Faculté de médecine vétérinaire

Université de Liège

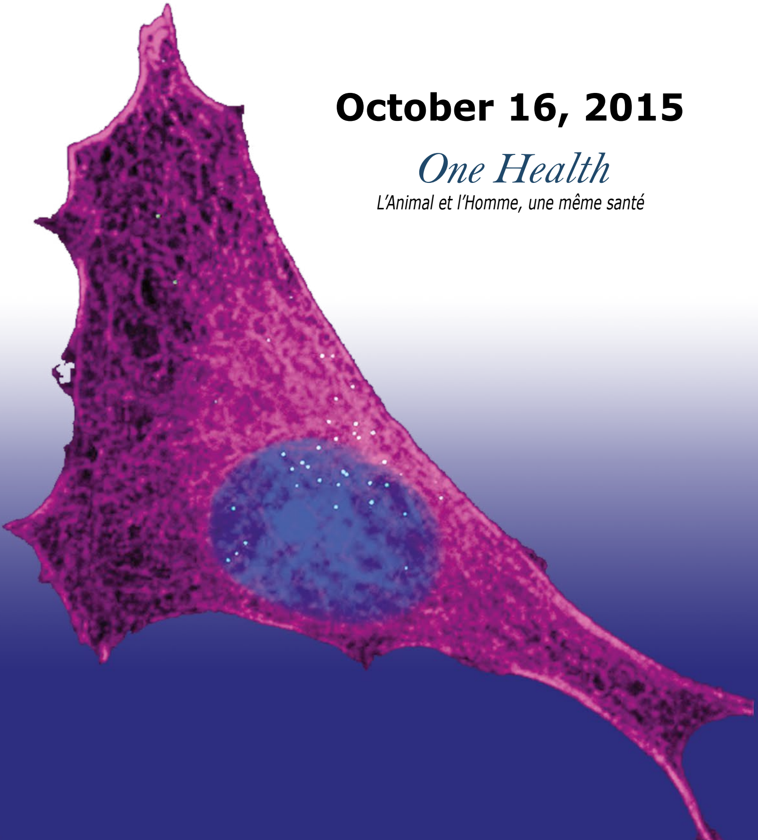
Proceedings of the 2nd FARAH-Day

Faculty of Veterinary Medicine
(University of Liege - Belgium)

October 16, 2015

One Health

L'Animal et l'Homme, une même santé



FARAH

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Faculty of Veterinary Medicine
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October 16, 2015

Edited by C. Bayrou, J.-F.Cabaraux, C. Delguste, C. Gatez, T. Jauniaux, C. Lété, D. Thiry,
D.-M. Votion, C. Zeippen
Presses de la Faculté de Médecine vétérinaire de l'Université de Liège
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COVER PICTURE CREDITS:

Muhammad Bilal LATIF and Dr. Laurent GILLET, Immunology-Vaccinology, FARAH
Confocal microscopy, Leica TCS/SP5
The Murid herpesvirus 4 (MuHV-4) entry into the BHK-21 cells

The MuHV-4 virions were bound to the adherent BHK-21 cells (2h, 4°C). Unbound virions were then removed by PBS wash. The cells were then incubated (1h, 37°C) to allow virion endocytosis and fixed. These cells were permeabilized and stained for the MuHV-4 ORF65 minor capsid protein, which is only revealed after envelope fusion (green) and alpha-tubulin (magenta). The nuclei were counter-stained with DAPI (blue). On this image, the virions which have released their capsids in the cytosol can be seen. These capsids reach the nucleus via the microtubule organization center.

Welcome to the 2nd FARAH Day

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

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

The founding principles of the FARAH incorporate the notion of interaction between scientists of the Centre and, as such, the annual meeting of the scientific staff gives us the opportunity to share our knowledge. Also, it is now under the auspices of the FARAH that the annual meeting will be held with the same organizers (i.e. members of the Scientific Staff). This edition gathers about 80 abstracts dedicated to fundamental, clinical and or applied researches.

Laurent Gillet, President of the FARAH

Frédéric Farnir, Vice-president

Dominique Votion, Secretary

Bienvenue à la 2nd journée du FARAH

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

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

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

Laurent Gilet, Président du FARAH

Frédéric Farnir, Vice-président

Dominique Votion, Secrétaire

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Program

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

09:00 - OPENING AND WELCOME SPEECH

DR CATHERINE DELGUSTE, *President of the Scientist Staff*

DR DOMINIQUE VOTION, *Board member of FARAH*

09:15 INVITED SPEAKER:

PR ALAIN VANDERPLASSCHEN

09:45 - Oral session 1

(lecture hall C, building B45)

CHAIRMEN: ***Marianne DIEZ*** *and* ***Etienne BAISE***

09:45 Use of a thorax-abdomen pressure gradient to improve the diagnosis of dynamic sliding hiatal hernia in 20 dogs with Brachycephalic Syndrome
Olivier Broux, Companion Animals Clinic, FARAH

10:00 Evaluation of diastolic function after a long distance endurance race in athletics horses
Simona Cerri, Equine Clinic, FARAH

10:15 Protective role of murid herpesvirus 4 infection on allergic asthma through modulation of alveolar macrophages
Michaël Dourcy, Immunology-Vaccinology, FARAH

10:30 Complete genome sequencing of Testudinid Herpesvirus 3 reveals a novel genome structure of Herpesvirales and a large region essential neither for viral replication in vitro nor for virulence in vivo
Frédéric Gandar, Clinic for Birds, Rabbits and Rodents, FARAH

10:45 - COFFEE BREAK AND POSTER SESSION 1 (Room P, building B45)

11:15 - Oral session 2

(lecture hall C, building B45)

CHAIRMEN: *Hélène AMORY* and *Axel MAUROY*

11:15 Exploring the potential of DNA vaccination to produce nanobodies in alpacas
Natacha Harmegnies, Immunology-Vaccinology, FARAH

11:30 Evaluation of the sensory quality of beef patties inoculated with Carnobacterium maltaromaticum strains with biopreservative potential
Pedro Imazaki, Food Sciences Department, FARAH

*11:45 **Short talks***
A protein conserved in cypriniviruses is a major virulence factor of Cyprinid herpesvirus 3
Maxime Boutier, Immunology-Vaccinology, FARAH

Galba schirazensis (Mollusca, Gastropoda) as a new potential intermediate host of Fasciola hepatica (Trematoda, Digenea) in Ecuador
Yannick Caron, Parasitology and Parasitic Diseases, FARAH

Nematode infection associated with elevated levels of helminth-related genital cytokines
Alisha Chetty, University of Cape Town

Q fever serological survey and associated risk factors in veterinarians, Southern Belgium, 2013
Fabiana Dal Pozzo, Epidemiology and Risk Analysis, FARAH

Helminth-induced inflammation controls murine γ-herpesvirus replication in the lung
Annette Dougall, Immunology-Vaccinology, FARAH

Development of a LC-MS analytical method for the simultaneous measurement of toxic aldehydes coming from polyunsaturated fatty acids degradation in animal feed
Caroline Douny, Food Sciences Department, FARAH

Comparison of two non-invasive 2% enilconazole infusion protocols for treatment of canine sinonasal aspergillosis and importance of debridement for treatment efficacy
Maud Girod, Companion Animals Clinic, FARAH

Link between milk fatty acids and non esterified fatty acids in the blood and it's implication on the diagnosis of negative energy balance in dairy cows
Emilie Knapp, Nutrition Unit, FARAH

12:30 - LUNCH AND POSTER SESSION 2 (Room P, building B45)

14:00 INVITED SPEAKER

DR NICOLAS ANTOINE-MOUSSIAUX

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(lecture hall C, building B45)

CHAIRMEN: ***Cécile CLERCX*** *and* ***Benjamin DEWALS***

14:30 Evaluation of the cardiomyotoxic effects of doxycycline overdose in calves using 2-dimensional speckle tracking
Laureline Lecoq, Equine Clinic, FARAH

14:45 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
Léa Morvan, Immunology-Vaccinology, FARAH

15:00 Characterization of egg-induced hepatic macrophages during Schistosoma mansoni infection
Marion Rolot, Immunology-Vaccinology, FARAH

15:15 Chronic toxic hepatitis in beef calves due to mycotoxins contamination in mixed feed
Léonard Theron, Bovine Clinic, FARAH

15:30 Effects of murid herpesvirus 4 infection on B-cell repertoire in mice
Xue Xiao, Immunology-Vaccinology, FARAH

15:45 - COFFEE BREAK AND POSTER SESSION 3 (Room P, building B45)

16:15 - Oral session 4

(lecture hall C, building B45)

CHAIRMEN: ***Céline LETE*** *and* ***Christian HANZEN***

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Murid herpesvirus 4 ORF63 is involved in the translocation of incoming capsids to the nucleus

Muhammad Bilal Latif, Immunology-Vaccinology, FARAH

Two-dimensional speckle tracking echocardiography in calves: feasibility, repeatability and variability study

Laureline Lecoq, Equine Clinic, FARAH

Equine infectious anaemia virus and its vectors: an assessment of their dispersion potential

Laetitia Lempereur, Parasitology and Parasitic Diseases, FARAH

Reverse genetic screen for loss-of-function mutations uncovers a frame-shifting deletion in the melanophilin gene accountable for a distinctive coat-color in Belgian Blue Cattle

Wanbo Li, Unit of Animal Genomics, GIGA-R

Evidence of an immune reaction in Tec4 inoculated SPF rabbits that survive to the challenge

Didier Marlier, Clinic for Birds, Rabbits and Rodents, FARAH

Seroprevalence of borreliosis in healthy equids living in Southern Belgium and in Luxembourg: a preliminary study

Amandine Piret , Equine Clinic, FARAH

A stop-gain in the Laminin A3 (LAMA3) gene causes recessive Junctional Epidermolysis Bullosa in Belgian Blue Cattle

Arnaud Sartelet, Bovine Clinic, FARAH

Identification and traceability of animal and human faecal contamination in bathing sites in Wallonia

Bernard Taminiau, Food Sciences Department, FARAH

Belgian wildlife as potential zoonotic reservoir of hepatitis E virus

Damien Thiry, Virology and Animal Viral Diseases, FARAH

Identification of cyprinid herpesvirus 3 envelope transmembrane proteins essential to viral growth in vitro

Catherine Vancsok, Immunology-Vaccinology, FARAH

Ecotoxicity's evaluation of 6 anthelmintic molecules used in cattle on insects feeding on dung

Caroline Vanvinckenroye, Parasitology and Parasitic Diseases, FARAH

Gp150 promotes sexual transmission of Murid Herpesvirus-4

Caroline Zeippen, Immunology-Vaccinology, FARAH

17:35 - Awards

Dominique VOTION, Board member of FAREH

17:45 - CLOSING SESSION

PR RUDI CLOOTS, *Associate Vice-Rector for Research*

PR LAURENT GILLET, *President of FARAH*

18:00 - COCKTAIL AND POSTER SESSION 4 (Room P, building B45)

20:00 - DINNER and DANCING PARTY (Room P, building B45)

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SUSTAINABLE LIVESTOCK PRODUCTION

COMPARATIVE VETERINARY MEDICINE

Oral presentations

1. Use of a thorax-abdomen pressure gradient to improve the diagnosis of dynamic sliding hiatal hernia in 20 dogs with Brachycephalic Syndrome

Broux O.[1], Clercx C.[1], Busoni V.[1], Claeys S.[1], Hamaide A.[1], Billen F.[1]

[1.] *FARAH, Department of Clinical Sciences of companion animals and equine, ULg.*

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Digestive symptoms are frequent in dogs with Brachycephalic Syndrome and are thought to be due to dynamic sliding hiatal hernia (SHH). However SHH is only rarely diagnosed. We hypothesized that artificially increasing the pressure gradient between the abdominal and thoracic cavities during diagnostic procedures could improve the detection of SHH.

The respiratory and digestive clinical signs of 20 dogs suffering from Brachycephalic Syndrome were evaluated and a respiratory and a digestive clinical score were assessed in each dog. Based on physical exam, thoracic radiographs and laryngoscopy, a respiratory lesion score was recorded in each dog.

The gastroesophageal junction (GEJ) was assessed using standard abdominal ultrasound, thoracic radiographs and digestive endoscopy. Each procedure was repeated using manual application of a pressure on the cranial abdomen (MAP), body tilted down at 30° (30°), and temporary complete endotracheal tube obstruction (ETO). Presence of SHH was assessed during each procedure with and without manipulations. A GEJ lesion score was calculated for each endoscopic (ENDO) manipulation.

Using standard procedures SHH was detected in 2 dogs. Manipulations during endoscopy allowed detecting SHH in 3 (ENDO-30°), 4 (ENDO-ETO) and 5 (ENDO-MAP) dogs. The severity of GEJ lesion scores was increased during the 3 manipulations compared to standard condition. Digestive clinical score was significantly correlated with the GEJ lesion score during ETO manipulation only ($p = 0.02$).

Conclusion: Increasing the thorax-abdomen pressure gradient increases the prevalence of GEJ lesions and improves SHH detection. ENDO-ETO is the sole manipulation revealing a correlation between digestive clinical signs and GEJ lesions.

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2. Evaluation of diastolic function after a long distance endurance race in athletics horses

Cerri S.[1], Sandersen C.[1], Farnir F.[1], Lecoq L.[1], Leroux A.[1], Amory H.[1]

[1.] *Equine Clinic, Faculty of Veterinary Medicine, ULg.*

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Exercise-induced cardiac fatigue (EICF) has been demonstrated in human athletes performing long duration exercise. A preliminary study showed left ventricular (LV) systolic dysfunction after a long distance endurance race in horses, but LV diastolic function has not yet been explored.

Clinical examination, venous blood analysis and two-dimensional time-motion mode, pulsed Doppler and tissue Doppler echocardiography were performed before and after an international endurance race (106-132 km) in 15 horses. Packed cell volume (PCV), total proteins (TP), electrolytes serum concentration and cardiac Troponine I (cTnI) were analyzed.

Echocardiography allowed to measure systolic and diastolic LV internal diameter (LVIDs and LVIDd), end-diastolic left atrial (LA), and aortic (Ao) diameter, peak transmitral early diastolic (E) and late diastolic (A) velocities, heart rate (HR), flow velocity integral (FVI), ejection time (ET) and pre-ejection period (PEP), and to calculate E/A ratio, stroke volume (SV) and PEP/ET ratio. LV longitudinal myocardial velocities were obtained during systole (S'), early diastole (E') and late diastole (A'). Isovolumetric relaxation (IVRT) and contraction (IVC) time were measured, E/E', E'/A' ratios were calculated.

After the race, PCV and TP were statistically significantly increased, while sodium and potassium plasmatic concentrations were decreased. Moreover, LVIDd, LVIDs, Ao, LA, E', FVI, SV and E'/A' were significantly lower and HR, A', IVRT, IVC, A, PEP, E/E', and PEP/ET were higher compared to pre-race values.

