agropastoralists (57.58%), and have the formal education system (84.85%). The herd size is between 5 and 160 heads per farm with an average of 54.48% dairy cows. The breeds exploited are generally imported: Holstein (67.45%) and Montbéliarde (21.79%). 66.72% of the farmers surveyed have acquired extensive experience in cattle farming (five years). They breed for purely economic purposes (60.60%) and combine cattle breeding with small ruminants (50%) and animals are generally kept overnight in the open air. The animals are watered twice a day (68.18%) and all farmers are monitored by a veterinarian. The quantity of milk produced varies according to the breed of cow: 14 to 18 kg/v/d or more at peak lactation for the Holstein breed.



## Posters

### 30. Current state of goat breeding in Algeria

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In Algeria, livestock farming is affected to four species: sheep, goat, cattle and camels. Goat represents the second animal component with five million heads; it is raised in different agro-ecological zones, mainly in arid regions where it plays a vital role in the livelihoods of poor rural households. In these areas, breeding systems are undergoing a significant mutation, with domination of sedentarization. El-Oued district is ranked the first with 10% of the national goat head. The evolution rate in the last decade is more than 33%. The goat/sheep ratio was 5.65 in 2017. The number of goat per inhabitant (0.12) allowed classifying Algeria in the category of underdeveloped countries in goat breeding. Breeds composition is very heterogeneous, composed of native breeds (Mekatia, Arabia, Dwarf of kabylia and Mozabite), exotic breeds (mostly, Saanen and Alpine) and their crossbreds. Goat milk production is insignificant compared to cow production. The evolution rate during the last decade was less than 1% for goat, which is significantly lower than cattle (20.11%). The red meat production is also dominated by cattle and sheep whereas goat meat remains negligible (4.25% of the national production). These results and findings show the poor contribution of goat sector in Algeria and require the orientation of policies towards this animal resource that could contribute significantly to the livelihood systems and food security of poor rural households, which represent 28.1% of the Algerian population.

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### 31. Parentage assignment of Striped Catfish (*Pangasianodon hypophthalmus*) with shallow whole genome data

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Pedigree information is important in estimating genetic parameters in selective programs and hatchery management of aquaculture. Several previous studies on parentage assignment have focused on using genetic data generated from micro-satellite markers and more recently from SNP arrays. In this study, we evaluate the performance of using shallow whole genome sequencing (SWGS) data to analyze parentage assignment of striped catfish (*Pangasianodon hypophthalmus*) in lieu of traditional array data. To prepare genetic data, we performed whole genome deep sequencing of one catfish with high fold coverage (~ 144X), and used this information to establish a de novo draft reference genome. For 59 parents (30 males and 29 females, leading to 870 full-sib families) and 500 offspring, we used SWGS with fold coverage of ~ 1 to 2X (parents) and ~ 0.5 X (offspring) per individual. We mapped SWGS data on the draft reference genome to identify genomic variants, including SNPs, that we will use for parentage assignment. The use of SWGS data raises two challenges: First, for low-coverage (e.g., < 2X), confirmed genotypes for offspring and parents are in most case not available. Second, read errors are common in next generation sequencing. To address these issues, we have developed a new parentage assignment algorithm based on a likelihood approach to identify the most suitable (*i.e* likely) set of parents for each offspring. In order to test this likelihood approach, we have simulated data and used the likelihood approach to try to reconstruct the families. The results show that quasi-perfect assignment can be obtained (with accuracy: 0.993) in the conditions of our experiment if at least 5.000 SNPs are used (assuming an error rate of 0.01). Further steps of this study will extract SNPs from 5.000.000 genomic variants (obtained from mapping genomic data from the 59 parents on the draft reference genome) to find the pedigree information of 500 offspring.

### 32. What are the alternatives to soybean meal for laying hens? Effect of two-grain legumes, peas and faba beans, on laying performance and egg quality

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Today, soybean meal is the main source of protein in the diet of laying hens. This feed ingredient is mainly produced abroad (Brazil, Argentina, USA), which makes the EU highly dependent on imports. Greater protein autonomy could reduce the economic impact (price fluctuations related to increased demand from China in particular) and the environmental impact (deforestation) related to the importation and cultivation of soybeans in the Amazon forest. A frequently proposed option for a few decades is to increase protein production in Europe through cultivation of grain-legumes such as pea, faba bean and lupine. However, the compositions of these grain-legumes differ from that of soybean meal: lower protein levels, presence of anti-nutritional factors, and poor amino acid profile. Therefore, they are more difficult to use. A lot of projects have been set up nationally and across Europe to promote the development of grain-legume crops, but they had little impact and the area under these crops have been decreased since the 90s. The aim of this work was to evaluate the potential effects of grain-legumes, namely pea and faba bean as an alternative to soybean meal in the diet of laying hens in terms of performance and physical and nutritional qualities of eggs. The experimental part of this work consisted evaluating the effects of a partial soybean meal substitution ration on the production performance of laying hens (laying performance, egg quality including the fatty acid profile) of two diets with two different protein sources: one with 22% of soybean meal and the other with 45% of grain-legumes (30% of pea and 15% of faba bean) plus 6% of soybean meal. The laying rate was significantly lower for the grain-legumes diet (51.79% vs. 60.63%,  $P < 0.05$ ). Physical parameters measured (egg weight, white weight, yellow weight and shell weight, shell thickness and strength), cholesterol and fatty acids were not significantly affected in both feeding groups.

