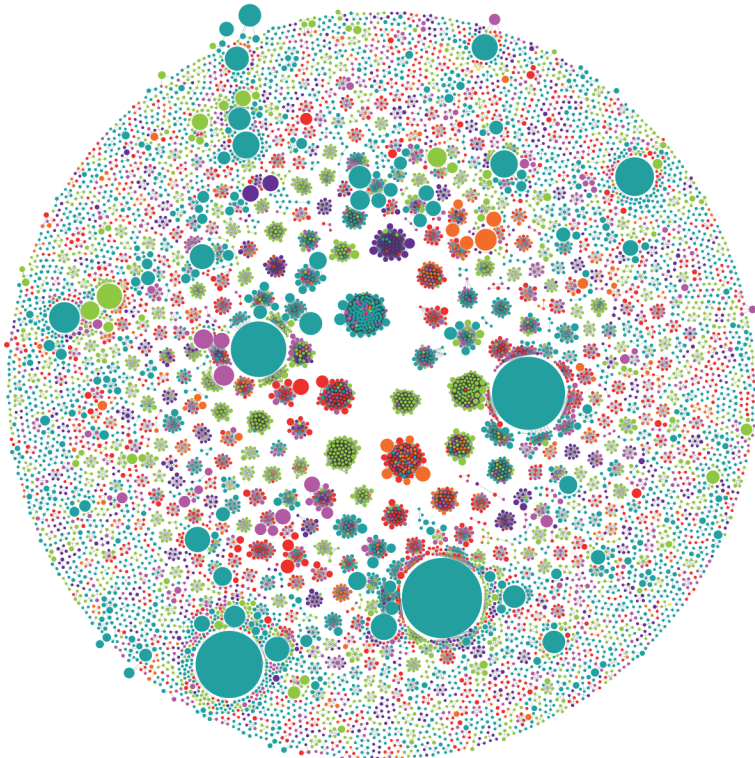




Proceedings of the 3rd FARAH-Day

Faculty of Veterinary Medicine
University of Liège - Belgium
October 21, 2016

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One Health

L'Animal et l'Homme, une même santé

Proceedings of the 3rd FARAH-Day

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

October 21, 2016

Edited by C. Bayrou, J.-F. Cabaraux, C. Delguste, C. Gatez, T. Jauniaux, C. Lété,
A. Sartelet, D. Thiry, D.-M. Votion, C. Zeippen

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COVER PICTURE CREDITS:

Dr Xue XIAO, Dr Céline LETE and Pr Laurent GILLET,
Immunology-Vaccinology, FARAH

B cell repertoire of a Murid Herpesvirus 4 (MuHV-4) infected mouse.

Network representation of the antibody repertoire from a MuHV-4 infected mouse (40 days post-infection). Each node represents a unique immunoglobulin heavy chain (IGH) sequence. The area of each node is proportional to the number of identical sequences. Isotype of the IGH sequence is indicated by colors as follow: cyan corresponds to IgM, green to IgA, violet to IgG1, red to IgG2a, orange to IgG2b, pink to IgG3, yellow to IgE and grey to IgD. Nodes are linked with edges (in light grey) when they share identical V and J assignment and 90% similarity (nucleotide level) in complementarity determining region 3 (CDR3). For this network, 100,000 IGH sequences were analyzed.

Welcome to the 3rd 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 more than 100 abstracts dedicated to fundamental, clinical and/or applied researches and is dedicated to the memory of Dr Etienne Baise.

Laurent Gillet, Chairperson of the FARAH

Frédéric Farnir, Vice-Chairperson

Dominique Votion, Secretary

Bienvenue à la 3^{ème} journée du FARAH

En 2012, le Personnel Scientifique de la Faculté de Médecine vétérinaire organisait sa première journée scientifique annuelle. Chaque réunion annuelle a été un grand succès avec, en moyenne, une centaine de résumés de recherche soumis dont une vingtaine étaient 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, et est dédiée à la mémoire du Dr Etienne Baise.

Laurent Gillet, Président du FARAH

Frédéric Farnir, Vice-président

Dominique Votion, Secrétaire

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Program

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

09:00 - OPENING AND WELCOME SPEECH

Dr CATHERINE DELGUSTE

Pr FRÉDÉRIC Farnir

09:15 - IN MEMORIAM Dr ETIENNE BAISE

09:45 - ORAL SESSION 1: (lecture hall C, building B45)

CHAIRMEN: Dr Axel MAUROY and Dr Carole CHARLIER

09:45 Comparative analysis of the respiratory microbiota of healthy dogs and dogs affected with canine idiopathic pulmonary fibrosis

Elodie Roels, Companion Animals Clinic, FARAH

10:00 Helminth-induced inflammation enhances control of γ -herpesvirus acute infection

Marion Rolot, Immunology-Vaccinology, FARAH

10:15 Gut microbiota involvement in blood pressure control : comparison between patients with normal blood pressure and patients suffering from high blood pressure

Bernard Taminiau, Food sciences department, FARAH

10:30 - COFFEE BREAK AND POSTER SESSION 1 (Room P, building B45)

11:00 - ORAL SESSION 2: (lecture hall C, building B45)

CHAIRMEN: Dr Frédéric BILLEN and Pr Tatania ART

11:00 Sexual transmission of Murid Hepresvirus-4 is reduced by the use of the nucleotide analogue cidofovir.

Caroline ZEIPPEN, Immunology-Vaccinology, FARAHA

11:15 Muscle-derived mesenchymal stem cells can be differentiated into cells of the cardiovascular system in vitro

Julien DUPONT, Equine clinic, FARAHA

11:30 Short talks

Synthetic virus-like particle – endocytosis and processing by dendritic cells for vaccine delivery

Rajni SHARMA, Immunology-Vaccinology, FARAHA

Outbreak investigations and molecular characterization of foot-and-mouth disease viruses circulating in Niger

Bachir SOULEY KOUATO, Epidemiology and Risk analysis, FARAHA

Identification of virulotypes and serotypes of enteropathogenic (EPEC) and Shigatoxigenic (STEC) Escherichia coli from healthy cattle at slaughterhouses in Wallonia.

Shino TATAKI, Bacteriology, FARAHA

The ORF27 gene of Cyprinid herpesvirus 3: a case of opposite selective pressures in vitro and in vivo

Catherine VANCOSK, Immunology-Vaccinology, FARAHA

First detection of Besnoitia bennetti (Protozoa: Apicomplexa) in donkey (Equus asinus) in Europe.

Yannick CARON, Parasitology and parasitic diseases, FARAHA

Comparison of buprenorphine, morphine and butorphanol effects on the European rabbit (O. cuniculus) gastrointestinal tract

Helene DEFLERS, Clinic for Birds, Rabbits and Rodents, FARAHA

Virulence acquisition in Infectious Salmon Anaemia Virus: the role of mutations in the hemagglutinin-esterase and fusion protein

Mickael FOURRIER, Marine Scotland Science, Aberdeen

12:15 - LUNCH AND POSTER SESSION 2 (Room P, building B45)

13:45 INVITED SPEAKER

Pr MUTIEN-MARIE GARIGLIANY

14:15 - ORAL SESSION 3: (lecture hall C, building B45)

CHAIRMEN: Pr Alain VANDERPLASSCHEN and Dr Dominique CASSART

14:15 Epithelial Rab guanine nucleotide exchange factor 1 (Rabgef1) deficiency increases susceptibility to DSS-induced intestinal inflammation in mice.

Sophie EL ABBAS, Cellular and Molecular Immunology, GIGA

14:30 Predictive abilities of genomic predictions for traits measured in Belgian Blue cattle genomic farms

Ann-Stephan GORI, Association Wallone de l'élevage, Ciney

14:45 A respiratory gammaherpesvirus infection protects against allergic asthma through alveolar recruitment of regulatory monocytes

Bénédicte MACHIELS, Immunology-Vaccinology, FARAH

15:00 Successful in vitro generation of genetically engineered pig embryos

Jessy M'BUMBA, Pathology and Necropsy, FARAH

15:15 Lung resident eosinophils represent a distinct cell subset with homeostatic functions

Claire MESNIL, Cellular and Molecular Immunology, GIGA

15:30 - COFFEE BREAK AND POSTER SESSION 3 (Room P, building B45)

16:00 - ORAL SESSION 4: (lecture hall C, building B45)

CHAIRMEN: *Dr Bénédicte MACHIELS and Pr Stefan DELEUZE*

16:00 **Short talks**

Association between vertebral heart score measurement and overweight condition in dogs

Marion FRANCOIS, Companion Animals Clinic, FARAHA

Computer-assisted study of animal behavior: a powerful technique for the development of an attenuated vaccine against Testudinid Herpesvirus 3 causing a lethal disease in several endangered tortoise species

Frédéric GANDAR, Clinic for Birds, Rabbits and Rodents, FARAHA

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

Emeline GOFFIN, Immunology-Vaccinology, FARAHA

Interferon response factor-3 promotes the pro-Th2 activity of mouse lung CD11b+ conventional dendritic cells in response to house dust mite allergens

Thibaut JANSSE, Cellular and Molecular Immunology, GIGA

*Prevalence of *Angiostrongylus vasorum* in Belgium, a coprological and serological survey.*

Laetitia LEMPEREUR, Parasitology and Parasitic Diseases, FARAHA

Antibiotherapy in pet rodents and rabbits : a preliminary analysis of practitioners' habits

Didier MARLIER, Clinic for Birds, Rabbits and Rodents, FARAHA

Individual monitoring of salmonid immune responses during immunisation and infection

Milena MONTE, University of Aberdeen

An important role for C-type lectin homolog-encoding A7 gene in the pathogenesis of malignant catarrhal fever.

Françoise MYSTER, Immunology-Vaccinology, FARAHA

Identification of bovine methicillin resistant staphylococci from Europe, Africa and North America by dot blot hybridization, PCR and antibiotic sensitivity.

Cyrille NGASSAM-TCHAMBA, Bacteriology, FARAHA

In vivo infectivity and in vitro replicative fitness regain of a recombinant murine norovirus

Louisa LUDWIG, Virology and Animal Viral Diseases, FARAHA

The use of cis-atracurum in horses as part of a balanced anaesthetic protocol. Partial results.

Alexandru TUTUNARU, Anesthesiology and Surgery, FARAHA

17:15 - AWARDS

Dr DOMINIQUE VOTION

17:30 - CLOSING SESSION

Pr RUDI CLOOTS

17:45 - COCKTAIL AND POSTER SESSION 4 (Room P, building B45)

20:00 - DINNER AND DANCING PARTY (ROOM P, BUILDING B45)

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Oral presentations

Oral presentations

Comparative analysis of the respiratory microbiota of healthy dogs and dogs affected with canine idiopathic pulmonary fibrosis

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Canine idiopathic pulmonary fibrosis (CIPF) is a parenchymal lung disease affecting mainly old West Highland white terriers (WHWTs). CIPF shares clinical and pathologic features with human IPF. The use of next generation sequencing technologies recently allowed to identify differences in the composition and diversity of the respiratory microbiota in human IPF. The objectives of the present work were to identify and characterize the microbiota present in the lung of healthy beagles and healthy WHWTs compared with the microbiota of WHWTs affected with CIPF. For this purpose, BALF samples were obtained from young and adult healthy research beagles, and client-owned healthy and CIPF WHWTs. Metagenetic analysis were performed on V1-V3 hypervariable region of 16S rDNA after total bacterial DNA extraction and sequencing on a MiSeq Illumina sequencer. Taxonomical assignation and microbiota community analysis were done with MOTHUR V1.35 with an OTU clustering distance of 0.03. Data analyses demonstrated that the same phyla predominated in all groups of dogs with Proteobacteria, Firmicutes, Actinobacteria, and Bacteroidetes being the most abundant. Bacterial species richness was significantly higher and evenness significantly lower in WHWTs, either healthy or affected with CIPF, in comparison with beagles, while there was no difference between groups for bacterial diversity. When comparing specifically CIPF WHWTs with healthy WHWTs, *Pasteurella*, *Conchiformibius* and *Bergeyella* spp. were found more abundant in CIPF dogs. In conclusion, results of the present study demonstrate the existence of a core airways microbiota in dogs that might be influenced by the breed, the environment or the disease status.

Keywords: Dogs, Microbiota, Lung

Helminth-induced inflammation enhances control of γ -herpesvirus acute infection

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In developing countries, helminth infections are highly prevalent. This is also the case for the lymphotropic gammaherpesviruses Epstein-Barr virus or Kaposi sarcoma-associated herpesvirus. Coinfections are therefore frequent and a better understanding of how our immune system faces multiple aggressions is essential. Various studies have shown that the impact of helminth infections on immune responses and inflammatory processes is variable. Interestingly, exposure to helminths was shown to induce reactivation of latent gammaherpesvirus infection in mice, using murid herpesvirus 4 (MuHV-4). However, it remains unknown whether pre-exposure to helminth affects gammaherpesvirus host colonization. Thus, we have investigated in the present study how helminth-induced Th2-type inflammation influences the control of MuHV-4 acute infection. We have monitored viral replication by in vivo imaging and observed that pre-exposure to *Schistosoma mansoni* (Sm) eggs enhanced the control of acute pulmonary infection and controlled the weight loss induced by MuHV-4. Interestingly, the enhanced protection was associated with a higher CD8+ T cell lung response, including higher cell numbers and better effector capacities and CD8 depletion impaired the enhanced control. The improvement of CD8+ T cell responses was dependent on Th2 responses, as signalling through interleukin-4 receptor α chain was essential to boost this response. CD8+ T cell gene expression profile will be investigated to better understand how Th2-type inflammation influences CD8+ T cell activation. Despite the enhanced protection against MuHV-4 infection, Sm eggs exposure did not affect MuHV-4 latency establishment. However, future experiments will investigate the impact of Sm eggs exposure on T cell memory formation, genital reactivation of latent MuHV-4 and transmission.

Gut microbiota involvement in blood pressure control : comparison between patients with normal blood pressure and patients suffering from high blood pressure

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High blood pressure (HBP) is a major public health risk in western countries. In some cases, HBP is a secondary symptom to another pathology. But it is more often "essential" as no other cause can be found. Treatment is an association drug therapy, physical exercises and diet. Despite these treatments, HBP physio-pathology remain largely unknown. Recently, a new blood pressure regulation pathway has been discovered in human kidney that is controlled by microbial short chain fatty acids (SCFA). The influence of propionate and acetate has also been demonstrated in a murine model. Our hypothesis is that HBP patients could harbor a different gut microbiota compared to matched healthy patients. Fifty male human volunteers were investigated. Ambulatory blood pressure were monitored for 24H and a sample of blood and feces were recovered. Feces were used for 16S amplicon profiling and blood for NMR metabolomic analysis. Patients were divided into HBP, borderline and control cohorts based upon blood pressure monitoring. V1-V3 hypervariable 16S amplicon profiling were obtained from MiSeq Illumina sequenced reads (10,000/sample). Metabolomics was performed on a 500 MHz NMR system and SCFA were measured. If global bacterial community for the 3 cohorts were similar in term of richness and diversity, HBP and borderline cohorts harbor a statistically higher abundance of *Prevotella* and *Subdoligranulum* genera. Metabolomics of blood SCFA shows a higher abundance of acetate and butyrate in the HBP cohort vs other groups. Investigation of *Prevotella* genus, known to be linked to TMAO production, will be further extended.

Sexual transmission of Murid Herpesvirus-4 is reduced by the use of the nucleotide analogue cidofovir

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The use of antiviral drugs to limit clinical signs associated with herpesviruses has been extensively documented in the past. In contrast, their capacity to block transmission remains unclear as herpesviruses are mostly shed and transmitted without any symptom and as most herpesvirus animal models do not allow transmission. Recently, we have shown that Murid Herpesvirus-4 (MuHV-4), a model for the highly prevalent human Epstein-Barr virus and Kaposi's Sarcoma associated Herpesvirus, transmits sexually from female to male in laboratory mice. This model is therefore a unique opportunity to evaluate the use of antiviral drugs to limit spread of herpesviruses that transmit sexually. In this study, we tested the capacity of the nucleotide analogue cidofovir to decrease MuHV-4 sexual transmission. On the one hand, we showed that a daily treatment of infected females can reduce genital shedding by 75%. On the other hand, a daily preventive treatment of naive males was sufficient to block viral transmission. Finally, a single post-exposure treatment reduced transmission but failed to block completely infection. Altogether, our results show that cidofovir is a powerful weapon to fight herpesviral infections as it can both reduce clinical signs and limit the transmission capacity of these viruses.

Keywords: Herpesvirus, Antiviral drug, sexual transmission

Oral presentations

Muscle-derived mesenchymal stem cells can be differentiated into cells of the cardiovascular system in vitro

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Mesenchymal stem cells have been isolated from skeletal muscle in horses, offering an alternative to commonly used bone marrow-derived mesenchymal stem cells. Aim: To differentiate equine muscle-derived mesenchymal stem cells (mdMSC) into endothelial cells and cardiomyocytes. Methods: Equine mdMSC have been achieved from a muscular micro biopsy. They have been grown by explant culture and isolated using a discontinuous Percoll density gradient. Cells have been expanded during several passages and after that they have been differentiated into endothelial cells using vascular endothelial growth factor, and into cardiomyocytes using dimethyl sulfoxide. Differentiation has been assessed by immunofluorescence. Results: After 2 weeks of explant culture, mdMSC have been isolated. A small amount of muscle (15-20mg) has allowed reaching approximately 60 millions mdMSC in 6 weeks. Cells cultured in differentiation medium have been efficiently differentiated into endothelial cells and cardiomyocytes as it has been highlighted by a positive staining for von Willebrand factor and cardiac troponin T respectively; mdMSC from the control group were negative. No morphological modification has been observed. Conclusion: A large amount of pluripotent equine mdMSC can be readily achieved in a micro-invasive manner. mdMSC can be a good alternative to bone marrow-derived mesenchymal stem cells whose sampling is painful and whose production yield is low. mdMSC and cells obtained by differentiation represent a valuable research material and offer original and promising therapeutic prospects in cardiovascular sciences. Acknowledgements: financial support from Fonds Leon Fredericq and Revatis s.a.

Keywords: mesenchymal stem cells, endothelial cells, cardiomyocytes

Epithelial Rab guanine nucleotide exchange factor 1 (Rabgef1) deficiency increases susceptibility to DSS-induced intestinal inflammation in mice

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Rab guanine nucleotide exchange factor (GEF)1 (Rabgef1) is a guanine nucleotide exchange factor for the endocytic GTPase Rab5, and also exhibits E3 ubiquitin ligase activity in vitro. In vivo functions of Rabgef1 remain largely unknown, but Rabgef1 is critical for health, as globally Rabgef1-deficient mice exhibit perinatal mortality and those surviving to adulthood spontaneously develop severe skin inflammation. This protein is highly expressed in murine intestinal epithelial cells (IECs). Objective: The aim of this study is to clarify the role of Rabgef1 in murine IECs. Materials and methods: We performed conditional deletion of Rabgef1 using the cre-lox system to obtain mice lacking Rabgef1 specifically in IECs (Rabgef1IEC-KO). Results: Rabgef1IEC-KO mice did not develop spontaneous intestinal abnormalities but showed an increased susceptibility to inflammation in dextran sodium sulfate (DSS)-induced colitis model. Indeed, compared to littermate controls, mice lacking Rabgef1 in IECs exhibited shorter and highly inflamed colons and higher inflammatory scores in histopathological examination of colons, suggesting that Rabgef1 expression regulates IEC function and is critical in limiting DSS induced inflammation and damage. In addition, we also showed that mRNA expression of pro-inflammatory cytokines, such as IL1b and Tnfa, was upregulated in IECs isolated from Rabgef1IEC-KO mice compared to the ones isolated from littermate controls. Conclusion: Taken together, these results suggest that Rabgef1 acts as a regulator of intestinal homeostasis, and that dysregulated Rabgef1 expression could contribute to intestinal barrier dysfunction in inflammatory conditions of the gut.

Keywords: Mucosal immunity, Epithelial cells, Rabgef1

Predictive abilities of genomic predictions for traits measured in Belgian Blue cattle genomic farms

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In order to implement genomic selection in Belgian Blue Beef cattle, the wallonian breeders organization (awé) decided to genotype a reference population (individuals with both genotypes and phenotypes) with a low density genotyping array interrogating ~7K SNPs in a set of 100 selected farms. Here we present the first study using this new generated data base. At the present time, 3488 animals were genotyped in the genomic farms and 8399 more were generated through other means (e.g. research projects, tests for genetic defects). As a result, the reference population ranged from 4208 to 7944 animals, according to the studied phenotype. Since the animals are genotyped on LD density arrays, we first imputed our data set to more than 30K SNPs. We then compared different genomic evaluation methods for traits measured at birth and others related to muscular development and size. Used methods were traditional evaluation (without SNPs), GBLUP (assuming that all the SNPs have small effects), BayesR (allowing to some SNPs to have large effects) and single step models (using simultaneously genotyped and ungenotyped animals). This last method performed best in most cases. In addition, increasing the reference population size had a clear effect on the accuracy of predictions. BayesR was sometimes superior to GBLUP, indicating that some variants had large effects as we showed in earlier studies (e.g. SQT, RNF11). Therefore, we are investigating whether the single step model can be improved by including some variants with large effects.

A respiratory gammaherpesvirus infection protects against allergic asthma through alveolar recruitment of regulatory monocytes

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The hygiene hypothesis postulates that augmentation of allergic diseases in developed countries could be linked to reduced exposure to infections during childhood. Surprisingly, the potential protective role of herpesvirus infections against allergy development has never been addressed in details. Here, we investigated how a gammaherpesvirus infection affects the subsequent development of allergic asthma. Our results demonstrate that respiratory infection by Murid herpesvirus 4 (MuHV-4) inhibits the development of House Dust Mites (HDM)-induced asthma by modulating functions of lung innate immune cells. Specifically, MuHV-4 infection induced the replacement of alveolar macrophages (AMs) by monocytes that were educated in the bone marrow to display markers associated with regulatory functions. These monocytederived AMs blocked the ability of dendritic cells to trigger a Th2 response against HDM. Collectively, our results reveal that replacement of embryonic AMs by regulatory monocytes is a major mechanism underlying the long-term training of the lung immunity after infections.