Results of this study suggest that not only a LV systolic dysfunction, but also a LV diastolic dysfunction, occurs in endurance horses, which suggests an EICF as demonstrated in human athletes.

3. Protective role of murid herpesvirus 4 infection on allergic asthma through modulation of alveolar macrophages

Dourcy M.[1], Machiels B.[1], Xiao X.[1], Mesnil C.[2], Sabatel C.[2], Zeippen C.[1], Javaux J.[1], Desmecht D.[3], Vanderplasschen A.[1], Dewals B.[1], Bureau F.[2], Gillet L.[1]

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The effect of the infection history is ignored in most animal models. However, no one is naive and our immune system is continuously shaped by environmental antigens. Gammaherpesviruses are highly prevalent pathogens that establish lifelong latency in their host. Yet, little is known about how these persistent viruses imprint the immune response of their host. Therefore, we used murid herpesvirus 4 (MuHV-4), a natural pathogen of wild rodents, to investigate the impact of gammaherpesvirus infections on house dust mites induced allergic asthma. Interestingly, we previously showed that MuHV-4 infection inhibits both the sensitization and challenge phases of allergic asthma. Here, we investigated the mechanisms that could explain this protection. Surprisingly, we observed by flow cytometry that alveolar macrophages (AM) display a dramatic change in their phenotype in MuHV-4 infected mice. Thus, we observed that the expression of key pro-Th2 markers such as Ym1, was reduced in MuHV-4 infected mice, suggesting that MuHV-4 imprinted AM are not able to acquire the typical "M2" functionnality otherwise observed in allergic inflammation. Moreover, we showed that MuHV-4 imprinted AMs were able to suppress TH2 orientation of bone marrow dendritic cells in vitro. Finally, transcriptomic analysis revealed a cluster of genes that are specifically upregulated in AMs after MuHV-4 infection. The role of these candidates is currently tested. Altogether, these results highlight that gammaherpesvirus infections could provide a long-term beneficial effect to their host by imprinting some innate immune cell populations. In the future, these results could allow the development of new therapeutics against asthma.

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4. Complete genome sequencing of Testudinid Herpesvirus 3 reveals a novel genome structure of *Herpesvirales* and a large region essential neither for viral replication *in vitro* nor for virulence *in vivo*

Gandar F.[1,2], Wilkie G.[3], Gatherer D.[4], Kerr K.[3], Marlier D.[2], Diez M.[5], Marschang R.[6], Dewals B.[1], Mast J.[7], Davison A.[3], Vanderplasschen A.[1]

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Testudinid herpesvirus 3 (TeHV-3) is the causative agent of a lethal disease affecting several species of tortoises. As TeHV-3 affect endangered species, there is a need for the development of prophylactic methods. The development of these methods requires fundamental knowledge on the virus such as its sequence. Here, we sequenced the two most studied strains of TeHV-3 (1976 and 4295 strains). The genome of the 1976 strain revealed a novel herpesvirus genome structure and demonstrated that TeHV-3 belongs to the *Scutavirus* genus of the *Alphaherpesvirinae* sub-family thereby establishing a phylogenetic relationship between tortoise and turtle herpesviruses. TeHV-3 1976 sequence also unraveled cellular homologues never described in alphaherpesviruses such as interleukin-10 and semaphorin homologues. Sequencing of the 4295 strain revealed that it consists of three related genome populations (called m1, m2 and M) exhibiting partially overlapping deletions ranging from 12.5 to 22.4 kb. Subcloning of these three genome populations demonstrated that they all replicated in cell culture comparably to the 1976 strain. With the goal in mind to test the potential of the 4295 strain (mixture of m1, m2 and M) as an attenuated vaccine candidate, Hermann's tortoises (*T. hermanni*) were inoculated intranasaly. Unfortunately, all inoculated subjects died from the infection. PCR analyses demonstrated the ability of the m2 and M forms to invade all tested organs (including the brain) while m1 was never detected. The data of the present study represent a major step toward the development of prophylactic methods against TeHV-3.

5. Exploring the potential of DNA vaccination to produce nanobodies in alpacas

Harmegnies N.[1], Boutier M.[1], Michel B.[2], Szpirer C.[2], Vanderplasschen A.[1]
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[2] *Delphi Genetics SA, Rue Antoine de Saint-Exupéry 5, Gosselies 6041, Belgium.*
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There is a continual need in biomedicine for antibodies. A diverse range of well-defined biomedical product already exist. In this market place, camelids have the exceptional potential to make the difference. Camelids produce both conventional antibodies and unique functional heavy-chain antibodies (HCAb). Single-domain antigen-specific recombinants derived from the variable region of HCAb are named Nanobodies (Nbs). Compared to other antibodies derived products, they present superior physico-chemical properties, reduced size (15kD), new binding capacities, and are easily expressed in microbial system.

This proof of concept study explore the use of alpaca, a member of the camelid family, for the production of Nbs using DNA vaccination. Advantages of DNA vaccination include: the potential to genetically engineer the plasmid encoding the antigen, the stimulation of both cellular and humoral responses, the improved stability and the absence of infectious agent. Here, we used the pStabyCMV-2-gD engineered DNA plasmid encoding the sequence of glycoprotein D (gD) of Suid herpesvirus-1 (SuHV-1), the causative agent of Aujesky's disease. This plasmid was chosen for his previously demonstrated safety and efficacy in mice to induce specific antibodies. The immunisation procedure was performed 3 times at 3 weeks interval on anesthetised alpacas. Intramuscular injection of the plasmid was combined with *in vivo* electroporation. Our preliminary results show that DNA immunisation by intramuscular injection combined with electroporation induce a significant immune response in the alpaca.

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6. Evaluation of the sensory quality of beef patties inoculated with *Carnobacterium maltaromaticum* strains with biopreservative potential

Imazaki P.H.[1], Jacques-Houssa C.[2], Kergourlay G.[2], Daube G.[2], Clinquart A.[1]
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Biopreservation is the use of naturally occurring microorganisms and/or their inherent antimicrobial compounds to extend shelf life and to enhance the safety of foods. The aim of the present study was to perform a hedonic sensory evaluation by assessment of individual attributes (appearance, odor, color, tenderness, flavor and juiciness) of beef patties inoculated with potentially biopreservative strains of *Carnobacterium maltaromaticum*. Three different strains of *C. maltaromaticum* (lab. ref.: CM_824, CM_827 and CM_829) isolated from vacuum packaged beef with long shelf life were selected for this study. An untrained panel was requested to make a sensory evaluation of raw and cooked beef patties 8 and 10 days after inoculation with the selected strains at 10^4 and 10^6 UFC/g and storage in high-O_2 atmosphere. After 8 days of storage, non inoculated samples (blank) were perceived as having the best studied sensory parameters. The samples inoculated with strain CM_827 had a sensory quality very close to the blank. After 10 days of storage, samples inoculated with the strain CM_827 at 10^4 UFC/g received the highest scores for appearance and color. This study permitted to evaluate the effect of three *C. maltaromaticum* strains on the sensory quality of beef patties. Strain CM_827 did practically not change the sensory attributes of beef patties. Samples inoculated with strain CM_824 and CM_829 received the worst scores for several of the tested parameters. Therefore, further research on the biopreservative capacity of *C. maltaromaticum* should be conducted with strain CM_827.

7. Evaluation of the cardiomyotoxic effects of doxycycline overdose in calves using 2-dimensional speckle tracking

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Doxycycline (DOXY) is associated with left ventricular (LV) dysfunction in calves in accidental overdose but not in experimental models when evaluated with conventional echocardiography. Two-dimensional-speckle tracking (2DST) is an innovative technique used to evaluate LV dysfunction in numerous species but not in cattle. The goal of this prospective study was to evaluate the cardiotoxic effects of an experimental overdose of DOXY using 2DST in calves. Ten healthy Holstein calves were used. Group DOXY: 5 calves received 5 times the recommended dose of DOXY (25 mg/kg) orally BID for 5 days. Group CONTROL: 5 calves received a placebo. Electrocardiography (ECG), conventional echocardiography and 2DST echocardiography were performed at day 1 and day 8. ECG traces were analysed for occurrence of arrhythmias. 2DST measurements included global and 6 segmental peak values for radial and circumferential strains (SR, SC), strain rates (SrR, SrC), rotation (Rot), rotation rates (RotR) and radial displacement (DR). ECG recordings and conventional echocardiography were unremarkable in both groups. Heart rate was never significantly different between groups. LV systolic function was affected in calves receiving an overdose of DOXY as shown by a significant decrease of segmental and global SR ($p<0.05$), SC ($p<0.05$) and DR ($p<0.05$) in treated calves compared to the placebo group. In calves, DOXY overdose induces a LV dysfunction in systole, and to a lesser extent, in diastole. A better comprehension of the pathophysiology involved in the DOXY overdose will help in the treatment of accidental cases.

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8. 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 sensors of nucleic acids. Nucleic acids are detected based on their structure, subcellular localization or sequence. Zalpha domains are 66 aa long domains which bind to left-handed dsDNA or dsRNA (Z-DNA/Z-RNA). The description of Zalpha domains in proteins belonging to 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 as a PAMP or DAMP. The present project aims to test this interesting and original hypothesis both *in vitro* and *in vivo* taking advantage of the Cyprinid herpesvirus 3 (CyHV-3)/ Carp model.

Carp, like all cypriniform fish, encodes 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-2α thereby blocking protein synthesis. This leads to accumulation of preinitiation complexes and later, to the organization of these into stress granules.

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 ORF112 gene is essential for replication of the virus in cell culture, that pORF112 co-localizes with stress granules (and potentially with dsRNA), and that only the full-length pORF112 is detected in infected cells. The interference of pORF112 with the protein synthesis pathway has yet to be demonstrated.

9. Characterization of egg-induced hepatic macrophages during *Schistosoma mansoni* infection

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Schistosomiasis is a parasitic tropical disease caused by the trematode *Schistosoma spp.*. According to WHO, more than 200 million people are infected worldwide. *Schistosoma mansoni* can infect human by skin penetration after a contact with contaminated water. Worms migrate and mature within the vasculature and pair in the mesenteric veins. Eggs cross the intestine wall and are excreted in the feces. Up to 50% of eggs are swept and trapped into the liver where they elicit an important type 2 immune response. They stimulate a granulomatous reaction involving T cells, eosinophils and macrophages. In this context of type 2 immunity, macrophages adopt an anti-inflammatory and repair phenotype called alternative activation. Alternatively activated macrophages (aaMφ) seem to protect from excessive granulomatous inflammation and fibrosis during *S. mansoni* infection. Here, we investigate this protective role of aaMφ. In this purpose, we wanted first to finely characterize macrophages populations found in the liver of *S. mansoni* infected mice. Wild-type mice as well as mice deficient in type 2 immune response ($Il4ra^{-/-}$) or mice which failed to alternatively activate their macrophages ($LysM^{Cre}$ $Il4ra^{-/lox}$) were used. Based on cell surface markers analysis by flow cytometry, we were able to identify two main populations ($CD11b^{+}$ $F4/80^{lo}$ $Ly6C^{+}$ and $CD11b^{lo}$ $F4/80^{+}$ $Ly6C^{-}$ macrophages) at different time points of the infection. Our objective is now to sort these populations and perform RNA sequencing to identify gene candidates likely to be involved in the immunoprotection mechanisms by which IL4R-dependent aaMφ control inflammation and fibrosis during *S. mansoni* infection.

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10. Chronic toxic hepatitis in beef calves due to mycotoxins contamination in mixed feed

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Mycotoxins intoxination is an emerging disorder in Belgium, although legal concentrations have been established for mycotoxins in the EU, farm forages are most of the time not tested (EU 2006/576/EC). In January 2015, a Belgian Blue farm referred a calf for unexplained fatal case of jaundice on a 2 months-old calf to the Clinic for Ruminants of the University of Liège for necropsy. It revealed generalized icterus, mild bilirubinuria, splenomegaly, but no precise etiology. On February a second 2 month old calf with jaundice is referred, lethargic, normothermic with a mild diarrhea, Calf shown generalized jaundice, anemia, elevation of leukocytes (neutrophils, eosinophils and monocytes), TGO, bilirubinemia, total biliary acids and Globulins (alpha2). He had also diminished erythrocytes and albuminemia. Copraemia, leptospirosis serology, leademia, hemoculture and pancreatic enzymes were within normal ranges. Abdomen ultrasonography revealed a mild hyperechogenicity of the liver but no gall bladder modifications.. A third calf was referred three days after in a worst clinical condition, with also a severely modified liver enzymes, but no anemia. Three days after, this calf died and a necropsy revealed petechiae and hemorrhages in the abomasum, congestive mucosae in the distal bowel, white depot in the kidney medulla, modified urine and splenomegaly. Liver histopathology revealed a severe liver degeneration compatible with chronic metabolic disorder. [...]The calves were fed a mixed feed (containing cereal mix, cocoa, beet pulp, soja, maize), assembled at the farm from primary product. The mix was tested for mycotoxins presence and ranged from 0.8 to 1.5 ppm of Desoxynivalenone (DON), and 115-215 ppb of Zearalenone (ZEA). Since any other hypothetic origin to this progressive hepatic intoxination was not demonstrated, we believe that the calves were chronically exposed to these toxins.

11. 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 read 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. Altogether, this study highlights that deep sequencing of immunoglobulin provides direct insight into the imprinting of γHVs infection on the immune system of their hosts.

Short communications

Short communications

1. 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, in the late 1990s, 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. This attenuated vaccine candidate is currently under development.

In the present study, we investigated the contribution of the two genes to the attenuated phenotype observed. To reach this goal, a series of recombinants deletedeither for ORF56 or ORF57 were produced. Deletions were designed in between predicted eukaryotic promoters of flanking ORFs. Sequences deleted were replaced by a galactokinase cassette. 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*. These recombinants were further tested *in vivo* and revealed that the attenuation observed for the ORF56-57 double deleted recombinant resulted mostly from the single deletion of ORF57.

In conclusion, this study demonstrates the role ORF57 as a major virulence factor of CyHV-3. Interestingly, ORF57 is conserved in cypriniviruses and could therefore represent a target for attenuated vaccine development in several other major fish pathogens.

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2. *Galba schirazensis* (Mollusca, Gastropoda) as a new potential intermediate host of *Fasciola hepatica* (Trematoda, Digenea) in Ecuador

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Fasciolosis is a widely distributed disease in livestock in South America but knowledge about the epidemiology and the intermediate hosts are scarce in Ecuador. During 3 months, lymnaeid snails were sampled (n=1482) in Pichincha province in two sites located in a highly endemic area. The snails were identified (based on morphology and ITS2 sequences) and the infection status was established through microscopic dissection and a multiplex PCR-based technique. If morphologic-based techniques were not useful to accurately named the one species collected, alignment study ascribed it to *G. schirazensis.* Rediae were observed in 1.75 % (26/1482) and *Fasciola sp.* DNA was detected in 6% (89/1482) of the collected snails. The COX1 region permitted the parasite species identification: *F. hepatica*. The relative sensitivity and specificity of the microscope related to the PCR results was 25.84% and 99.78% respectively. The mean size of the snails recorded positive for *F. hepatica* through crushing and microscopy was significantly higher than the mean size of negative snails. There was not such difference in PCR positive snails. The role of *G. schirazensis* as an intermediate host of *F. hepatica* in Ecuador is discussed and a hypothesis of an adaptation of the snail to the trematoda is formulated. For the first time, an epidemiological survey, based on molecular biology-based techniques assessed the role of lymnaeid snail in the epidemiology of fasciolosis in Ecuador.