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### 33. Agriculture and forestry in the district of Chemini, Algeria: Duality and complementarity

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Algeria is characterized by the great diversity of its pedoclimatic conditions. It has the natural resources necessary to attain sustainable development. The management of the resources is ensured by agreements between the different stakeholders using these resources. Nevertheless, conflicts between the different users can appear and lead to a break in the eco-systemic balance. The present work is a case study of livestock and forest management in the region of Ath Waghli in Basse Kabylia (Algeria). It describes the biodiversity of domestic animals raised in this region of Kabylia and the management of the Akfadou forest. Livestock production in the region of Ath Waghli is mainly oriented towards the intensification of dairy cattle, poultry farming and extensive exploitation of sheep and goat farming. The agricultural practice of the community is of a subsistence type carried out with rudimentary techniques. Arboriculture (fig trees, olive trees in particular) has been highly concentrated in the region for centuries. Vegetable crops are also widely practiced. The situation of Akfadou forest is worrying. Wood is increasingly used as a source of energy because of the increased price of gas following the liberalization of the sector in response to the injunctions of the IMF and the WTO, which have accelerated the deforestation. In addition to the agro-pastoral activities, the undergrowth and soil in forest are the source of many traditional activities widely practiced. These include exploitation of (1) wood (alder, elm, cork oak, eucalyptus, pine) and cork oak in particular extraction of oil from the olive tree, (2) forage plants (ivy, cork oak acorns, olive leaves, calycotum, grasses, etc), (3) forest products such as pine nuts, mushrooms, arbutus, myrtle, bay leaves, etc, (4) medicinal and aromatic plants, and (5) harvest of wild honey. The sustainability of the Akfadou forest requires a reasonable and sustainable management of its resources. The management of these common resources must be conceptualized following the concept of Garret Hardin named "tragedy of the commons".

## Posters

### 34. Management of genetic resources goats in Algeria: case of the "kabyle dwarf" breed in the area of Tizi Ouzou

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Characterized by an extraordinary pedoclimatic diversity, Algeria has an exceptional animal genetic wealth. In the livestock production, the goat population is out the second rank after sheep. Through a survey conducted with 36 kabyle dwarf goat breeders 2017, this study investigates the main characteristics of the management of the Kabyle dwarf goat breed in the wilaya of Tizi Ouzou, The questions focused on genetic diversity and management of production and reproduction of kabyles dwarf goats. The results showed that goat breeds identified were the kabyle dwarfs (100% of the breeders), the Saanen (41,66% of the breeders) and the alpine (30,55% of the breeders). Breeders attach importance to Kabyle Dwarf goats for its resistance and hardiness. The majority of breeders surveyed (80,55%) choose breeding stock. Selection criteria for breeding males are conformation (72,22%), horn size (50%) and breed membership (19%). With regard to the criteria for the selection of females, we find the milk production (75%), the prolificity (61,11%), a good conformation of the udder (19%) to avoid injuries during grazing. The Kabyle Dwarf breed is considered threatened by anarchic interbreeding with other indigenous and exotic breeds.

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### 35. Effect of stinging nettle (*Urtica dioica*) powder on Hematological and Serum Biochemical Parameters of Turkey Broiler

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This study aimed to evaluate the effects of stinging nettle powder (SNP) on the growth performances, hematological and serum biochemical parameters of turkey broiler chickens. A total of 90-day-old turkey chicks were randomly allocated to three dietary treatments with three replicates of 10 chicks per replicate. Experimental diets were formulated in such a way that there was no SNP in control group (SNP0), 2% SNP in SNP1 group, and 4% SNP in SNP2 group. The feeding experiment was carried out for 35 days during which feed and water were offered ad-libitum. The blood samples were taken on the 35th day of the experiment. The weights of the turkeys of the different groups were similar (SNP0: 1430, SNP1: 1410, SNP2: 1490,  $P > 0.05$ ). The values of red blood cells -SNP0: 2.21, SNP1: 2.38, SNP2: 2.26, SEM: 0.10 ( $\times 10^6/L$ )-, hemoglobin -SNP0: 10.96, SNP1: 11.36, SNP2: 11.32, SEM: 0.29 (g/dl)-, white blood cells -SNP0: 192.20, SNP1: 194.20, SNP2: 195.40, SEM: 2.81 ( $\times 10^3/L$ )-, prepared cell volume -SNP0: 36.00, SNP1: 37.30, SNP2: 37.40, SEM: 0.39 (%)-, mean corpuscular hemoglobin concentration -SNP0: 30.21, SNP1: 31.42, SNP2: 32.96, SEM: 0.70 (g/dl)-, mean corpuscle hemoglobin -SNP0: 50.92, SNP1: 51.46, SNP2: 51.65, SEM: 1.08 (pg)-, mean corpuscular volume -SNP0: 166.34, SNP1: 158.06, SNP2: 169.44, SEM: 8.46 (fl)-, total serum protein -SNP0: 3.72, SNP1: 3.76, SNP2: 3.77, SEM: 0.03 (g/dl)-, serum albumin -SNP0: 1.44, SNP1: 1.40, SNP2: 1.38, SEM: 0.05(g/dl)-, serum globulin -SNP0: 2.36, SNP1: 2.28, SNP2: 2.40, SEM: 0.10 (g/dl)-, glucose -SNP0: 232.40, SNP1: 229.80, SNP2: 227.20, SEM: 1.04 (mg/dl)-, urea -SNP0: 3.70, SNP1: 3.66, SNP2: 3.64, SEM: 0.05 (mg/dl)-, Cholesterol -SNP0: 157.40, SNP1: 153.00, SNP2: 152.40, SEM: 3.12 (mg/dl)-, creatinine -SNP0: 0.06, SNP1: 0.05, SNP2: 0.06, SEM: 0.10 (mg/dl)-, sodium -SNP0: 116.40, SNP1: 117.20, SNP2: 116.60, SEM: 0.60 (mmol/l)-, potassium -SNP0: 5.52, SNP1: 5.36, SNP2: 5.56, SEM: 0.07 (mmol/l)-, chloride -SNP0: 77.60, SNP1: 77.40, SNP2: 78.00, SEM: 0.93 (mmol/l)-, bicarbonate -SNP0: 28.20, SNP1: 28.80, SNP2: 28.20, SEM: 0.058 (mmol/l)-, Alkaline phosphatase -SNP0: 24.46, SNP1: 25.54, SNP2: 25.05, SEM: 0.59 (ALP) (IU/I)-, SGPT -SNP0: 11.01, SNP1: 11.40, SNP2: 10.80, SEM: 0.23 (IU/I)-, SGOT -SNP0: 40.40, SNP1: 40.20, SNP2: 39.10, SEM: 1.40 (IU/I)- were similar ( $P > 0.05$ ) in all three groups. *Urtica dioica* had no negative effect on health, daily weight gain, biochemical and hematological parameters in turkey poults.