Keywords: viral imprinting, lung immunology, regulatory monocytes

Successful in vitro generation of genetically engineered pig embryos

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We have previously generated genetically engineered (GE) mice by pronuclear injection of a bacterial artificial chromosome (BAC) expressing the complete *Bos taurus* antiviral Mx system. It turned out that these mice display exceptional anti-viral activities against lethal infections with influenza A viruses. As swine are highly susceptible to infection with both avian and human influenza A viruses, new potentially life-threatening variants can appear by reassortment in the pig lung. For testing the hypothesis that expressing a Mx isoform endowed with strong antiinfluenza activity would reduce the risk of disseminating reassortants, we aim to generate bovine Mx1-expressing pigs. The first step to complete such initiative was to establish (from scratch) a suite of technologies generating ready-for-transfer GE porcine embryos from BAC DNA, frozen sperm, and ovaries collected in local slaughter plants. Here, we report the efficiency of our current process for generating such embryos after complete in vitro production in defined media, successively including (i) in vitro maturation of oocytes, (ii) BAC-loading of sperm, (iii) in vitro fertilization and (iv) in vitro development of zygotes until the blastocyst stage. Fertilization and genetic modification were obtained via sperm-mediated gene transfer (Watanabe et al., 2012). About 2.6% of slaughterhouse-derived oocytes gave blastocysts. This rate is relatively low at first sight but, contrary to other teams, we used defined media only, which guarantees the reproducibility and biosafety of the process over time. Further, 64% of these blastocysts had incorporated at least a fragment of the BAC, of which about two thirds had incorporated the complete sequence of the BAC. Generating ready-for-transfer GE pig embryos is thus feasible using the suite of technologies we currently have in hands. All blastocysts produced since several months are now cryopreserved by vitrification for a future transfer in surrogate sows.

Keywords: genetically engineered pig, spermmediated gene transfer, boMx1

Lung resident eosinophils represent a distinct cell subset with homeostatic functions

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Increases in eosinophil numbers are associated with infection and allergic diseases, including asthma, but there is also evidence that eosinophils contribute to homeostatic immune processes. In mice, the normal lung contains resident eosinophils (rEos), but their function has not been characterized. Here, we have reported that steady state pulmonary rEos are IL-5- independent parenchymal Siglec-FintCD62L+CD101low cells with a ring-shaped nucleus. During house dust mite-induced airway allergy, rEos features remained unchanged and rEos were accompanied by recruited inflammatory eosinophils (iEos), which were defined as IL-5- dependent peribronchial Siglec-FhiCD62L-CD101hi cells with a segmented nucleus. Gene expression analyses revealed a more regulatory profile for rEos than for iEos, and correspondingly, mice lacking lung rEos exhibited an increase in Th2 cell responses to inhaled allergens. The elevation of Th2 responses was linked to the ability of rEos, but not iEos, to inhibit the maturation, and therefore the pro-Th2 function, of allergen-loaded dendritic cells. Finally, we determined that the parenchymal rEos found in non-asthmatic human lungs (Siglec- 8+CD62L+IL-3Rlow cells) were phenotypically distinct from the iEos isolated from the sputums of eosinophilic asthmatic patients (Siglec-8+CD62Llow-IL-3Rhi cells), suggesting that our findings in mice are relevant to humans. In conclusion, our data define lung rEos as a distinct eosinophil subset with key homeostatic functions.

Short communications

Short communications

Synthetic virus-like particle – endocytosis and processing by dendritic cells for vaccine delivery

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Dendritic cells (DCs) employ several endocytic routes for processing antigens, driving forward adaptive immunity. Different nano/micro scale particulate vaccine carriers have been proven to be promising vaccine or drug delivery vehicles. However, information on the process of particle interaction with DCs and the endocytic routes utilised for their trafficking and processing within DCs is limited. In order to characterize the interaction of such particles with DCs we employed synthetic virus like particles (SVLPs). These self-assembling SVLPs efficiently induce adaptive immunity without requirement for an adjuvant, raising the question of how DCs interact with them. DCs rapidly bind SVLPs within 1 minute. Confocal microscopy demonstrated an association of internalized SVLPs with ovalbumin, high molecular weight dextran, and cholera toxin B, implying a clathrin independent mode of endocytosis. While SVLPs were readily internalised by immature DCs, with maturing DCs the uptake was reduced but not abolished. These results demonstrated that SVLPs are internalised via different endocytic routes, of which caveolin-independent, lipid raft-mediated macropinocytosis is identifiable as primary. We further evaluated if SVLPs can be structurally modified in order to achieve both targeting and activation of DCs. DCs rapidly and efficiently bound and internalised all types of SVLPs, implying that structural manipulations did not impact interaction of SVLPs with DCs. SVLPs carrying P3Cys or P2Cys moieties were capable of DC activation, measured by up-regulation of CD86. The present characterization allows for a definition of how DC handle virus-like particles showing efficacious immunogenicity, elements valuable for novel vaccine design in the future.

Keywords: Dendritic cells, Endocytosis, Synthetic virus like particles

Outbreak investigations and molecular characterization of foot-and-mouth disease viruses circulating in Niger

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In Niger, the epidemiological situation regarding foot-and-mouth disease (FMD) is unclear and the majority of outbreaks are not reported. The objectives of this study were to i) identify current FMDV strains circulating in cattle herds, ii) identify exposure risk factors associated with FMD seropositive animals and iii) test the relationship between one FMDV isolate from Niger and reference vaccine strains. Epithelial tissues (n=25) and sera (n=202) were collected from cattle in eight districts of the western part of Niger. 142 out of the 202 tested sera were positive for the presence of antibodies against FMD, using the non-structural protein (NSP) test. Using the Liquid Phase Blocking ELISA (LPBE) test, 125 of 142 sera tested for antibodies to structural proteins of four FMDV serotypes (A, O, SAT 1 and SAT 2) scored positive for one or more serotypes. The multivariate logistic regression analysis with NSP positive revealed that only the herd composition (presence of both cattle and small ruminants) was significantly associated with FMDV seropositivity (p=0.006). Antigen-ELISA and VP1 coding sequence analysis revealed the presence of FMDV serotype O that were characterised within the O/WEST AFRICA topotype. The antigenic relationship between one of the FMDV isolates from Niger (O/NGR/4/2015) and three reference vaccine strains was determined by the twodimensional virus neutralization test, revealing a close antigenic match between the field isolate from Niger and vaccine strains tested. These findings may help to develop effective control/preventive strategies for the disease in Niger as well as in other countries of West Africa.

Identification of virulotypes and serotypes of enteropathogenic (EPEC) and Shigatoxigenic (STEC) *Escherichia coli* from healthy cattle at slaughterhouses in Wallonia

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Escherichia coli producing the attachment-effacement (AE) lesion (EPEC) and/or Shiga toxins (STEC) cause enteritis and (bloody) diarrhoea in young calves and in humans, and are also present in the intestines of healthy cattle. The aim of this study is to identify the virulotypes and the O serotypes of EPEC and STEC isolated from healthy cattle at slaughterhouses in Wallonia. A total of 245 feces of cow were sampled in 2 slaughterhouses in 2014 and grown overnight at 37°C in Lauryl sulfate Enterobacteriaceae selective broth. The enrichment broths were assayed with a stx1, stx2 and eae triplex PCR and positive broths were inoculated onto 4 different media. A total of 2563 coliform isolates were sub-cultured and tested by the colony hybridization assay (stx1, stx2 and eae genes). The triplex PCR was again performed on all probe-positive isolates. The PCR-positive *E. coli* were subsequently assayed with two pentaplex PCR targeting the specific genes coding for ten O se rogroups. A total of 744 isolates from 62 animals tested positive with the colony hybridization assay. Of them, 687 isolates from 59 animals were positive with the triplex PCR and the results of both tests were in agreement for 617 isolates. The most frequent virulotypes were eae+, eae+stx1+ and stx2+. AE-STECS, EPEC and STEC are excreted by 25% of the healthy cattle at slaughterhouses in Wallonia and different virulotypes can be excreted by the same animal. The results confirm that EPEC and STEC, which could represent a public health hazard, are observed in healthy cattle at slaughterhouse and also show that the colony hybridization is useful for large-scale surveillance.

Keywords: *Escherichia coli*, slaughterhouse

The ORF27 gene of Cyprinid herpesvirus 3: a case of opposite selective pressures in vitro and in vivo

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Sequencing of CyHV-3 genome revealed the ORF25 family: a family of 6 paralogous sequences encoding type I transmembrane proteins containing an immunoglobuline-like domain. Using mass spectrometry approaches, four ORF25 family members were identified as envelope proteins. ORF27, initially described as a pseudogene, was not detected in the laboratory strains but was subsequently identified as a structural protein in field strains. The focus was made on this ORF27, based on the assumption that it could negatively influence viral growth in vitro, and positively influence viral growth and/or transmission in vivo. Sequence alignments performed with nine strains of CyHV-3 revealed that mutations occurring in ORF27 during in vitro culture mainly lead to its non-expression. This conclusion was confirmed by indirect immunofluorescent staining using polyclonal and monoclonal antibodies raised against pORF27. For functional studies, four CyHV-3 ORF27 recombinant strains were produced encoding a wild-type (WT) OR truncated (TR) ORF27; with OR without luciferase expression cassette (inserted in a non coding intergenic region) allowing in vivo detection of infected cells using In Vivo Imaging System. Co-cultures (ORF27 WT - ORF27 TR) performed in vitro showed a progressive increase of the proportion of ORF27 TR genotype (from 20% at passage 0 to 98% at passage 4). Multiple growth curve assay and plaque size assay performed in vitro led to the observation that ORF27 WT genotype produces less infectious particles than ORF27 TR but produces larger plaque size. Finally, comparison of the titres of infectious particles obtained after normal incubation versus spinoculation revealed a significantly higher increase for the ORF27 TR genotype, suggesting a possible function of pORF27 in viral attachment.

Keywords: Cyprinid herpesvirus 3, Protein, Recombinant virus

Short communications

First detection of *Besnoitia bennetti* (Protozoa: Apicomplexa) in donkey (*Equus asinus*) in Europe

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A two year old male donkey was purchased in May 2016 in poor body condition (cachexia, alopecic areas, pruritus, dirty hair) by the present owner in Le Roeulx (Belgium) from a donkey farm aimed at milk production in Frasnés-lez-Buissin (Belgium). Shortly after its purchase, the donkey was shorn and showed crusts, and hyperkeratosis with no other clinical signs except anorexia and cachexia. A treatment with phoxim was given without improvement; a cutaneous biopsy of hyperkeratotic skin was performed in July and showed a perivascular eosinophilic infiltrate with a large thick walled cyst located in the dermis. The cyst was filled with numerous bradyzoites. This was highly suggestive of besnoitiosis. A daily treatment based on sulfamethaxazole and trimethoprim was given orally and some improvement was noticed. Further clinical examination performed on August highlighted scleral pin-head sized cysts in right eye and between nares. Haematologic evaluation was unremarkable. Several skin biopsy samples were obtained for qPCR analysis and confirmed the presence of *Besnoitia* spp.'s DNA. Another ten year old female donkey purchased in France and sharing the same accommodation showed a good clinical condition. Punch-biopsy and haematology were realized on July but were unremarkable. A deepest clinical examination on August in this second animal showed the presence of cysts on the inner face of upper labial mucosa. Further laboratory diagnosis tests are in progress through sera (Western Blot) and rDNA sequencing to confirm *Besnoitia bennetti* aetiology. To the authors' knowledge, this is the first detection of *B. bennetti* in donkeys in Europe.

Keywords: *Besnoitia bennetti*, scleral and labial cysts, donkey

Comparison of buprenorphine, morphine and butorphanol effects on the European rabbit (*O. cuniculus*) gastrointestinal tract

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Opioids are mainly known for their analgesic properties. They also decrease gastrointestinal motility in humans and animals, acting on mu and/or kappa opioid receptors expressed by enteric neurons and intestinal muscle cells. Therefore their use in rabbits presented with ileus is still being discussed. Recently, it was demonstrated that buprenorphine did not slow down gastrointestinal transit in healthy rabbits contrary to morphine. Butorphanol is known for its short analgesic action and is a mu receptors antagonist and an agonist on kappa receptors. In order to assess the specific effects of those opioids on gastrointestinal motility in rabbits, noninvasive methods were employed. Forty New Zealand White rabbits were followed during 24 hours without treatment and after an intramuscular injection of 100 µg/kg of buprenorphine or 10 mg/kg of morphine or 5 mg/kg of butorphanol. Rabbits underwent barium follow through and intestinal contraction counting by ultrasound. Buprenorphine does not slow down gastro intestinal transit in rabbits contrary to morphine and butorphanol. However, pyloric contractions are significantly increased under opioid treatment. Duodenal contractions are significantly increased with buprenorphine and are decreased with butorphanol treatment. Morphine does not modify duodenal contraction number. Morphine is a more potent depressor of the gastrointestinal transit in European rabbits than butorphanol, even if local motility seems to be less affected.

Keywords: opioids, rabbits, gastrointestinal tract

Virulence acquisition in Infectious Salmon Anaemia Virus: the role of mutations in the haemagglutinin-esterase and fusion protein

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Infectious Salmon Anaemia (ISA) is a viral disease which has resulted in substantial economic losses to the salmon industry worldwide. It is caused by an orthomyxovirus with a genome consisting of eight RNA segments. Segments 6 and 5 encode the two major surface glycoproteins, the Haemagglutinin Esterase (HE) and Fusion (F) protein, respectively. The HE is the receptor binding protein while the F protein orchestrates viral membrane fusion. Since the discovery of an avirulent HPR0 variant and the fact that deletions in the segment 6 Highly Polymorphic Region (HPR) are systematically associated with pathogenic strains, there has been a strong suspicion that ISAV surface glycoproteins play an important role in determining virulence. However, functional analyses of these surface proteins are lacking which has hindered our ability to understand the mechanisms leading to virulence acquisition. We recently performed two extensive studies using point mutations on both the HE and F proteins from wild types ISA strains and fusion assays based on ghost erythrocyte techniques. Results indicated that both segment 6 HPR deletions and segment 5 mutations work in conjunction to promote viral fusion. More accurately, these mutations influence specific stages of this viral process, such as the activation of the F protein by the HE or proteolytic cleavage. These findings provide the first indication that avirulent HPR0 ISAVs constitute a reservoir from which pathogenic strains arise in farmed Atlantic salmon. This work also sheds some light on how the combined effect of HE and F protein mutations may determine ISAV virulence.

Keywords: Atlantic Salmon, ISAV, Viral fusion

Association between vertebral heart score measurement and overweight condition in dogs

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Introduction: Vertebral heart score (VHS) is used to assess heart size on thoracic radiographs. The aim of this study is to determine if VHS is influenced by overweight condition in dogs. The hypothesis is that VHS is influenced by the body condition score (BCS) and T4 ratio.

Methods : Recruitment criteria for this retrospective study were the presence of 2 thoracic radiographic views and a normal echocardiographic examination. BCS, breed were extracted, VHS and T4 ratio were calculated. Dogs from breeds with a normal maximal VHS < 11 and from breeds with a normal maximal VHS > 11 were separated. A Spearman test was performed to assess correlation between BCS and T4 ratio. A linear model was used to evaluate the influence of BCS and of T4 ratio on VHS in each group.

Results: Fifty-one dogs were included in the study. BCS ranged from 3 to 9. Thirteen dogs had a VHS value superior to the normal range and all of them had BCS ≥ 4. A weak positive correlation was found between BCS and T4 ratio ($P \leq 0.001$, $R^2=0.4$). There was a positive relationship between BCS and VHS ($P \leq 0.003$) for each group and between T4 ratio and VHS for each group ($P \leq 0.001$).

Conclusion: This study confirms that the T4 ratio is correlated to BCS. This ratio could be used to assess overweight condition on thoracic radiographs. VHS is influenced by BCS and the T4 ratio; therefore, VHS should be used with caution in overweighted dogs.

Keywords: heart, VHS, overweight

Short communications

Computer-assisted study of animal behavior: a powerful technique for the development of an attenuated vaccine against Testudinid Herpesvirus 3 causing a lethal disease in several endangered tortoise species

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Testudinid herpesvirus 3 (TeVH-3) is the causative agent of a lethal disease affecting several endangered species of tortoises. The threat that this virus poses to endangered animals is focusing effort on the development of vaccines. As various inactivated vaccines were proved to be ineffective, we aimed to develop a live attenuated vaccine. With that goal in mind, the genome of different strains and subclones of TeVH-3 was sequenced recently (Gandar et al., 2015, Journal of Virology, 89:11438-11456). This approach revealed the existence of mutants exhibiting deletions ranging from 12.5 to 22.4 kb. Importantly, inoculation of Hermann's tortoises suggested the potential of some of these mutants as attenuated vaccine candidates. Attenuated vaccines must be safe and efficacious. Here, we developed a platform to investigate the safety of vaccine candidates using computer-assisted study of animal behavior. In this system, tortoises are housed individually in longitudinal arena with a floor encompassing a temperature gradient of 24°C to 32°C. The behavior of the animals is monitored constantly using adapted automatized video-tracking software. This system allows to measure and to analyze statistically various behavioral parameters for each subject according to time. Examples of these parameters are the thermal preferendum of the animals and their mobility (distance covered, mean speed, top speed, ...). In the near future, this powerful multi-translation approach will be used in addition to clinical examinations to test the safety of different attenuated vaccine candidates.

Keywords: Herpesvirus, Tortoise, Behavior

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

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Oral vaccination offers many immunological and practical advantages. Nevertheless, oral immunization may be hampered by oral tolerance mechanisms. A solution to this problem lies in the use of viral vectors. Since the 1970s, alive, orally administered adenovirus serotype 4 and 7 vaccines are effectively used to protect U.S. military personnel from severe respiratory diseases caused by these viruses. Replication-competent adenoviruses appear therefore as promising vectors for the development of oral vaccines. Until now, as human adenoviruses replicate efficiently only in a highly restricted host range, researches on this topic have suffered from the lack of reliable animal models. In this study, we used mouse adenovirus type 1 (MAV-1) to develop a small animal model for oral replicative adenovirus vaccines. We firstly characterized adenovirus oral infection in mice. No clinical signs were observed following MAV-1 oral administration. Nevertheless, viral DNA was detected by qPCR in various organs, showing that the virus efficiently infects by this route. Furthermore, this infection generated a specific and neutralizing humoral response. We then evaluated the protection induced by MAV-1 oral infection against a respiratory homologous challenge. Our observations showed that oral immunization prevent the weight loss due to an intranasal infection. Moreover, histological and qPCR analysis showed a protection against lungs inflammation and viral replication. Altogether, these results show that MAV-1 offers a reliable model for oral vaccination based on replicative adenoviruses. This model provides a valuable tool to assess the potential of adenoviruses as oral vaccine platforms.

Keywords: Adenovirus vector, Oral vaccination, Mouse model

Interferon response factor-3 promotes the pro-Th2 activity of mouse lung CD11b+ conventional dendritic cells in response to house dust mite allergens

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Only few transcription factors have been identified that are required for antigen-presenting cells (APCs) to induce T helper type 2 (Th2) responses. Because lung CD11b+ conventional dendritic cells (CD11b+ cDCs) are responsible for priming Th2 responses in house-dust mite (HDM)-induced airway allergy, we used them as a model to identify transcriptional events regulating the pro-Th2 activity of cDCs. Transcriptomic profiling of lung CD11b+ cDCs exposed to HDM in vivo revealed first that HDM triggered an antiviral defence-like response and second that the majority of HDM-induced transcriptional changes depended on the transcription factor Interferon Response Factor-3 (Irf3). Validating the functional relevance of these observations, Irf3-deficient CD11b+ cDCs displayed reduced pro-allergic activity. Indeed, Irf3-deficient CD11b+ cDCs induced less Th2, more regulatory T cell, and similar Th1 differentiation in naïve CD4+ T cells compared to their wild-type counterparts. The altered APC activity of Irf3 CD11b+ cDCs was associated with reduced expression of CD86 and was phenocopied by blocking CD86 activity in wild-type CD11b+ cDCs. Altogether, these results establish Irf3, mostly known for its role in antiviral responses, as a transcription factor involved in the induction of Th2 responses through the promotion of pro-Th2 costimulation in CD11b+ DCs.

Keywords: Dendritic cells, Th2, interferon response factor 3

Prevalence of *Angiostrongylus vasorum* in Belgium, a coprological and serological survey

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Canine angiostrongylosis, a gastropod borne helminthic infection, is considered as emergent in many European countries. In dogs, *Angiostrongylus vasorum* may cause a wide spectrum of clinical signs with respiratory distress as the most frequent symptom. The aim of the present study is to gain additional information on the distribution, prevalence and risk factors associated with *A. vasorum* infection in the dog through the combined use of in-house detection of circulating specific antigen and coprology in two different canine populations: dogs with clinical signs compatible with angiostrongylosis and dogs presented for unrelated conditions (control). Forty six (46) dogs out of 979 (4.7%) had *A. vasorum* circulating antigen. However there was a highly significant difference between the two populations (3.6 and 8.6 % in control and symptomatic dogs respectively). L1 of *A. vasorum* were found in 7 out of 24 serological positive control dogs and 6 out of 17 serological positive symptomatic dogs. Eighteen (18) sera of dogs with conflicting results between serology and coprology were analysed by Elisa (Ag and Ab). Out of 17 *Angio detect*™ IDEXX positive dogs with negative or no performed Baermann test, 1 dog was positive for both Elisa (Ag and Ab) and 1 dog was positive for Ag Elisa. This study demonstrated a fairly high seroprevalence in Southern Belgium for *A. vasorum*. The *Angio detect*™ IDEXX was found to be suitable in this context. However, coproscopy remains a useful tool notably for the identification of other canine lung nematodes such as *Crenosoma vulpis*.