3. Nematode infection associated with elevated levels of helminth-related genital cytokines

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Helminth infections are highly immunogenic and can have systemic effects on host immunity. Helminth infections can also exert important influences on diseases that affect tissues not colonized by the parasite. The female reproductive tract represents an anatomical location that is not generally colonized by parasitic nematodes, and the influence of these nematode infections on immunity in the female reproductive tract is not known. Here, we determined parasitic nematodes exposure in women residing in an area with high worm burden, and whether exposure was associated with specific changes in the cytokine profile in the female genital tract. In 48 HIV-negative women from Gugulethu, Cape Town, we determined plasma antibody responses against two common nematode infections *Ascaris lumbricoides* (ASL) and *Trichuris trichiura* (TT) by ELISA and CAP RAST, and genital cytokine concentrations by Luminex. Observational analysis of genital cytokine responses in women was confirmed in the reproductive tract of BALB/c mice infected with *Nippostrongylus brasiliensis*. In women, plasma ASL-specific IgG4 responses, indicating chronic parasite exposure, were positively associated with concentrations of IL-13 and IL-1β in cervicovaginal lavages. In female BALB/c mice infected with *N. brasiliensis*, levels of IL-4 and IL-13 were elevated in genital tissue. Our findings suggest that chronic exposure to helminths may increase in helminth-related Th2 cytokines in the female genital tract. We hypothesize that this association may be due to a systemic alteration in immunity by the parasite.

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4. Q fever serological survey and associated risk factors in veterinarians, Southern Belgium, 2013

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A sero-epidemiological survey was organized among veterinarians working in Southern Belgium in order to estimate the seroprevalence of Q fever and the risk factors associated with exposure. A total of 108 veterinarians took part to this cross-sectional study, with a majority practicing with livestock animals. The overall seroprevalence was 45.4%, but it increased to 58.3% among veterinarians having contact with livestock. Three main serological profiles were detected (relatively recent, past and potentially chronic infections). The contact with manure during the prior month was the risk factor associated with seropositivity after multivariate logistic regression analysis. Classification and regression tree analysis identified the age as a predictive variable to exclude potentially chronic infection in apparently healthy seropositive veterinarians. In conclusion, livestock veterinarians practicing in Southern Belgium are highly exposed to Q fever, a neglected zoonosis for which serological and medical examinations should be envisaged in at risk groups.

5. Helminth-induced inflammation controls murine γ-herpesvirus replication in the lung

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Traditionally, host-pathogen interactions are studied in isolation. Although in reality hosts are often subject to concurrent infections in the natural environment. Schistosomiasis caused by numerous species of trematode from the genus *Schistosoma*, affect some of the world's poorest populations. Schistosomiasis overlaps geographically with human γ-herpesviruses such as Kaposi's sarcoma-associated herpesvirus, also responsible for severe malignancies. In this study we sought to determine how acute γ-herpesvirus infection and host colonization could be affected by preliminary helminth infection. We used the lung model to generate *S. mansoni* egg-induced inflammation and typical Th2-type responses in mice, this was followed by intranasal infection with a MuHV-4-luc virus. We observed that the parasite egg-induced inflammation caused a significant reduction of MuHV-4 replication in the lung at day 5 and 7 post-infection (p.i.) using *in vivo* imaging and viral titration. Additionally, the egg-induced inflammation in the lung protected from the weight loss caused by the acute MuHV-4 infection. Using a MuHV-4-eGFP virus, we further observed that the egg-induced inflammation was associated with significantly reduced numbers of alveolar macrophages supporting viral infection. At day 7 p.i. with MuHV-4, flow cytometry analyses revealed that the presence of *S. mansoni* eggs in the lung was associated with reduced numbers of neutrophils, while increased numbers of eosinophils, dendritic cells, RELM-α$^+$ macrophages, CD4$^+$ effector T-cells and importantly virus specific CD8+ T-cells. These results demonstrate that *S. mansoni* egg induced-inflammation controls acute MuHV-4 infection in the lung. Whether IL-4-dependent Th2-type responses and/or type-I interferon responses are involved are currently investigated.

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6. Development of a LC-MS analytical method for the simultaneous measurement of toxic aldehydes coming from polyunsaturated fatty acids degradation in animal feed

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Omega-3 fatty acids can be found in a large variety of food and animal feed, but unfortunately, those essential polyunsaturated fatty acids (PUFA) are known to be easily oxidized by light, temperature, etc, during food storage or processing. PUFA oxidation leads to the formation of primary oxidation products (hydroperoxydes) or secondary degradation compounds (aldehydes or ketones). The formed aldehydes are relatively stable and have been shown to be cytotoxic and genotoxic by reacting with proteins and nucleic acids. They are usually detected by the TBARS method. This method measures the total content of aldehydes, expressed in malondialdehyde content. There is a need of having a more specific analytical method to assess the content of each of those compounds. Therefore, a LC-MS method has been developed to evaluate the concentration of aldehydes in feed samples: malondialdehyde, 4-hydroxy-2-nonenal (4-HNE), 4-hydroxy-2-hexenal (4-HHE), crotonaldehyde, acrolein, hexanal, 2,4-nonadienal, 2,4-decadienal and benzaldehyde. Separation and detection of aldehydes were performed using a ThermoFinnigan Spectra System P4000 HPLC system and a ThermoFinnigan LCQ Deca ion trap mass spectrometer, with an Electrospray source. The analysis was performed in MS/MS mode, with positive ionization for malondialdehyde and negative ionization for the other compounds. The aldehydes were detected by following specific mass fragmentations. Isotopic dilution technique was used for quantification, using methylmalondialdehyde, acrolein-^{13}C, methylcrotonaldehyde, benzaldehyde-^{13}C, 4-HNE-D_3 and hexanal-D_{12} as internal standards. A method validation is being conducted according to the criteria and procedure described in Commission Decision 2002/657/EC.

7. Comparison of two non-invasive 2% enilconazole infusion protocols for treatment of canine sinonasal aspergillosis and importance of debridement for treatment efficacy

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Topical infusion therapies are widely used in canine sinonasal aspergillosis (SNA) but are time-consuming. The main goal of the present study was to compare the efficacy of a simplified infusion protocol (D15E) with 1-hour infusion protocol (D60EB). D60EB consisted in endoscopic debridement followed by 60 minutes 2% enilconazole infusion and 1% bifonazole cream depot into the affected frontal sinus. For D15E protocol, after debridement, enilconazole infusion was shortened to 15 minutes and bifonazole cream was not used. Adjunctive oral itraconazole was prescribed in both protocols. Effective debridement of fungal plaques is considered as an essential therapeutic step but has never been assessed as such. Therefore, the second aim of this study was to evaluate the effectiveness of debridement on success rate. Fisher's exact test was used to assess the difference in success rate between both protocols and in function of full debridement. Twenty-eight dogs were treated with D15E and 25 dogs with D60EB. First treatment success rate was 68% for D15E and 60% for D60EB and did not differ between both protocols. Both protocols had an overall success rate of 96% after 2 procedures. Completeness of debridement was assessed in 48 dogs (28 treated with D15E and 20 with D60EB). Complete debridement had a significant effect on first treatment success rate (79%) compared to incomplete debridement (40%). In conclusion, the simplified infusion protocol offers a favourable alternative for treatment of canine SNA and completeness of debridement is an important step for treatment success.

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8. Link between milk fatty acids and non esterified fatty acids in the blood and it's implication on the diagnosis of negative energy balance in dairy cows

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Monitoring the energy metabolism in the early lactation of high producing cows is essential for the management of the herd as a high fat mobilization can cause major economic losses. Blood and milk samples were obtained on 61 cows from 7 farms on a monthly basis starting from calving. The FA data sets, analysed by gas chromatography, were divided in categories based on plasma non esterified fatty acids (NEFA) (< 0,4 mmol/l, 0,4-0,6 mmol/l and > 0,6 mmol/l) and a mixed procedure was applied (SAS 9.1). In proportion, the milk short chain FA produced de novo in the mammary gland, were significantly lower in fat mobilizing cows even with mild mobilization (15.1 vs 19.5% $P< 0.001$). However, with the increase of the fat content in the milk due to high NEFA concentration (40,7 g/l vs 46,5 g/l $P<0,01$), the quantity of short chain FA was not significantly different. On the other hand, the milk C18:1 was largely higher ($P<0.001$) in proportion and content even in the mild mobilizing cows (30,4 vs 24,7 % of FA and 15.1 vs 13.2 g/l). The C18:1 was the most interesting FA . First because the excretion of the FA in the NEFA fraction was not linear and the C18:1 was the first FA excreted massively. Secondly, the correlation between the C18:1 in milk and in the NEFA fraction was very high (r^2:0,87). Because the mammary gland takes FA from the blood, especially from NEFA, milk sampling could be a useful method to diagnose cows with a negative energy balance in early lactation.

9. Murid herpesvirus 4 ORF63 is involved in the translocation of incoming capsids to the nucleus

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Gammaherpesviruses are important human and animal pathogens. Despite they display the classical architecture of herpesviruses, the function of most of their structural proteins is still poorly defined. This is especially true for tegument proteins. Interestingly, a potential role in immune evasion has recently been proposed for the tegument protein encoded by ORF63. However, this study did not involve the construction of an ORF63 knockout strain and the significance of these results remains unknown. In this project, we wanted therefore to better define the importance of ORF63 in the Murid Herpesvirus 4 (MuHV-4) lifecycle. We showed that a lack of ORF63 was associated with a severe viral growth deficit both *in vitro* and *in vivo*. The latter deficit was mainly associated with a defect of replication in the lung but did not appear to be due to a reduced ability to establish latency. On a functional point of view, inhibition of caspase-1 or inflammasome did not restore the growth of the ORF63 deficient mutant suggesting that the observed deficit was not associated with the immune evasion mechanism identified previously. Moreover, this growth deficit was also not associated with a defect in virion egress from the infected cells. In contrast, it appeared that MuHV-4 ORF63 deficient mutants failed to address most of their capsids to the nucleus during entry, suggesting that ORF63 plays a role in capsid movement along the microtubule network. In the future, ORF63 could therefore be considered as a target to block gammaherpesvirus infection at a very early stage.

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10. Two-dimensional speckle tracking echocardiography in calves: feasibility, repeatability and variability study

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Two-dimensional speckle tracking (2DST) is a non-invasive technique used in many species to evaluate global and regional left ventricular (LV) function; however it received little attention in the bovine species. We wanted to assess the feasibility and reliability of 2DST for the evaluation of circumferential and radial LV wall motions in calves. 12 Holstein black calves (age: 62 ± 11.6 days; body weight: 75.25 ± 5.4 Kg) were used in this observational study. Right parasternal short axis views at the level of the papillary muscles were recorded and subsequently analysed by 2DST for global and regional radial and circumferential strains and strain rates, radial displacement, rotation and rotation rate. Echocardiographic examinations were performed in unsedated, standing calves by 2 different observers to evaluate intra- and interobserver variability expressed as coefficient of variation. 2DST was feasible in all calves. Automated tracking was better in systole than in diastole. Intraobserver repeatability was good to moderate for most systolic global and segmental peak values. Systolic peak values for radial strain and strain rate were more repeatable than circumferential strain, circumferential strain rate and diastolic measurements. Variability of the interobserver measurements was greater than the intraobserver measurement. Two-dimensional speckle tracking is feasible in calves and as in other species systolic radial wall motion can be more reliably evaluated than circumferential and diastolic wall motion.

11. Equine infectious anaemia virus and its vectors: an assessment of their dispersion potential

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The recent outbreaks of equine infectious anaemia (EIA) in Belgium have dramatically increased the concern about this viral disease and its transmission in our country. Mechanical transmission seems to be the major route of spread of EIA and Tabanidae and Muscidae are considered as the main arthropod vectors. The potential dispersion of unfed or partially fed Tabanidae and Muscidae was assessed using challenging and innovative techniques such as *in vitro* feeding and mark-recapture experiments. 3600 biting flies (*Stomoxys calcitrans, the stable fly*) and 2900 tabanids of the genus Haematopota were collected in farm and on horses respectively. These flies and tabanids were colored using fluorescent dyes and half of them were partially fed with heparinized blood using an *in vitro* technique. They were released at increasing distances from bait horses surounded by traps in order to assess the distance that unfed and partially fed vectors could fly and consequently the potential dispersion of the virus. This experiment was repeated several times without other animals in the vicinity and under appropriate weather conditions. These experiments demonstrated that the present control measures based on a 200 meters buffer zone applied in case of introduction of the virus in Belgium are unsufficient in order to prevent virus transmission.

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12. Reverse genetic screen for loss-of-function mutations uncovers a frame-shifting deletion in the melanophilin gene accountable for a distinctive coat-color in Belgian Blue Cattle

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In the course of a reverse genetic screen in the Belgian Blue cattle breed, we uncovered a ten base-pair deletion *(c.87_96del)* in the first coding exon of the *melanophilin (MLPH)* gene. It introduces a premature stop codon *(p.E32fsX1)* in the same exon, truncating 94% of the protein. Recessive damaging mutations in the MLPH gene are well known to cause skin, hair, coat or plumage-color dilution phenotypes in numerous species, including human, mice, dog, cat, mink, rabbit, chicken and quail. Large-scale array genotyping was undertaken to identify *p.E32fsX1* homozygous mutant animals. It revealed a mutation frequency of 5% in the breed and allowed for the identification of ten homozygous mutants. As expression of colored coat required at least one wild-type allele at the co-dominant Roan locus encoded by the *KIT Ligand (KITLG)* gene, homozygous mutants for the *p.A227D* corresponding missense mutation were excluded. The six remaining colored calves displayed a distinctive dilution phenotype as anticipated. This new coat color was named "*cool grey*". It is the first damaging mutation in the *MLPH* gene described in cattle. It extends the already long list of species with diluted color due to recessive mutations in *MLPH* and broadens the color palette of grey in this breed.

Running Title: *Fifty shades of grey in Belgian Blue.*

13. Evidence of an immune reaction in Tec4 inoculated SPF rabbits that survive to the challenge

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The serum from five SPF rabbits inoculated with TEC4 that survived the challenge and from five control animals were collected before and 28 days after inoculation. The antibody production of those 10 rabbits were compared by ELISA using fractions of TEC 4 (supernatant of centrifugation and whole bacteria) or purified bacterial strains (*Clostridium perfringens, Entecococcus faecium, Pseudomonas luteola*) isolated from this inoculums as antigens. Despite the very limited changes in optical density and the few blood samples used in this study, some significant ($P < 0,05$; U test of Mann-Whitney) differences in antibodies level were found between control and inoculated animals when "whole bacteria" and "*Enterococcus faecium*" were used as antigens. Analysis conducted with the supernatant as antigens were at the limit of signification ($P = 0,06$; U test of Mann-Whitney).