### 36. Effect of olive oil supplementation on performance and selected blood biochemical parameters in Japanese quails

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The objective of this study was to evaluate effects of the olive oil's incorporation in to the diet on growth performance at five weeks old and selected serum biochemical parameters.

A total of 360, one-day old Japanese quails (*Coturnixcoturnixjaponica*) were randomly divided into three groups, consisted of 12 quails in each of ten replications during a growth period of five weeks. The control group received a basic diet containing 0% olive oil (T) and two other groups received 2 (B) or 4% (A) olive oil. Live weight and feed intake were recorded on a weekly basis and feed conversion efficiency was calculated. Blood samples were taken at 5<sup>th</sup> week old on the day of slaughter and serum samples were analyzed for glucose, triglycerides, total cholesterol, total protein, uric acid and creatinine. The results obtained showed that quails receiving olive oil in their rations have recorded better zootechnical performances (average daily gain, average body weight and the feed conversion ratio) in comparison with the sample batch, without any signification effect. The live weight at the fifth week is 184,09g for the batch B; 181,62g for A and 178,11g for T group, with an average daily gain of 5,06 ; 4,99 and 4,89 g respectively. The biochemical analyses that the addition of the oil in the induced feeding has an unsigned significative elevation rise on blood glucose, triglycerides, total protein, uric acid and creatinine, however no effect on cholesterol and urea levels that are similar to control group. In conclusion, the present study demonstrated that the olive oil's incorporation on diets of quails has not negative effect on growth performances and on serum blood parameters and should be continue the experimentation to evaluate the olive oil incorporation effect on egg production.

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### 37. Knowledge of household on rabies and socioeconomic factors affecting the decision of vaccinating pets against rabies in Bobo-Dioulasso (Burkina Faso)

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Rabies is a zoonosis. WHO estimates that 90% of human rabies cases occur through rabid dog bite. It causes more than 60,000 deaths each year worldwide. Despite the implementation of some mass vaccination campaigns since 1999 in Burkina Faso, the context is still characterized by low immunization coverage of pet animal. The study aimed to identify socioeconomic constraints to the adoption of rabies vaccination of pet animals. A total of 391 households were randomly selected and then surveyed. Data on animal ecology, the socio-economic rationale of owners were collected through a questionnaire interview with the head of household or another adult person. A total of 141 pets have been identified in the selected households including 127 dogs and 14 cats. In dogs, 40% of the them were vaccinated (vaccination certificate up to date) while no cat was vaccinated. Financial (53%) and geographical (23%) inaccessibility of immunization services, carelessness (18%) and lack of information on rabies risk (8%) were constraints to the adoption of vaccination in households.

## Posters

### 38. An update on PRRS seroprevalence in Southern Belgium

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In 2018, a PRRS monitoring program started in Belgium. In this perspective, in Wallonia, where only 6% of Belgian pigs are kept, PRRS seroprevalence was assessed on both breeding and fattening pig farms. Serum samples (n = 1786 from 585 sows and 1201 piglets and/or growing pigs) collected in 2016 were tested with Elisa Idexx PRRS X3 on a maximum of 10 breeding and/or 5 feeder pigs per farm (i.e. 106 farrow-to-feeder/finish and 203 growing/finishing farms). An individual result was considered negative if the S:P ratio was < 0.4; a herd was considered positive if at least one pig had an S:P ratio ≥ 0.4. A survey addressed to these 309 Walloon pig owners gave 121 usable answers from 75/106 sow and 46/203 pig(let) owners. Overall, in farrow-to-feeder/finish farms, the apparent herd, individual and intra-herd prevalence were respectively 35% (95% c.i.: 26-43%), 36% (95% c.i.: 33-39%) and 38% (95% c.i.: 35-41%). In growing/finishing farms, the apparent herd, individual and intra-herd prevalence were respectively 60% (95% c.i.: 53-67%), 54% (95% c.i.: 51-58%) and 85% (95% c.i.: 82-88%). This study showed that approximately 2/3 of Walloon breeding farms appear to be PRRS free. The next step will be to identify risk and protective factors from the survey. In the perspective of starting a national PRRS control program, these indicative results, even though they need to be confirmed, will be helpful in making specific regional decisions.

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### 39. Interest of the essential oil of *Rosmarinus Officinalis* (L.) in the protection of rooster sperm mobility parameters during 4 °C short-term storage

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Short term storage at 4°C of avian sperm is widely used in poultry production within artificial insemination programs to optimize the management of genetically superior males. However, sperm parameters (mobility, viability and concentration) decreased rapidly after collection, thus reducing the level of fertility. Recently, the use of some essential oils extracted from medicinal plants in the conservation of semen of breeding cock improved significantly semen quality and fertility.

The aim of the present study was to evaluate the effect of rosemary (*Rosmarinus officinalis*) essential oil on short-term storage of rooster sperm at 4°C. Pool sperms were collected from ten 45-weeks old Hubbard commercial broiler reproductive cocks. Three concentrations (8.7, 87 and 870 µg/ml) of rosemary essential oil were tested during the experiment. The different sperm mobility parameters were evaluated using a Computer Assisted Semen Analysis (CASA) and measurements were carried out at 0, 6 and 24 h of conservation in the refrigerator at 4 °C.

The results showed a significant effect of rosemary essential oil on progressive mobility compared to the control group. In term of sperm velocity parameters (VCL, VAP, VSL, ALH, BCF), the highest values were recorded with the concentration of 87 µg/ml after 6 and 24 hours of storage.

Overall, the use of *Rosmarinus officinalis* essential oil in the conservation of rooster sperm showed a protective effect on mobility parameters after 24 hours of storage at 4 °C. The protective effect was dose-dependent where the low concentrations of essential oil (8.7, 87 µg / ml) were those showing the most efficiency.