Keywords: *Angiostrongylus*, Dog, Belgium

Short communications

In vivo infectivity and in vitro replicative fitness regain of a recombinant murine norovirus

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Recombination can create considerable changes in viruses, mediating antigenic shifts, host jumps and pathogenesis and fitness modifications. Mathijs et al. recently isolated a recombinant murine norovirus (RecMNV) of reduced replicative fitness in vitro after coinfection of murine norovirus strains MNV1-CW1 and -WU20 in permissive cells. We (1) evaluate in vivo infectivity of RecMNV and (2) follow its replicative and genetic adaptations over serial in vitro passages. (1) In vivo infectivity of RecMNV was compared to its parental strains in Balb/cByJ mice via weight loss measurement and viral load estimation in faeces, tissues and organs 48 and 72 hours post-infection using plaque assay and RT-qPCR. Body weights of RecMNV- infected mice were generally higher than those of parentally- infected mice. Viral loads were detected in all examined organs, suggesting that, like its parental strains, RecMNV can disseminate beyond the digestive tract to produce systemic infections. (2) RecMNV progenies resulting from the first (RecE) and tenth (RecL) in vitro passages were compared and their sequences determined. RecL showed significant lysis plaque diameter increase and faster replication kinetics than RecE. Molecular analysis yielded several nucleotide- and amino acid changes both between RecE and its parental strains and between RecE and RecL. Our data suggest that recombination occurring in vitro between homologous murine norovirus strains can give rise to a chimeric strain, which shows similar biological properties to its parental strains and is capable of productive in vivo infection. We demonstrate viral adaptation and in vitro replicative fitness regain after a recombination event.

Keywords: Norovirus, Recombination, Fitness

Antibiotherapy in pet rodents and rabbits : a preliminary analysis of practitioners' habits

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An electronic sondageonline survey questionnaire was created to assess which antibiotic were used in pet rodent and rabbit medicine. Forty-five respondents completed the questionnaire and 110 antibiotic treatments were analysed. Fluoroquinolone was given as first-line treatment in 65.5% of the cases. The frequency of fluoroquinolone used as first-line treatment was significantly lower (P value for Chi square test = 0.03) in practices in which exotic pet species were considered as important or main part of their cases. The frequency of fluoroquinolone used as first-line treatment almost significantly differed (P value for Chi square test = 0.08) according to the practice type (small animal practice, mixed practice, large animal practice, exotic pet practice, equine practice, wildlife practice). Respective percentages of fluoroquinolone as first-line treatment did not differ significantly by countries (P value for Chi square test = 0.49) nor by veterinarian age class (P value for Chi square test 0.46). Fluoroquinolones are clearly overused in exotic small mammals at least in France and Belgium. Compared to other companion animal the fluoroquinolone utilisation rate is 4 to 6 times higher in exotic small mammals than in dogs or cats. To understand factors influencing antibiotic prescribing habits some important questions remains such as on which basis was an antibiotic treatment decided and why was a specific medicine chosen? The questionnaires included these items that are under analysis.

Keywords: antibiotherapy, exotic mammals, quinolone

Individual monitoring of salmonid immune responses during immunisation and infection

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Aquaculture currently provides nearly half of all fish consumed globally and is the fastest growing sector of food and animal production. Infectious diseases are the most significant threat to the future expansion of this industry. Vaccination is widely considered the best prevention strategy and much effort is focussed upon the development of new and more efficacious fish vaccines. Most research groups use a vaccination-challenge strategy to evaluate immune protection in terminally-acquired tissue samples. However this approach requires large numbers of animals to obtain sufficient statistical power providing limited information on the kinetics of the protective response. To address this, we established a non-lethal sampling method (by withdrawing small amounts of blood repeatedly from the same individual) to monitor salmonid immunity during infection or immunisation. This enabled us to monitor (by real-time PCR) key immune markers from a small number of animals during pathogen challenge. Furthermore, due to the limitations of mRNA-level validation, we are currently developing a high-throughput proteomics platform to allow the rapid and accurate quantification of immune-responsive proteins in plasma samples during immunisation. Thus, we are optimising targeted and shotgun mass spectrometry approaches, performed on a Q Exactive hybrid quadrupole-Orbitrap, using rainbow trout (*Oncorhynchus mykiss*) as our study model. By applying a non-lethal sampling we were able to individually monitor changes in immune markers during the course of an immune response. Such information will allow a better understanding of fish immunity and might be applied in the future to reduce the number of animals required in vaccine development.

Keywords: Non-lethal sampling method, Rainbow trout, Immune responses

An important role for C-type lectin homolog-encoding A7 gene in the pathogenesis of malignant catarrhal fever

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Alcelaphine herpesvirus 1 (AIHV-1) persists in wildebeest asymptomatically but induces malignant catarrhal fever (MCF), a fatal lymphoproliferative disease upon transmission to several ruminants, including cattle. High-throughput sequencing of attenuated strain WC11 revealed a genomic sequence very similar to virulent strain C500. Among the 73 ORFs identified in the strain C500 genome, only four were not conserved in WC11, including the A7 gene which is fully deleted. A7 is a positional homolog of Epstein-Barr virus (EBV) BZLF2 encoding the C-type lectin-like glycoprotein gp42. Likewise, A7 has been predicted to encode a glycoprotein containing a C-type lectin-like domain. Gp42 is expressed in the envelope of EBV virions and mediate entry into B cells. Our recent data have demonstrated that AIHV-1 infects CD8+ T-cells causing their proliferation and MCF lesions. The absence of A7 could participate in the attenuation of WC11 through a defect of virus entry into target cells. To determine the role of A7 in MCF, we constructed a recombinant A7-nonsense (A7NS) strain impaired for A7 expression using the C500 BAC clone. A7NS virus showed significant increased viral growth *in vitro* compared to the wild-type (WT) virus. Following intranasal infection of rabbits as a model, A7NS virus induced hyperthermia with a significant delay compared to the WT virus but we did not observe any expansion of CD8+ T cells nor enlargement of lymphoid organs. Thus, the lack of A7 significantly alters the development of MCF. Additional analyses are in progress to investigate whether A7 could orientate viral tropism for CD8+ T-cells.

Short communications

Identification of bovine methicillin resistant staphylococci from Europe, Africa and North America by dot blot hybridization, PCR and antibiotic sensitivity

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Staphylococci are the most prevalent bacterial mastitis pathogens worldwide. Antimicrobial treatment of mastitis has led to the selection of resistant staphylococci, of which the Methicillin Resistant Staphylococcus (*aureus*) MRS(A) are the most studied ones. The aim of this study is to compare genetic and phenotypic typing methods for MRS(A) isolated from bovine mastitis in different continents. A total of 1168 staphylococci isolates were tested genetically by hybridization assay for *mecA* and *mecC* genes and phenotypically by growth on "Chrom MRSA ID®" agar. Isolates positive at both or either tests were further studied by PCR targeting the same two genes and by the disk diffusion assay to oxacillin and ceftioxin. A total of 265 isolates were positive, including 27 *S. aureus* for both test, 32 *S. aureus* for hybridization *mecA* only and 169 *S. aureus* growing on "Chrom MRSA ID®" plates only. No non-*aureus* tested positive for both tests and no isolate were tested positive for *mecC* genes. In addition, 15 non *aureus* were hybridization *mecA*+ only and 22 non *aureus* were MRSA ID + only. The high difference between the results of both tests could be explained by the weak specificity of phenotypic tests comparing to genetic tests. The positive isolates for the two methods can be considered as MRSA mediated by the *mecA* gene. However, results of PCR and disk diffusion assay will confirm respectively the presence of *mec* genes and which of the two methods is the most suitable for identifying MRS(A) from bovine mastitis.

Keywords: MRS(A), Bovine, identification

The use of cis-atracurium in horses as part of a balanced anaesthetic protocol.

Partial results

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Neuromuscular blocking agents (NMBA) induce skeletal muscle paralysis by acting at the neuromuscular junction. Cis-atracurium is a short acting non-depolarising NMBA, a more potent isomer of atracurium with fewer side effects.

Objective: To assess two different doses of cis-atracurium in order to obtain a moderate neuromuscular block (NMB).

Materials and method: Twelve horses were part of the study and were randomly split in two groups. All horses were anaesthetised using the same anaesthetic protocol and an anaesthetic sheet was recorded every five minutes. Cis-atracurium was only administered after we reach a stable plane of anaesthesia. First group (8 horses) received cis-atracurium 50µg/kg IV and the second group (4 horses) received cis-atracurium 100µg/kg IV. NMB was monitored by producing a train-of-four (TOF) stimulation pattern using acceleromyography. Two needle electrodes were placed over the superficial peroneal nerve and the acceleromyograph crystal was attached on the dorsal part of the cannon bone. Both TOF-ratio and T-count results were recorded on a special NMB sheet every 15 seconds.

Main results: Three of eight horses (38%) in the first group failed to obtain a moderate NMB and a supplementary dose of cis-atracurium was needed. However, all horses in the second group successfully obtained moderate NMB.

Conclusion: The present study shows that a moderate NMB may be successfully induced with cis-atracurium at doses of 100 µg/kg. However, the use of cis-atracurium at doses of 50 µg/kg may require supplementary doses of cis-atracurium to obtain the same level of NMB.

Keywords: neuromuscular block, cis-atracurium, horse

Posters

Veterinary public health

1. Reemerge of the Schmallenberg virus associated triad hydranencephaly-micromyeliaarthrogryposis in a newborn calf in Belgium, 2016

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Schmallenberg virus (SBV) emerged in Germany in 2011, then spread rapidly across Europe, causing an enzootic outbreak of abortion, stillbirth and birth at term of lambs, kids and calves with neurological signs and/or musculo-skeletal malformations. SBV-associated disease in newborns disappeared in Belgium in 2013. Here, we describe a SBV genomic RNA-positive malformed calf born in May 2016. It reveals the return of SBV circulation during the fall 2015 in the said area.

Keywords: Schmallenberg, Reemerge, Calf

2. Zebrafish: a small fish to tackle alternative routes toward anti-diabetic treatments

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Diabetes is a chronic metabolic disease becoming a leading health issue within not only the human population worldwide but also pet animals such as cats and dogs. This disease occurs when the body undergoes insulin resistance and/or deficiency. Pancreatic insulin-producing beta cell mass is dramatically reduced in diabetic patients. Besides present therapeutic strategies aimed at improving insulin treatments or engrafting new beta cells, a promising alternative would be to replenish the pancreas with functional beta cells by triggering intrinsic regenerative mechanisms from other pancreatic cells. However, the plasticity of such cells, though reported, is quite limited in mammals thereby hampering efforts to investigate these mechanisms with the goal to stimulate them. The zebrafish is a small laboratory animal that displays the remarkable capacity to regenerate many cell types and tissues. Thanks to its genetic tractability and to numerous similarities with mammals, it is also becoming a popular model for many diseases such as diabetes. We recently identified the cellular origin of regenerated beta cells using a zebrafish diabetic model able to restore its beta cells after destruction. Our work focuses now on the understanding of the molecular mechanisms underlying this process, through transcriptomic profiling, gene editing and treatments with pharmacological inhibitors/activators. We are beginning to identify genes and pathways that might be harnessed to improve beta cell regeneration.

Keywords: zebrafish, human disease model, gene editing

3. Metabolites from media supplemented with 3'-sialyllactose and fermented by bifidobacteria have an antiviral effect against intestinal pathogens

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Complex oligosaccharides from human milk (HMO) promote growth of *Bifidobacterium bifidum*. Oligosaccharides from cow milk (BMO), similar to HMO, are mainly represented in colostrum by 3'-sialyllactose (3'SL). *Bifidobacterium crudilactis*, a species from bovine origin and encoding for β -galactosidases and α -glucosidases, could be able to metabolise them. Also, fermentation products could have antiviral activity against intestinal pathogens. This study focused on capacity of bifidobacteria to metabolise 3'SL and on potential antiviral effect of cell-free spent media (CFSM) against pathogenic bacteria. *B. bifidum* BBA1 and *B. crudilactis* FR/62/B/3 isolated respectively from breastfed children feces and cow raw milk cheese were grown on media supplemented with 3'SL as sole source of carbon. Next, CFSM effects were tested against virulence gene expression using *ler* and *hilA* promoter activity of luminescent constructs of *Escherichia coli* 0157:H7 ATCC 43888 and *Salmonella Typhimurium* SA 941256, respectively. The effect was confirmed on wild type strains of *E. coli* O157:H7 ATCC 43890 and *S. Typhimurium* ATCC 14028 using RT-qPCR. Both strains were able to grow in presence of 3'SL. CFSM resulted in under-expression of *hilA* and *ler* genes for the luminescent constructs and in under-expression of *ler* (ratios of -15.4 and -8.1) and *qseA* (ratios of -2.1 and -3.1) genes for the wild type strain of *E. coli* O157:H7. No effect was observed with *S. Typhimurium*. Little is known about CFSM metabolites and they have to be isolated and identified. The potential synbiotic effect between 3'SL and bifidobacteria will be tested using the Shime®, a human gastrointestinal model.

Keywords: Bifidobacteria, 3'-sialyllactose, antiviral effect

4. A protein conserved in cypriniviruses is a major virulence factor of Cyprinid herpesvirus 3

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Cyprinid herpesvirus 3 (CyHV-3) is the causative agent of a lethal disease in common and koi carp. Since its emergence, CyHV-3 has caused severe economic losses worldwide creating a need for a safe and efficacious vaccine. In a previous study, we showed that a recombinant strain deleted for ORF56 and ORF57 exhibited a safety/efficacy profile compatible with its use as an attenuated recombinant vaccine. In the present study, we investigated the relative contribution of the two genes to the attenuated phenotype observed. To reach this goal, a series of recombinants deleted either for ORF56 or ORF57 were produced. These recombinants were characterized in vitro for their correct molecular structure. In addition, immunofluorescence staining showed that the deletion of ORF56 did not abrogate the expression of ORF57, and vice versa. In vivo experiments demonstrated that the deletion of ORF57 explains most of the attenuation observed for the ORF56-57 deletion. Furthermore, we observed that ORF57 deletion induced in vitro a growth defect (reduction of both the production of infectious particles and the size of viral plaque). Orthologue of CyHV-3 ORF57 in Anguillid herpesvirus 1 (AngHV-1) has been shown to be a tegumental protein. Interestingly, using both qPCR and western blot based approaches, we demonstrated that the particles produced by the ORF57 deleted mutant are less infectious than those of the wild type virus. In conclusion, this study demonstrates that ORF57 is a major virulence factor of CyHV-3. Importantly, as ORF57 is conserved in cypriniviruses, its orthologues could therefore represent a target for production of attenuated vaccine against several other major fish pathogens such as AngHV-1 and Cyprinid herpesvirus 2 (CyHV-2).

Keywords: Cyprinid herpesvirus 3, Vaccinology, Virology

5. Swimmer's itch in Belgium: first recorded outbreaks, identification of the parasite species and intermediate hosts

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Cercarial dermatitis or swimmer's itch is a skin condition in humans due to the larval forms of bird schistosomes of the genus *Trichobilharzia*. The life cycle of these schistosomes requires freshwater snails and waterfowls. Repeated exposures to cercariae can lead to skin sensitization with the induction of pruritic skin lesions. We describe here several outbreaks of human cercarial dermatitis at the Eau d'Heure Lakes, Belgium. In July and August 2012, a total of respectively, 78 and 10 people reported a sudden skin rash accompanied by pruritus following recreational activities in the Plate Taille Lake. However no ocellate furcocercariae were detected following light exposure of the snails collected between September 2012 and September 2013 (n= 402). No outbreaks were recorded in 2013 and 2014. In August 2015, about 30 new cases were recorded at the same place. Snails were collected (n= 270) in different locations around the lake. After light exposure, seven *Radix* spp. (2.6%) shed ocellate furcocercariae. Molecular identification based on the rDNA ITS-2 sequence ascribed the infected snails to *R. balthica* (= *R. peregra* = *R. ovata*) (6/7) and to *R. auricularia* (1/7). Based on the amplification of the D2 domain of the 28S rDNA the cercariae, were shown to belong to two different haplotypes of *Trichobilharzia franki*. This is the first record in Belgium of *T. franki* and associated skin condition.

Keywords: *Trichobilharzia franki*, Ocellate furcocercariae, *Radix* spp.

6. Abdominal mass due to *Echinococcus multilocularis* in a two and half years old hard haired dachshund

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An abdominal mass was detected in a two and half years old male hard haired dachshund. This mass was situated in the left part of the cranial abdomen and seemed evolutive. No other clinical signs were recorded except exhaustion following exertion, and soft faeces. Abdominal echography showed a hepatomegaly with a wide histological modified aspect, multiple hyperechogenic spherical important masses (till 5.15 x 5.7 cm) with fluidic anechogenic central zone and mineralised points. Microscopical examination of smears from hepatic mass and fluid puncture revealed paucicellular samples with protoscolex suspect elements. In front of the importance of the hepatic lesions, euthanasia was realized. Necropsy revealed a severe multilocular chronic active hepatitis with a beginning of right ventricular dilatation. Bacteriological cultures of the mass (Columbia agar plates with 5% sheep blood and specific agar plates for Enterobacteriaceae) were negative. The screening of the intestinal tract for parasites was negative, probably because of previous treatment (milbemycin oxime, praziquantel; Milbemax®). Based on histopathology (numerous multilocular degenerating cysts lined by hyaline membrane, containing eosinophilic debris, mineralized granular material, and one parasitic structure partially fitting with a protoscolex) a diagnosis of cystic hydatidosis due to *Echinococcus multilocularis* was done. The dog was living in the Ardennes region in Belgium (Hastière) known as endemic area of *E. multilocularis*. The high density of foxes (*Vulpes vulpes*) probably explains the occurrence of the disease. Indeed, 1 of the 5 (20%) intestinal scraping of foxes found dead in this area in 2016 was positive for *E. multilocularis*. Fox faeces coprophagia is probably the best explanation for this dog cystic hydatidosis.

Keywords: Abdominal mass, Severe multilocular chronic hepatitis, *Echinococcus multilocularis*

7. Equine Ocular Habronemiasis in Belgium

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Members of the genus *Habronema* are parasitic in the stomach of the horse. The chief importance of these parasites is as a cause of cutaneous habronematidosis or « summer sore », generally observed in warm countries. When larvae (L3) are deposited by the intermediate host (dipteran flies) on a skin wound or around the eyes, they can invade the tissues and may cause granulomatous conjunctival and skin lesions. A few cases of conjunctival *Habronema* granulomas are diagnosed yearly. More severe cases were observed very recently in Belgium. Three warmblood horses presented varying degrees of conjunctivitis with granulomas. One pony presented severe blepharo-conjunctivitis and conjunctival granulomas causing ulcerative eosinophilic keratitis. Surgical treatment consisted of granuloma excision and corneal contact bandage lens. Medical treatment consisted of 2 moxidectin administrations at 2 weeks interval as well as ocular and systemic antibiotics and anti-inflammatory. Histopathology and PCR were done to confirm the aetiology. *Habronema* infestation should be considered as one possible cause of conjunctivitis and keratitis in the horse. Cases of ocular habronemiasis are observed in Belgium, but it seems we are facing an increasing incidence of this may be re-emergent disease.

Keywords : *Habronema* sp., conjunctival granuloma, moxidectin

8. Monitoring and modelling of microbial ecosystem in Belgian white pudding

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Objective: In order to control food losses and waste, studies highlight the importance of monitoring the microbial diversity of food products because, during process and storage, some microorganisms present metabolic activities which can lead to spoilage. Studies using metagenetic approach based on high-throughput 16S rRNA gene sequencing revealed a much higher resolution than culture-based methods. This work proposed to study the bacterial microbiota of white-pudding, a typical Belgian pork meat product, using culture-dependent and independent methods. Material and Methods: Daily during 28 days aging tests, the absolute abundance of strain was monitored by combining total count on plate agar and metagenetic analysis for each temperature (at 4 °C, 8 °C and 12 °C) and packaging conditions (modified atmosphere packaging, 30% CO₂ – 70% N₂, food wrap, and vacuum packaging). Main results: *Brochothrix thermosphacta* and *Pseudomonas* sp. were the dominant strain into the microbial ecosystem, the others strains were present at lesser proportions (<10 % of reads). The growth of each dominant species was modelled to Sym'Previous software and R software, where μ_{pref} were obtained: a higher growth parameter was observed in vacuum packaging condition, in contrast to modified atmosphere packaging for both bacterial strains. Validation of tertiary modelled curves using data of Com'Base, a combined database online, show a correct prediction under identical experimental conditions. Conclusions: Thereby, these results highlighted the importance of combining metagenetic analysis and classical methods, with predictive microbiology, to offer a new tool for follow intrinsically the evolution of each strains on the bacterial ecosystem.

Keywords: Microbial ecosystem, Predictive microbiology, Metagenetics

9. Causes of death of harbour porpoises (*Phocoena phocoena*) stranded on the Belgian coast (1990-2015)

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Since the end of the 1990s, a significant rise of harbour porpoise (*Phocoena phocoena*) strandings occurred along the southern North Sea. The aim of the study is to present the main lesions and causes of death of porpoises stranded on the Belgian coast and their evolution between 1990 and 2015. Porpoises were selected for necropsy and sampling (histopathology, toxicology, microbiology) following a standard procedure. Frequent observations included net marks on the skin, sub-cutaneous and muscular bruises, emaciation, pulmonary (blood vessels and airways) and gastric parasitism, acute pneumonia, and pulmonary congestion and edema. The two main causes of death were bycatch and infectious disease. Bycatch was mainly observed in animals stranded in March and April and appeared to be increasing (from 20% to 35% of all animals investigated). Infectious disease (40% of all animals investigated), mainly acute pneumonia associated with severe parasite infestation, occurred throughout the year. The infection by the bacteria *Brucella ceti* was reported in 8% of the porpoises for which it was investigated. Two other causes of death have recently emerged: (1) severe emaciation combined with lung edema and an absence of other lesions, and (2) grey seal predation with typical lacerations of the skin and the blubber. The two main reasons for the increase in porpoise strandings as observed during the last two decades are linked with the southward shift of the population within the North Sea. Increasing numbers of porpoises lead to increased numbers of stranded animals, with the influence of factors such as the emerging phenomenon of predation by grey seals (with populations increasing), bycatch and starvation to be assessed.

Keywords : Harbour Porpoise, Southern North Sea, Strandings, Causes of death

10. The EEL4EVER project: a multidisciplinary initiative aiming to develop an attenuated vaccine against Anguillid herpesvirus 1 compatible with reintroduction program of glass eels

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The stock of European eel (*Anguilla anguilla*) is severely depleted reaching less than 1% of what it was in the 1980s. In 2007, the European commission initiated "the eel management plan" aiming to rebuild the stock of this diadromous fish species. This plan relies on three main axes: (i) limiting fisheries; (ii) facilitate fish migration through the rivers and (iii) restocking suitable inland waters with young eel (glass eel) captured during their migration from sea water to fresh water. Unfortunately, this plan totally neglected the impact of infectious diseases. Anguillid herpesvirus 1 (AngHV 1), a member of the Cyprinivirus genus has been suggested as one of the main infectious causes of eel decline. AngHV-1 is phylogenetically related to Cyprinid herpesvirus 3 (CyHV-3 also called Koi herpesvirus (KHV)). Recently, we developed a safe and efficacious attenuated vaccine against CyHV-3. This vaccine is compatible with mass vaccination of young carp. The goal of the EEL4EVER project is to develop a similar vaccine for eel against AngHV-1. This vaccine should be compatible with the reintroduction program of glass eel. Here, we describe the main lines of this ambitious and multidisciplinary project which has been selected recently by the "Fonds européen pour les affaires maritimes et la pêche (FEAMP)".