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14. Seroprevalence of borreliosis in healthy equids living in Southern Belgium and in Luxembourg: a preliminary study

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The aim of this study was to evaluate the seroprevalence of borreliosis in horses living in the southern Belgium and in Luxembourg. To be included in the study, the horses had to be older than 1 year, and in the past 12 months, to have lived in Wallonia or in Luxembourg without discontinuation, and to have not showed any clinical signs suggestive of Lyme disease. For each horse, the sex, age, province of living and detection of infestation by ticks in the previous 12 months were noted.

A blood sample was collected and centrifuged within 1 to 12 hours after collection. Sera were isolated and frozen at -80°C. The antibody title against borreliosis was measured using an immunofluorescence test (Fluo Borrelia burgdorferi Horse, Biopronix), and the result was considered positive when the titers was ³ 1/512. The effect of sex, age, presence of ticks, or province of living on seropositivity was tested using a general linear model using the The R® program.

A total of 60 horses were used in this preliminary study, including 6 stallions, 16 gelding and 38 mares which were aged from 1 to 28 years old. Ticks were previously detected by the owner on 13 horses. There was no statistically significant association between seropositivity and sex, age, presence of ticks, or province of living. Globally, the seropositivity was 22% in Belgium and 17% in Luxembourg. These results are in good agreement with previously reported results in other European countries and in northern Belgium. However, the study should be extended to a larger number of hoses.

15. A stop-gain in the *Laminin A3 (LAMA3)* gene causes recessive Junctional Epidermolysis Bullosa in Belgian Blue Cattle

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Four newborn purebred Belgian Blue calves presenting a severe form of epidermolysis bullosa were recently referred to our heredo-surveillance platform. SNP array genotyping followed by autozygosity mapping located the causative gene in a 8.3 Mb interval on bovine chromosome 24. Combining information from (i) whole-genome sequencing of an affected calf, (ii) transcriptomic data from a panel of tissues and (iii) a list of functionally ranked positional candidates, pinpointed a private G to A nucleotide substitution in the *LAMA3* gene. It creates a premature stop codon *(p.R2609X)* in exon 60, truncating 22% of the corresponding protein. The *LAMA3* gene encodes the _3 subunit of the heterotrimeric laminin-332, a key constituent of the *lamina lucida* that is part of the skin basement membrane connecting epidermis and dermis layers. Homozygous loss-of-function mutations in this gene are known to cause severe junctional epidermolysis bullosa in human, mice, horse, sheep and dog. Overall our data strongly support the causality of the identified gene and mutation.

Running Title: *Junctional Epidermolysis Bullosa in Belgian Blue.*

16. Identification and traceability of animal and human faecal contamination in bathing sites in Wallonia

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Bathing water site is defined as a place where water is expressly authorised. There are 37 bathing sites in Wallonia whose microbial quality must be compliant to the European Commission directive 2006/7/EC. In 2014, several bathing sites still showed unsatisfactory level of faecal contamination based on *Escherichia coli* and enterococci plate counts. The source of faecal contamination in various biotopes is multifactorial, ranging from fishes, birds to the wild/domestic mammals and humans ; making thus microbial quality management more difficult. As a culture-independent methodology, 16S metagenetic analysis has been applied in a survey of four problematic bathing sites in Wallonia. During three weeks between August and September 2014, 4 sampling points per bathing sites (the bathing site and three uphill spots) were investigated by classic microbiology and 16S metagenetic analysis. 16S profiling and classic microbiology revealed a significant proportion of bacterial population of faecal origin in the samples. The classic microbiology biomarkers (*E. coli and Enterococci*) corresponds only from 0.001% to 10% of the faecal population identified by metagenetics. Among the faecal population, we selected three abundant bacterial genera for which we collected all the available 16S genetic and performed phylogenetic analysis in order to in order to identify and trace the host sources of contamination. We were able to identify the rodents and the human as the major sources of contamination and to pinpoint these to specific sampling spots, establishing our methodology as a powerful alternative for more accurate faecal biomarkers detection and improved microbial quality management.

17. Belgian wildlife as potential zoonotic reservoir of hepatitis E virus

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Hepatitis E is an acute human liver disease in healthy individuals but may become chronic. It is caused by the hepatitis E virus (HEV) and can have a zoonotic origin. In this study, 383 sera from wild boars were selected for serology; for virology, 69 sera and 61 livers from young wild boars were used. A total of 189 and 235 sera of respectively red and roe deer were collected for serological analysis. For virology, 84 and 68 sera and 29 and 27 livers from respectively red and roe deer were sampled. An apparent seroprevalence of 34% was found in wild boars, 1% in red deer and 3% in roe deer. In order to assess the ELISA screening prevalence, Western blot (WB) analyses and a ROC curve analysis were performed. Different scenarios with varying ELISA specificities relative to WB were analysed. In wild boar, seroprevalence remained high whatever the scenario; 4 out of 69 sera and 4 out of 61 livers were detected as positive for HEV RNA. All sequences obtained from sera belonged to genotype HEV-3. HEV RNA, belonging to genotype HEV-3, was detected in one red deer liver. Wild boar can be considered as a host reservoir of the virus in Belgium. However, the low prevalence in deer makes these species an unlikely reservoir. This evidence needs further investigation in order to determine in which situation deer can serve as reservoir and raise the question of the dynamics of HEV infection between wild fauna, domestic pigs and humans.

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18. Identification of cyprinid herpesvirus 3 envelope transmembrane proteins essential to viral growth *in vitro*

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Cyprinid herpesvirus 3 (CyHV-3) is the aetiological agent of an emerging and lethal disease in koi (*Cyprinus carpio koi*) and common carp (*C. carpio carpio*). Its genome consists of a linear, double-stranded DNA of 295 kb encoding 155 open reading frames. Viral envelope proteins expressed on the surface of virion play key roles in viral entry, egress and pathogenesis. In the present study, we aimed to identify CyHV-3 envelope proteins that are essential to viral growth *in vitro*. To reach this goal, a two steps approach was developed. First, two-dimensional liquid chromatography tandem mass spectrometry (2D-LC MS/MS) were performed on purified CyHV-3 virions and led to the identification of 16 transmembrane proteins. Second, for each gene encoding these 16 transmembrane proteins, we produced a recombinant deleted strain using bacterial artificial chromosome cloning and prokaryotic recombination technology; and subsequently, tested the ability of the recombinant virus to replicate in common carp brain (CCB) cells. Eight of transmembrane proteins turned out to be essential to viral growth *in vitro*. Reversion of the deletion restored the ability of the virus to replicate. In the next step, expression on cell surface of these essential envelope proteins and their potential as target for neutralisation will be investigated using specific antibodies. In contrast, the 8 remaining proteins were not essential to viral growth *in vitro*. *In vitro* growth phenotype of these proteins will further be characterized using growth curves and plaque size assays.

19. Ecotoxicity's evaluation of 6 anthelmintic molecules used in cattle on insects feeding on dung

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The first article dealing with the environmental consequences of animal treatment with ivermectin was written in 1987. Since then, over one hundred scientific articles reported lethal and sublethal macrocyclic lactone residues in diptera and beetles feeding on dung. Therefore, the use of some anthelmintics can, through these different effects, reduce the size of the population of many insects with a life cycle linked to feces. An assay was set up in Wallonia to test the effects on these insects of six anthelmintic molecules (ivermectin, closantel, moxidectin, oxfendazole, febantel and levamisole) frequently used in the Belgian farms. The objective was to obtain enough entomological samples to compare the numbers of different groups of insects in the dung from control and treated animals. Thirty five dungs (5 animals per molecule + 5 for control) in duplicate were collected 3, 7, 14 and 28 days after treatment administration. The 280 dung samples were placed on the meadow and emerging insects were collected via a locally developed emergence trap. These traps were harvested 2 or 3 times per week during 3 months. Statistical analysis of these data doesn't permit to conclude about the toxicity of one of these molecules on the 71 insect's families identified. However, control dungs were richer in number of insects than treated dungs sampled 3 days after administration. It also appeared that dung from animals treated with ivermectin and closantel were particularly affected. Further more focused experiments are needed to evaluate in the field the environmental impact of these two molecules.

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20. Gp150 promotes sexual transmission of Murid Herpesvirus-4

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Gammaherpesviruses are important pathogens in human and veterinary medicine. During co-evolution with their hosts, they developed many strategies allowing them to shed infectious particles in presence of immune response. Understanding these strategies is likely to be important to control infection. Interestingly, we recently observed that Murid herpesvirus 4 (MuHV-4), a gammaherpesvirus infecting laboratory mice, could be sexually transmitted between mice. This model offers therefore the opportunity to understand the mechanisms underlying natural transmission. Some of these mechanisms could rely on the glycoprotein 150 (gp150), which could at least limit virus neutralization and promote the release of infectious particles from cells. In this study, we tested therefore the importance of gp150 in the context of MuHV-4 sexual transmission. Briefly, female mice were infected with WT or gp150- strains expressing luciferase. They were then imaged with an *in vivo* imaging system to follow infection. When lytic replication was observed in the genital tract, infected females were mated with naïve males to compare the capacity of transmission of the two strains. Our results show that, while the gp150- strain has no deficit in reaching and replicating in the female genital tract, it displays a major deficit of sexual transmission in comparison with WT virions. Interestingly, this deficit appears to reflect a deficit of virions release from vaginal epithelial cells. Altogether, our results show that, while gp150 is not required for efficient dissemination and maintenance of MuHV-4 within its host, it is essential for efficient transmission, at least by the sexual route

Posters

Veterinary public health

1. Equine cadaver ligaments : A new promising source of stem cells

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The isolation of mesenchymal stem cells (MSCs) from cadaveric tendon or ligament has not been achieved yet. This approach should be explored and could generate new insights and provide future treatment strategies in regenerative medicine. Four suspensory ligaments, received within 48-72 hours of post-mortem (from horses 18-20 years old) were used for this study. Equine cadavers (EC) MSCs were isolated, cultured and evaluated for viability, proliferation, expression for the pluripotent transcription factor (OCT-4) and the surface-based pluripotent marker (SSEA-1), as well as being characterized by transmission electron microscope (TEM). EC-MSCs were successfully expanded *in vitro*, and maintained for 20 passages with high cell viability and proliferation. Phase contrast microscopy revealed that cells with fibroblast-like appearance were the predominant population in the culture. Immunofluorescence analysis showed a high percentage of positive cells for OCT-4 and SSEA-1. Furthermore, ultrastructural analysis using TEM confirmed the stemness of EC-MSCs. To the best of our knowledge, this work represents the first attempt, in both animal and human, to isolate and characterize MSCs from cadaveric tendon or ligament. Our research may benefit the development of an advantageous source of stem cells for regenerative medicine. EC-MSCs may provide a low cost solution in ready supply for the development of novel therapeutic strategies for equine tendon or ligament injuries.

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2. Optimisation of culture media for *Bifidobacterium bifidum* and *Bifidobacterium crudilactis* and study of the antimicrobial effect of the culture supernatants

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Complex oligosaccharides from human milk (HMO) contribute to infant health. *Bifidobacteriumbifidum* mainly found in breast-fed infant microbiota has all the enzymatic machinery for degradation of HMO. On the other hand, whey is rich in complex bovin milk oligosaccharides (BMO) very similar to HMO, including 3’-sialyllactose (3’SL). They are very likely to be metabolised by *B. bifidum* too, but also by *B. crudilactis*, a bovine origin strain. Fermentation of HMO or BMO by bifidobacteria can result in production of metabolites modulating virulence expression of several pathogenic bacteria.

Two strains of bifidobacteria were used in this study: *B. bifidum*, isolated from breastfed infant feces and *B. crudilactis*, isolated from bovine raw milk. The ability of those strains to metabolise culture media enriched in glucose, whey and 3’SL has been assessed. Then, the obtained culture supernatant has been tested against virulence genes expression of *E. coli* O157:H7.

Both strains were able to grow in presence of BMO and 3’SL. *B. crudilactis* presented the best growth on all media. All culture supernatants obtained after supplementation with 3’SL resulted in significant under-expression of genes *ler* and *qseA*. The trend of genes *stxB2* and *luxS* was also toward a down-regulation.

BMO combined to some bovine or human origin bifidobacteria strains could be interesting synbiotics to maintain or restore the intestinal health of young children. These effects observed *in vitro* require further investigations to ensure repeatability in humans and to identify the exact nature of molecules obtained from fermentation media by *B. bifidum* and *B. crudilactis*.

3. The use of 16S rDNA metagenetic monitoring of refrigerated meat products for understanding the kinetics of microbial subpopulations at different storage temperatures: the example of white pudding

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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. According to this, this work proposed to study the bacterial microbiota of Belgian white-puddings during storage at different temperatures, representative of good or bad storage practices, using culture-dependent and independent methods. This product was inoculated with a mix of strains: *Carnobacterium maltaromaticum, Lactobacillus fuchuensis, Lactobacillus graminis, Lactobacillus oligofermentans, Lactococcus lactis, Leuconostoc mesenteroides, Raoultella terrigena* and *Serratia sp.* Daily during 16 days, the relative abundance of each inoculated strain was monitored by combining total plate count and metagenetic analysis, which were then compared with qPCR analysis. These data allowed the bacterial strains subdivision into three classes based on criteria of growth parameters for the studied temperature: the "dominant", the "subdominant" and the "inhibited" bacterial species, according to their maximal concentration (Nmax, log CFU/g), growth rate (μmax, /h) and time to reach the stationary phase (days). These data have permitted to follow the evolution of each dominant bacteria currently with the behaviour of the whole microflora in each temperature conditions. These results highlighted the importance of keeping a standard low temperature constant during storage of meat products. This work shows also that the reliability of the Jameson effect can be discussed.

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4. Cercarial dermatitis (swimmer's itch) in Vietnam: A morphological and molecular approach to determine intermediate and definitive hosts and responsible trematode species

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Cercarial dermatitis (swimmer's itch) is a zoonotic disease caused by the penetration of human skin by avian schistosome cercariae. This disease is found worldwide but it has not been reported in Vietnam so far. Therefore, the aim of the present study was to identify the potential intermediate and definitive hosts of trematode species known to be responsible for this condition in man. Two lymnaeid snails (*Austropeplea viridis* n= 4231 and *Radix auricularia* n=5513) were collected in rice-fields and channels in Hanoi city and examined by the crushing method. Twenty five domestic ducks (*Anas platyrhynchos*) were collected in the same area and the liver were dissected. Adults and larval stages of avian schistosome were collected for microscopy and DNA extraction. A morphological description of trematodes was performed. The ITS2 sequence was selected as marker. The results showed that the prevalence of the parasite in duck was high (64.0%). Ocellate brevifurcate-apharyngeate cercariae were observed in *A. viridis* (1.53%; 65/4231) with morphological characteristics: oval body, two eye spots located between acetabulum and head organ, penetration glands present, the inner surface of ventral sucker curved spine, tail distinctly longer than furcae. The morphological characteristics of adult and cercariae *schistosomatids*' were similar with description of *Trichobilharzia franki*. In addition, sequences analysis of the ITS2 region confirmed that they are identical to *T. franki*. This study indicates that the agents of cercarial dermatitis are present in Vietnam. The medical impact of these pathogens should be assessed by the medical profession.