#### 40. Improvement of rooster sperm quality using the essential oil of *Artemisia herba alba* during 4 °C short-term storage

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To the best of our knowledge, the use of essential oils *in vitro* as a supplement in semen extenders has never been investigated, all animal species included. The aim of the present study was to investigate the effect of the essential oil extracted from the aerial part of *Artemisia herba alba* on rooster sperm mobility parameters at 4°C. Qualitative analysis of the essential oil was conducted by Agilent 2890 HP gas chromatography coupled with mass spectrometry. The collected sperm was stored after treatment with three concentrations of the essential oil, A, A5 and A10 (corresponding to the dilutions 1/100, 1/500 and 1/1000 in Tris-based extender, respectively). The control extenders were a commercial extender (IMV) and a Tris-based extender without any supplementation. Treated sperms were stored in a refrigerator (4°C) and analyzed at 0, 6, and 24 h using a computer aided sperm analyzer (CASA). The results of the phytochemical analysis of the essential oil revealed the presence of 36 bioactive compounds including three major molecules: camphor (31.9%),  $\alpha$ -pinene (13.16%) and camphene (12.69%). The percentage of progressive spermatozoa was significantly ( $p < 0.05$ ) higher in A5 and A10 after 24 h of storage with rates of 47.9 and 54.14, respectively. After 24 h of storage, VCL (curvilinear velocity) values were 28.46, 31.36, 40.99 and 47.81  $\mu\text{m/s}$ , in the control extender (Tris), commercial extender (IMV), A5 and A10 extenders, respectively. The highest concentration of the essential oil (A: 1/100) expressed a spermicidal activity, especially after 24h of storage. In terms of lipid peroxidation, sperms treated with A5 and A10 showed lower levels of malondialdehyde (MDA) than control, whereas the highest MDA level was observed in sperm treated with the highest concentration of essential oil (870 $\mu\text{g/ml}$ ). In addition, the evaluation of the total antioxidant capacity (TAC), after 6 hours of storage, showed that sperm treated with A5 (87 $\mu\text{g/ml}$ ) expressed the highest antioxidant activity. The current results revealed a real potential effect of *Artemisia herba alba* essential oil in the conservation of rooster sperm at 4°C, probably by expressing a powerful antioxidant activity.

# **Comparative veterinary medicine**

#### 41. Normal or mild increased C-reactive protein values in 16 dogs with bronchial and pulmonary infection with *Bordetella bronchiseptica*

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C-reactive protein (CRP) has been recently shown to be useful in discriminating bacterial bronchopneumonia from other lung diseases in dogs but dogs infected by *Bordetella bronchiseptica* (Bb) have not been distinguished and amplitude of increase of CRP in naturally-infected dogs with Bb has not been described. Aims of this study were to describe CRP values in dogs with lower airways Bb infection and compare values with dogs diagnosed with bacterial aspiration bronchopneumonia (ABP). Sixteen dogs with lower airways Bb infection and 36 dogs with ABP were selected. For each dog, a CRP value and thoracic radiographs at diagnosis were available. Bb infection was confirmed by culture or PCR on bronchoalveolar lavage fluid. ABP was diagnosed based on history, physical examination, radiographic lesions and favorable evolution on antimicrobial therapy.

Eleven dogs with Bb infection had alveolar lesions on radiographs with only one having another bacterial coinfection. CRP value was mildly elevated in 11/11 and 1/5 dogs with and without alveolar lesions respectively ( $p=0.002$ ); the median CRP value was significantly higher in dogs with alveolar lesions than in dogs without alveolar lesions (20 mg/L [14-38] and 5 mg/L [5-11],  $p=0.002$ ). Duration of clinical signs was longer than 2 weeks for Bb dogs and was not different between dogs with normal or elevated CRP as well as between dogs with or without alveolar lesions. In dogs with Bb infection and alveolar lesions, median CRP value was significantly lower than in dogs with ABP (17 versus 118 mg/L,  $p<0.001$ ).

Regardless of the presence of alveolar lesions, Bb may thus be suspected in coughing dogs with normal to slightly elevated CRP.

#### 42. The BARF diet for pets: a survey of vet opinions and attitudes

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A survey was conducted to know the opinions and attitudes of vet's about BARF (Bones And Raw Food) in companion animals. An online questionnaire was built using Qualtrics [1] and the link was sent to French-speaking vets in 2019. According to the vets, the BARF diets described by the owners were 1)a raw meat diet with or without bones (55 % of the vets), 2)a diversified BARF diet including vegetables and supplements (22 %) 3)a whole-prey diet (10%) or 4)a commercial frozen "BARF" hamburger (8 %). Forty-seven % of the vets declared that 1 to 5 % of the dogs presented in 2017 were fed with BARF diets but 27 % declared this for 6 to 10 % of dogs and for 15 % of vets, even 11 to 15 %. Compared to 2017, vets thought that feeding BARF increased or was similar in 2018. Vet's knowledges about BARF came from continuing education lectures (21%), internet forums (20 %) and curriculum (19%). Forty-nine % of vets declared that health troubles were associated with BARF diet in dogs (n=227) and in cats (n=40). In dogs, the top 3 of health troubles were digestive problems, poor general condition and injuries associated to feeding bones. Considering the risks of BARF diet, the vets cited in the following order, nutritional and metabolic troubles, risks associated to bones feeding and finally sanitary risks. The majority of vets thought that BARF is contraindicated during growth (65%), reproduction in females (14%) and in senior dogs (10%). Results found that the raw meat (only) diet is the first "BARF" diet, which is particularly unsafe. There is a need to teach extensively the BARF diet and its consequences during the curriculum and in continuing education. [1] <https://www.qualtrics.com>.



## Posters

### 43. Vegetarian and vegan diets for pets: a survey of vet opinions and attitudes

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As unconventional diets (BARF -Bones And Raw Food -and vegetarian/vegan diets) [1] become very popular in companion animals, a survey was conducted to know the opinions and attitudes of vet's about those practices, using Qualtrics [2]. Comparing to 2017, 44 % and 23 % of vets thought that the vegetarian and vegan practices were increasing, respectively in the dog and in the cat in 2018 while 34 and 25 % thought that the frequency is similar. Less than 2 % of vets thought that the frequency is decreasing. As expected, the estimated frequency of pets fed vegetarian diets was higher than pets fed vegan diets, with differences between the 2 species. Vets were 18% to report consecutive troubles to vegetarian/vegan diets, mainly poor body condition and digestive problems. In a general way, vets warned owners of the troubles associated with a plant-based diet (40%) or strongly argued against (41%); only 17% tried to balance the diets. Commercial supplements appeared to be useful for 40 % of the vets. The majority of the practitioners (81 % for dogs and 90 % for cats) do not want to sell vegetarian/vegan diets but the others could eventually consider it. The vets declared to be in majority omnivorous; only 3 % are vegetarian or vegan. In conclusion, according to vets, vegetarian and vegan practices increased in the pet population in 2018, compared to 2017. Vegetarian diets, including eggs and milk products, can be balanced in the 2 species. By contrast, vegan diets for pets are difficult to balance and may be also considered as being unethical. References: [1] Dodd et al. 2019. PLOS ONE <https://doi.org/10.1371/journal.pone.0210806> ;[2] <https://www.qualtrics.com>

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### 44. Continuing education in companion animal nutrition: what do vets expect?