Keywords: Vaccination of wildlife, European eel, Anguillid Herpesvirus-1

11. The use of antibiotics in cattle livestock in the north Benin, a real factor of the development of bacterial resistance in the cattle and the food chain

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This study aim is to inventory antibiotics used in cattle livestock in north Benin and assess risk practices that could be the cause of both food chain contamination by antibiotic residues and selection of antibiotic-resistant bacteria in animals and humans. Survey was conducted in the Commons of Banikoara, Kandi, Bembereke and Kalale in north Benin, where 98 cattle farmers were chosen by the "snowball sampling" process. Semistructured interviews were conducted following farmer status, breeding system, antibiotics and use practices. Descriptive statistics were performed with Excel software while multiple correspondence analysis and hierarchical classification analysis of breeder typology were performed with the software "R". Seventy-one percent of farmers were "Fulani" and 97 % of them were uneducated. Cattle herds were composed by the breeds "Borgou" (76 .4 %) and "Fulani Zebu" (16 %); some herds were mixed. Antibiotics groups used in cattle breeding were tetracyclines, beta-lactams, sulfonamides, aminoglycosides and macrolides by respectively 100, 88, 56, 44 and 35 % of farmers. These drugs were purchased in local market (58 %) and veterinary pharmacy (39 %). They were used against bacterial diseases (respiratory, podal, mastitis, omphalitis and neonatal enteritis diseases) and skin diseases. Only 49 % of farmers seek veterinary services to treat animals and 93 % of them did not respect antibiotic withdrawal times. Farmers were classified in three groups: high-risk, medium and low risk group. These practices suggest high risk of on one hand, contamination of bovine meat with antibiotic residues, and on another hand, selection of resistant bacteria, both resulting in adverse health effects on consumers.

Keywords: cattle farmers, antibiotics, Benin

12. Evaluation of the quality of antibiotic pharmaceuticals used in cattle breeding in northern Benin, through their ability to inhibit *Bacillus subtilis* growth

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In Benin, veterinary antibiotics used in cattle breeding were more purchased in local markets than veterinary pharmacies. This study aim is to assess the quality of these antibiotics used in northern Benin, through their ability to inhibit *Bacillus subtilis* growth. Twenty-eight antibiotic drugs were collected in drug stores of Banikoara, Kandi and Parakou as well as in the cattle market of Tourou (Parakou), in North Benin. The sampled veterinary drugs contained oxytetracycline (17 pharmaceuticals), sulfamethazine (4), tylosine (2), penicillin G (2) and mixture of penicillin and streptomycin (3). Microbiological inhibition tests on culture media containing *Bacillus subtilis* strain was used to analyse each pharmaceutical, diluted at two levels of concentrations, as well as standard solutions of the corresponding antibiotic. NaOH, Penicillinase and 4-aminobenzoic acid solutions were used for identification of oxytetracycline, penicillin G and sul famethazine antibiotics, respectively. Inhibition areas were measured to estimate antibiotic concentrations, by comparison with standard antibiotics calibration curves. From a qualitative point of view, all identification tests of antibiotic active substances showed that the veterinary drug contained the active substance declared on the label. Quantitatively, the 24 products containing oxytetracycline, tylosine, penicillin G and mixtures of penicillin G and streptomycin can be qualified of good quality as they showed measured levels of antibiotics ranging between 72% and 141% of the declared concentrations, including 17 products (out of 24) showing measured levels above 90% of the declared concentrations. On the contrary, products containing sulfamethazine (4) showed bad results, with only 20% of the declared concentration. **Keywords:** Antibiotic pharmaceuticals, cattle breeding, Benin

13. The modified New Two Plates Test for detecting tetracycline, beta-lactams and sulfonamides antibiotic residues in kidney and muscle of cattle slaughtered in North Benin

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In Benin, veterinary antibiotics are widely used for cattle breeding. This livestock contributes to 57 % of the locally produced meat. The aim of this study is to assess the impact of the large use of antibiotics on the contamination level with antibiotic residues of bovine meat, in North Benin. Kidney and muscle samples taken from 50 bovine carcasses in the two principal slaughterhouses of Parakou town were analyzed for the detection of antibiotic residues by a screening method developed in this study, which was inspired by the "New Two Plates Test". Some positive and negative samples were subjected to liquid chromatography-mass spectrometry for the confirmation of tetracycline and sulfonamide residues. Sixty percent of carcasses were containing antibiotic residues. Tetracyclines residues were identified in 54 % of cattle carcasses while beta-lactams and sulfonamides residues were present in 2 % and 6 % of selected bovines, respectively. The liquid chromatography-mass spectrometry confirmed the presence of oxytetracycline, epi-oxytetracycline, tetracycline and epi-tetracycline at maximum levels of 1380, 350, 190 and 230 µg/kg respectively, in the kidney. Sulfamethazine residues were confirmed in one of cattle carcass at very high doses of 3900 µg/kg in kidney and 2220 µg/kg in muscle. The observed results were above the maximum residue limit applied in European legislation in 12 % of the carcasses. These high contamination levels of antibiotic residues are firstly a potential threat to the health of consumers, and secondly, a high risk of selection of antibiotic-resistant bacteria in animals and in human.

Keywords: cattle meat, antibiotics residues, Benin

14. Development of an analytical method for the simultaneous measurement of nine biogenic amines in food

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The formation of biogenic amines is essentially the result of enzymatic decarboxylation of specific amino acids, due to microbial enzymes. Knowing that biogenic amines, particularly histamine and tyramine, can cause health problems in predisposed consumers, their presence in food is not desired. What is more, quantification of biogenic amines in meat is suitable for detecting the stage of deterioration of meat and their concentration may be associated with the freshness and the level of degradation of proteins of the product. Therefore, a UPLC-PDA-Fluorescence method has been developed to evaluate the concentration of nine biogenic amines in food: methylamine, tryptamine, 2-phenylethylamine, putrescine, cadaverine, histamine, tyramine, spermidine and spermine. The dansylated amines were analysed on a UPLC Acquity system integrated autosampler, solvent delivery system and column heater coupled to a Acquity PDA detector and an Acquity Fluorescence detector (Waters Corporation). The column used was a Acquity UPLC BEH C18 (2.1 x 100 mm, 1.7 µm), with a UPLC BEH C18 VanGuard precolumn (2.1 x 5 mm, 1.7 µm) (Waters Corporation). The amines were identified by comparing the retention times of their chromatographic peaks with those of the corresponding standards. Until now, the developed method was applied to quantify biogenic amines in fish and meat products. The limits of quantification (LOQ) are ranging from 0.75 to 10 mg/kg. A method validation is being conducted according to the criteria and procedure described in Commission Decision 2002/657/EC. Acknowledgments: The authors acknowledge the Walloon Region.

Keywords: biogenic amines, UPLC, food

15. A Gammaherpesvirus Infection Protects the Host from Pneumovirus-Induced Immunopathologies

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Gammaherpesviruses are highly prevalent pathogens that establish lifelong latency. However, little is known about how these viruses imprint the immune system of their host. Here we used murine herpesvirus 4 (MuHV-4) to investigate the impact of gammaherpesvirus infections on the development of pneumovirus-induced immunopathologies. Firstly, a respiratory Th2 vaccine-enhanced disease was induced in mice by subcutaneous vaccinations with formalin-inactivated antigens of pneumonia virus of mice (FI-PVM) followed by an intranasal challenge with wildtype PVM. This homologous host-pathogen model was used to mimic the historical human respiratory syncytial virus vaccine-enhanced disease that had occurred in children during past vaccinal trials. We observed that MuHV-4 infection, either before or after the FI-PVM vaccination, prevents the development of the PVM-induced Th2 immunopathology while the vaccinal protection against PVM infection was unaffected. Notably, reduced levels of total leukocytes, eosinophils, and Th2 cytokines were observed in MuHV-4 imprinted mice. This protective impact against the vaccine-enhanced disease was maintained over time and required pulmonary MuHV-4 replication. Furthermore, in non-vaccinated mice, we also observed that MuHV-4 confers striking heterologous clinical protection against the lethal wildtype PVM infection. This protection was associated with a highly improved PVM-specific cytotoxic CD8 T cell response that was observed in lungs of MuHV-4 imprinted mice. Altogether, these results open new perspectives for prevention of pneumovirus-associated diseases and highlight that some so-called pathogens could be revealed in the end as beneficial for their host.

Keywords: Gammaherpesvirus, Pneumovirus, Immunopathologies

16. Genome sequencing of Cyprinid herpesvirus 3: a basis for the study of its evolution and of the pathogenesis

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Since its emergence in the late 1990s, CyHV-3 has had great ecological impact and induced severe economic losses in the common and koi carp industries. Until now, the genomes of 4 different strains of CyHV-3 have been available in the Genbank. Even if these strains represent the two existing lineages (Asian and European) described for CyHV-3, genome sequencing of additional strains is required to estimate the genetic diversity within this viral species. Here, we sequenced the complete genome of 7 additional strains. The 11 genome sequences were then used to investigate the phylogeny of the strains and the genetic diversity of different genes studied in our laboratory (16 genes encoding envelope transmembrane proteins, ORF112 encoding a Zalpha domain protein, ORF56 and ORF57 loci which are deleted in the attenuated recombinant vaccine developed by our laboratory). For most of these genes, the conservation of the expression of a protein product was investigated in cells infected by 6 representative strains. The data obtained confirmed the non-essentiality of some ORFs and different levels of conservation for the others. Finally, full length genome analyses revealed interesting features on the evolution of CyHV-3.

Keywords: Cyprinid herpesvirus 3, genome sequencing, viral evolution

17. Investigation of traditional processing methods used to produce hanlan, a cured pork meatbased product from Benin

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Pork is an unprocessed meat from pig (*Sus scrofa* or *Sus scrofa domestica*) commonly consumed in Benin. Pork meat consumption is around 1,2 Kg/person/year with 4968000 tons of annual production in 2013 in Benin. Cured pork meat is an appreciated food in Beninese diet. As regard to data on chemical hazard from scientific reports, curing process presents healthy risk due to polycyclic aromatic hydrocarbons (PAHs), including benzo(a)pyrene, a carcinogenic compound. Polycyclic aromatic hydrocarbons formation depend on raw material, smoking methods, fuel type, combustion temperature. This study aims to give a better understanding on traditional cured pork meat production and to assess factors influencing benzo(a)pyrene and other PAHs production during curing pork meat process. The study was carried out as field investigation using questionnaire for individual interviews, focus group and observations of actors on processing sites. Consumers (372) and processors (188) were surveyed in five municipalities of southern Benin according to Dagnelie, (1998). Indigenous pig breed, crossbreed and exotic breed were processed by 93.6%, 71.8% and 38.3% of processors respectively. Direct curing with one room grill was the most commonly used, but indirect curing using two rooms grill was also recorded. Wood (76.1 %) was the most used fuel and came from four main tree species: *Acacia auriculiformis*, *Tectona grandis*, *Mangifera indica* and *Anogeissus leiocarpa* used by 38.3 %, 36.2 %, 17.6 % and 13.3 % of processors respectively. Future investigations need to be undertaken to characterize cured pork meat on chemical hazards aspects.

Keywords: Processing, Curing, Pork

18. Assessment of Polycyclic Aromatic Hydrocarbons contamination in cured pork consumed in Benin

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In Benin, traditionally produced cured pork presents contamination risk with polycyclic aromatic hydrocarbons (PAHs) due to smoke which contains large amounts of PAHs, or to fat content dropping along in the fire during the processing, resulting in PAHs formation. This study aims to assess PAHs contamination of cured pork produced and consumed in southern Benin. Twelve samples of cured pork meat were randomly collected from processing sites according to main process and consumption forms identified during a previous study. Fifteen PAHs, selected as priority PAHs in food by European Union in 2002, were analysed on freeze-dried samples through extraction with accelerated solvent extraction (ASE), purification on solid phase extraction (SPE) columns cartridges, and finally, injection on a Ultra High Performance Liquid Chromatography coupled with Fluorescence detector (UPLC/ FLD). The PAH's limit of quantification ranged between 0.12 and 0.16 µg/kg fresh weight (depending on the humidity of the sample), except for Indeno[1,2,3-cd]pyrene and Benzo[j]fluoranthene which display an LOQ ranging between 0.48 and 0.65 µg/kg. The results showed high concentrations of benzo(a)pyrene (BaP), exceeding nine times the limit of 2 µg/kg set by the European Commission (EC). Likewise, the sum of 4 PAHs, ranged between 20.4 and 110.5 µg/kg, and were above the 12µg/kg maximal limit set by EC. These results showed evidence of public health issue when consuming smoked pork and further studies of process reengineering are needed to decrease the PAH contamination of these kind of food products.

Keywords: Cured pork, Polycyclic aromatic hydrocarbo, Benin

19. Effect of aging time, aging technique (dry- vs. wet-aging) and packaging on tenderness, pigment and lipid stability of Belgian blue beef

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The aim of this study was to compare the effect of aging technique (dry-aging and wet-aging), aging time (0, 21, 42 and 63 days) and packaging during display (vacuum and shrinkable film wrapping) on pH, tenderness, pigment and lipid stability of beef. Three longissimus dorsi muscles from two Belgian Blue cows were dry- or wet-aged for up to 63 days at 2 °C. At different times, part of these samples was cut into steaks and repackaged in vacuum bags or shrinkable film, and stored during 4 days at 4 °C + 8 days at 8 °C (simulated retail display). The following parameters were evaluated at different intervals: pH, tenderness (Warner-Bratzler shear force), color (CIE L*a*b*), myoglobin oxidation (K/S 572/525 ratio) and lipid oxidation (TBARS). The aging technique and the packaging during simulated retail display had an effect on pH (dry-aging > wet-aging, $P < 0.05$; shrinkable film > vacuum, $P < 0.05$). An increase of tenderness was observed during the first 21 days of aging ($P < 0.05$). The sensitivity of samples to pigment oxidation was influenced by the packaging during display (shrinkable film > vacuum, $P < 0.05$). Aging time and packaging during display increased lipid oxidation (42 and 63 > 0 and 21 days, $P < 0.05$; plastic wrap > vacuum, $P < 0.05$). This study contributes new knowledge about Belgian Blue beef behavior whether it is wet- or dry-aged. Further research will be conducted to study the antioxidant capacity of these meats in order to better understand the oxidation process.

Keywords: aging, oxidation, Belgian Blue

20. Gut microbiota involvement in blood pressure control : comparison between patients with normal blood pressure and patients suffering from high blood pressure

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High blood pressure (HBP) is a major public health risk in western countries. In some cases, HBP is a secondary symptom to another pathology. But it is more often "essential" as no other cause can be found. Treatment is an association drug therapy, physical exercises and diet. Despite these treatments, HBP physio-pathology remain largely unknown. Recently, a new blood pressure regulation pathway has been discovered in human kidney that is controlled by microbial short chain fatty acids (SCFA). The influence of propionate and acetate has also been demonstrated in a murine model. Our hypothesis is that HBP patients could harbor a different gut microbiota compared to matched healthy patients. Fifty male human volunteers were investigated. Ambulatory blood pressure were monitored for 24H and a sample of blood and feces were recovered. Feces were used for 16S amplicon profiling and blood for NMR metabolomic analysis. Patients were divided into HBP, borderline and control cohorts based upon blood pressure monitoring. V1-V3 hypervariable 16S amplicon profiling were obtained from MiSeq Illumina sequenced reads (10,000/sample). Metabolomics was performed on a 500 MHz NMR system and SCFA were measured. If global bacterial community for the 3 cohorts were similar in term of richness and diversity, HBP and borderline cohorts harbor a statistically higher abundance of Prevotella and Subdoligranulum genera. Metabolomics of blood SCFA shows a higher abundance of acetate and butyrate in the HBP cohort vs other groups. Investigation of Prevotella genus, known to be linked to TMAO production, will be further extended.

21. Florpro project: Selection of beneficial bacteria from chilled foodstuffs to protect them from bacterial spoilage and to increase their shelf life

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Microbial food spoilage is a major issue in the food industry. It has been estimated to be responsible for 25 % of the world's food supply losses. Meanwhile, the desire of industrials to reduce the use of preservatives in their food preparation is becoming stronger. In this context the FLORPRO project was developed in partnership with Belgian food industrial partners. The original approach is to study, select and promote beneficial bacterial flora from chilled food products in order to protect them from spoilage and increase their shelf life. A wide range of food products has been selected for this project: fresh meat, cooked meat, pasta, cheese and prepared dishes. The strategy implemented was, first, to study microbiota of different batches of naturally contaminated food product and to monitor the development of the main species using a 16S rDNA metagenetic approach and, then, to isolate bacteria from the product. The most interesting species were then selected on their abilities to grow at the required temperature gradient standing for consumer's fridge. During challenge-tests in our pilot plant selected strains were, then, tested on naturally contaminated food products. The effect of these strains on the organoleptic quality of the product were assessed with sensory and biochemical analysis. The dynamic of the most interesting strains in the natural ecosystem of the product was evaluated with metagenetic analysis and especially, their effect on the spoilers. Some biopreserved food products showed a better sensory evaluation than those naturally contaminated after the end of the usual shelf life. The potential of these strains as bio-preservative was confirmed with the implementation of challenge-test in real processing conditions on the production line of the industrial partners and complemented with antagonist activity tests of these strains against food pathogens.

Keywords: Biopreservative, Food microbiology, Metagenetics

22. Effects of Murid Herpesvirus 4 infection on B cell repertoire in mice

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To confer protection, the adaptive immune system produces a highly diversified repertoire of antibodies that are selected and expanded in response to specific antigens. While pathogens can affect the antibody repertoire of responding B cells, their effect on the whole repertoire is mostly unknown. This is especially true for persistent viruses such as Gammaherpesviruses (γHVs) which are ubiquitous in human and animal populations. Indeed, although γHVs induce a polyclonal B cell activation as a normal part of their life cycle, the consequences of these infections on the host's antibody repertoire are still largely unknown. Recent advances in high-throughput DNA sequencing technologies enabled characterization of the antibody repertoire. In this study, we used murid herpesvirus 4 (MuHV-4), a γHV infecting laboratory mice, to study the imprinting of a γHV infection on the antibody diversity of its host. Briefly, we developed in mice a consensus sequencing approach that incorporates unique barcode labels on each starting RNA molecules and therefore allows us to reduce rate of sequencing error and to quantify transcripts. Based on this technique, the subsequent bioinformatics analysis of antibody heavy chain sequences allowed us to compare the diversity, the isotype frequency, the level of somatic hypermutation and the lineage structure of the antibody repertoire in MuHV-4-infected and uninfected mice. Using a reporter virus, we also investigated the antibody repertoire of MuHV-4-infected and uninfected cells. Altogether, this study highlights that deep sequencing of immunoglobulin transcripts provides direct insight into the imprinting of γHVs infection on the immune system of their hosts.

Keywords: Gammaherpesvirus, Antibody repertoire, Next generation sequencing.

23. Influence of suppression of sodium nitrite or incorporation at a reduced level on the growth of *Clostridium botulinum* in cooked ham

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Sodium nitrite (NaNO₂) is used in meat products manufacturing for its antimicrobial effect on *Clostridium botulinum*, responsible of botulism by production of neurotoxins. Its use is associated with nitrosamines production, considered as carcinogenic substances. The objective of this study is to compare the growth of *C. botulinum* Group II non-proteolytic in a "cooked ham model" in function of the NaNO₂ incorporation rate (0 and 80 ppm) by comparison with the conventional rate (120 ppm). Ground pork was mixed with NaNO₂ and commercial ingredients, stored (24h, 4°C), inoculated with spores of Group II non-proteolytic *C. botulinum* SR398 (3 log CFU/g) and vacuum packed in portions of 50g. Cooking and cooling were applied in conditions simulating the core temperature of a 7kg industrial cooked ham. A second experiment ("worst case" chilling conditions) was performed by applying a storage at 27°C for 24h before chilling. The products were stored under two conditions (52 days, 8°C or 31 days, 4°C + 21 days, 8°C). No growth of *C. botulinum* was observed by qPCR and by classical culture methods during the process and the storage whatever the conditions were. It can be concluded that 0 or 80 ppm incorporation rate of NaNO₂ has no impact on the growth of *C. botulinum* by comparison with conventional rate. However the production of neurotoxin has not been checked in the present experiment; it can therefore not be totally excluded.

Keywords: *Clostridium botulinum*, Sodium nitrite, challenge test

24. Metagenomic and metatranscriptomic analysis of beef meat products stored under modified atmosphere packaging

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Meat is one of the most perishable food products on the market thus resulting in economic losses. Different parameters have an impact on the meat spoilage such as the initial microbial load, post-harvest processing, packaging method and the storage temperature. A metagenomic approach was used to monitor changes in bacterial populations during the refrigerated storage of 2 different batches of naturally contaminated raw beef meat under modified atmosphere packaging. Total bacterial genomic DNA and total mRNA samples were extracted from the different batches at 5 different time points during storage and used for, respectively, metagenomic and metatranscriptomic analysis. In parallel, microbial plate counting and sensory analysis were conducted. In addition, more than, 80 bacterial strains were isolated from the samples and identified using 16S rDNA Sanger sequencing. Phylogenetic analysis based on the high-throughput sequencing of 16S rRNA genes indicated the dominating members of the spoilage microbiota such as *Lactobacillus algidus*, *Leuconostoc gelidum*, *Lactococcus piscum*, as well as, *Brochothrix* and highlighted their dynamic during storage. In order to complement the metagenomic study, metatranscriptomes will be analyzed using RNAseq to describe the global gene expression of the dominant species.