5. Use of RAL Stainer for the detection of *Mycobacterium* genus in veterinary medicine. Comparison with the reference

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Tuberculosis and paratuberculosis are two diseases with economic impact as well in public than as animal health. Besides considerable decreases in meat and milk production, there are a zoonotic and epidemiological aspects. Indeed, some wild animals can be reservoir and transfer the pathogen (genus *Mycobacterium*) to humans, pets and breed animals. It is therefore necessary to detect these diseases in breeding farms but also in wildlife (including wild boar, badgers, roe-deer and red-deer) to assess health risks. The genus *Mycobacterium* is part of acid fast bacteria resistant and bacterioscopic method reference is the Ziehl-Neelsens hot protocol (ZN). Its main inconveniants are the toxicity of phenol vapors and the staining time. The RAL company developed a stainer (Gram, Ziehl and Giemsa) using a cold variant of ZN and able to color 20 slides at a time. If this device has been validated in human medicine, its use in veterinary medicine is rare. In addition, animals samples (fecal materials, mesenteric or mandibular lymph nodes, etc) are more contaminated than humans samples (sputum), with no bacterian elements (especially in the case of ruminant feces) that can interfere with staining. This study aims to compare the coloration of different samples (breed animals / wild fauna, diverse biological origins) using the RAL Stainer with the reference method in order to validate its routine use.

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6. Wild boar's mandibular lymph nodes microbiota: a 16S ribosomal DNA-based characterisation

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Wildlife diseases have gained considerable attention in recent years due to their impact on public health, economy and wildlife conservation. The role of wildlife as reservoirs of pathogens transmissible to livestock are problematic in countries where overabundance of wild species and extensive management of livestock increase the risk of interspecies transmission. The aim of this study was to characterize the microbiota of wild boar mandibular lymph nodes by a metagenomic analysis targeting the V1V3 hypervariable region of 16S ribosomal DNA using MiSeq sequencer with in-house bioinformatic pipeline. We identified various bacteria and especially some known for their possible zoonotic transmission: *Streptococcus suis, Streptococcus dysgalactiae, Hafnia alvei, Erysipelothix rhusiopathiae, Moraxella catarrhalis, Staphylococcus aureus, Corynebacterium ulcerans* and *Brucella spp*. Overall, our results suggest wild boar may a play a role in spreading pathogens that may have an impact on human and animal health.

7. Generating germline chimera by intra-cardiac injection of genetically modified primordial germ cells in the chick embryo: 1st success

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The production of transgenic chickens has increasing applications in biotechnology, providing an excellent model organisms for developmental biology research and bioreactors for pharmaceutical proteins production. Increasing public health safety by generating poultry with engineered disease resistance and/or improved production traits should have an even greatest global impact. Germ line transgenesis in avian species has been described as a process involving the injection of genetically modified PGCs into the blood stream of embryos to produce germline chimera (van de Lavoir *et al.* 2006*). Since the injection is performed at 65h post-incubation, providing a favorable environment for chick embryos during the procedure and for the subsequent development is challenging. A counterintuitive but operative solution is the transfer of the chick embryo into a surrogate eggshell prior to the injection until hatching. Here, we report our in-house experience with surrogate turkey eggs and the successful hatching of control and putatively chimeric chicks.

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8. 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 could lie in the use of viral vectors. Since the 1970s, alive, orally administered adenovirus serotype 4 and 7 vaccines are effectively used to protect United States military personnel from severe respiratory disease 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 murine adenovirus type 1 (MAV-1) to characterize adenovirus oral infection in mice. No clinical signs were observed following oral administration of the virus. Nevertheless, viral DNA was detected by qPCR in various organs, showing that virus efficiently infect mice by the oral route. Furthermore, we observed an increase in MAV-1 specific and neutralizing antibodies over time, indicating that oral infection generates a humoral response against the virus. We finally evaluated the protection induced by MAV-1 oral infection against a respiratory challenge with the same virus. Clinical observations and histological analyses showed that orally immunized mice were protected against severe symptoms observed after intranasal infection of naive mice. Altogether, these results show that MAV-1 offers a reliable model for oral vaccination based on replicative adenoviruses. This model provides a precious tool to study the potential of orally administered adenoviruses as vaccine platform.

9. Use of metagenetics and classical microbiology to assess the bacterial superficial contamination in cattle classically slaughtered or following the halal ritual

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In Europe, two cattle slaughtering protocols exist: the classical method, that encompasses a stunning step before the sticking procedure, and the halal method, combining the stunning and the sticking in one single step. In the halal protocol, a single cut with a sharp knife is practiced directly on live cattle, instead of two cutting steps in the classical slaughtering technique. The aim of this study was to seek if the two slaughtering techniques were similar regarding the superficial contamination of carcasses, swabbed between 2 and 4 hours after the killing step. Classical microbiological tests (TVC and *Enterobacteriaceae*) and 16S rDNA metagenetic analysis were carried out from 20 cattle carcasses (swabbing of "legal" zone – 1,600 cm2 – and in the neck area – 200 cm2). The microbiological results revealed no significant differences between the two slaughtering practices. Statistical analysis of pyrosequencing data showed that differences in bacterial population abundance between slaughtering methods were mainly found in the "legal" swabbing zone compared to the neck area. Bacterial genera belonging to Actinobacteria (*Brevibacterium*, *Corynebacterium*) were more abundant in "Halal" samples whereas populations from the Proteobacteria (*Caulobacteraceae*, *Comamonadaceae*, *Bradyrhizobiaceae*) and Firmicutes (*Lactobacillus*) were more abundant in the "classical" group. The analysis of OTU abundance of bacteria from the digestive or respiratory tract revealed no differences between groups. In conclusion, the slaughtering method does not influence the superficial microbiological pattern in terms of specific microbiological markers of the digestive or respiratory tract, the legal swabbing zone being still the best sampling zone compared to the neckline.

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10. Molecular epidemiology and modeling the dynamic of spread of Foot-and-Mouth Disease in Niger

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Foot-and-Mouth Disease (FMD) is one of the most important animal diseases which occur worldwide. The causative agent is a picornavirus, member of the genus Aphthovirus belonging to the Family *Picornaviridae*. Seven serotypes (A, O, C, Asia-1, Southern African Territories (SAT)-1, SAT-2 and SAT-3) exist and there is no cross protection between them. FMD is endemic in Niger and cattle are mainly being involved. Reviews of submissions to World Reference Laboratory suggested that serotypes O, A and SAT 1&2 have been circulating in Niger from 2000 to 2006. At this time, there is no vaccination against FMD virus and no restriction of animal movement in the affected areas. In addition, there is an under-reporting of FMD outbreaks and the identified cases are not mainly confirmed by laboratory analysis. Considering that the FMD virus has huge antigenic variation, substantial care must be exercised in selecting vaccine strains that are appropriate for a particular outbreak. In this context, an intensive molecular study of different serotypes regularly suspected is required. However, descriptive epidemiology is essential for a better interpretation of molecular study. Little study of FMD was performed in Niger which nevertheless due its geographical position constitute a risk zone for other regions like the North Africa. The number of outbreaks reported does not fully take into account neither the size of the population at risk or the spatial distribution of FMD. Therefore, it is clear that, this approach does not assess the risk associated with the onset of disease. Therefore, there is need to analyse the relative risk of FMD and its spatial and temporal distribution.

11. Bovine herpesvirus 4 modulates its beta-1,6-N-acetylglucosaminyltransferase activity through alternative splicing

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Carbohydrates play major roles in host-virus interactions. It is therefore not surprising that, during co-evolution with their hosts, viruses have developed sophisticated mechanisms to hijack to their profit different pathways of glycan synthesis. Thus, the Bo17 gene of Bovine herpesvirus 4 (BoHV-4) encodes a homologue of the cellular core 2 β-1,6-N-acetylglucosaminyltransferase-mucin type (C2GnT-M) which is a key player for the synthesis of complex O-glycans. Surprisingly, we show in this study that, as opposed to what is observed for the cellular enzyme, two different messenger RNAs are encoded by the Bo17 gene of all available BoHV-4 strains. While the first one corresponds to the entire coding sequence of the Bo17 gene, the second results from the splicing of a 138 bp intron encoding critical residues of the enzyme. Antibodies generated against the Bo17 C-terminus showed that the two forms of Bo17 are expressed in BoHV-4 infected cells, but enzymatic assays revealed that the spliced form is not active. In order to reveal the function of these two forms, we then generated recombinant strains expressing only the long or the short form of Bo17 and showed by glycomic analyses that BoHV-4 uses alternative splicing to markedly regulate the cellular core 2 branching activity of infected cells. We therefore postulate that the relative abundance of active/inactive forms of pBo17 in Golgi oligomeric complexes may define the global level of C2GnT-M activity in the infected cell. These results suggest the existence of new mechanisms to regulate the activity of glycosyltransferases from the Golgi apparatus.

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12. Orbivirus screening on dried blood spots from captive Oryx in United Arab Emirates stresses the importance of pre-import measures

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Following reintroduction and conservation programs of the Arabian Oryx (*Oryx leucoryx)* and the scimitar horned Oryx (SHO, *Oryx dammah*) in the United Arab Emirates (UAE), import of animals from the United States of America (USA) is not uncommon. The objective of this study was to estimate the prevalence of Bluetongue virus (BTV) and epizootic haemorrhagic disease virus (EHDV) in Arabian Oryx and SHO from captive herds in the UAE. Dry blood spots from 175 SHO and 16 Arabian Oryx were collected. The latter were imported from Texas (USA) two weeks before sampling. Elutes from blood spots were tested for BTV and EHDV antibody detection with indirect and competitive ELISAs, respectively. Samples were also tested by pan-BTV RTqPCR detecting all BTV serotypes. Serotype specific end-point RT-PCR to detect BTV2, BTV8, BTV10, BTV11, BTV13 and BTV17 were also performed on RTqPCR positive samples. Eight out of 16 Arabian Oryx were found BTV seropositive. BTV genome was detected in 5/16 of the Arabian Oryx. Serotype could not be identified by end-point PCR. There were no EHDV positive samples. At least 15 different BTV serotypes were reported in the USA and at least 10 in the Middle East, thus the Oryx could be infected by a serotype not tested so far. The sensitivity of end-point RT-PCR might be insufficient to detect BTV out of eluted blood spots. These results stress the need for pre-import risk assessment and implementation of biosecurity measures when considering translocation of wild ruminant species susceptible to BTV and EHDV.

13. Nociceptive innervation in the anterior horn of the equine medial meniscus: an immunohistochemical study

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In horses, diseases leading to lameness are of major importance due to the loss of performance and the economic concerns. Although that, in the hindlimb, stifle lesions are frequent due to the large and complex joint and that meniscal tears have been identified as the most common soft tissue injuries in this joint, little is known about the mechanism that generate the painful sensation and thus the lameness. The aim of our study was therefore to highlight any nociceptive fibers in healthy anterior horns of the equine medial meniscus, which is the most common localization reported for equine meniscal injuries. Immunohistochemical stainings were performed using antibodies against substance P in order to identify nociceptive fibers and against glial fibrillary acidic proteins in order to identify Schwann cells.

From a purely fundamental point of view, our work highlights for the first time the presence of nociceptive fibers in equine meniscus. They were found in the abaxial part of the anterior horn of the equine medial meniscus. This study suggests thus that when the abaxial part is injured, the meniscus itself could be the source of pain in meniscal tear. In order to alleviate painful sensation in equine meniscal tears, this finding provide a better understanding of the sensory innervation of the equine meniscus which could serve as a basis for the establishment of new therapeutic strategies.

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14. Serological tests for detecting bovine brucellosis: a systematic review

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The occurrence and spread of zoonotic diseases such as brucellosis are major public health concerns. Surveillance programs combined with efficient serological detection tests have allowed countries to become free of brucellosis (for example: Northern Europe or Canada). Unfortunately false positive serological reactions often occurs mainly due to cross reactivity between the lipopolysaccharide (LPS) of *Brucella* and *Enterobacteriaceae* such as *Yersinia enterocolitica*. The aim of this study was to conduct a systematic review of the literature focusing on serological tests used to detect brucellosis in cattle. A total of 3306 articles were retrieved using 3 online databases: PubMed, Scopus and Cab Abstracts. Inclusion and exclusion criteria were applied and 47 articles were selected and analyzed. Indirect ELISA and competitive ELISA are the most used diagnostic tests followed by the Fluorescence Polarization assay. The aforementioned two first tests used the LPS, outer membrane proteins (OMPs) or synthetic oligosaccharides as antigens whilst the third test used the O-polysaccharide. These antigens provide good results in term of sensitivity and specificity. However the choice of a test should rely also on the seroprevalence of the country of interest. The lack of details regarding the cross reactivity with other bacteria (e.g. *Yersinia enterocolitica*), study area, repeatability and reproducibility of the test being assessed lead to difficulties to interpret the results and consequently to the misunderstandings of the conclusions. This systematic review encourages authors to provide detailed information about all characteristics of tests in order to be reproduced by others or to develop better serological tests for the detection of bovine brucellosis.

15. Comparison of different identification approaches of mammo-pathogenic bacteria: typing and characterization of staphylococci

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The aim of this study is threefold: bacteriological identification of mastitis-associated bacteria from 178 milk samples; comparison of the bacteriological results with those of the quantitative (q) PCR and establishment of the antibiotic resistance profile for these bacteria.

178 milk samples were collected from cows with mastitis in 11 farms between January and June 2014. Each sample was inoculated onto Columbia blood agar and 3 selective agar plates. After overnight growth at 37°C, colony counts were performed. Isolates with >100 CFU/ml were identified using appropriate API sets.

Out of a total of 233 isolates growing on Columbia blood agar, 130 also grew on Chapman's agar, 68 on Edwards' agar, and 17 on Mc Conkey's agar. The 18 isolates growing only on Columbia blood agar were bacilli and yeasts.

So far the results from 9 farms have been compared excluding positive samples for mycoplasma or algae to the PathoProof®. A total of 160 isolates that have been compared showed that 120 (75%) samples yielded positive results for both tests. Of these 120 samples (positive Pathoproof-BC), a total or partial matching between the results of the two tests has been found for 69 samples (57%).