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As a large number of lectures in continuing education are delivered each year by the department, it appeared useful to gather the opinion of practitioners about their expectations. An online survey about BARF (Bones And Raw Food) and vegetarian/vegan diets was conducted in February 2019 using Qualtrics [1]. The last question was: "In continuing education in pet nutrition, what topics would be of interest in the next 5 years?". Sixty-eight % of the vets (n=136) did answer and most of them made several proposals. The proposed topics in continuing education were 1) homemade diets (25%), BARF (22%), commercial diets (13%), nutrition and diseases (10%), vegetarian/vegan diets (8 %), cereals (8 %), communication to the owner (8%) and cycle of life or others (new companion animals, organic food, zerowaste, ...) (<5%). In conclusion, the answers were probably influenced by the topics of the survey. Nevertheless, vets did express a large interest for unconventional diets, especially BARF and homemade (47% for these 2 topics). Surprisingly, they were less interested in clinical nutrition. This is the reflection of the clients concerns in daily practice, and it is necessary to provide continuing education and/or tools to cover those topics to ensure that the public benefits from the relevant expertise.

References: [1] [2] Diez et al., 2019 a,b, ESVCN Congress 2019

#### 45. Comparison of measurements of the laxity index with 3 versus 5-point circles on stress radiographs performed with the Vezzoni- modified Badertscher hip distension device

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The laxity index (LI) obtained with Vezzoni-modified Badertscher distension device (VMBDD) presents many advantages compared to the distraction index obtained on the PennHIP distraction radiograph (only 2 X-rays, only a single operator, no legal obligations...). In a recent study, they described a measurement method of the LI on stressed radiographs performed with VMBDD of which a step consisted to delimit the femoral head by a circle from 5 predefined points. However, the majority of image analysis software package allows 3-point circles with the exception of Digimizer which only the trial version is free. The aim of this study was to compare the LI obtained with measurements made with 3 versus 5-point circles.

The radiographic images used in this study were acquired on 15 dogs presented to the Veterinary University Clinic in Liège, for obligatory hip screening and lameness from the hips. The 30 LI were measured independently by a last year student and a senior clinician in radiology with 3 and 5-point circles. Non-parametric Wilcoxon tests were used to compare the measurements.

The LI measurements performed by the two operators were not significantly different ( $p=0,45$  and  $0,1$  for the left and right hip, respectively). The LI made from 3-point circles are not significantly different from those to 5 points ( $p=0,5$  for both hips).

These results suggested that usual image analysis software package with 3-point circles allowed correct measurements. They confirmed that a high degree of experience in radiographic assessment is not necessary to perform the LI.

#### 46. Analysis of the lung microbiota in dogs with *Bordetella bronchiseptica* infection and correlation with culture and quantitative polymerase chain reaction

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Infection with *Bordetella bronchiseptica* (*Bb*) can be confirmed using bacterial culture or quantitative polymerase chain reaction (qPCR). The lung microbiota (LM), which has been described in healthy experimental dogs, has not yet been studied in dogs with lower respiratory infection. In the present study we aimed to analyze the LM in dogs with *Bb* infection compared with healthy dogs, and to correlate the 16S rDNA amplicon sequencing results with culture and qPCR results. Twenty dogs with a diagnosis of *Bb* infection obtained either by qPCR and/or culture and 4 healthy dogs were included. 16S rDNA sequences were obtained from naïve bronchoalveolar lavage fluid (BALF) after DNA extraction, PCR targeting the V1-V3 region of the 16S rDNA and sequencing.

Sequencing results showed the presence of *Bb* in all diseased dogs. About half of the dogs were co-infected, the majority with *M. cynos*. In diseased dogs, a shift in the  $\beta$ -diversity of the LM was observed ( $P=0.002$ ); the richness and the  $\alpha$ -diversity were significantly lower ( $P=0.012$  and  $0.006$ ) and the bacterial load higher ( $P=0.004$ ) compared with healthy dogs. *Bb* qPCR level and culture results were positively correlated with the relative abundance of *Bb* species after sequencing ( $r=0.56$ ,  $P=0.028$  and  $r=0.70$ ,  $P=0.002$ ).

*Bb* induced a major dysbiosis of the LM, characterized by high bacterial load, low richness and diversity and increased abundance of *Bb*, in comparison with healthy dogs. Results of the LM analysis highly correlate with results obtained by qPCR and culture and show that results of LM can be reliable for identification of potentially causal bacterial microorganism involved in lung infectious diseases.

## Posters

### 47. The modification of microbiota after intoxication with hypoglycin A: preliminary study

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Hypoglycin A (HGA) is a toxin found in the *Sapindaceae* botanical family. This toxin causes atypical myopathy in equids, Jamaican vomiting sickness and encephalopathy in human. Eukaryotic cells metabolize HGA in its toxic metabolite. The intestinal microbiota is able to metabolize branched amino acids through an enzymatic system similar to that used to metabolize HGA in mammals. Thus, two questions arise: does the HGA modify the microbiota and, is the toxin metabolized by the microbiota? The purpose of this preliminary study was to answer to the first question owing to the Simulator of Human Intestinal Microbial Ecosystem (SHIME®) that enables to study microbiota in different parts of the intestinal tract. After the introduction of a microbiome and a stabilization period, purified HGA (6000 µg) was added to the daily diet of the SHIME® for one week. Samples were taken in ascending colon (AC), transverse colon (TC) and descending colon (DC). Alpha diversity indices were used to quantify diversity status. Bacterial taxonomy profiling was obtained by V1V3 16S amplicon sequencing. The concentration of volatile fatty acids (VFA; acetate, propionate, butyrate) were analyzed by solid phase microextraction, gas chromatography and mass spectrometry. After the stabilization period, the bacterial phyla and the ratio in VFA were similar from those described in the literature. After HGA intoxication, a dysbiosis was noted with (1) a general decrease of VFA, (2) a decrease in diversity index in TC and DC, (3) a sharp decrease in *Lachnospiraceae* family and an increase in *Enterobacteriaceae* family and (4), a variation in the ratio between acetate and propionate. In conclusion, HGA modified the microbiota of the SHIME®.