Keywords: spoilage, meat, metagenomic

25. Identification of bovine and porcine colistin-resistant *mcr1*-positive *Escherichia coli*

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Polymyxins have been used for years in veterinary medicine and as last resort in human medicine. For years, only chromosome-mediated resistance to colistin was identified. Recently, a plasmid-located gene (*mcr1*) was identified in Gram - enterobacteria. This study aimed to compare phenotypic and genetic for the detection of resistance to colistin and of the *mcr1* gene in a collection of *E. coli* isolated from different animal species and humans. More than 3000 *E. coli* were tested for resistance to colistin by growing them on agar plates with colistin. The Minimal Inhibitory Concentrations (MIC) and the presence of the *mcr1* gene were determined using the E test® and colony hybridization assay. The probe-positive isolates were tested by PCR. A total of 410 *E. coli* isolated grew on colistin-containing agar plates. MIC of 273 isolates was ≥ 1 mg/ml of colistin. Of those 410 isolates, 34 hybridized with the *mcr1* gene-derived probe: 5 from pigs and 11 from bovines gave black spots, while 17 from pigs and one from bovine gave grey spots. Fifteen "black spot" probe-positive isolates tested positive with the *mcr1* PCR as did 3 porcine "grey spot" probe-positive isolates, while the remaining 16 isolates repeatedly tested negative. This study confirms that the results of phenotypic assays for the detection of colistin resistance cannot be always trusted; the *mcr1* gene is not the only one mechanism of resistance to colistin; *mcr1* variants may exist that cannot be detected by the classical PCR. Presence of *mcr1* gene and putative variants will be confirmed after WGS that will also allow comparing the *mcr1*-positive plasmids and isolates from pigs and cattle to similar human *E. coli* isolates.

Keywords: *Escherichia coli*, Antibioresistance, Colistin

26. The Cyprinid herpesvirus 3 – carp model: a unique model to test the roles in anti-viral innate immunity of Zalpha domain proteins detecting unusual nucleic acid conformations

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The innate immune system relies on numerous molecules that act as nucleic acid sensors, which are detected based on their structure, subcellular localization or sequence. Zalpha domains are 66 aa long domains which bind to left-handed dsDNA (Z-DNA) or dsRNA (Z-RNA). The description of Zalpha domains in proteins of the host innate immune system but also in viral proteins suggests that even the conformation of the nucleic acid could be exploited by the innate immune system. This project aims to test this fascinating hypothesis taking advantage of the Cyprinid herpesvirus 3 (CyHV-3)/Carp model. Carp, like all cypriniform fish, encode PKZ, a paralogue of the dsRNA-dependent protein kinase (PKR) expressed by all vertebrates. While PKR possesses dsRNA binding domains, PKZ has Z-DNA/Z-RNA binding domains. Both proteins, once activated by binding to the appropriate nucleic acids, phosphorylate eIF-2 α thereby blocking protein synthesis. Recently, it was demonstrated that ORF112 of CyHV-3 encodes a Zalpha domain protein (pORF112) over-competing the binding of PKZ to Z-DNA. So far, experiments conducted on this topic show that the Zalpha domain, in addition to being conserved throughout the different strains of CyHV-3, is essential for replication of the virus in cell culture. More importantly, the essential component has proven to be the Zalpha domain of ORF112 and its function, since replacement of ORF112 by its Zalpha domain alone or by that of another organism leads to the rescue of viral growth. Additionally, pORF112 co-localizes with Z-DNA and dsRNA respectively in the nucleus and cytoplasm of CyHV-3 infected cells. These results further support the interest of the CyHV-3/carp model to test the roles in anti-viral innate immunity of Zalpha domain proteins detecting unusual nucleic acid conformations.

Keywords: Zalpha, Innate immunity, Z-DNA/Z-RNA

27. In vitro study of antibacterial activities of plant extracts and commercial products against two strains of *Aeromonas hydrophila* isolated from red tilapia (*Oreochromis sp.*) in Vinh Long province (Vietnam)

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The aim of this study was to examine the in vitro antibacterial activities of 20 herbal extract samples and 3 commercial products for the main purpose of pre-selection for their further application as antibiotic alternatives or to improve seafood and aquaculture products preservation. The antimicrobial capacity against two strains of *Aeromonas hydrophila* was tested using a colorimetric indicator (resazurin) for the minimum inhibitory concentration (MIC) determination. Both bacterial strains were isolated from red tilapia (*Oreochromis sp.*) in Vinh Long province (Vietnam). Four plant extracts (*Phyllanthus amarus* Schum. et Thonn., *Piper betle* L., *Psidium guajava* L. and *Euphorbia hirta* L.) and one commercial product showed moderate antimicrobial activities against the first strain, with MICs values ranging between 156 and 625 µg/mL, while the rest of the samples showed weak or no antibacterial activity (MIC ≥ 1250 or 2500 µg/mL, respectively). The growth of the second strain was also inhibited by *Phyllanthus amarus* Schum. et Thonn., *Piper betle* L. and *Alba*, but at higher concentrations of plant extracts (MIC = 625 µg/mL). *Psidium guajava* L. and *Euphorbia hirta* L., which inhibited the growth of the first strain at a concentration of 312 and 625 µg/mL respectively, needed a concentration of 1250 µg/mL to inhibit the second strain of *Aeromonas hydrophila*. The results showed that both strains of *Aeromonas hydrophila* display the same pattern of sensitivity towards tested plant extracts, but the first strain is more sensitive than the second one. These findings of a significant antibacterial activity of two plant extracts (*Phyllanthus amarus* Schum. et Thonn., *Piper betle* L.) against two strains of the pathogenic bacteria *Aeromonas hydrophila* could be useful for the selection of natural alternative to antibiotics as well as to prevent bacterial growth in fish products during storage.

28. In vitro investigation of antioxidant capacity of herbal extracts and commercial products used to improve aquaculture products quality

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This study was conducted to determine the in vitro antioxidant activities of 20 herbal extract samples and 3 commercial products for initial selection of potential application in seafood and aquaculture products storage. The methods of Folin-Ciocalteu (FC) and 2,2-diphenyl-1-picrylhydrazyl radical scavenging (DPPH) were applied to evaluate the antioxidant activity. The results showed that eleven out of twenty three plant extracts displayed antioxidant activity, with concentrations able to inhibit half of the maximum response (IC₅₀) ranging from 6 to 49 µg/mL. *Phyllanthus amarus* Schum. et Thonn. showed the strongest radical scavenging effect (IC₅₀=6 µg/mL). The remaining 4 samples showing a high antioxidant activity (i.e. IC₅₀ < 30 µg/mL) were in the following order: *Piper betle* L. > *Psidium guajava* L. > *Euphorbia hirta* L. > *Mimosa pudica* L.. A group of six samples, including *Zingiber officinale* Rosc., *Hepamin*, *Eclipta prostrata* (L.) L., *Alba*, *Annona reticulata* L., *Houttuynia cordata* Thunb., showed an intermediate antioxidant capacity (i.e. 30 µg/mL < IC₅₀ < 50 µg/mL). There was a positive correlation between total phenolic content (expressed as gallic acid equivalents) and antioxidant activity (expressed as 1/ IC₅₀) (R²=0.9137). The next step will be to test compounds with high in vitro antioxidant activity for their capacity to inhibit fatty acid oxidation in feed used in aquaculture and aquaculture products.

Keywords: Antioxidant activity, Folin-Ciocalteu, herbal extracts

29. Release of Neutrophils Extracellular Traps as a main trigger for asthma onsetRadermecker C.¹, Sabatel C.¹, Johnston S.L.², Toussaint M.², Bureau F.¹, Marichal T.¹¹ *Laboratory of Cellular and Molecular Immunology, GIGA-R, ULg*² *Airway Disease Infection Section, National Heart and Lung Disease Institute (NHLI), Imperial College London, London, UK*

Neutrophils Extracellular Traps (NETs) release (also called netosis) is an important component in organism defense against pathogen invasion but has also been identified as central effector of immune disorders like erythematous systemic lupus, gout, rheumatoid arthritis. Asthma is an important TH2 associated immunopathology. If the pathology of the disease is well described, its etiology is largely unknown. Nevertheless, neutrophilic environmental conditions like viral infections increase the risk of disease inception. In this study, we analysed the role of neutrophils, and particularly NETs, as potential asthma inducers during viral infections. We first designed a model of virus-induced asthma. In this model, only infected mice developed asthma when they were sensitized by injecting house dust mite (HDM) during the peak of infection. At the peak of infection, we observed a recruitment of neutrophils associated with a release of free DNA in bronchoalveolar fluid lavage. The association of this free DNA with netosis was confirmed by confocal microscopy and western blot. We then tested the role of netosis in this model by using two NETs inhibitors. Both inhibitors significantly decreased all the parameters of asthma in our model. Finally, to address how NETs could lead to a TH2 immune response, we analysed by flow cytometry the subpopulations of lung dendritic cells (DCs) in our virus-induced asthma model. We observed, during netosis phase, a recruitment of Ly6C+ DCs, described to be responsible of allergic sensitization. In conclusion, we have demonstrated a role for NETs in asthma onset by recruiting lung Ly6C+ dendritic cells.

Keywords: Neutrophils, Extracellular Traps, Asthma**30. TRIM24 suppresses infection by Murid Herpesvirus 4 infection**Rathakrishnan A.^{1*}, Vidick S.^{1*}, Palmeira L.¹, Xiao X.¹, Tischer C.², Pepperkok R.², Neumann B.², Gillet L.¹

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Gammaherpesviruses are archetypes of persistent viruses that have been identified in a range of animals from mice to man. They are host-range specific and establish lifelong latency of immunocompetent hosts. Most of the gammaherpesvirinae members are associated with neoplastic diseases. For example, the best studied gammaherpesviruses are Human herpesvirus 4 and 8 that are respectively associated with Burkitt's lymphoma and Kaposi's sarcoma. By opposition to its human counterparts, Murid herpesvirus-4 (MuHV-4) is able to replicate to high titres in cultured cells and is therefore an excellent candidate for studying the cycle of these viruses. Here, using a whole genome RNAi screen, we identified 65 genes that appeared to be necessary for the completion of the MuHV-4 cycle and likely the initial steps of infection. In parallel, silencing of 110 genes increased MuHV-4 infection suggesting a possible anti-viral function. Among these, TRIM24, a member of the RING-type E3 ligase family, exhibited strong antiviral activity against MuHV4. Interestingly, silencing of TRIM24 expression appeared to significantly increase MuHV-4 binding to the host cell. In the future, we want to investigate how TRIM24 exerts its antiviral effect and if it also blocks infection by other viruses.

Keywords: MuHV-4, TRIM24, RNAi

Posters

31. Study of the potential zoonotic transmission of *Clostridium difficile* in Belgian cattle farms

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Introduction: Zoonoses are infectious that can be transmitted between animals and humans through direct contact, close proximity or the environment. Since food animals frequently test positive for the bacterium, it seems plausible that *C. difficile* could be zoonotic. **Purpose:** This study aimed to determine the epidemiology of *C. difficile* in cattle farms and the possible spread of the bacterium among animals and farmers.

Methods: A total of 176 faecal samples of cattle were collected from 5 different Belgian farms, from November 2015 to February 2016. A stool sample of each farmer was also requested. Detection of *C. difficile* was performed by classical culture on *C. difficile* selective medium (cycloserine cefoxitin fructose cholate). Isolates were characterised by PCR-ribotyping and Genotype Cdiff test (Hain Lifescience), which allows the detection of all toxin genes, mutations in *gyrA* gene and the deletion in the regulator gene *tcdC*. Toxic activity was confirmed by a cytotoxic assay on MRC-5 cells.

Results: *C. difficile* was detected in 14/178 (7.9%) animal samples. Isolates were grouped into five different types, including PCR-ribotype 015 (this ribotype is one the most encountered in hospitals in Belgium). The other types were UCL46A, UCL24*, UCL24, UCL33. All of them were identified as toxigenic by cytotoxicity assay and toxin genes profile. In contrast, none of the 5 farmers studied were positive for the bacterium.

Conclusions: Results obtained indicate that PCR-ribotypes commonly isolated from hospitalised patients are also present in cattle, indicating an animal reservoir. However, a zoonotic transmission could be not demonstrated in this preliminary study.

Keywords: *Clostridium difficile*, cattle, zoonosis

32. Association of targeted metagenetic analysis and classical microbiology for *Clostridium difficile* detection and microbial ecosystem mapping of surfaces, hands and foodstuffs in a meat processing plant

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Objective: Proper hygiene practices in meat processing plants are essential for prevention of food-borne disease outbreaks. This study aimed to evaluate the hygienic level of a meat processing plant and to identify possible sources of cross contamination. A microbiological detection scheme was performed along with an overall microbial biodiversity study of the samples by metagenetics. Detection of the pathogenic bacteria *C. difficile* was also performed.

Methods: The production line of two meat products (pork tomato sauce and white pudding) was monitored. Samples from operator hands (n=8), surfaces (n=9) and products (n=11) were collected at different points of the production. All samples were analysed by classical microbiology to determine the levels of total aerobic viable counts, Enterobacteriaceae, *Escherichia coli*, *Staphylococcus aureus* and to detect the presence of *C. difficile*. Metagenetic analysis was targeted on the V1-V3 hyper-variable region of 16S rDNA. Taxonomical assignment was performed with Mothur and Blast algorithms.

Results: None of the samples were positive for *C. difficile*. Using international standards, all of the samples contained acceptable levels of the other bacteria studied. Metagenetics revealed the presence of some taxa in the final products that were not detected in the intermediate products, including populations from the *Acinetobacter*, *Proteus* and *Staphylococcus* genera.

Conclusions: Results indicated that *C. difficile* contamination of the meat products studied was unlikely. High-throughput sequencing reveals a cross contamination in the production line. Further studies are needed to improve taxonomic identification.

Keywords: metagenetics, contamination, meat processing plants

33. Bacterial CpG-DNA prevents asthma by expanding lung interstitial regulatory macrophages from local and splenic reservoir monocytes

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Living in a microbe-rich environment reduces the risk of developing asthma. Unmethylated CpG-DNA from bacteria is particularly efficient in reproducing these protective effects when administered to humans or animals, suggesting a major contribution of CpG-DNA to microbe-induced asthma resistance. However, how CpG-DNA confers protection remains elusive. Here, we show, in mice, that CpG-DNA has the unique ability to expand lung interstitial regulatory macrophages (IM). Experiments in clodronate-treated, chimeric, parabiotic and splenectomized wild-type mice and mice lacking CCR2, a receptor required for classical monocyte mobilization from the bone marrow, unexpectedly demonstrated that CpG-DNA-induced IM (IMCpG) developed from monocytes residing in the lung or recruited from the spleen independently of CCR2. Furthermore, IMCpG produced much higher levels of the immunosuppressive cytokine interleukin-10 than their steady-state counterparts and recapitulated the protective effects of CpG-DNA when adoptively transferred to mice before allergen sensitization or challenge. By identifying CCR2-independent pulmonary and splenic reservoir monocytes as CpG-DNA-responsive precursors for hypersuppressive IM, our study reveals a possible mechanism by which exposure to the environmental microbiome, or treatment with synthetic CpG-DNA, protects against asthma.

Keywords: regulatory macrophages, CpG-DNA, asthma

34. Exploring the role of CD169+ lymph node macrophages in alum adjuvant activity

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In mice, aluminium-adjuvanted vaccination promotes on the one hand inflammatory dendritic cell-mediated canonical T helper type 2 response and IgE production, and on the other hand IgG1 production by mechanisms that remain poorly understood. Such antigen-specific IgG1 production is known to be dependent on the draining lymph node-associated follicular B cell response, but the mechanisms leading to native antigen capture by these B cells are not fully elucidated. B cells-rich areas are separated from the lymphatic sinus by an endothelial wall containing CD169+ subcapsular macrophages. These macrophages are increasingly thought to play a determinant role in the capture of high molecular weight or particulate antigens and their presentation to the surrounding B cells, leading us to hypothesize that alum could act on these macrophages to boost adaptive immunity – particularly IgG1 production – directed against the co-administrated antigen. Here we used transgenic CD169-DTR mice, in which diphtheria toxin treatment leads to depletion of CD169+ cells. Using a mouse model of intramuscular alum-adjuvanted vaccination against bovine serum albumin (BSA) or ovalbumine (OVA), we found that CD169+ lymph node macrophages are required for antigen-specific IgG1 response against BSA, but not OVA, during an alum-adjuvanted vaccination. This project could potentially lead to the better understanding of the implication of CD169+ lymph node macrophages on the antigen's spatiotemporal distribution and the efficacy of alumboosted humoral IgG1 response, and therefore could contribute to the development of future efficient vaccination strategies.

Keywords: Alum, CD169+ macrophage, Vaccination

35. Microbiota analysis of the upper and lower respiratory airways of kennel healthy dogs

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The advent of culture-independent microbiology has made a radical change in host-bacteria interactions, as the microbiological presence and biological importance in every host interface is now extensively explored. Long thought as being sterile, upper and lower airways of humans are now reported to be colonized by stable and consistent microbiota. We report here the exploration of microbiota relations between upper and lower airways of healthy kennel Beagle dogs with 16S amplicon profiling. Twelve adult kennel Beagle dogs were investigated. Bronchoalveolar lavage fluid (BALF), nasal and pharyngeal swabs were recovered and used for global bacterial DNA extraction. V1-V3 hypervariable 16S amplicon library were prepared for each sample and sequenced with MiSeq Illumina sequence (V3 kit). A total of 120,000 reads were further analyzed. Taxonomical assignation and clustering were performed with Mothur against SILVA database. Results show that Proteobacteria is the predominant phylum in the study followed by Firmicutes. The dominant families were Pseudomonadaceae, Moraxellaceae and Pasteurellaceae. Ecological and statistical analysis reveal that each microbiota clusters independently from the others and that nasal and oropharyngeal microbiota harbor higher species richness than in the BALF. Distance analysis of the 3 microbiota underlines the proximity between nasal and lung microbiota compared to the pharyngeal's one ; this is surprisingly different from observations from human studies. Recent reports in human put in relation the lung microbiota stability and healthy status. A good knowledge of animal lung microbiota is thus warranted for further exploration of microbial implications in respiratory diseases.

36. Isolation of bacteriophages against *Klebsiella pneumoniae* and in vivo activity

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Klebsiella pneumoniae is a bacterial pathogen able to induce severe healthcare-associated or community-acquired infections in humans and animals. The constant emergence of antibiotic resistant strains reinforces the need to find alternatives to antibiotic treatments. The aim of this study was to isolate bacteriophages directed against *K. pneumoniae* strains and to test their efficacy in a murine model. Bacteriophages against 5 different *K. pneumoniae* were isolated and purified from waste water. Phage titers were determined on their respective hosts as well as on 18 other *Klebsiella* strains. Kinetics of bacterial lysis were monitored during 15h at 3 multiplicities of infection, in triplicates. For in vivo experiment, a total of 10 mice were inoculated with *K. pneumoniae* by oral gavage and monitored for 10 days. Five mice did not receive any treatment and 5 other mice received a cocktail of 3 bacteriophages at day 4 post-inoculation. A total of 54 bacteriophages were isolated and purified. The host range study showed a specificity related to the capsular type of their bacterial host. Lysis kinetics of bacteria suggested that different phages were isolated. Despite difficulties with the murine intestinal model, evidence was obtained that bacteriophages are able to reduce intestinal carriage. Our results show that bacteriophages isolated against *K. pneumoniae* are specific for a given capsular type, although further studies are necessary to provide more details on this capsular specificity and its molecular determinants. To fully address the in vivo potential of phages, a reliable mouse model of intestinal carriage of *K. pneumoniae* strains needs to be established.

Keywords: *Klebsiella pneumoniae*, Bacteriophages, in vivo

37. Sarcoptic mange infection in red foxes (*Vulpes vulpes*) in Belgium

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Eight cases of sarcoptic mange in foxes were reported during summer 2014 (n=3) and winter 2015 (n= 5) in Belgium. All animals came from the same restricted forest zones near urbanized areas and some of them were discovered in private gardens (Forest District of Thuin, Hainaut, Belgium). Three of them were found dead, the others were shot for sanitary reasons. At necropsy, all of them presented large areas of alopecia and extensive skin lesions such as hyperkeratosis and suppurative lacerations. Animals presented poor body condition and some of them were severely emaciated (absence of visceral fat). Mange has profound influences on population since, if untreated, death follows in four to six months. In the present cases, cutaneous scrapings and histopathological examinations were systematically performed. Numerous parasites were observed in skin scrapings and marked dermo-epidermatitis with several parasites was observed in histopathological sections. These results suggest an outbreak of sarcoptic mange in red foxes in a limited area of the country. This is a first record for the country. Furthermore, investigations on lungs and digestive tracts performed on 3 of these foxes revealed they were also infected by parasites transmissible to pets and/or humans: *Angiostrongylus vasorum* was observed in the respiratory tract of one fox whereas *Toxocara canis*, *Uncinaria stenocephala*, *Taenia* spp. and *Echinococcus multilocularis* worms were detected in the digestive tract. In conclusion, urban foxes represent a source of parasites of public health and veterinary importance and foxes surveillance should be strengthened in these areas.

Keywords: *Sarcoptes scabiei*, red fox, hyperkeratosis

Sustainable livestock production

38. The aggregation of experts as a tool to detect genetic interactions

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The last decades have seen major developments in the field of genetics. These advances have led to the emergence of a wealth of biological information, allowing new strategies to be applied in many fields of the biological research. Although these approaches have been successful, many elements in the genetic landscape of the studied traits are still unknown and uncharacterized. A potential track to new findings, that could help to better understand complex determinisms, is to detect interactions between regions rather than single region association. Although promising, this raises serious modeling, computational and statistical concerns, especially when large GWAS are to be used. While many methods have been proposed for detecting such interactions and despite the success of these methods to solve some problems and detecting some of the genetic interactions, there is currently no gold standard method able to detect interactions in all situations, and the relative performances of these methods remains largely unclear. Our work is a contribution to this field: rather than devising a new method from scratch, we have attempted to find a comprehensive method, aiming at extracting the information from a set of already defined methods. The idea is that proposed in the "aggregation of experts" theory: methods outputs are combined to provide a prediction gathering the information of the various methods ("experts"). Our first experimental results, based on simulated data and on a few popular methods, show that the aggregated predictor can produce better performances than each individual predictor to detect the genetic interactions, and is consequently a useful addition to the various methods available to tackle this complicated problem.