The antibiogram on different isolates revealed quite variable resistance values for staphylococci (including potentially resistant isolate to methicillin). This resistance is well marked for streptococci / enterococci (including a potentially resistant isolate to vancomycin), whereas they are relatively low in Enterobacteriaceae.

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16. Characterization and relatedness of *Clostridium difficile* strains isolated from animals, meat and humans in Belgium

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Clostridium difficile is an anaerobic spore-forming bacterium recognised as a major cause of nosocomial colitis and antibiotic associated diarrhea. Over the past few years, several studies have focused on the possible role of animals and food as contamination routes for human *C. difficile* infections. The aim of this study was to isolate and compare the *C. difficile* strains circulating in animals, food and humans in Belgium. Faecal samples of newborn pigs and calves were collected from breeding farms. Intestinal contents and carcasses samples were collected from cattle and pigs at slaughterhouse. Raw meat was obtained from the retail trade. Horse faecal samples were collected from hospitalized animals. Human *C. difficile* isolates were obtained from care home residents and hospitalized patients. *Clostridium difficile* strains were compared with respect to the toxin gene profile, PCR-ribotyping, antimicrobial activity, multilocus sequence typing (MLST) and multiple-locus variable number tandem repeat analysis (MLVA). A neighbour-joining phylogenetic three was constructed in order to determine the correlation between human, animal and food isolates. A total of 127 isolates belonging to 32 different PCR-ribotypes were collected. The PCR-ribotypes most prevalent in terms of number of isolates were 078, 014 and 027. For a given PCR-ribotype, strains presented a similar susceptibility to the antimicrobials tested, irrespective of the isolation source. Phylogenetic analysis showed that human, meat and animal isolates with the same PCR-ribotype cluster in the same lineage. The overlap between strains from animal, food and human origins suggest a potential risk of interspecies and foodborne transmission.

17. Carriage of *Clostridium difficile* in hospital patients in spain, including molecular characterization and antimicrobial susceptibility of the isolates

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Increasing age, several co-morbidities, environmental contamination, antibiotic exposure and other intestinal perturbations appear to be the greatest risk factors for *C. difficile* infection (CDI). Therefore, hospitalized patients are considered particularly vulnerable to CDI. The main objective of this study was to evaluate the prevalence of *C. difficile* in a Spanish hospital and to characterize the isolates with respect to the PCR-ribotype, antibiotic resistance and toxin activity. In addition, multilocus sequence typing (MLST) was performed in order to study clonal relationships between *C. difficile* isolates obtained from two different European countries.

Culture of samples was performed in a selective medium cycloserine cefoxitin fructose cholate. An identification of the isolated colonies was done by PCR detection of *tpi*, *tcdA*, *tcdB* and *cdtA* genes. Toxic activity was confirmed by a cytotoxic immunoassay. Further characterization was performed by PCR ribotyping and MLST was used in order to determine genetic relationships between Spanish and Belgian *C. difficile* isolates recovered from hospital patients in both countries.

Clostridium difficile was frequently detected in hospitalized patients. The isolates belonged to different PCR ribotypes, including type 027. Most of the strains obtained harboured *tcdA* and *tcdB* genes. The number of positive faecal samples considerably increased among elderly patients over 65 years old.

This study reveals the circulation of toxigenic *C. difficile* in a Spanish hospital. The relatedness between Belgian and Spanish isolates indicates a common source or a lack of diversity from some PCR-ribotypes. Continuous inter-country surveillance is suitable to understand the spread of *C. difficile* isolates among hospitalized patients.

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18. Canine idiopathic pulmonary fibrosis is not associated with herpes virus infection

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An association between gammaherpesvirus infection and pulmonary fibrotic disorders has been suggested in humans, horses and rodents. In dogs, canine idiopathic pulmonary fibrosis (CIPF), a fibrotic lung disease of unknown origin and poorly understood pathophysiology, occurs principally in West Highland white terriers (WHWTs) at an advanced age. The present study was intended to investigate the potential association between herpesvirus infection and CIPF. PCR assays, using either degenerate or deoxyinosine-substituted primers targeting highly conserved regions of the DNA polymerase gene (DPOL) of herpesviruses were used on lung samples from WHWTs affected with CIPF and control dogs from various breeds. DNA of gammaherpesvirus-positive murine (Murid herpesvirus-4) and bovine (Bovine herpesvirus-4) spleen samples were included as positive controls. Water samples were tested as negative controls. Herpesvirus DPOL sequence could not be amplified from the 46 lung samples (28 CIPF-WHWTs and 18 controls) tested. Results of the present study are not in favor of an association between herpesvirus infection and CIPF. Investigation of other etiologic agents is warranted.

19. A herpesvirus alters the behavior of its host to enhance its replication and transmission

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When infected by pathogens, endotherms and ectotherms can both increase their body temperature to limit the infection. Ectotherms do so by moving to warmer places, hence the term "behavioral fever". Previous studies of the regulation of behavioral fever in ectotherms at the level of the central nervous system have demonstrated its evolutionary relationship with fever in endotherms. However, the function of cytokines as peripheral mediators of this process is still unknown. It is also unknown whether pathogens can alter this behavior to increase their fitness. Here, we show that common carp express salutary behavioral fever in response to cyprinid herpesvirus 3 (CyHV-3) infection and that Tnfα1 is a key mediator of this innate mechanism in the CyHV-3/carp model. Our results demonstrate that fever in endotherms and behavioral fever in ectotherms are evolutionarily and functionally related through cytokine mediators that originated more than 400 million years ago. We also demonstrate that CyHV-3 is able to delay the migration of infected fish to warmer environments and thereby promotes its replication and transmission. The mechanism of this inhibition was found to rely on the expression of ORF12 encoding a viral soluble Tnfα-receptor. Surprisingly, ORF12 deletion did not exhibit a phenotype under standard laboratory conditions but only revealed its function under conditions mimicking temperature gradients of natural environments. Remarkably, this study demonstrates for the first time the ability of a vertebrate virus to alter host behavior through the expression of a single gene.

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20. Anatomical and histological study of the sheep brain

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The study of normal structures of brain is very important to understand the vital and pathological functions. The aim of this work is to improve anatomical and histological knowledge of sheep brain. In this study, five heads of adult sheep were opened and immerged in formalin about 10 days. After good fixation, the brains were extracted and dissected. Transversal, frontal and sagittal sections were performed and stained by the method of Mulligan for macroscopic examination. Two different histological techniques of staining (the hematoxylin-eosin and the cresyl violet) were compared. Gross morphological examination showed the detailed anatomy of the different parts of brain. The microscopic analysis permitted to determine the different layers of the cerebral and cerebellar cortex. the layers of the cerebral cortex were more visible by using the cresyl violet. The hematoxylin-eosin allowed to identify clearly the layers of the cerebellar cortex and dendrites of neurons. The present work lays the bases for future studies on the physiological and pathological processes in sheep brain.

21. Etude de la diversité microbienne de poudre de lait dopée par analyse métagénomique: Quantification des bactéries vivantes par exclusion de bactéries mortes

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L'étude de la diversité bactérienne et la quantification des taxons composant cette diversité a été menée sous différentes conditions sur des poudres de lait dopées de bactéries faisant l'objet d'un essai interlaboratoire organisé par le Réseau d'Analyses et d'Echanges en Microbiologie des Aliments (RAEMA), Paris, France. Les échantillons ont subi trois types de traitements : une extraction classique, une extraction mécanique avec des billes et une extraction précédée d'un traitement permettant l'exclusion des bactéries mortes. Au départ de L'ADN bactérien, la région hypervariable V1-V3 de l'ADNr 16S des bactéries a été séquencée en utilisant la technologie de séquençage haut débit Illumina Miseq. L'analyse métagénétique du profil 16S révèle une très grande diversité bactérienne composée des genres *Lactococcus, Lactobacillus, Leuconostoc, Streptococcus, Pseudomonas, Geobacillus, Anoxybacillus, Acinetobacter, Aerococcus, Corynebacterium, Chryseobacterium* et les bactéries inoculées. Une grande proportion des séquences d'ADN identifiées (55,5 % et 57,7%) est attribuée à l'espèce *Lactococcus lactis subsp. cremoris* SK11 respectivement dans les échantillons suite au traitement classique et celui avec les billes. Cependant lorsque l'exclusion de l'ADN des bactéries mortes est incluse avant l'extraction, cette proportion, représentant de l'ADN issu de bactéries inactivées passe à 26,3%. Par ailleurs, la proportion d'*Entecoccus faecalis* passe à 36,6% (précédemment 1,8%), espèce qui constitue la flore majoritaire inoculée, et de *Staphylococcus aureus*. Ces résultats démontrent que le prétraitement appliqué permet de masquer une importante proportion de l'ADN des bactéries mortes et de mieux suivre l'ensemble des populations de germes ajoutées. Cependant, l'effet du prétraitement est variable sur les différentes espèces bactériennes.

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22. Progress in the etiological diagnosis of bovine abortions: the contribution of 16S rRNA metagenetic analysis

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Failures in bovine reproduction due to abortions cause heavy economic losses in this sector. Although the determination of the cause of abortion is often difficult, an etiologic diagnosis is necessary to develop appropiate sanitary measures. Cases of bovine abortions were submitted to the laboratory of the Regional Association of Identification and Animal Health (ARSIA) through the surveillance of bovine brucellosis. These aborted fœtuses were all submitted to a standardized panel of analysis covering a large spectrum of pathogens. In this study, the direct involvement of at least one pathogen was demonstrated in half of analyzed fœtuses, suggesting an important progress marging in the diagnostic efficiency. In this perspective, a method to identifying bacteria that are difficult to isolate by classical culture methods (intracellular or slow growing species) or non-viable germs (due to an antibiotic treatment or a poor sample conservation) has been prospected.

16S rRNA sequencing is an "universal" approach and does not require targeting bacteria with specific primers in order to detect them. Therefore, the application of this technique within the abortion surveillance protocol would increase the sensitivity and thus reduce the proportion of unresolved cases. Indeed, some abortive pathogens that require a particular growing medium were identified by 16S rRNA sequencing in the absence of a positive result on culture. This technique has broadened the etiologic diagnosis and opens new horizons in the diagnosis of abortion cases.

23. Porcine reproductive and respiratory syndrome virus (PRRSV) serosurveillance in the wild boar (Sus scrofa) population of Southern Belgium, 2014

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A recent study has shown that, at herd level, the apparent seroprevalence of PRRSV in Walloon pig farms is 48% (IC95%: 39 – 57%). In that region of low density of pig production (0.05 pig farms/km²), wild boars are largely distributed, with an estimated population of 25 000 animals over 16 844 km². As wild boars share a variety of pathogens with farm animals, they theoretically constitute a source of contamination. It is therefore important to evaluate the fraction of the wild boar population that eventually hosts the PRRSV as they could (re)introduce it, especially in outdoor pig farms. The present study had for objective to determine the global seroprevalence of PRRSV in the wild boar population of Wallonia. 434 wild boars from 31 forest districts were sampled during the hunting season 2014. Animals were necropsied in the field, within 2-3 hours after shot. Postmortem examination included determination of sex, age, body weight, and body condition. Blood was collected in dry tubes by veni- or cardiac puncture. Samples were transported to the lab within 12 hours. After centrifugation, sera were stored at −20°C until analysis. An ELISA (IDEXX-PRRS-X3) was used to screen the presence of IgG anti-PRRSV. An individual result was considered negative if the s/p ratio was <0.4. A total of 6/434 wild boars were found serologically PRRS-positive. The overall apparent prevalence was 1.38% (IC95%: 0.29 – 2.47%). Our results provide indirect evidence of the presence of PRRS virus in wild boars. A screening at larger scale is needed in order to confirm this low seroprevalence in the region.

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24. O serogroups of enteropathogenic (EPEC) and Shigatoxigenic (STEC) *Escherichia coli* from <1 month-old diarrhoeic calves 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. Besides the O157:H7 serotype, EPEC and STEC can belong to more than sixty O serogroups. Of them, 8 have been most frequently identified worldwide: O5, O26, O103, O111, O118, O121, O145 and O165, with some also causing diarrhoea in young calves. This study aimed at identifying the pathotypes and the O serogroups of STEC and EPEC isolated from <1-month-old diarrhoeic calves in Wallonia. A total of 233 enterohaemolysin-producing *E. coli* were isolated at ARSIA between November 2008 and Februari 2014 from diarrhoeic calves after growth on EHLY Medium®. They were tested with a triplex PCR targeting the *stx1*, *stx2* (Shiga toxins) and *eae* (AE lesion) genes. Triplex PCR-positive *E. coli* were assayed with two pentaplex PCR targeting the specific genes coding for the nine O serogroups listed above and for the O104 serogroup. In conclusion, 206 isolates tested positive with the triplex PCR. The most frequent pathotypes were *eae+stx1+* (102 isolates), *eae+* (78 isolates) and *eae+stx1+stx2+* (13 isolates). The most frequent serogroups of EPEC and STEC were O26 (57 isolates) and O111 (36 isolates). A few additional isolates tested positive for the O103, O5, O145, O121 and O157 serogroups. The future is to compare these EPEC and STEC with those isolated from healthy cattle and from humans, to identify host- and age-specific properties.

25. Comparative study of experimental infection of piglets with a field strain of wild boar HEV, a wild boar HEV strain previously passed in porcine model and a swine HEV strain

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Domestic pig and wild boar are reservoirs for hepatitis E virus (HEV). This study aims to investigate the infection of pigs with HEV strains from wild boar and to compare the behaviour of a wild boar strain to a pig strain *in vivo*. The objective is to contribute to the elucidation of the crossing barrier between wild boar and pig with this zoonotic virus. A total of 12 specific pathogen free piglets were divided into four groups and orally inoculated respectively with a wild boar HEV strain previously passed in pigs (WbHEV), a wild boar HEV (WbHEVs), a swine HEV (SwHEV) and a negative control group. One pig from each group was euthanized 15 days after inoculation. The remaining pigs were sacrificed on day 56. A serological monitoring by ELISA was realized throughout the experiment, the viral load was determined in different organs by qRT-PCR. Viral RNA was found in several organs and tissues of the inoculated pigs. Most of the pigs were HEV positive at the 15th day and no clinical signs were observed during infection. Liver enzymes (ALT and AST) remained within the reference values. This study provides experimental evidence of the swine infection with a strain of HEV isolated from wild boar and previously passed in pig. Furthermore, these data indicate the possibility of the transfer of the virus from wild boar to pig, for example, in the context of outdoor pig breeding.