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### 48. Handlers' practices in feeding canicross dogs

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Canicross is a human-animal teamwork in a cross-country run. The dog is harnessed and hitched to the runner by a 2 meters elastic leash. This new sport becomes popular as a leisure and competitive sport. Nevertheless, data for the canicross dogs' nutritional requirements are scarce. The aim of this study was to collect data on handlers' practices. A paper survey was distributed to canicross runners, during competitions in Belgium, from January to June 2017. Dogs' data were collected from 156 questionnaires and the composition of 67 dry food-based diets was studied. Dogs were  $3,5 \pm 2$  y old; the sex ratio was 60% males and 40% females; the half being neutered. The mean ( $\pm$  SD) dogs' BW was  $25 \pm 8$  kg. The mean BCS was of  $2.4 \pm 0.7/5$ . Owners reported  $11 \pm 8$  h/week of total activity. The mean composition of the dry diets was (% dry mater (DM)): crude proteins (CP)  $32 \pm 6$ , nitrogen-free extract (NFE)  $34 \pm 11$ , lipids  $19 \pm 4$ , metabolizable energy (ME)  $394 \pm 33$  kcal/100g. Proteins provided  $27 \pm 5\%$  ME, lipids  $41 \pm 6\%$  and NFE  $32 \pm 9\%$ . Dogs received  $132 \pm 38$  kcal ME/kg BW<sup>0.75</sup>/day (80 – 222). This variability can be explained by at least the differences in activity level. Extra lipids were given to 45% of the dogs and 74% received leftovers and treats. Handlers used to give a variety of energy supplements just before or after the race. According to the owners, from the moment they start canicross, 42% of dogs had no BW change; 30% lost weight and 25% gained muscles. In conclusion, each handler has his own feeding and training practices but no nutritional guidelines are available. Dogs receive high energy food but the study shows that nearly half of the dogs are underweight.

#### 49. Infection with *Babesia canis* in dogs in the Algiers region: parasitological and serological study

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Canine Babesiosis is a vector disease transmitted by ticks of the *Ixodidae* family. The effects of infection in dogs can range from the subclinical to the lethal severe form. In this work the main objective is to make an original contribution to the knowledge of circulating species of *Babesia spp* in dogs in the region of Algiers as well as mechanisms and risk factors for their transmission. For this purpose, an epidemiological study was carried out on 189 blood samples taken from dogs from April 2015 to January 2016. The samples taken underwent parasitological and serological analyzes. The parasitological results of the Giemsa stained blood smears revealed the presence of two groups of parasites of the genus *Babesia*: Large *Babesia* (1.6%) and Small *Babesia* (11.64%). Serological analysis by the IFAT test at a dilution of 1/32 showed an overall seroprevalence with *Babesia canis* of 17.98% (95% CI 11.53-22.46). The distribution of the antibody titres for the positive samples showed that of the 34 positive sera with a titre  $\geq$  1/32, 28 sera remained positive at a dilution of 1/64 (14.81%), 22 at a dilution of 1/128 (11.64%) and 15 sera remained positive at a dilution of 1/256 (7.93%). Although seroprevalence varied according to canine population (20% and 19.49% in pet dogs and canine pound dogs respectively and 6.66% to 0% in Farm dogs and Hunting dogs respectively), Statistical analysis showed no significant differences between populations. The antibody titers obtained after several dilutions showed that 22 canine pound dog sera remained positive at a dilution of 1/128 compared to pet dogs and farm dogs which ceased to be positive at the dilution of 1/64. The comparison between the two diagnostic methods showed a strong agreement between the parasitological examination by FS and the serological method by IFAT. However, IFAT was much more sensitive. The analysis of risk factors, which may influence *Babesia canis* seroprevalence, has shown the influence of age, tick presence and season. Finally, of the 242 ticks collected from a total of 59 dogs, only one tick species was identified, *Rhipicephalus sanguineus*. Furthermore, the identification of a new species *Rhipicephalus senegalensis* never recorded in North Africa is to be confirmed by the molecular tool.

#### 50. Assessment of mitochondrial dysfunction by blood mononuclear cells analysis

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Mitochondrial dysfunction has emerged as a ubiquitous cause of disease in human and animals. Using respirometry, a so-called "mitochondrial syndrome" has been reported in equine atypical myopathy, which is characterized by a severe decrease of mitochondrial respiratory capacity in affected individuals. Up to now, respirometric studies have been conducted on skeletal muscles samples. However, the muscle biopsy procedure is rather invasive and therefore difficult to use in equine clinical routine. Despite the fact that peripheral blood mononuclear cells (PBMC) have been used in human medicine to assess mitochondrial function, there is only one study comparing PBMC and skeletal muscle oxidative capacity in animals, i.e. monkeys, so far. Therefore, the aim of this study was to test whether mitochondrial function could be assessed on equine blood cells with respirometry. Whole blood was collected in 9 ml EDTA tubes in four horses. Cells of interest (PBMC) were isolated with a density-separation medium (Lymphoprep™) following a standardized procedure. Electron paramagnetic resonance as well as the respirometric analysis were performed within the following 4 hours. With this isolation technique more than  $1 \times 10^6$  PBMC/ml of whole blood were obtained. Respirometric analysis with PBMC taken from the same horses indicated a lack of reproducibility. Therefore, a correlation between skeletal muscle fiber and PBMC mitochondrial bioenergetics in the equine species is, up to now, not possible to be established. The cause of variation within the same individual is still under examination.