Keywords: aggregation of experts, gene-gene interaction, genome wide association study

39. Comparison of performance in weaned pig fed either maize grain silage or a standard starter diet

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An experiment was performed to evaluate maize grain high moisture ensiled (MGHE) inclusion in a weaned pig diet. The trial was performed using a concentrate mixed 1:1 with MGHE compared to a standard starter diet (SD). Concentrates were produced to obtain diets with equal nutrient levels on a dry matter basis; the dry matter content of MGHE was 61%. 144 piglets weaned at 28 days were allotted in 12 pens of 12 piglets. During 28 days, after a transition period, 6 groups of pigs were fed with MGHE and 6 groups of pigs were fed with SD. Pigs were individually weighed at the beginning and at the end of the trial as well as every week. The feed consumption was measured every week at the pen level.

The average weight of piglets at weaning was 7.6 ± 1.0 kg. At the end of the transition period, one piglet died. During the trial, 3 more pigs fed with the standard starter diet died. The average daily weight gain of piglets fed MGHE was significantly higher: 0.341 ± 0.031 kg vs. 0.300 ± 0.019 kg ($P=0.021$). The mean feed intake (corrected for a 87.5% dry matter content) was not significantly different (0.594 kg/piglet.day). The difference between the mean feed conversion ratio was highly significant, i.e. 1.71 ± 0.07 with MGHE vs. 2.02 ± 0.14 with the standard starter diet ($P=0.001$).

Conclusion: significantly better performance was obtained in piglets fed with maize grain silage.

40. Technical and institutional innovations in farming systems Ndama cattle in Senegal

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Objective of this study is to characterize the technical and institutional innovations on the Ndama cattle breeding systems introduced in the farmer and the reasons for their adoption. Participatory surveys in the form of focus groups were conducted. In total 6 focus groups in four rural municipalities have been carried out. 22 major innovations mentioned by farmers were classified in six main areas: food, animal health, reproduction, breed choice, infrastructure and agricultural materials and institutional. They were also classified according to their origin. Thus, 29% of innovations are developed by farmers themselves and other exogenous origin introduced by NGOs, research and other development actors. Correspondence analysis Factor between innovation and the reasons for their adoption reported that factors explain 58% of the total variance. It allowed classifying innovations into two groups. The first group is related to the increase incomes, economic and social factors and facilitating to work. The second group is related to climate and environmental change, low costs of innovation and increasing animal performance.

Keywords: Innovations, Ndama cattle, Reasons of adoption

41. Surgical treatment of a mucometra in an Alpine Goat

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Pseudopregnancy is one of the main causes of temporary infertility in goats, especially in dairy herds where the incidence varies around 15 %. The persistence of corpus luteum seems to be the cause of accumulation of aseptic liquid in the uterus without viable embryo. The etiology of hydrometra or mucometra is not totally understood, but failure of luteal regression can be the result of ovulation without fertilization in a non-mated cycling animals or an early embryonic mortality. The most common treatment for pseudopregnancy is the injection of prostaglandin (PGF2alpha) or synthetic analogs, two injections 12 days interval.

We herein report a 7-years old non-mated overweight pet goat with a recurrent mucometra. She was referred in 2015 for depression and anorexia. A mucometra was diagnosed by X-rays and ultrasonography. The medical treatment with PGF2 alpha was efficient. One year later, she developed a mucometra non-responding to prostaglandin associated to a low blood level of progesterone. Abdominal radiography and ultrasonography revealed a distended uterus, containing anechoic liquid, occupying an important part of the abdomen and hindering the normal function of the gastrointestinal tract. In agreement with the owners, an ovariectomy using a midline celiotomy was performed under general anesthesia without complications. Five months later, despite the fact that the goat exhibit arthrosis clinical signs, there is no complication regarding the ovariectomy.

The number of pet goats presenting pseudopregnancy increased in the Clinic for Ruminants. Like in buck or in dogs or cats, elective spay is recommended in female pet goats.

42. Biomass production and use of silvopastoral areas in northern Morocco: A case study of western Rif

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In northern Morocco, silvopastoral areas provide diverse needs for local population and livestock. These pasture represent the most important feed sources for goats in Rif Mountain. This study aims to evaluate biomass production and investigate the use of a silvopastoral area in two representative pastoral areas (Derdara and Loubare) exclusively used by goatherds. For biomass production, we used the method of the reference module. The surveys, on using pasture by breeders, were realized during the biomass evaluation period. The pastoral shrubs, mainly *Arbustus unedo*, *Cistus crispus*, *Cistus monspeliensis*, *Erica arborea*, *Lavendula stoeches* and *Mentha pulegium*, are the main diet of goats. Breeders use pasture throughout the year, except on rainy days where they resort to limbing. The pasture was characterized by an average level of diversity of pastoral flora in Derdara (94 species) and poor floristic diversity in Loubare (65 species) dominated by shrubs. The phytomass productions at Derdara and Loubare were estimated at respectively 1867 kg and 373 kg for dry matter per hectare. The continuous use of pastoral resources and lack of pasture management has considerably reduced the palatable pasture species and has allowed the appearance of low pastoral value species. Statistically significant differences were observed depending on season, with a maximum in the spring and a minimum in the winter, and according to sampling sites are differentiating by intensity of canopy cover. Management actions and use of silvopastoral resources, including a reduction in grazing pressure, should be developed to ensure better productivity and sustainability of these resources.

Keywords: Northern Morocco, Silvopastoral, Goat

43. Use of new technology to estimate the grazing behavior of goats in northern morocco

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In northern Morocco, goats are exclusively or partially conducted in extensive farming systems in which most nutrients from forest pastures. Grazing is associated with daily activities significantly different from those of animals in confinement, such as grazing behavior and distance. Currently, new techniques have been proposed to characterize the feeding behavior of goats on rangelands and throughout the day. These measures are now determined mainly by GPS collars and IceTag, which are used to monitor activity, location and movement in grazing areas. This study was conducted in two representative pastures (Derdara and Loubare) in western Rif. The study concerned the characterization of movement of goats on a silvopastoral pasture through using new technology. GPS collars have allowed us to estimate a distance travelled at 8 km for 10 hours of grazing per day. With IceTag (attached to a rear leg of goat), we estimated the number of steps and time spent standing, lying, and active. IceTags and GPS collars data was used simultaneously to estimate times spent grazing/eating, as well as other behaviors. The study also revealed that the goats consumed mainly of palatable vegetation at ground level. The intersection of several parameters of pastoral assessment with the study of feeding behavior of goats on rangelands will allow us in future to manage better the herd in time and space.

Keywords: Northern Morocco, Goat, Behavior

Posters

44. Effect of the breeder calving detection on C-section complications in Belgian blue cattle breed

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Caesarean section (c-section) is a very common surgery in cattle. In Belgian blue cattle breed, the first indication is the fetomaternal disproportion. In order to prevent dystocia, early elective C-section is systematically performed in this breed (96 %).

Different methods are used to detect early calving. In order to evaluate the different methods and their impact on c-section complications, a survey of 34 questions answered by 73 breeders was conducted in Wallonia.

It appears that 26,03 % of farmers monitor ligamentous laxity, 6,85 % monitor decrease of rectal temperature and 58,90 % perform both. Vaginal probe (4,11 %) and surveillance camera (4,11 %) are not frequently used. The monitoring of rectal temperature and ligamentous laxity are reliable and not expensive comparatively to the automated method. Seventy five percent evaluate cervix dilatation by vaginal palpation. The vaginal palpation is performed with clean disposable gloves (58,93 %) or multiple used gloves (3,85 %), others farmers perform vaginal search with a bare hand with (23,21 %) or without (14,07 %) disinfection. Vaginal palpation is significantly associated with more complications than the others technics. The most frequents are retained foetal membranes (77,42 %), surgical abscesses (75 %) and peritonitis (66,67 %). Breeders performing a vaginal palpation using gloves have more complications than those using bare hand after disinfection.

We herein demonstrate that vaginal palpation is the most frequent technic to evaluate calving and is often associated with postoperative complications due to the lack of hand hygiene.

Keywords: calving detection, ceasarean section, cattle

45. Effect of the method and the period of oil extraction on chemical composition of olive cake in northern of Morocco

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Coding and non-coding variants in HFM1, MLH3, MSH4, MSH5, RNF212 and RNF212B affect recombination rate in cattle

In the northern of Morocco, goat population is the most dominant. His diet is based on forest rangelands. Therefore, it is necessary to diversify and improve its feed calendar. Olive cake is by-product of oil extraction that widely available in the region with important production. It can be a feed resource of ruminant. This resource is produced by three methods and for limited period. This work aims to determine the effect of different oil extraction method and period on the chemical composition of olive cake in northern of Morocco. Twenty-seven samples of olive cake were collected from 3 mills by each extraction method (mechanical pressure, centrifugation 2 and 3 phases) during three periods (November, December and January). The chemical composition (dry matter, fat, mineral matter, crude protein and fibers) of the collected samples was determined by AOAC methods (1997). The extraction method had a significant effect on all parameters of the chemical composition with the exception of the fat and crude protein ($P < 0.05$). The olive cake obtained by centrifugation 2 phases method had lower dry matter and fibers. The period had no effect on chemical composition, which eases the introduction of olive cake in the diet of ruminants. The olive cake of 2 phases is the most suitable for feed but has a reduced shelf life, which requires the development of a suitable conservation mode.

Keywords: Olive cake, Extraction method, Extraction period

46. Innovation along the pig value chain in Benin: an assessment of prevailing discourses through the Q method

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Sustainability and resilience reinforcement drive farmers in Africa to use locally available resources for innovating in order to enhance their own practices. Social innovation may be defined as all new know-how that tackles existing social, economic and environmental challenges for people's advantage. In Benin, the pig value chain (PVC) is a leading chain at the national level. Nevertheless, the recurrent African Swine Fever epizootics since 1997 as well as the lack of organization of PVC actors have been undermining the sector. Therefore innovations, both of technical and organizational natures, are needed to help the sector taking advantage of the present rise in pig meat consumption. The Q-method is a tool mobilized in social sciences to investigate the discourses at play on complex topics in a particular social setting. It is here proposed to use this approach to characterize the perceptions of innovation along the PVC in the Oueme and Plateau provinces in South-east Benin. Firstly, a list of statements about innovations origins, benefits, risks, and social impact was established through focus group discussions (3 groups of 5 persons), of which 55 were selected based on the level of agreement between actors, estimated through the calculation of the Kendall's coefficient on proportional piling exercises. Then, 25 actors along the PVC were selected for individual interviews and statements classification along an 11-grades agreement scale (from -5 to 5). The method allowed describing 3 discourses, distinguished by their level of optimism, criticism and cautiousness facing innovations. This study should usefully shed light on the factors to take into account in the organization of actors to foster innovation facing the present PVC challenges in Benin.

Keywords: Pig value chain, Innovation, Q-method, Discourses, Benin

47. Coding and non-coding variants in HFM1, MLH3, MSH4, MSH5, RNF212 and RNF212B affect recombination rate in cattle

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We herein study genetic recombination in three cattle populations from France, New Zealand and the Netherlands. We identify 2,395,177 crossover (CO) events in 94,516 male gametes, and 579,996 CO events in 25,332 female gametes. The average number of CO was found to be larger in males (23.3) than in females (21.4). The heritability of global recombination rate (GRR) was estimated at 0.13 in males and 0.08 in females, with a genetic correlation of 0.66 indicating that shared variants are influencing GRR in both genders. A genome-wide association study identified seven QTL for GRR. Fine-mapping following sequence-based imputation in 14,401 animals, pinpointed likely causative coding (5) and non-coding (1) variants in genes known to be involved in meiotic recombination (HFM1, MSH4, RNF212, MLH3, MSH5) for 5/7 QTL, and non-coding variants (3) in RNF212B for 1/7 QTL. This suggests that this RNF212 paralogue might also be involved in recombination. Most of the identified mutations had significant effects in both genders with three of them each accounting for approximately 10% of the genetic variance in males.

Keywords: meiosis, cross over, cattle

48. Contribution to the study of some forage species: Nutritional value and effect on the physico-chemical composition of goat milk in Laghouat, Algeria

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The objective of this work is to study the chemical composition and energy value of some fodder plants selected and commonly consumed by Arabia breed goats in Tadjmout area and to determine their effects on the physico-chemical characteristics of raw milk produced by these goats in early lactation. This study encompassed: (1) Forage analysis of 14 steppe plants, one cultivated fodder and 2 complementary feed mixes, to determine the dry matter (DM), mineral matter (MM), organic matter (OM), total azotic matter (MAT) and crude fiber (CF). Also, the energy value was calculated to estimate the energy intake of the consumed diet. (2) Nine goats milk was collected every 15 days from parturition until the 90th post-partum. The milk analysis concerned the total solids extract (EST), the not fatty solids (NFS), the density (D), the freezing point (FP), the fat (FC) and protein contents (PC). The results show a considerable floristic diversity (9 families identified). Plants 1 (unidentified), 2 (*Rubia peregrina*), 3 (*Launea* sp), 4 (*Malva sylvestris*), 5 (family of *Chenopodiaceae*) and 6 (*Foeniculum vulgare* Mill) presented a good energy value (>1UFL/kg of DM), a high MAT rate (15-30%) and low CF (9-16%). The total energy provided by different types of feed (steppe, cultivated and complementary) equals 1.54 UFL/day (this will allow the production of only 2 kg of milk, according to INRA tables). The results of milk physico-chemical analysis showed low PC and variable FC depending on the milk sampling time. The evolution of FC allowed the estimation of the peak lactation at 1 month (minimum value registered in this period equals 3.07%). The feeding effect on the quality of local goat milk in Laghouat is an initial investigation that should be continued on a larger animal sample, throughout the lactation period. The knowledge of environmental factors affecting the quantity and quality of milk, especially season and feeding, is crucial in order to determine the genetic potential of Arabia goat for milk production. **Keywords:** Goat, Arabia, Steppe plants, Milk, Nutritional value, Fat and Protein contents.

49. Participatory evaluation of immunization services against Newcastle disease in village poultry in Kasangulu (R.D.Congo)

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Livestock production in the Democratic Republic of Congo (DRC) meets only 16% of national demand. Imports therefore supply the major cities while rural areas are exposed to recurrent food crises. The farming of chickens by households plays a crucial role in food security in the country. This study proposes the evaluation through participatory approaches a paid vaccination campaign for local chickens against Newcastle disease (ND) and estimated demand for the sustainability of such a service in the town of Kasangulu. The survey included 50 farmers (individual interviews) and 8 persons of public veterinary services (focused group discussion). For proportional piling, it is estimated by the different actors that about 50% of households have chickens Kasangulu, averaging 20.3 hens per household in our sample. The MN is shown as an annual, reaching 76.7% on average mortality rates. The MN creates a largely affirmed concern and almost all of the surveyed farmers (96%) are willing to pay for a vaccination. The first vaccination campaign reached 10 breeders in our sample (20%), of which 8 are convinced of the effectiveness of vaccination. Public veterinary services believe that this campaign has enabled them to gain a working tool, generate financial gain and to have an opportunity to work with small farmers. Factors taken into account in the description by breeders desired immunization services are primarily the route of administration and the qualification of the vaccinator. The maximum possible price of approximately 200 CF dose, the prices (50 to 150 FC) appear as accepted.

Keywords: Participatory evaluation, immunization services, village poultry

50. The "FMV small animal husbandry unit" for students and research

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The "small animal husbandry unit" (SU) of the Veterinary Medicine Faculty (FMV) of the University of Liege is located at the Experimental and Pedagogic Farm (EPF) of the said faculty. Since its creation in 2014 by the Department of Animal Production and with the support of the Dean and the Management of FMV, SU has proposed teaching and research activities. As teaching activities, SU offers FMV students the opportunity to look after and manipulate a variety of animal species (guinea pigs, chicken, rabbits, carrier pigeons, and mini-ponies). Besides, a specific learning activity is organised during which students learn about zootechnical and biological specificities of each species. These learning activities can be coupled to visits to the "smaller animal husbandry" in which black soldier flies (BSF), earthworms and snails are raised. A second objective of the SU is the preservation of native breeds under threat of extinction such as the Ardennaise chicken, Bleu de Ham rabbits and Cravaté Liegeois pigeons. Research activities conducted at SU include various experiments, e.g., studies of growth performances and meat quality of chicken fed soybean meal substituted by BSF larvae, earthworms or other ingredients. These studies necessitate the collaboration between EPF and various FARAH units. Other research activities concern studies carried out by students during their graduate work, e.g., genetic study of chicken combs, effect of herbal diet on growth of guinea pigs, effects of food supplements in laying hens. These experiments received approval from the Ethics Committee of the University. Actually, new pedagogic activities and research projects are examined to better assist the FMV community.

Keywords: The small animal husbandry unit, guinea pigs, chicken, rabbits, pigeons, ponies, Black soldier fly, Snai

51. Inbreeding and genomic runs of homozygosity in Vietnamese local chickens

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Most researches on genetic variety evaluation in Vietnamese local chicken breeds have been based up to now on microsatellite markers (Cuc et al., 2011; Pham et al., 2013). Thanks to the genotyping of ~580,000 SNPs on 95 chickens, this study aimed to assess the genetic diversity in four Vietnamese local breeds (Mia, Mong, Ho and Dong Tao). Inbreeding was examined through the detection of runs of homozygosity (ROH; Curik et al., 2014). The ROHs are long stretches of homozygous genome that are likely to reflect autozygosity. Given that Ho chickens have a small effective population size, more inbreeding and consequently longer ROHs are expected. Conversely, Mia chickens, with a larger effective population size should show less consanguinity and shorter ROHs. As expected, Mia chickens led to less observed homozygosity and less ROHs than the three other breeds. Moreover, when ROHs were subdivided in different lengths, the proportion of individuals having at least one ROH longer than 5Mb was significantly higher in Ho (63%) than in Mia chickens (33%). Given that very long ROHs are particularly associated with recent inbreeding, it seems that many Ho chickens have consanguineous parents. However, no significant difference was observed between the fourbreed concerning the total length of long ROHs (> 5Mb). In conclusion, the analysis of ROHs reported less consanguinity in Mia than in Ho chickens. In each breed, some individuals are found more consanguineous than expected. This study also confirms that ROHs are a useful tool in inbreeding research.

Keywords: Genetic diversity, Inbreeding, Runs of homozygosity

52. Utilisation of Rice Distiller's By-product for Swine Production in Northern Vietnam

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The objective of this study was to investigate the production of rice distiller's by-product and its use as feed for pigs in three provinces (Hai Duong, Hung Yen and Bac Giang) of Northern Vietnam to identify annual supply resource of the by-product as pig feed. The nutrition components of this by-product were analysed. A total of 120 rice alcohol producers classified by production scales (large, medium and small) were interviewed from January to August 2015. Additionally, forty-two rice distiller's by-product samples were collected from investigated areas to determine nutrition components and the effect of storage time (from the first to the seventh day) on quality. Annual rice distiller's by-product production was 17.26, 6.26 and 3.59 tons per household for large, medium and small scales respectively ($P < 0.001$). The dominance of swine quantity in large-scale alcohol producing households in comparison with smaller number of pigs raised in medium and small ones ($P < 0.05$) proved a clear relation between number of pigs in household and alcohol production scales. The amount of by-product used in daily diet of sows in all three scales gradually reduced from pregnant to lactating sows. The utilisation of by-product for fattening pigs was significantly diversified among different scales (33.6%, 29.3% and 25.3% for large, medium and small scales respectively) ($P < 0.05$). The rice distiller's by-product was a rich source of crude protein (26.2%), neutral detergent fiber (33.7%), lactic acid (2.27 %) and gross energy (20.41 MJ/kg DM). Furthermore, its nutrition values were stable under ambient condition during a week ($P > 0.05$).

Keywords: rice distillers' by-product, pig production, Northern Vietnam

53. The effects of timing of insemination and GnRH treatment on pregnancy rates of N'Dama females after induction of oestrus with progesterin

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Given its rustic nature, its butchery quality and its trypanotolerance, the N'Dama breed represents a significant potential for developing African cattle breeding. His genetic improvement involves the use of artificial insemination. The aim of this clinical trial was to quantify the results of pregnancy after treating N'Dama female cattle ($n=168$) using a CIDR® (1.38g of progesterone) inserted for seven days. An intramuscular (IM) PG injection (500µg of cloprostenol) was administered two days before removal of the device. Subsequently, an IM injection of 400UI of eCG was administered when the device was removed. The animals were inseminated respectively 48 (Group 1) and 72 hours (Group 2) after removal of the CIDR®. In both groups, half the animals were treated with 4.2µg of busserlin acetate and the other half with 1ml of physiological saline. Four protocols were thus tested: CIDR-PG-eCG/IA48h, CIDRPG- eCG/IA48h/GnRH+, CIDR-PG-eCG/IA72h, and CIDR-PG-eCG/IA72h/GnR H+. A pregnancy diagnosis has been done by echography 60 days after insemination. The average pregnancy rate was 37.5% ($n=168$). That of the adult cows (43.2%, $n=111$) was significantly ($P < 0.03$) higher than that of the heifers (26.3%, $n=57$). The pregnancy rate observed in animals in Group 2 (48.8%) was significantly higher ($P < 0.002$) than that observed in the animals in Group 1 (26.2%). Physiological condition and the injection of GnRH at the time of insemination had no significant impact on the pregnancy rate. The hormonal protocol using CIDR-PGeCG (400UI) and a timed artificial insemination 72 h later improves the pregnancy rates in the N'Dama female.

Keywords: N'Dama, Reproduction, Progesterins

54. Variation of some metabolic parameters between mating and parturition of nulliparous pregnant Algerian local rabbit does

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Gestation in rabbits lasts for between 28-32 days and when properly managed, female metabolic status of pregnant nulliparous Algerian local rabbit does on average led to five litters per year.

To study the metabolic status of pregnant local rabbit does, blood samplings were performed on twenty-three (23) pregnant nulliparous local rabbit does. Animal were housed individually and raised between mating and parturition. Pregnancy diagnosis was performed by abdominal palpation at 10 days post mating. Once a week, blood samples were collected from the margin ear vein to evaluate serum metabolites (glucose, total protein, urea, triglycerides and cholesterol).

Results showed that serum glucose decreased significantly and progressively between mating and parturition ($p < 0.001$) while serum total protein remained unchanged between mating and day 21, then decreasing significantly at day 28 of pregnancy ($p < 0.05$).