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26. Phylogenomic comparison of 16 O5 Shigatoxigenic *Escherichia coli* (STEC) isolated from young calves and humans with diarrhoea

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In developed countries, *Escherichia coli* producing Shiga-toxins (STEC) cause enteritis and diarrhoea in young calves and humans, the human haemolytic–uremic syndrome, and are present in the intestines of healthy cattle. Besides the most frequent serotypes, STEC can belong to several subdominant serotypes, like O5:H- that is present in young calves and, more rarely, in humans and healthy cattle. The aim of this study is to analyse the phylogeny of the most important virulent factors detected in 12 calves and 4 human O5 STEC belonging to different pathotypes: *eae+stx1+* (14), *eae+stx1+stx2+* (1) or *stx1+* (1). Genomic DNA was extracted from a growing colony and sequenced on the Illumina MiSeq platform. The raw sequences were assembled into scaffolds and subjected to an automated annotation pipeline (RAST2.0). The scaffolds were used to identified virulence genes and 7 housekeeping genes (VirulenceFinder1.2, MLST1.7). Nucleotide homologies were calculated for the main selected virulence genes on the 16 isolates and an O5 bovine reference strain; both were aligned within BioEdit. Phylogenetic relationships including additional Enterobacteriacea genomes were inferred with MEGA6. More than 99% of identities were observed in the alignment between the best matching virulence genes in the VirulentFinder1.2 and the corresponding sequences of the 16 isolates. Same results were observed for the MLST using MLST1.7. A high nucleotide homology (95–100%) was observed in most of the selected virulence genes and MLST sequences. No significant difference was observed in the main virulence genes and MLST between the O5 STEC isolated from human and young calves.

27. Long term culture, cryopreservation and genetic modification of chicken primordial germ cells

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Avian primordial germ cells (PGCs) are precursor of gametes and appear during early stages of embryonic development. Under appropriate culture conditions, these cells can keep their germ cells properties in vitro and are foreseen as promising tools for developing efficient avian genetic engineering and preservation of germplasm. We propose original methods that allow long term expansion, efficient cryopreservation and genetic modification of primary cultures of undifferentiated PGCs. PGCs are collected from embryonic blood during their migratory period and grown in cell-culture insert in the presence of feeder cells (BRL). This physically separated co-culture system along with selective culture medium promoted emergence, selection and proliferation of PGCs lines. Forty percent of blood samples gave rise to lines originating from three commercial layer and two Belgian endangered breeds. PGCs lines were characterized for the expression of the stem cells and PGCs marker SSEA-1 by FACS. RT-PCR confirmed expression of germ-line specific markers (CVH, CDH, DAZL), pluripotency markers (cPouV, cSox2, cNanog), telomerase and CXCR4 receptor. All lines were male although isolated from pooled male and female blood samples. Two cryopreservation methods were developed based upon slow-freezing and aseptic vitrification. Both have shown a similar effectiveness in allowing storage without phenotype drift. Stably expressing lines were obtained by Lipofectamine® mediated transfection of a GFP plasmid. PGCs were subsequently injected in recipient embryos. Persistence of exogenous PGCs in the developing gonad of recipient embryos confirmed that PGCs retain their gonadal colonisation ability, both after long-term culture and after cryopreservation.

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28. A retrospective study of causes of mortality in free ranging red deer (Cervus elaphus) found dead or shot for sanitary reasons in Southern Belgium 2010-2014 (n=190)

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The objective of this retrospective study was to report the results of 190 red deer pathologic examinations. One hundred and nine of them were found dead whereas the others (n= 81) were shot for sanitary reasons and all of them came from southern Belgium. The study was performed from January 2010 to December 2014. *Postmortem* examinations were performed at the Veterinary Faculty of Liège according to a systematic protocol on the basis of unusual gross lesions: targeted microbiological analysis, histopathological analysis and eventually Xrays examinations (for traumatic injuries for exemple). Regarding the found dead animals transported at the Faculty (n = 109), the distribution of causes of death was as follows: traumatic 62/109, infectious 20/109, parasitic 10/109, miscellaneous 9/109 and undetermined causes 8/109. Traumatic injuries mainly involves road accidents and poaching trials whereas the main infectious cause includes paratuberculosis (14/20) far ahead from other infectious diseases (6/20) like enterotoxemia, Pasteurellosis, septic arthritis or meningo-encephalitis. The 10 cases of heavy parasitism mainly involve lungworms infestations. Miscellaneous cases include intern foreign bodies (plastic) and intoxication suspicions. Among culled animals (n = 81), two main causes are traumatic injuries (n = 40), and infectious diseases (n = 34) including 30 cases of paratuberculosis, whereas miscellaneous, including neoplasms, and undetermined causes remain minor causes. Overall, traumatic injuries and infectious diseases are the major causes of mortality in red deer in Southern Belgium. Furthermore, selective culling of cervids suffering from paratuberculosis allow to eliminate potential excretors and in this way prevent the disease from spreading.

29. Characterization of fish consumers in households of North Benin districts

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North Benin is a region of intensive cotton culture for which large quantities of pesticides (especially insecticides) are used. These pesticides contaminate the fauna and the flora from the environment, in particular surface water ponds occurring next cotton fields. These ponds are sources of fish popularly consumed by local households, with possible negative consequences on their health, because of pesticides residues in fish flesh.

This study aims to make the typology of fish consumer households. For this, two hundred and ten households were surveyed in three districts of study in Northern Benin that are Banikoara, Kandi and Gogounou. Principal Component Analysis (PCA) was used to classify households according to four criteria, which are the average income level of the household per year, share of fish consumption expenditure in total food expenditure, share of fish from water retention in relation to the total amount of fish consumed and average number of fish consumers in the household. The distribution of observations on a PCA graph using the listed classification criteria enabled to distinguish four types of fish consumer households (H1, H2, H3 and H4) in the area studied.

The surveyed households were divided following the four types of fish consumer households according to their District of residence. The independence test of chi-square used showed that the distribution of household types varied from one District to another.

Sustainable livestock production

30. KNN-MDR: a learning approach for improving interactions mapping performances in genome wide association studies

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Under the emergence of a wealth of biological information, finding epistatic interactions in a genome-wide association study (GWAS) with the nowadays available large volume of SNP data is a challenging and unsolved issue. Few previous studies could handle genome-wide data due to the intractable difficulties met in searching the combinatorial explosive search space and statistically evaluating high-order epistatic interactions given a limited number of samples. Our work is a contribution to this field, where we propose a novel KNN-MDR approach using K-Nearest Neighbors (KNN) and Multi Dimensional Reduction (MDR) for detecting gene-gene interactions as a possible alternative, especially when the number of involved determinants is potentially high.

The idea behind our method is to replace the status allocation used in classical MDR methods by a KNN approach: the majority vote occurs in the k (a parameter that must be tuned and depends on various parameters such as the extent of linkage disequilibrium, for example) nearest neighbors instead of within the (potentially empty) cell reached when using the tested attributes of the individual to be classified.

The experimental results on both simulated data and real genome-wide data from Wellcome Trust Case Control Consortium (WTCCC) show that KNN-MDR significantly outperforms its recent competitors.

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31. Forest and pastoral areas change: A case study of northern Morocco (1984-2014)

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Land cover and land use change has become a central component in current strategies for managing natural resources and in environmental change monitoring. This study aims to investigate the trend evolution of forest and pastoral areas in Rif Mountain, between 1984 and 2014, on Landsat imageries using remote sensing and GIS techniques. The forest and pastoral areas declined from about 4548 km² in 1984 to about 3588 km² by 2014, while the non-forest and non-pastoral areas increased by some 7,7% from 7912 km² in 1984 to about 8872 km² by 2014. Deforestation, expansion of agricultural lands, increase of rural population, overgrazing have been identified as the main factors that contribute to the forest and pastoral areas degradation. To ensure the sustainability of pastoral and forest resources, management and protection actions should be undertaken.

32. Estimation of genetic parameters for semen characteristics of stress negative Piétrain in the tropics: the case of Vietnam

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A total of 1225 ejaculates collected from 41 boars between 2009 and 2014 from 2 farms in Northern Vietnam (Dong Hiep pig farm and Experimental farm at Vietnam National University of Agriculture) was used to estimate genetic parameters for ejaculate volume (VOL), spermatozoa motility (MO), sperm concentration (CO) and total number of spermatozoon in ejaculate (NT) of stress negative Piétrain pigs. The restricted maximum likelihood methodology was used for estimations with MTDFREML software. The mixed model was used including 3 fixed effects (farm, year and season of the year) and 1 random effect of boar (direct additive genetic) for all traits. Estimates of genetic variances and heritabilities for VOL, MO, CO and NT were 2814, 19.3, 71.9, 356 and 0.41, 0.23, 0.24, 0.21 respectively. These genetic parameters suggest that semen characteristics of stress negative Piétrain could be improved by selection using direct effect under tropical conditions as Vietnam.

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33. Evaluation of chemical composition available by-products in the North of Morocco

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In the northern region, goat population is the most dominant. His diet is based on forest rangelands. So it is necessary to diversify and improve its food calendar. This work aims to characterize the chemical composition of agricultural by-products to be introduced in the diet of herds. Twelve kinds of agricultural by-products was collected in the region to be analyzed. The chemical composition of the collected samples is determined by AOAC methods (1997). From results, dry matter of by-products is greater than 15% except cactus rackets with reduced content (<8%). Their mineral content is very interesting with 22.08% and 23.62% respectively for young and old rackets. Among studied by-products, potatoes tops are high content of total nitrogenous matter (> 12%). Olive leaves and cake have a higher fat content. The fiber content is high in cactus rackets, sorghum residue and faba bean straw. In conclusion, the studied by-products have interesting compositions and can take their place in herds food calendar in the region.

34. Participatory tools for the characterization of the swine meat value chain in the Oueme-Plateau, Benin

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The swine commodity chain is identified in Benin as a dynamic economic sector due to the increase in the country of pig meat specialty restaurants. This value chain appears as an additional income source for different socio-professionals categories. In Benin, livestock development policies have lacked a sufficient involvement of the different stakeholders. However, the present national strategy aims at better involving these stakeholders in the design and implementation of development actions and policies. In order to realize this willingness, new tools of economic analysis are needed. In this prospect, this paper proposes to mobilize participatory approaches for the analysis of the swine meat commodity chain in the department of Ouémé-Plateaux, Benin. Participatory tools allowed for the involvement of actors in both the collection and analysis of qualitative and semi-quantitative data. Kendall coefficients (W) were calculated to express the level of agreement between actors. Multiple Correspondence Analysis (MCA) and Hierarchical clustering (AHC) was used to identify different groups of breeders and pig butchers according to their innovative practices. Stakeholder participation allowed to collect relevant information and to propose tracks for further investigation.

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35. Genetic diversity of goats in Laghouat region, Algeria

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In Algeria, goat breeding is of major social and economic importance. The goat genetic resources consist of a mosaic of breeds and mixed populations involved in a large diversity of farming systems. To establish a strategy for conservation and improvement of these resources, it is necessary to know their precise constitution as well as the farming systems harbouring them. Surveys were conducted with 100 goat breeders in the Laghouat region in order to determine the phenotypical diversity. Local and exotic breeds and their crossbreds were recorded. Local breeds were mainly represented by the Arabia, that is a resistant and rather growth-oriented breed kept in extensive system with commercial purpose. The second local breed, the Mekatia, is less resistant but shows a better dairy production ability and is kept for traditional and cultural motives. The Arabia breed includes a variety called Cherguia, the ears of which are long and pendulous, with a higher milk production potential compared to the main Arabia variety. The Mekatia breed also includes a particular variety that differs from the main breed by longer hair on the legs. Exotic breeds are mainly Saanen, Alpine and recently Chamia (from Syria), introduced for commercial purpose and milk production for home consumption. Despite good production abilities, the Chamia is not very appreciated by farmers for aesthetic motives. The crossbreds result from uncontrolled crossbreeding. Thus, a high phenotypic diversity is observed. These crossbreds are highly appreciated by breeders (mostly crossbreds Saanen x Arabia), because their kids are sold at high prices on markets, being renowned for the quality of their meat. Also, crossbreds have better milking ability. Finally, another breed known as Demene was found in 5 farms. However, its origin (local or exotic), morphology and performance are not yet identified. These results provide a working basis for undertaking the phenotypic and genetic characterization in order to better identify the goat genetic diversity in Laghouat.

36. Goat breeding in the rural district of Chemini (Algeria)

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The Kabyle goat breed represents 10.52% of the total Algerian market (3.8 million heads). A survey of 69 farmers has been carried out in order to characterize it and its breeding in the District of Chemini. The questions focused on household agricultural activities, including breeding of goats, cattle, sheep, rabbits, chicken, turkeys, honeybees and the production of olive oil and figs. The goat morpho-biometric characterization was based on 18 corporal measurements. The Hierarchical Cluster Analysis (HCA) of the farm structures defined four groups of farms, variance between groups accounting for 55.7% of total variability. The average number of goats in groups 1 to 4 was 7.2±2.8, 11.1±3.5, 22.3±1.4 and 3.4±1.0, respectively. Group 3 (n=6), showed the greatest number; it consists of older farmers (67 years old or over) not practicing arboriculture. They also have the largest numbers of sheep (48.67), rabbits (50.83), chicken (48.33) and turkeys (42). Group 4 (n=9), with the lowest number of goats, was the group of young farmers (39 years old or less), more dedicated to cattle breeding (~52 heads) and the production of olive oil (~207 trees) and figs (~47 trees). The 18 morpho-biometric variables were significantly higher in males than in females ($p<0.05$). The Kabyle goat is small (male: 68.23±0.97cm and female: 65.41±0.55cm) with long hair (male: 12.24±0.51cm, and female: 9.51±0.29cm). Its ears are drooping, its convex profile has a slightly pronounced nasal split and its dress color ranges from dark brown to black. This adapted genetic resource should be key in the development of a local production, based on a strong commitment of farmers inside a breed association, for the production of specimens corresponding to a standard, to be determined collectively.

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37. Ho chicken breed: morpho-biometric characteristics and economic efficiency of production

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This study was carried out on 34 rural households who raised Ho chickens from December 2012 to April 2013 to find out information on development status and economic efficiency of this chicken breed. A total of 181 individuals (46 cocks and 135 hens) were used to identify morpho-biometric characteristics according to FAO standards. The Ho chicken population is still very limited with 30.78 chickens per household. Age of the first laying is rather late (7.42 months) with 11.97 eggs in a laying cycle and 76.32% of hatchability rate. These numbers are low because Ho chickens are heavy and clumsy, and therefore they could easily step on and break their eggs. The body weight of a cock is 3.79kg at 9 months old while a hen is 2.63kg at 12 months old. The body length, neck length, back length, thigh length of cocks are significantly higher than those of hens ($P<0.05$). Production of these chickens does not require much initial investment costs. The most important cost items in the structure of the fixed costs are the chicken housing and breed. To operate chicken production, the farmers had to pay the costs of 0.89 million VND and 0.65 million VND respectively for the chicken housing and breed. Both of them accounted for approximately 90% of the total fixed costs. The operation of a chicken farm was estimated to cost about 12.16 million VND per year (Min=2.65, Max=7.77), while the average revenue was found to be around 30.85 million VND (Min=7.20, Max=90.20).