## Posters

### 51. High seroprevalence against *Lawsonia intracellularis* in horses living in Belgium

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*Lawsonia intracellularis* (LI) is an obligate intracellular gram-negative rod causing equine proliferative enteropathy (EPE). Occasional cases of EPE in foals have been reported in Belgium, but the seroprevalence of equine LI in the country is unknown. The aim of this study was to determine the seroprevalence of LI in adult healthy horses in Belgium.

The target population included clinically healthy adult horses, housed in the same premises for the previous 12 months. Serum samples from horses living throughout Belgium were collected and analysed for specific IgG antibodies against LI using a blocking ELISA test. Results were expressed as Percentage of Inhibition (PI). Samples that had a PI <20% were judged as negative, those between 20 and 30% as inconclusive and those >30% were considered positive.

A total of 356 blood samples were analysed with 352 horses (98.8%) testing positive, 2 horses (0.56%) testing negative and 2 horses (0.56%) showing inconclusive results. The high prevalence of positive samples precluded any statistical analysis to test the impact of province of residence, age, breed or gender on the results.

In conclusion, the large percentage of seropositive samples obtained in this study confirms a widespread exposure of Belgian horses to LI. Seroconversion is rarely associated to clinical disease, but suggests that EPE should be part of the differential diagnosis in horses with compatible clinical signs (ill-thrift, hypoproteinemia, edema, and diarrhea). These results are in accordance with results of prevalence reported in neighbouring countries (The Netherlands, Germany), whilst other countries (Israel) report a much lower prevalence.

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### 52. Inadequate mare-foal bonding restored by adoption of her own foal - a case report

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Difficulties in mare-foal bonding are mostly consequences of an abnormal maternal behaviour. Despite current therapies usually based on restrain and sedation of the mare, the mare-foal bonding is not always created. However, adoption of foster foals and establishment of the maternal behaviour is often successful. Procedures of adoption mimic the transient increase of oxytocin during delivery and its effects by cervical and vaginal stimulation (Ferguson reflex), or by pharmacological simulation of the high concentrations of prostaglandins observed during parturition. Even if the mechanism is uncertain in mares, both hormones seem to have a direct effect on the brain control of maternal behaviour. A primiparous 7 years old mare was presented at the Equine Clinic showing aggressive behaviour towards her 4 days filly especially when the foal attempted to suckle. No evident cause of pain at suckling was found. A hormonal adoption procedure of her own foal was implemented after several attempts to restore the mare-foal bonding by restrain and sedation of the mare. After 3h of fasting and isolation of the foal, the mare received an im injection of 750µg of cloprostenol. The foal was carefully presented to the mare when the secondary effects of the prostaglandins were visible. The mare accepted the foal and allowed suckling rapidly. During the next 24 hours under supervision, the mare showed a very normal maternal behaviour. After 3 days of normal bonding, mare and foal were discharged from the clinic. Protocol for adoption with the use of cloprostenol could be a valuable option in the treatment to establish or restore the normal bonding between a mare and her own foal.

### 53. Liver lobe torsion in a cat presented with hemoabdomen

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An 11-month-old spayed female domestic shorthair cat was presented in emergency for evaluation of acute severe lethargy. The cat was hypothermic, bradycardic with pale pink mucous membranes and a distended abdomen. Abdominal point of care ultrasound showed significant abdominal effusion. Abdominocentesis revealed a hemoabdomen. Most notable bloodwork changes were regenerative anemia (9,8%) and hyperlactataemia (10,5mmol/L). After a first transfusion, the cat was stable with a systolic pressure of 90mmHg but few times later, the cat was again hypotensive at 60mmHg. A crystalloid fluid bolus did not really improve blood pressure and a colloid fluid bolus raised transiently the pressure to 90 mmHg. At this moment, the microhematocrit was 15% and lactate was 6,5 mmol/L. A second canine xenotransfusion was realized without any complications. Complete abdominal ultrasonography then revealed a large volume of free abdominal echogenic fluid and a suspicion of right medial lobe and gallbladder torsion. Exploratory laparotomy revealed torsion of the right medial and squared hepatic lobes together with the gallbladder. A lobectomy of the affected lobes and a cholecystectomy was performed using a surgical stapler. The cat was discharged after 4 days. Histopathologic examination of the liver concluded a pseudocyst with torsion and hemorrhagic infarction associated with a mucocele. One month postoperatively, the cat had totally recovered. This case report describes an uncommon presentation of a hemoabdomen in a young cat secondary to liver lobes and gallbladder torsion, managed with blood transfusions and successfully treated by one stage liver lobe lobectomy and cholecystectomy.

### 54. Prevalence and Antibiotic resistance profile of Enterobacteriaceae isolated from clinical mastitis of algerian dairy cows

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The aim of this study was to investigate the prevalence and antimicrobial susceptibility of *Enterobacteria* strains that were isolated from clinical Bovine Mastitis in Bordj Bou Arreridj region. Bacteria were cultured from 260 quarter milk samples obtained from cows presented clinical signs of an acute mastitis (n = 240). These samples were provided from 100 dairy farms. The bacteria were identified using colony morphology, Gram staining and biochemical characteristics. Susceptibility testing was performed by the disc diffusion method according to the guidelines of the Antibiogram Committee of the French Society for Microbiology (CA-SFM). Over a 2 years period, 60 *Enterobacteriaceae* isolates were collected from milk samples. The most frequently characterized bacteria was *E. coli* (15%), followed by *Enterobacter cloacae* (8%), *Klebsiella pneumonia* (2%) and *Serratia marcescens* (2%). Forty-four percent of them were resistant to at least one antibiotic. Most resistance was observed against Ampicillin (80%) and tetracycline (75%). The resistance patterns of *Enterobacteria* spp isolated during the study are concordant with antimicrobial usage in the study herd. This is in agreement with the generally accepted notion that selection pressure from the use of antibiotics is a main factor in development of antibiotic resistance.