Serum urea increased between day 0 and day 7, remained steady until day 14, then decreased at day 28 of pregnancy ($p < 0.05$). Serum cholesterol unchanged between days 0 and 21 post mating and decreased thereafter ($p < 0.001$), while serum triglycerides increased significantly between days 0 and 21 then decreased at 28 days of pregnancy ($p < 0.001$).

In conclusion, during pregnancy, the metabolic status of Algerian local does vary according to the time. This suggests some ways to improve the control of these metabolites during the reproductive period.

Keywords: Local rabbit does, metabolic status, pregnancy.

55. Effects of dietary protein levels on productivity of pregnant nulliparous local Algerian rabbit does

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In rabbit does at first pregnancy, diet is one of the most important factors for better reproductive success. During this period, does have to fulfil maintenance requirement, fetal growth, and also body growth as rabbit enters into reproduction when reaching 75% of adult weight. In this context, fifty-two (52) nulliparous local rabbit does were used to study the effect of different dietary protein contents on females' performance and their litters at the first parturition. Does received one of the three experimental diets characterized by similar digestible energy (DE) content (2600 kcal/kg) and different digestible protein (DP) contents (15, 17 and 19 % DP/kg for diet L, M and H respectively). Rabbit does' weight and feed intake were controlled each week between mating and first parturition. Diets were supplied individually on an ad libitum basis. Protein level neither showed effects on does live weight between mating and first parturition, nor on weight gain. The litters size and weight at partum were not affected by the treatments. It must be mentioned that the litter size, used as covariate, affected feed intake during pregnancy ($P < 0.05$ at the 2nd week of pregnancy, and trends at the 3rd week and at pre-partum). In conclusion, dietary protein level appears not to affect performance of nulliparous pregnant rabbit does, or characteristics of first litter at partum. Owing to the different physiological status of rabbit does during the next pregnancies; the results should be confirmed on subsequent parities subjected to similar treatments.

Keywords: Local rabbit doe, diet, digestible protein, performance, pregnancy.

Comparative veterinary medicine

56. Post-operative analgesic administration to non-human primates in animal experimentation

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It is widely accepted that animal undergoing invasive experimental procedure should receive an appropriate analgesic regimen consistent with the nature of the procedure and the aims of the research protocol. We reviewed the reported use of analgesics in non-human primates used in research procedures. Pubmed online database was searched for publications from 2010 to 2015 using the key words "macaca" and "surgery" and the results screened according to predetermined inclusion and exclusion criteria. 257 papers were selected. Clear statement about the use of post-operative analgesia was made in only 73 publications but 21 of these did not specify the analgesic agent. When used as a sole analgesic agent, 16 papers reported the use of NSAIDs and 18 of an opioid. Carprofen and buprenorphine were the most frequently used agents. Multimodal analgesia, combining opioids and NSAIDs, was also noted and represented 22% of the totality of analgesia regimen reported. Overall of the 184 publications that did not report any use of analgesia, 117 made a reference to relevant guidelines such as the National Institute of Health Guide for the Use and the Care of Laboratory Animals or the European Directive, both of which require use of analgesics unless they are specifically contraindicated. This review highlights the lack of reporting of analgesia in the peer-reviewed literature, which represents either underreporting or underuse. A similar lack of reporting has previously been noted in laboratory rabbits, rodents and ruminants. Appropriate feedback from reviewers and editors of scientific journals adopting reporting practices such as those outlined in the ARRIVE guidelines are essential.

Keywords: analgesia, experimentation, non human primate

57. Determination of canine long bone strain at failure by digital image correlation

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Introduction: Creating models and simulating long bone fractures by finite element analysis require description of bone behaviour by mechanical parameters. The purpose of this study was to determine the strain at failure (or rupture criterion) of cortical bone tissue issued from canine long bones by digital image correlation (DIC). **Materials and methods:** Canine radii, humeri and femora were harvested from adult dogs (n=4) weighing 20-30 kg, 1,5-8 years of age, euthanized for reasons unrelated to this study. Soft tissues were removed. Bones were wrapped in saline-soaked sponges, stored at -20°C and thawed at room temperature overnight before mechanical testing in compression (radius, femur) and tension (humerus). Canine radii and femora were left intact. The humeral diaphyses were trimmed down to a Dumbbell shaped strip of bone, plotted in conical resin blocs. All specimens were coloured with DIC specific colours (white: Mop 04036; black: Mop 04031) and placed centered in a rotulated jig into the electrohydraulic testing machine (Zwick, 100kN maximum load). Three high speed cameras (AVT Pike, type f032B, Kodak KAI340 - 640X480 pixels cells) were positioned in front of the specimen at three different angles. Testing was performed at a constant speed of 0.01mm/s (trimmed tensile samples), 0.2mm/s (intact radius) and 0.1mm/s (intact femur) in compression. Images and videos were recorded at 100 images/s. Analysis was performed at a resolution of 9x9x2 pixels (radius/ humerus) and 11x11x2 pixels (femur): nine points of maximum strain were selected at maximal load before breakage. **Results:** All specimens fractured through a sudden brittle crack within the thinned part (humeri) or diaphysis (radii and femurs). All specimens fractured in tension. Strain at failure was 1.745% ± 0,091 for humeri in tension, 1.724% ± 0,079 for femora and 1.718% ± 0,079 for radii in compression. Mean strain at failure for all bones tested was 1.73% ± 0,083. **Conclusion:** Strain at failure for canine bone was 1.73%, which is comparable to values obtained in younger men. Based on our work and others, DIC is a suitable and valuable method to measure strain at failure. A strain-based rupture criterion will be useful to create a realistic finite element model of canine long bone fracture pattern for further studies.

58. Utility of quantitative PCR on bronchoalveolar lavage fluids in the diagnosis of Angiostrongylosis in dogs with negative rapid in-clinic assay (Angio Detect Test®)

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Canine angiostrongylosis is now considered as an emerging condition in Belgium. Faecal detection of first-stage larvae is the most frequently used diagnostic method. Because of irregular faecal shedding, sensitivity of this test is suboptimal. Parasite circulating antigens can be detected in blood using an in-house rapid ELISA assay (AngioDetect Test ®). High sensitivity was found in naturally infected dogs when Baermann analysis was considered as gold standard, although the test might be negative in early cases. However, no information about infected cases with negative Baermann test was provided. Moreover, the clinical usefulness of qPCR on broncho-alveolar lavage fluid (BALF) has been recently described in naturally-infected dogs. The aim of this study was to report results of qPCR on BALF, Baermann analysis and in-house rapid test in a small series of dogs diagnosed with pulmonary angiostrongylosis, using qPCR on BALF as gold standard. Six dogs with angiostrongylosis diagnosed by qPCR on BALF were retrospectively included. In each dog, a serum sample was frozen at diagnosis and was retrospectively thawed to perform the in-house rapid test. The rapid device test was positive in only 2/6 dogs. Among the dogs with negative rapid test, Baermann analysis was also negative in 2 cases. In conclusion, even though faecal and rapid tests are currently available and safe, they might be negative in some cases which can be detected by qPCR on BALF. qPCR on BALF should therefore be considered in clinically-suspected cases in which rapid test and Baermann analysis are negative.

Keywords: angiostrongylosis, dog, bronchoalveolar lavage fluid

59. Detection of *Aspergillus fumigatus* by quantitative polymerase chain reaction assays in the bronchoalveolar lavage fluid of dogs with eosinophilic bronchopneumopathy

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Eosinophilic bronchopneumopathy (EBP) is characterized by eosinophilic infiltration of the lung and bronchial mucosa. Hypersensitivity to aerosolized antigens is suspected, while the exact inciting antigens remain presently unidentified. In humans, *Aspergillus* spp. has been proposed as trigger for allergic bronchial disease. In dogs, the association between *Aspergillus* and inflammatory bronchopneumopathy has not yet been explored. The aim of this study was to investigate by quantitative PCR (qPCR) the presence of *Aspergillus* spp. and *Aspergillus fumigatus* in bronchoalveolar lavage fluid (BALF) samples obtained from dogs affected with EBP, compared with healthy dogs and dogs with chronic bronchitis (CB). For this purpose, BALF samples collected from 23 dogs with EBP (mean age = 4.5 years), 14 healthy dogs (6.3 years), and 21 dogs with CB (7.2 years) were retrospectively included. qPCR results were expressed as Ct values; Ct value above 32.1 corresponding to very low DNA copy numbers. *Aspergillus* spp. qPCR yielded positive results in two EBP dogs (Ct = 34 and 33), 1 CB dog (Ct = 36) and one healthy dog (Ct = 33), while *Aspergillus fumigatus* qPCR was only positive in the same 2 EBP dogs (Ct = 32), but not in healthy and CB dogs. Based on these results, an association between *Aspergillus* and canine EBP appears unlikely. Nevertheless, absence of *Aspergillus* genetic material in BALF samples cannot definitively rule out its implication in EBP. Future studies should include investigation of serum *Aspergillus*-specific antibodies in order to definitively elucidate the role of *Aspergillus fumigatus* in canine EBP.

Keywords: aspergillus, eosinophilic bronchopneumopathy, dogs

60. Oxygraphy as an indicator for differentiation state of stem cellsCeusters J.^{1,2}, Franck T.², Niesten A.², Graide H.^{1,2}, Dupont J.², Serteyn D.^{1,2,3}¹ *RevaTis SA, Rue de la Science 8, Aye, Belgium*² *Center for Oxygen, Research and Development (CORD), FARAH, ULg*³ *Department of Clinical Sciences, Equine Surgery, Faculty of veterinary medicine, FARAH, ULg*

Introduction: Mitochondria are the main source of energy in aerobic conditions. Each type of cells have their own metabolism but what about the differentiation state of stem cells?

Aims: To show if the shift from glycolytic to oxidative metabolism when stem cells differentiate will be observable in oxygraphy and if the aging of the cells will modify these parameters.

Method: Undifferentiated equine skeletal muscle derived stem cells obtained by a patented method [WO2015091210] were cultured from passage 5 to 10. Cells from each of these passages were in parallel differentiated into osteoblasts by culturing them in specific differentiation medium during 5 to 7 days. Undifferentiated and differentiated cells were collected and a phosphorylation control protocol was applied in oxygraphy to evaluate their routine respiratory state (Routine), their non-phosphorylated (Leak, after addition of oligomycin) and their noncoupled (ETSmax, after titration with carbonyl cyanide *p*-(trifluoromethoxy) phenylhydrazine (FCCP)) respiratory capacities.

Results: The Routine, Leak and ETSmax respiratory parameters were respectively about 3, 4.8 and 3 times higher for osteoblasts than for undifferentiated stem cells. Between passage 5 and 10, we observed also an augmentation of Routine, Leak and ETSmax respectively about 2.5, 1.4 and 1.8 times for osteoblasts and about 3, 2.6 and 2.8 for undifferentiated stem cells.

Conclusion: The oxygraphy allows us to observe the metabolic shift happened during the differentiation of stem cells. The aging of cultured cells can also be monitored by oxygraphy and seems to be more important for undifferentiated stem cells, which could reflect a loss of pluripotency.

Keywords: oxygraphy, stem cells, differentiation

61. Teaching veterinary radiology: does comparison helpEtienne A.L.¹, Delguste C.², Busoni V.¹¹ *Diagnostic Imaging Section, Department of clinical sciences, Faculty of Veterinary Medicine, FARAH, ULg*² *General Services of Faculty of Veterinary Medicine, FARAH, ULg*

As described in human medicine, by comparing radiographs with and without pathology, veterinary students could learn to discriminate relevant information to recognize the disease. We hypothesized that exposure to a training by side-by-side comparison of normal to abnormal radiographs would lead to higher visual diagnostic accuracy and possibly to a higher ability to describe the features of a known disease on canine thoracic radiographs. Twenty veterinary students were randomly assigned to either a group that compared radiographs showing thoracic disease with normal images (group 1) or to a group that only studied abnormal radiographs (group 2). The procedure consisted in three experimenter-supervised phases: 1. training, 2. visual recognition test, 3. feature description test. In the training phase, each screen showed two radiographs with the name of the disease present in each. In the group 1, a radiograph of a patient and a normal image were shown next to each other. In the group 2, two radiographs of the same disease were shown next to each other. A Mann-Whitney test was used to compare the success rates of groups of students. On visual recognition test, there was no statistical difference in visual diagnostic accuracy between groups. Students of group 1 had a higher accuracy for normal thoracic radiographs. On feature description test, no significant effect of comparison learning was found, but focal diseases were better described than diffuse diseases. Results show that comparison with normal images did not help in recognizing or describing thoracic pathologies but helps in identifying normal images.

Keywords: radiology, teaching, comparison

62. NDS27, the water-soluble derivative of curcumin, is better than curcumin to inhibit NADPH oxidase activity of equine neutrophils

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Many horse pathologies with fatal issue are associated with uncontrolled activation of PMNs that involves the activation of NADPH oxidase (Nox2) responsible for superoxide anion (O₂⁻) from which derives other reactive oxygen species. PMNs stimulation leads to the assembly of the cytosolic subunits of NADPH oxidase with the membrane flavocytochrome b558, forming the active enzyme.

The aim of the study was to compare the effects of curcumin alone solubilised in DMSO with NDS27, a water-soluble form of curcumin being complexed with lysine and hydroxypropyl- β - cyclodextrin (HP β CD), on the Nox2 activity.

The activity of Nox2 on whole PMNs was measured by the extracellular ROS production using lucigenin-dependent chemiluminescence. A cell-free assay and an innovative technique (EquiNox2) were used to evaluate the effect of curcumin and NDS27 on the Nox2 assembly.

Likewise, curcumin and NDS27 inhibit the extracellular ROS production suggesting that lysine and HP β CD do not affect the antioxidant potential of NDS27. The cell-free assay showed that the presence of curcumin or NDS27 inhibits the Nox2 assembly. But, at 5.10⁻⁵M, both compounds had different behaviours: a complete inhibition (100 %) for NDS27 and a lower (45%) for curcumin. Equinox2 showed that curcumin, NDS27 but also HP β CD disturb the membrane fraction of Nox2 with an impact on its activity. Curcumin alone by its antioxidant and lipophilic properties have already the ability to inhibit the Nox2 action. But, thanks to HP β CD, NDS27 seems to improve the curcumin incorporation into the PMN membranes resulting in a better disturbance of Nox2 assembly and activity.

Keywords: NDS27, curcumin, NADPH oxidase

63. Triglyceride concentration and body condition score are markers of fat-induced metabolic changes in healthy non-obese dogs

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Obesity is a health issue in dogs. The body condition score (BCS) system allows the assessment of fat accumulation, but is rather subjective and imprecise. Serum triglyceride (TG) concentrations increase in obese dogs and can be accurately measured. This study aimed at comparing BCS and TG as independent markers of metabolic disruption in healthy dogs. 534 dogs from 9 breeds were included in the study. They were aged from 2 to 6 years old. A BCS (using a 1-to-9 scale) was assigned and those under 2 or over 7 were excluded. Health status was checked. Serum concentrations of free fatty acids (FFA), cholesterol, TG, insulin, fructosamine, glucose, cortisol, aldosterone and C-reactive protein (CRP) were measured. Two different two-way ANOVA analyses were used to compare dogs, firstly, divided in BCS categories ("overweight", with BCS>5, versus "lean", with BCS= \leq 5) and, secondly, in TG categories ("hypertriglyceridemic", with triglycerides >0.5g/L, versus "normotriglyceridemic", with triglycerides= \leq 0.5g/L). Both analyses were corrected for the effect of the breed. A Fisher's exact test was performed in order to look for an association between the BCS and triglyceride categories. Independently of the breed, "overweight" dogs had significantly higher serum cholesterol and insulin and were older than "lean" dogs. "Hypertriglyceridemic" dogs had significantly lower FFA and significantly higher cholesterol, insulin and fructosamine than "normotriglyceridemic" dogs. Being "hypertriglyceridemic" or "normotriglyceridemic" was not associated with a specific BCS category. Both BCS and serum TG concentration allow, independently, recognition of alterations in lipid and glucose metabolism in healthy dogs.

Keywords: Obesity, Dog, Triglycerides

64. Hydrosoluble Curcumin Enters in Equine Myoblasts and Protects their Mitochondria Against the Adverse Effects of Myeloperoxidase

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Objective: Patented hydrosoluble form of curcumin, NDS27, has the ability to inhibit the activity of myeloperoxidase (MPO). Previous study reported that active MPO can enter into equine myoblasts and decrease mitochondrial respiration. The aim of this study was to test if curcumin from NDS27 can enter into myoblasts and protect their mitochondrial functions.

Methods: Primary equine myoblasts were incubated 2h with equine MPO (250 ng/ml), NDS27 (10-5 M) or their combination. Control was made with non-treated cells. The mitochondrial function was assessed by oxygraphy. Myoblast fractions (membranes/nuclei, mitochondria and cytosol) were separated by differential centrifugations. The activity of MPO, determined by SIEFED, and the assay of curcumin content, performed by HPLC, were evaluated in each fraction.

Results: MPO activity increases in membrane/nuclei and mitochondrial fractions of MPO treated cells. When N DS27 was added to MPO, this activity decreases (23%) in the mitochondrial fraction. Oxygraphy results highlight that NDS27 partially inhibits the negative effect of MPO on mitochondrial routine (23%) and maximal respirations (31%). HPLC dosage shows that curcumin is mainly present in membranes/nuclei (94.5%) then in mitochondria and cytosol (3% and 2.4% respectively).

Conclusion: These results showed that curcumin from NDS27 enters in muscle cells, interacts with membranes and could protect mitochondria against the adverse effect of MPO on oxidative phosphorylation. These abilities open perspectives for using NDS27 as therapeutic control of inflammatory pathologies, vehicle for drug delivery and regenerative medicine.

Keywords: curcumin, equine skeletal myoblasts, myeloperoxidase

65. The Isoflurane-Sparing Effects Induced By Constant Rate Infusions Of Fentanyl-Lidocaine-Ketamine And Sufentanil-Lidocaine-Ketamine In Dogs Undergoing Total Ear Canal Ablation with Bulla Osteotomy – A Preliminary Study

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The total ear canal ablation with bulla osteotomy (TECABO) is the gold standard treatment for end-stage otitis in dogs. However, it often induces a painful stimulus that is poorly controlled by local anaesthetics and systemic opioids and that requires a high end-tidal isoflurane concentration (ETIso). The multimodal analgesia provided by constant rate infusions (CRI) of fentanyl-lidocaine-ketamine (FLK) and sufentanil-lidocaine-ketamine (SLK) may result in an isoflurane-sparing effect, although this hypothesis had never been tested. This study aimed to compare the ETIso during CRI of FLK and SLK in dogs undergoing TECABO. Four dogs were premedicated with a bolus followed by a CRI of lidocaine (3.6 mg/kg; 3.6 mg/kg/h) and ketamine (0.6 mg/kg; 0.6 mg/kg/h) associated with fentanyl (FLK; 3.6 µg/kg; 3.6 µg/kg/h) or sufentanil (SLK; 0.72 µg/kg; 0.72 µg/kg/h). After 5 minutes, anaesthesia was induced with propofol and maintained with isoflurane in 100 % oxygen (4 L/min). During dissection, the ETIso was decreased by 0.2% every 10 minutes until observing spontaneous movements and/or a 20% increase in heart rate, arterial pressure or respiratory rate. At this point, propofol (1 mg/kg) was administered and the ETIso was increased in 0.1% from the former value, which was maintained throughout. The ETIso was assessed prior to the beginning of surgery (baseline) and every 10 minutes during surgery. During FLK, the ETIso increased from 1.35±0.07% at baseline to 1.45±0.07%, and during SLK the ETIso decreased from 1.25±0.07% at baseline to 0.63±0.04%. The preliminary results suggest that SLK, but not FLK, may have an isoflurane-sparing effect.

Keywords: opioid, pain, multimodal analgesia

66. Effect of unipodal vs bipodal stance on radiographic evaluation of forefeet in horses

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Introduction/Purpose: Lifting the contralateral forelimb is often used as a method of restraint in horses. This experimental study was conducted to evaluate the effect of unipodal vs bipodal stance on several radiographic parameters in equine forefeet.

Methods: Seven non-lame horses were randomly selected. Lateromedial (LM) and dorsopalmar (DP) projections were obtained on both forefeet, squarely placed on blocks, using 2 x-ray generators in a fixed lateral and dorsal position. Radiographs of each foot were made in a bipodal stance, immediately followed by the same protocol in an unipodal stance. Several measurements were made: distal interphalangeal joint (DIPJ) space width on both projections; mediolateral joint balance on DP projections; extensor process-to-middle phalangeal condyle (PE-P2) distance and deep digital flexor tendon (DDFT) angle on LM projections. A matched pairs design, student's t-test with a 95% confidence level and ANOVA were used to test for statistical significance.

Results: Compared to a bipodal stance, lateral DIPJ space width was significantly reduced on unipodal DP views, whereas mediolateral joint imbalance and to a lesser extent medial DIPJ space width were significantly increased. The PE-P2 distance as well as the DDFT angle were significantly increased in unipodal LM views, indicating a higher degree of DIPJ flexion in that condition.

Discussion/Conclusion: Unipodal stance significantly affects the mediolateral balance of the DIPJ on DP radiographs and significantly alters the phalangeal axis on LM radiographs. These findings suggest that stance should be carefully taken into consideration when radiographically evaluating equine forefeet, especially if assessing foot balance and conformation.

67. Cardiovascular response to training and competition high-level eventing horses

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International eventing competitions require extreme fitness of the horse. To prepare horses for eventing competitions appropriate training is mandatory. The purpose of this study was to identify parameters measured during training and competition, which provide information on fitness and adequacy of training used. Data of 187 1* to 4* eventing horses were collected during 410 training sessions and during 916 cross country competitions. Duration, covered distance and speed during cross country respectively training were measured by GPS devices. Heart rate (HR) was recorded continuously and blood lactate concentration (BLa) was determined 10 minutes after the effort. Statistical analysis included ANOVA with a level of significance set at $p \leq 0.05$. The exercise during competition and training are significantly different between levels. Duration and distance during training increase with increasing level. The competition level has a significant effect on HR and BLa. In competition, HR and BLa increases with increasing level. During training, the HR and BLa decrease with increasing level. On 1* and 2* level, the BLa after training exceeds that after competition, while on 3* and 4* level it is considerably lower compared to competition. In previous investigations, lower exercise intensities during training than in competition have been observed, suggesting that horses were inappropriately trained. Conversely, horses investigated here underwent a training which seemed appropriate. The continuous recording of HR during cross country shows that the majority of metabolic requirements during cross country is anaerobic. Evaluation of physiological response to training and competition through implementation of measurement technology allows an assessment of fitness and appropriateness of training.

Keywords: horse, eventing, fitness

68. Physiological response to training and competition in 1-star to 4-star eventing horses

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Purpose: Appropriate training and competition management of horses competing at international eventing competitions necessitates information about exercise intensities during training and competition as well as fitness. The purpose of this study was to identify parameters whose measurement can be largely integrated into the daily training and competition routine and which provide information on adequacy of training used.

Material and Methods: Training and competition intensities were examined using data from 181 eventing horses over a period of six years. Data were collected from 410 training sessions and 863 Cross Country rides, including distance and speed, continuous heart rate (HR) and blood lactate concentrations (BLC). The altitude profile was recorded at each session.

Results: The ANOVA indicated a significant effect of competition level on HR ($p < 0.001$) and BLC ($p < 0.001$). Under competition conditions, HR and BLC increased from 1-star to 3-star level, while under training conditions, they decreased with increasing level. On 1-/2-star level, BLCs after training exceeded those after competition. Reverse applied for 3-/4-star level. At 3-star level, the percentage of HRs above 200 bpm was considerably greater than on lower levels (1-star: 33%; 2-star: 54%; 3-star: 94%). The altitude profile had a significant effect on HR ($p < 0.001$) and BLC ($p < 0.001$).

Conclusion and practical application: Implementation of measurement technology allows an assessment of the different impacts on exercise intensities and should be more commonly used to assess appropriateness of training and competition management in eventing horses. Thereby a valuable contribution to health maintenance and welfare of the horses could be made.

Keywords: exercise physiology, horse, eventing

69. Intradiscal injection of muscle-derived mesenchymal stem cells in the intervertebral disc of dogs affected by natural disc degeneration: clinical safety and intervertebral disc imaging assessment

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Introduction/Purpose: The effects of mesenchymal stem cell (MSCs) transplantation on intervertebral disc (IVD) degeneration (IVDD) has been evaluated by pre-clinical animal trials. According to preliminary histological results and to IVD imaging assessment, MSCs injection stops IVDD. However, a systematic study on canine naturally IVDD using autologous muscle-derived MSCs is still lacking. The aims of this study were to evaluate the clinical effects of intradiscal injection of muscle-derived MSCs and its effects on IVD imaging features. **Methods:** Eight experimental Beagles dogs were included with the approval of the University's Animal Care and Use Committee. Inclusion criteria were a naturally degenerated lumbosacral IVD detected on magnetic resonance (MRI) images and the obtaining of 3×10^6 autologous muscle-derived MSCs. Mesenchymal stem cells diluted in 0.2 ml of Hypothermosol-FRS were injected in the lumbosacral IVD. Thirteen IVD imaging parameters were assessed on computed tomography (CT) and MRI examinations, before and 2 months after the procedure. A linear model with a mixed procedure was used for statistical analysis ($P\text{-value} \leq 0.05$). Clinical examinations were performed regularly for one month after the procedure. **Results:** Six dogs met the inclusion criteria. The remaining 2 dogs did not undergo intradiscal injections, but were used as control group. No complications were recorded. No statistically significant changes of IVD imaging features were noticed. **Discussion/Conclusions:** Lumbosacral intradiscal injection of muscle-derived MSCs is clinically safe and it is not associated with any progression of the imaging features of IVDD. Further studies are needed to assess its efficacy as treatment for IVDD.

Keywords: Mesenchymal stem cell, Intradiscal, injection

70. Modified parasacral approach of an ultrasound-guided sciatic nerve block in beagle dogs

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Objective: Develop and describe a new ultrasound-guided proximal approach to block the sciatic nerve at the parasacral level in beagle dogs.

Materials and method: In vitro: Left and right sciatic nerves of seven beagle cadaver were located using an out-of-plane ultrasound-guided approach. After parasacral localisation of the sciatic nerve, methylenblue solution (0.2ml) was injected perineurally. The sciatic nerves were dissected to complete accuracy of the injections. In vivo: The localisation of the sciatic nerve was repeated in seven experimental beagle dogs. After randomisation, 0.2 ml/kg of levobupivacaine 0.5% (1 mg/kg) was injected perineurally (Leg L) and 0.2ml/kg of saline 0.9% (Leg C) was injected on the contralateral leg. Locomotion, proprioception (knuckling test) and sensitivity (pinprick test) was evaluate on each leg.

Results: In vitro: Out of the 14 injections, 9 (64%) were considered positive as the dye was staining the sciatic nerve over ≥ 0.5 cm. In 4 injections (29%) perineural staining of tissue surrounding the nerve was observed. In one case (7%) the injection was considered negative. In vivo: It was not possible to localise and perform the injections in one obese dog (body condition score 8/9). In Leg L, proprioception was slightly delayed in 3 dogs, reaction to pinprick was decreased in all dogs and locomotion was impaired in all but one dog. No changes in any of the test were observed in Leg C. All tests had returned to baseline values latest 8 hours after sciatic nerve block.

Conclusion: The modified parasacral approach to the sciatic nerve seems suitable in beagle dogs. Further studies should evaluate this approach in clinical cases.

Keywords: parasacral, sciatic, dog

71. Impact of feeding and housing on the development of osteochondrosis in foals - a longitudinal study

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Osteochondrosis is a developmental orthopaedic disease (OC) due to a failure of the endochondral ossification in epiphyseal plates and joint cartilage. This may induce the presence of fragments, fissures or subchondral bone cysts within the growth cartilage. The detachment of these fragments in the articulation is referred to as osteochondrosis dissecans (OCD). Overall one third of the equine population may be affected but prevalence as high as 44% have been reported in Europe. Affected horses commonly show pain, lameness and reduced performance. OCD is influenced by an interaction of different factors. Housing conditions and type of feeding of the foals and pregnant mares have been identified as major risk factors. A diet rich in concentrates (high energy content food) during pregnancy leads to an increased incidence of OCD. The aim of this study was to investigate the evolution of radiological OCD status (recovery or presence of the disease) in foals from 6 to 18 months in relation to the type of feeding and housing conditions. These factors and OCD status were obtained by a questionnaire and radiological examination, respectively. This allowed dividing the foals into four groups according to the initial OCD status and the evolution of the condition. As a result, foals fed with concentrates show a higher probability to develop OCD lesions ($p=0.06$), while foals not receiving concentrates, have a higher probability to heal from existing OCD lesions ($p=0.001$). This study supports the theory that management factors such as feeding or housing may influence the evolution of OCD.

Keywords: Osteochondrosis, Horse, orthopedic

72. Evaluation of distal interphalangeal joint synovial effusion on radiographs: an ex-vivo study on 12 equine feet

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Introduction/Purpose: This study aimed to establish sensitivity, specificity, predictive values and accuracy of radiographs in detection of DIPJ synovial effusion on 12 isolated feet and to correlate degree of effusion to a radiographic score. **Methods:** Seventy-two radiographs were obtained on 12 distal forelimbs of horses euthanized for reasons unrelated to the study. Isolated limbs were placed to simulate standing position. The joint was emptied and 4 consecutive lateromedial views were taken before and after filling the joint with a total of 3, 6 and 12 ml of water. Slightly oblique views were also taken after maximal joint filling. The radiographs were randomized and presented to 4 readers for blinded scoring (0 - 3). Sensitivity, specificity, predictive values and accuracy were calculated on lateromedial views for absence or presence of effusion. Correlation between injected volume and radiographic scores was also calculated. **Results:** Radiographs identified synovial effusion with a sensitivity of 0.81, specificity of 0.83, positive predictive value of 0.90 and negative predictive value of 0.69. Accuracy was calculated at 0.82. There was a positive correlation between injected volume and radiographic scores. Nondistended joints had a higher percentage of correct scoring compared to distended joints. A trend toward underestimation of distension was seen on oblique views. **Conclusion:** Results of this study suggest that lateromedial view of the equine foot can be considered reliable to assess presence and degree of DIPJ effusion on isolated limbs. However, on clinical cases radiography does not allow to discriminate fluid effusion from proliferation of the synovial membrane.

Keywords: equine DIPJ, synovial effusion, radiography

73. A water soluble form of curcumin (NDS27) reacts with superoxide anion produced by SIN-1 and KO₂: Optical and EPR studies

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As primary reactive oxygen species (ROS), superoxide anion (O₂⁻) plays a crucial role in redox process causing cellular damages with subsequent reactivity towards molecules of interest like polyphenols. Curcumin, a yellow powder turmeric, is known for its antioxidant, antiinflammatory and anti-carcinogenic properties. However, due to the low solubility and bioavailability of curcumin, we have prepared a water soluble form of curcumin (NDS27: complex of curcumin lysinate with hydroxyl-propyl- β -cyclodextrin) and demonstrated its good antioxidant and antiinflammatory activities on cell-based assays and on in vivo animal model. The key factor responsible of the activity of curcumin is its ability to scavenge ROS and RNS but controversial works on its direct O₂⁻ scavenging effect were reported. This study aimed to investigate the precise mechanism of action of NDS27 towards O₂⁻ radical produced from two main producers: potassium superoxide (KO₂) and 3-Nmorpholinolinosydnonimine (SIN-1) by optical and electron paramagnetic resonance (EPR) spectroscopies. By absorption, addition of 1 mM KO₂ in methanol/H₂O (60:40, v/v) mixture containing 2.5 10⁻⁵ M NDS27 resulted in the appearance of new peak (475 nm) compared to NDS27 alone (430 nm). Similarly, a typical EPR spectrum (DMPO-OOH) was obtained by addition of 1 mM KO₂ in MeOH/water which was dose-dependently inhibited by NDS27. Kinetic studies of 10⁻⁵ M SIN-1 decay were carried out overtime at room temperature and 37° C in aqueous media (pH 7.5 and 8) by UV-visible. Kinetics show changes overtime with decrease at 292 nm and increase at 278 nm. The presence of 10⁻⁵ M NDS27 strongly reduced the SIN-1 decay.

Keywords: superoxide anion, curcumin, EPR

74. Sonoelastography of the canine patellar tendon: feasibility, repeatability and reproducibility

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Objective: The aims of the present study were to describe the sonoelastographic characteristic of the patellar tendon in clinical healthy dogs, to investigate if sonoelastography (EUS) is a feasible, repeatable and reproducible technique for the evaluation of the patellar tendon and to establish the normal elastogram of the patellar tendon in healthy dogs. **Materials and Methods:** 14 dogs were considered in this study. Each dog had a normal physical and orthopaedic examination, a normal blood workup and a normal B-mode patellar tendon ultrasonographic examination in both legs. EUS was performed by two operators. Ultrasonography and EUS were carried out using an Esaote My Lab™ Class C ultrasound machine equipped with the ESAOTE ElaXto software and a 12-18 MHz linear transducer. ROIs were drawn in order to measure the softness (Eix-t%*sft*) of the tissue. Categorical, qualitative data analysis was performed using a weighted kappa statistic for repeated evaluations by the same and by a different observer. **Main results:** Qualitatively, the 28 patellar tendons evaluated were predominantly soft (red), with a mean \pm SD Eix-t%*sft* of $94.9\% \pm 9.3$. Repeatability was 86.2%, with a weighted kappa of 0.64 (good), for the former operator and 83.3%, with a weighted kappa of 0.53 (moderate), for the latter. Reproducibility was 86.2%, with a weighted kappa of 0.65 (good). **Conclusion:** This study suggests that the normal canine patellar tendon showed a highly soft and homogeneous elastogram, and that EUS is a feasible, repeatable and reproducible imaging modality to evaluate tissue strain and softness/stiffness in the patellar tendon in dogs.

Keywords: sonoelastography, ultrasound elastography, canine patellar tendon

75. Can serum progesterone be used to decide altrenogest supplementation discontinuation in problem pregnancies in mares?

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Use of oral altrenogest as exogenous progesterone (P4) in pregnant mares is a widespread practice, but timing when it can safely be stopped is frequently uncertain. The aim of this study was to determine if timing of altrenogest administration discontinuation could be based on the blood concentration of P4.

Data were obtained from 7 mares in seasonal anoestrus and in dioestrus. Mares were treated during 12 days with 0.044 mg/kg of Regumate® (Intervet, Boxmeer, Netherlands) and blood was collected at day 0, 6, 12, 18. P4 concentration was obtained by radioimmunoassay and altrenogest by liquid chromatography-mass spectrometry. Differences in P4 and in altrenogest between different sampling days were determined by Friedman non parametric test.

Concentrations of altrenogest before treatment were almost not detectable. At day 6, they were significantly higher ($p < 0.05$), and were back to basal at day 18, in both groups. P4 concentration was basal (< 0.4 ng/ml) all through the experiment in the anoestrus group. In the dioestrus group, progesterone was variable from one mare to another, but it was significantly higher in mid-dioestrus as compared to the time of the impending next oestrus.

No cross-reaction between P4 and altrenogest assays was observed. Despite the treatment, the variation of P4 over time was as expected in both groups. Altrenogest was no longer detectable 6 days after the last administration.

Our experiment shows that P4 levels assayed by RIA can be trusted to evaluate if the secondary CL₁ can maintain the pregnancy while altrenogest supplementation is discontinued.

76. Methylenecyclopropyl acetic acid-CoA but not hypoglycin A alters the mitochondrial function of equine skeletal muscle cells: preliminary studies

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Atypical myopathy (AM) in grazing horses results from ingestion of hypoglycin A (HGA) contained in seeds and seedlings of *Acer pseudoplatanus*. Once ingested, HGA is metabolized into methylenecyclopropyl acetic acid-CoA (MCPA-CoA) which inactivates short-chain and medium-chain dehydrogenases of the β -oxidation system and competitively inhibits the carnitine-acyl CoA transferase system required by long chain fatty acids to enter the mitochondria. Therefore, AM results from muscle energy deprivation due to inhibition of the lipid metabolism within the mitochondria. Despite the central role of this organelle in AM pathophysiology, the impact of HGA poisoning on the mitochondrial oxidative phosphorylation (OXPHOS) system has been overlooked. Objective – To test the effect of HGA and MCPA on mitochondrial respiration while initiating OXPHOS by feeding the electron transfer system (ETS) with NADH and FADH₂ linked substrates which are used by the mitochondrial OXPHOS to produce ATP. Materials and method – High-resolution respirometry (HRR) was applied to measure oxygen consumption of cultured equine skeletal muscle cells in response to different energetic substrates (examples: pyruvate, succinate, glutamate...). Then, the effect HGA and MCPA directly added into the respiration medium was evaluated. Results – In cultured equine myoblasts, MCPA but not HGA markedly inhibited ADP-stimulated respiration supported by substrates that do not undergo β -oxidation. Conclusion – These results indicate that MCPA toxicity is not restricted to alteration of the lipid metabolism and suggest a direct action of MCPA on the ETS.

Keywords: Equine atypical myopathy, *Acer pseudoplatanus*, mitochondria

77. Computed tomographic findings of a far lateral lumbar disc extrusion

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Introduction: The aim of this case report is to describe computed tomographic (CT) findings of a far lateral lumbar disc extrusion (FLLDE) in a dog.

Methods: A six-year-old Beagle was presented with a left hind limb pain and shivering. Clinical examination revealed only a left hind proprioceptive deficit without pain at palpation.

Results: A pre- and post-contrast CT scan and a myelogram of the lumbosacral spine were performed. A small homogeneous hyperattenuating (350HU) ovoid structure, well circumscribed by an even hyperattenuating rim (1000HU), was observed at the left lateral aspect of L6-L7 intervertebral disc space. The L6-L7 annulus fibrosus had a normal attenuation and seemed intact apart from a very thin hyperattenuating line next to the ovoid structure. Perineural fat was not observed and contrast enhancement and thickening were visualized at the level of the left sixth lumbar nerve root. No compression of the spinal cord was observed at this level on the myeloCT. These findings were suggestive of a dystrophic mineralization or an osteochondromatosis. At surgery some mixed gelified calcified material consistent with disc material was removed at the level of the left L6-L7 nerve root tract. The histological analysis confirmed the presence of degenerated herniated vertebral disk.

Discussion/Conclusions: In dogs, CT is often used to assess spinal diseases. This case report is the first describing CT findings of a FLLDE in dogs. In conclusion, FLLDE should be included in the differential diagnosis of a calcified ovoid structure lateral to the spine even if the annulus fibrosus appears normal.

78. Assessment of chemokine CCL2 in canine urogenital tumours and its potential as target for treatment

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Introduction: Chemokine CCL2 is a chemotactic cytokine recruiting monocytes, releasing growth factors and promoting adhesion in vascular endothelium. Elevated serum and urinary CCL2 levels as well as expression of its receptor (CCR2) have been associated with oncogenesis of urinary malignancies in humans. The implication of CCL2 has not been investigated in canine urinary transitional cell carcinoma (TCC). The aim of this study was to compare CCL2 serum and urine levels (expressed as creatinine ratios) in control dogs, dogs with urogenital non-neoplastic disease, and dogs with TCC.

Materials and methods: CCL2 ELISA test was performed on urine and serum of healthy dogs (n=20), dogs with cystitis (n=16), prostatitis (n=10), benign prostatic hyperplasia (BPH) (n=10), and TCC dogs with (n=14) or without metastasis (n=9). Mann-Whitney tests were performed to detect significant differences.

Results: CCL2 serum and urine levels were significantly higher in diseased dogs compared to controls (both $P < 0.001$). In diseased animals, dogs with TCC had significantly higher serum and urine CCL2 (both $P = 0.001$) than dogs with cystitis or BPH/prostatitis. No significant difference in serum and urinary CCL2 level was found between dogs with cystitis and dogs with BPH/prostatitis. Surprisingly, animals with metastases showed a significantly lower serum CCL2 compared to the non-metastasised tumour group ($P = 0.007$). Weight, age and sex were not related to serum and urinary CCL2.

Conclusions: CCL2 is elevated both in serum and urine in dogs with all kind of urogenital diseases; however, higher levels are found in dogs with urinary TCC. As such, the CCL2/CCR2 axis might serve as a potential therapeutic target.

Keywords: transitional cell carcinoma, CCL2, dog

79. Imaging findings of a stifle osteosarcoma in an immature dog

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INTRODUCTION: Osteosarcoma is the most common primary bone tumour in dogs. This tumour is highly malignant and has a high metastatic rate. The large and giant breeds are predisposed, especially Rottweilers and Great Danes. The 7 to 9 years old dogs are more concerned with a peak at 7 years old. A small peak also exists at 2 years old.

CASE REPORT: A 15-month-old female neutered Golden Retriever was referred to the University of Liège with a one-month history of left pelvic limb lameness. On physical examination, there was a severe lameness with a swelling of the left stifle and a positive cranial tibial thrust. A cranial cruciate ligament incompetence was suspected and radiographs were performed. A monostotic aggressive bone lesion was observed in the distal metaphysis of the femur. Ultrasound-guided fine needle aspirates were made but they were inconclusive. Surgical biopsies were then obtained and histopathology confirmed the osteosarcoma. The owners declined chemotherapy and amputation. The dog was discharged with a symptomatic treatment and he was euthanized one month later because of deterioration of his general condition.

DISCUSSION/CONCLUSIONS: Osteosarcoma should be considered in young dogs with lameness. The treatment is the same for both young and old dogs but the prognostic is worse for young dogs compared to older ones.

Keywords: osteosarcoma, dog, imaging

80. Non-implementation of relevant guidelines in peri-anaesthetic care of rats used in stroke models

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Numerous studies using rats in stroke models have failed to translate into successful clinical trials in humans. The Stroke Therapy Academic Industry Roundtable (STAIR) has produced guidelines on the rodent stroke model for preclinical trials in order to promote the successful translation of animal to human studies. These guidelines also underline the importance of anaesthetic and monitoring techniques. The aim of this literature review is to document whether anaesthesia protocols (i.e., choice of agents, mode of ventilation, physiological support and monitoring) have been amended since the publication of the STAIR guidelines in 2009. A number of articles describing the use of a stroke model in adult rats from the years 2005 and 2015 were randomly selected from the PubMed database and analysed for the following parameters: country where the study was performed, strain of rats used, technique of stroke induction, anaesthetic agent for induction and maintenance, mode of intubation and ventilation, monitoring techniques, control of body temperature, vascular accesses, and administration of intravenous fluids and analgesics. Results show that there were very few differences in the anaesthetic and monitoring techniques used between 2005 and 2015. In 2015, significantly more studies were performed in China and significantly fewer studies used isoflurane and nitrous oxide. The most striking finding is that the vast majority of all the studies from both 2005 and 2015 did not report the use of ventilation; measurement of blood gases, end-tidal carbon dioxide concentration, or blood pressure; or administration of intravenous fluids or analgesics. The review of articles published in 2015 showed that the STAIR guidelines appear to have had no effect on the anaesthetic and monitoring techniques in rats undergoing experimental stroke induction, despite the publication of said guidelines in 2009.

Keywords: animal experimentation, guidelines, lack of compliance

81. Analysis of endochondral ossification process in Ship2 deficient-mice, a model for human opsismodysplasia?

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Opsismodysplasia is a human skeletal dysplasia characterized by delayed bone maturation. Patients have facial anomalies with shortened long bones associated with mutations in the gene that codes for the phosphoinositide 5-phosphatase Ship2. These symptoms are shared by Ship2Δ/Δ mice of which this enzyme has been catalytically inactivated in all cell types. The goal of this study is to analyze in these deficient mice the endochondral ossification process as the mechanism by which the lack of Ship2 affects this process in patients with opsismodysplasia is unknown. First results of histological analysis of Ship2Δ/Δ mice confirmed shortening of the long bones (femur and tibia) and the reduced size of the tibial growth plate as well as the number of cells. We discovered that Ship2 protein is expressed in chondrocytes and we generated mice in which Ship2 can be inactivated only in this specific cell type (Ship2^{flox}/flox Col2a1 CreERT2). Following stimulation with IGF-1, the most obvious alteration is a hyper-activation of the MAPK pathway (pErk1/2) when Ship2 is pharmacologically inactivated in primary murine chondrocytes, compared with normal cells.

Keywords: Ship2, Opsismodysplasia, Phosphoinositide

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