## Posters

### 55. Assessment of nasal microbiota in healthy dogs of different breeds

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Dolichocephalic breeds are predisposed to sinonasal aspergillosis while brachycephalic dogs are not affected. The aim of this study was to describe and compare the composition of the nasal microbiota in healthy dogs of various breeds based on the hypothesis that differences in predisposition to nasal disease in dogs could be associated with differences in core nasal microbiota between breeds. Forty-six healthy dogs were included and categorized in 3 groups: 22 dogs from dolichocephalic breeds, 12 brachycephalic dogs and 12 terrier dogs. Nasal swabs were obtained under anesthesia and banked at -80°C. A PCR targeting the V1-V3 region of the 16S rDNA was performed. The nasal microbial population was predominantly composed of the phyla *Proteobacteria* (mainly represented by the family *Moraxellaceae*), *Actinobacteria*, *Firmicutes* and *Bacteroidetes*. Analysis of ecological indexes showed that bacterial richness and  $\alpha$ -diversity were significantly higher in the brachycephalic group compared to the two other groups. The AMOVA analysis also indicated that the brachycephalic group was different compared to the Dolichocephalic and Terrier groups. Nine distinct species were found as indicators of discrimination among which 8 species in the brachycephalic group. Our study mainly demonstrated significant differences in the nasal microbiota in the brachycephalic group. Such differences might be associated to their particular facial morphology and/or breathing pattern. We did not identify nasal microbiota breed-differences that would be in favor of a breed susceptibility of dolichocephalic dogs for nasal diseases.

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### 56. Echocardiographic diagnosis of canine cardiac effusion: changing incidence in underlying cause?

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Neoplasia and idiopathic pericarditis are the most common causes of pericardial effusion. Echocardiography is the preferred clinical diagnostic test for pericardial effusion and tamponade. Determining the underlying cause is pivotal for treatment and prognosis. The reported incidence of cardiac neoplasia in dogs with pericardial effusion varies between 30% to 62%.

Our aim was to evaluate diagnosed underlying etiologies at our institution.

Echocardiography is accepted as clinical reference diagnosis when performed by a cardiologist for the diagnosis of pericardial effusion and underlying etiology. Right sided congestive heart failure appears to be a rare cause of cardiac tamponade. The incidence of cardiac tamponade due to neoplasia before 2014 (9/15; 60%) was lower than after 2014 (40/55; 72%). However, this difference in incidence is statistically non-significant (chi-square) in this population.

This difference could be due to the increased ability of the observer to detect neoplasia, or be a coincidence. Technical improvements however seem unlikely, as the same machine was used throughout the study.

Cardiac tamponades of neoplastic origin appear more common than described. Atrial ruptures do not always present with tamponade, while right sided congestive heart disease may induce tamponade. Findings of this study must be conformed in multicenter studies before drawing conclusions.

## 57. Measured and predicted oxygen uptake in healthy adults

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Measurement of maximal oxygen uptake ( $\dot{V}O_{2max}$ ) is considered to be the gold standard (GS) to determine aerobic capacity in human. Its determination is not easy to perform in untrained or unhealthy individuals. Several tests are designed to predict  $\dot{V}O_{2max}$  while reducing the risk and costs linked to its direct measurement. Among them, the one-mile track walk test (1 mile-WT) does not require specialized equipment. Also, a non-exercise based-equation model (NE-BEM) has been proposed to estimate  $\dot{V}O_{2max}$  in healthy individuals. This study tests the validity of the 1 mile-WT and NE-BEM to estimate  $\dot{V}O_{2max}$  in a group of healthy adults.

All 12 participants performed a maximal graded exercise on a treadmill up to exhaustion. Expired air during exercise was analyzed (Cosmed K4b<sup>2</sup>) for O<sub>2</sub> content. The test began at a speed of at 7 km/h for 5 minutes to be increased by 2 km/h every 3 minutes. For the 1 mile-WT, participants were instructed to walk as fast as possible. The time to perform 1 mile was included in an equation to predict  $\dot{V}O_{2max}$  taking into account individual characteristics and final heart rate (HR). The NE-BEM estimates  $\dot{V}O_{2max}$  based on a calculation including gender, age, body mass index, resting HR, and self-reported physical activity. Six statistical methods were used to estimate level of agreement between measured and estimated  $\dot{V}O_{2max}$  values.

The mean ( $\pm$ SD)  $\dot{V}O_{2max}$  was  $41.2 \pm 4.1$  with the GS vs  $44.6 \pm 5.4$  and  $34.7 \pm 5.1$  mL.kg<sup>-1</sup>.min<sup>-1</sup> as estimated by the 1 mile-WT and the NE-BEM, respectively. There was no agreement between the tests. Estimating  $\dot{V}O_{2max}$  in a group of non-homogenous individuals was not valid using the prediction equations referenced in the literature.



## Posters

### 58. Prediction of maximal oxygen consumption using simple field tests in healthy adults

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Numerous training programs aim to promote health in the general population. A field test that could be applied to individuals of various training levels and age might be of particular interest to assess efficacy of fitness programs. Fitness level may be estimated from the one-mile track walk test (1 mile-WT) but also from non-exercise based-equation models (NE-BEM). The NE-BEM take into account factors known to be predictive of fitness (e.g. gender, age, body size etc.) and self-reported physical activity levels. This study compares values of  $\dot{V}O_{2max}$  estimated from a 1 mile-WT vs NE-BEM in a large group of healthy individuals.

All 84 participants completed a questionnaire to obtain data necessary for  $\dot{V}O_{2max}$  prediction by the two models. The 1 mile-WT was performed on an athletics track. Subjects were instructed to walk as fast as possible and heart rate was recorded every 400 m. Six statistical methods, with different assumptions, were used to estimate the level of agreement between values of  $\dot{V}O_{2max}$  estimated from the two tests.

On average, the 1 mile-WT required  $12.9 \pm 1.3$  minutes to be performed. The mean  $\dot{V}O_{2max}$  was  $41.8 \pm 7.5$  vs  $32.7 \pm 7.5$  mL.kg<sup>-1</sup>.min<sup>-1</sup> as estimated with the 1 mile-WT and the NE-BEM, respectively. There was no agreement between the different tests.

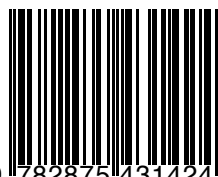
The 1 mile-WT is a simple test that can be performed by any kind of healthy subject and that requires no specialized equipment. A previous study indicated that its value to estimate  $\dot{V}O_{2max}$  in a group of people not linked by fitness level and/ or age is doubtful but its usefulness to follow improvement of individuals following training programs remains to be tested.

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