Comparative veterinary medicine

38. Retrospective study of 160 cases of nephrosplenic entrapment referred to the University Liege

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Nephrosplenic entrapment (NSE) is a common cause of colic in horses. Large size warmblood geldings and stallions have been reported to be predisposed to this disease, and prognostic values have been described for medical treatment with phenylephrine and for surgical correction. The aim of this study was to describe risks factors for NSE and success rates of the medical treatment with epinephrine and of the surgical treatment in our hospital-based population of horses.

Files of 160 horses treated for NSE in the University of Liege between 2004 and 2014 were reviewed and categorized according to their breed, sex, age, body weight, clinical scoring and type of treatment. Risks factors were tested by comparing horses suffering from NSE with 3274 horses admitted to the equine clinic for other problem than NSE between 1994 and 2011. Then clinical scoring of horses with NSE which have undergone surgical treatment were compared with those which have only undergone medical treatment ($p<0.05$ significant).

The results of this study were in accordance with those previously described in the literature. NSE was mainly encountered in large warmblood geldings. Horses which have undergone surgery had significantly severer abdominal distension and cardiovascular shock index than horses which have undergone only medical treatment. Observed success rates were high for both treatments (86% for medical treatment and 89% for surgical treatment). However the use of epinephrine to treat medically NSE seemed to be less efficient than the previously described use of phenylephrine whether or not associated with exercise or rolling.

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39. Development of a field exercise test adapted to an interval training session and identification of new parameters of interest in the sports follow-up of Standardbred horses

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Standardised exercise tests are known to allow evaluating fitness and detecting subclinical pathologies in standarbred horses. However, most of these tests are based on strict schedules with fixed number of steps run at increasing pre-determined speeds.

The aims of our study were:

- to develop a field exercise test completely adapted to an interval training session followed by trotters without interfering with the habits of the trainer,
- to determine new parameters of interest in the follow-up of these horses.

Eight standardbred horses were followed during a training period of 3 months. The field test consisted of a warm-up phase followed by two or three steps run at maximal speed. A recovery period at a slow-trot was allowed between each step and after the last step. Velocity and heart rates (HR) were recorded during the entire test and lactate blood concentrations were mesured 2 minutes after the end of the test (La2). A recovery index (RC) was calculated by dividing mean HR during each recovery period by the mean velocity of the recovery period and a final recovery index (FRC) was calculated by dividing the HR measured 2 minutes after the end of the test by the mean velocity of the interval steps. The effect of training was tested on each parameter ($p<0,05$ significant). The velocity of the intervals was significantly increased by training, whereas RC and FRC were significantly decreased and La2 tended to be also decreased. This study confirmes the possibility to adapt field exercise tests to interval training sessions and highlights new parameters of interest in the follow-up of race horses.

40. Stifle intra-articular lipomatous lesion in a dog

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Introduction: In human medicine, intra-articular lipomatous lesions are rare and main differentials include intra-articular lipoma and lipoma *arborescens*. The present case describes clinical and imaging features of a canine stifle joint lipomatous lesion. **Materials and methods:** A 7 year-old female Labrador Retriever was presented for chronic and intermittent left hindlimb lameness, deteriorating over the 3 previous weeks. Palpation revealed a bilateral stifle joint swelling, more marked on the left side. **Results:** Radiographs showed an adipose, ovoid mass at the cranio-medial aspect of the left distal femur, with cranio-medial displacement of the patella and marked bilateral degenerative joint disease. CT revealed an ovoid, well-defined, mildly heterogeneous, non-enhancing, septated fat attenuating mass (-70HU). On US the mass had smooth regular margins and an echogenic homogeneous aspect. Cytology revealed well-differentiated adipocytes. Our findings were consistent with intra-articular lipomatous lesion, most likely true intra-articular lipoma. The owners refused the excisional surgery. The dog currently receives chondroprotectors and is not lame. **Discussion:** In humans, final diagnosis of intra-articular lipomatous lesion is based on magnetic resonance imaging (MRI). True lipoma is considered to have smooth, well-defined margins, while a nodular "finger-like" appearance is considered pathognomonic of lipoma arborescens. In this case MRI was not performed. However, the typical "finger-like" appearance was not found, making intra-articular lipoma the most likely diagnosis. Although histopathology is not available, complete resolution of clinical signs is suggestive of a benign lesion. To the authors' knowledge, this is the first description of intra-articular lipomatous lesion in dogs.

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41. Urethral intussusception following traumatic catheterization in a male cat

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In cats, differential diagnoses for urethral obstruction include urolithiasis, idiopathic diseases, neoplasia, urethral stricture, anatomic malformations, foreign bodies and urethral injuries.

A 8-year-old 4.2-kg castrated male European shorthair cat was referred for lower urinary tract obstruction causing anuria. The cat had presented multiple episodes of signs of feline lower urinary tract diseases within the past 2 years. Urinalysis revealed struvite crystalluria and the presence of Escherichia Coli urinary tract infection. The cat was catheterized and referred for further investigation.

During physical examination, a urethral mass was identified per rectal palpation and confirmed on retrograde cysto-urethrography caudal to a focal and ampullar dilation of the pelvic urethra. Surgical approach via a perineal urethrostomy was planned in order to relieve the obstruction and resect the suspected mass.

Once the location of the suspected urethral mass was identified, intussusception of the urethra with a complete avulsion of the penile part of the urethra and partial rupture of the urethra at the site of intussusception could be visualized. After removal of the caudal part of the urethra and penis, the perineal urethrostomy was carried on.

This is the first report of urethral intussusception following traumatic catheterization in a cat. Urethral intussusception can create a mass effect on abdominal ultrasonography and retrograde cysto-urethrography. This should be taken into account in the differential diagnoses of a urethral mass.

42. Mindfulness training for veterinary students: report of one-year experience.

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Objective: The purpose of this poster is to share data about development of a mindfulness training for veterinary students and to illustrate students' participation and feedback after on year experience at the Faculty of Veterinary Medicine of Liège.

Method: The available data about 3 groups of students attending the training at 3 different times between April 2014 and May 2015 and their feedback were examined for descriptive analysis.

Results: A total of 39 students voluntarily participated to the training. Thirty-seven students attended all 4 sessions, 2 students participated twice, and 21 responded voluntarily to a feedback questionnaire. Students were from all years of the curriculum with a higher prevalence of students from the last 2 clinical years. Feedbacks indicated that practices during sessions were highly appreciated and that relation to the group of participants was important. Answers suggest an increased awareness of relation to thoughts and of interpersonal communication mechanisms after the training. Home practice was difficult for the majority of participants. Most of feedback respondents asked for more than 4 sessions and thanked for having received the possibility to follow a free mindfulness training during their veterinary curriculum at the Veterinary Faculty.

Conclusion: Results suggests the interest in further developing and integrating mindfulness programs in the veterinary curriculum.

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43. Identification of a new prognostic tool for atypical myopathy

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European atypical myopathy (AM) is an acute rhabdomyolysis resulting from energetic impairment by a toxic compound named methylenecyclopropyl acetic acid-CoA (MCPA-CoA) which disrupts fatty acid β-oxidation and amino acid metabolism. Effects of MCPA-CoA lead to accumulation of acylcarnitines (AC) in blood. Although particularly high rates of creatine kinase activities contribute to diagnosis confirmation, they have no prognostic value for survival. Despite intensive supportive therapy, about 74% of AM affected horses die, most of them during the first two days of onset of clinical signs.

This study investigated the serum AC profile of AM cases in order to identify prognostic parameters. The AC profile (*i.e.* 33 different AC measured) of AM survivors (n=12), deceased AM (n=38) and healthy controls (n=44) were determined and compared. Sera of AM considered in this study were the earliest available from the onset of clinical signs. Unpaired t test has been used to compare the data ($P < 0.05$) and logistic regression to seek for prognostic parameters.

All AC parameters of AM cases significantly differed from those of controls. Survivors differed significantly from deceased horses by several AC enabling the calculation of a prognostic factor (PF) ranging from 0 (100% of chance to die) to 1 (100% of chance to survive). For example, based on 3 selected AC, the PF for deceased AM horses was 0.18 ± 0.05 *vs*. 0.81 ± 0.08 for survivors.

In conclusion, AC profiling contributes to the diagnosis of AM and helps to assess prognosis early in the process of the disease.

44. Atypical myopathy: a preliminary study to identify the site of hypoglycine A metabolism

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The cause of European atypical myopathy has been recently discovered. This myopathy results from hypoglycine A (HGA) ingestion contained in the seeds from Acer pseudoplatanus. Once ingested, HGA is metabolized into the toxic compound methylenecyclopropyl acetic acid-CoA (MCPA-CoA) which disrupts fatty acid β-oxidation and amino acid metabolism. Severe rhabdomyolysis ensues and currently, there is no cure for this frequently fatal disease. Up to now, the site of HGA metabolism into MCPA-CoA is unknown. A preliminary in vitro study was designed to test the hypothesis of HGA metabolism into blood. Blood samples were taken from the jugular vein of a healthy horse and were dispatched into plain, EDTA and heparin tubes. Then HGA was added in blood samples as well as in a tube containing NaCl 0.9% to reach a concentration compatible with natural intoxication (*i.e.* HGA 1300 ng/mL of serum). The HGA concentration was measured using modified aTRAQ® assay before and after addition of HGA at several time points in serum, plasma or water for 48 hours. Samples were gently agitated and kept at 37°C for the whole procedure. In all blood samples but not in NaCl 0.9%, HGA concentration decreased by more than 35% in the first 24 hours. Results of this preliminary study suggest that HGA is metabolised, at least partially, in the blood. However further experiments are needed to exclude HGA capitation into the red blood cell and to confirm concurrent MCPA formation. Identification of the site of HGA metabolism might be of interest for therapeutic perspectives.

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45. Assessment of ways to fight against equine summer eczema

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Equine summer eczema (SE) is a hypersensitivity reaction to bites of insects such as *Culicoides spp* (midges). Equine SE induces pruritus, typically at the mane, basis of the tail and lower part of the abdomen. Lesions are described as crusting, alopecia and excoriations or even skin folds in chronic cases. This epidemiological survey aimed at describing factors associated with the clinical expression of SE, the treatments used by the owners of horses and their efficacy.

Owners of horses with at least one animal suffering from SE were recruited by means of an Internet survey. On-line questionnaires enabled to obtain data from 88 horses suffering from SE and from 74 co-grazers considered free of SE by their owner; these healthy horses constituted the control group. Questions included information about individual horses and pasturing. Depending on the parameter of interest, a chi-square (χ^2) or a Fisher's exact test was used to compare the SE group to controls ($P < 0.05$).

At the level of horses, an increased risk of developing signs of SE has been identified for ponies (in particular Shetland), working horses (especially if the work is frequent). At the level of the environment, wind exposure and the spreading of manure significantly decrease the risk of SE. According to owners of SE affected horses, the disease affects seriously the welfare but not the sport performance of the animal. The majority of the owners uses a combination of mechanical and topical protections to which they attribute an average effectiveness. Desensitization was rarely cited.

46. Heart rate variability as a measure of comfort in the anaesthetised horse

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Despite considerable progress in the past years, anaesthesia of horses remains a challenging task to the veterinarian. Determining the correct anaesthetic depth is crucial to safe and comfortable anaesthesia, but lacks objective measures. Heart rate variability (HRV), a surrogate of the patient's comfort, explores variations of beat-to-beat intervals and reflects the regulation of the autonomic nervous on the cardiovascular system. The aim of the present study was to compare HRV in the form of parasympathetic tone activity (PTA) index to common anaesthetic parameters in anaesthetised horses.

Twelve horses, aged between 3 months and 12 years and anaesthetised for various indications were included in the study. After premedication with xylazine intravenously, anaesthesia was induced with midazolam and ketamine intravenously and maintained with isoflurane in oxygen/air delivered via orotracheal intubation by a large animal circuit. Positive pressure ventilation was applied to maintain normocapnia, dobutamine was administered when required to maintain normotension. Among the recorded anaesthetic parameters heart rate (HR), end-tidal concentration of isoflurane (IsoET) and mean arterial pressure (MAP) were compared to PTA index.

Poor correlation between HR and PTA indicates that HRV is not HR-dependent. Poor correlation between PTA and IsoET indicates that the autonomic nervous system is not suppressed at higher therapeutic doses of isoflurane. The lack of correlation between PTA and MAP needs further analysis as the catecholamine dobutamine administered to maintain normotension might have affected autonomous regulation of the heart itself. Further studies are necessary to determine the usefulness of PTA as surrogate of intra-anaesthetic comfort in horses.

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47. Clinical safety of computed tomography-guided lumbosacral transforaminal and translaminar epidural and facet joint steroid injections in dogs

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Introduction: To test the clinical safety of computed-tomography guided transforaminal and translaminar epidural and facet joint steroid injections in dogs. **Materials and methods:** Fifteen healthy Beagles were assigned to three groups and underwent computed tomography-guided transforaminal and translaminar epidural injections and facet joint injections of 0.1mg/kg of methylprednisolone acetate. During the procedures, vital parameters were recorded. Clinical examinations of the lumbosacral region were performed 1, 3, 7 and 10 days after the injections. In the presence of neurological abnormalities, a control examination was performed 24 days after the procedure. **Results:** Steroid injections were performed in 14/15 dogs. In 1/15, vascular puncture occurred and the injection was not performed. No complications were reported during the procedure. Only mild transient hyperthermia and mildly altered patellar withdrawal, cranial tibial and perineal reflexes were occasionally and temporary noticed. Overall, altered reflex responses were observed in 11/14 dogs, during 27/65 clinical examinations. **Discussion:** Computed-tomography guidance allows to verify the position of the needle and to avoid injections into the vascular system during epidural and lumbosacral facet joint injection. Furthermore, this study demonstrates that steroid injections in the epidural and facet joint space are clinically safe and that their use can be suggested for clinical trials.

48. Clinical safety of computed tomography-guided lumbosacral transforaminal and translaminar epidural and facet joint steroid injections in dogs

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Introduction: To test the clinical safety of computed-tomography guided transforaminal and translaminar epidural and facet joint steroid injections in dogs. **Materials and methods:** Fifteen healthy Beagles were assigned to three groups and underwent computed tomography-guided transforaminal and translaminar epidural injections and facet joint injections of 0.1mg/kg of methylprednisolone acetate. During the procedures, vital parameters were recorded. Clinical examinations of the lumbosacral region were performed 1, 3, 7 and 10 days after the injections. In the presence of neurological abnormalities, a control examination was performed 24 days after the procedure. **Results:** Steroid injections were performed in 14/15 dogs. In 1/15, vascular puncture occurred and the injection was not performed. No complications were reported during the procedure. Only mild transient hyperthermia and mildly altered patellar withdrawal, cranial tibial and perineal reflexes were occasionally and temporary noticed. Overall, altered reflex responses were observed in 11/14 dogs, during 27/65 clinical examinations. **Discussion:** Computed-tomography guidance allows to verify the position of the needle and to avoid injections into the vascular system during epidural and lumbosacral facet joint injection. Furthermore, this study demonstrates that steroid injections in the epidural and facet joint space are clinically safe and that their use can be suggested for clinical trials.

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49. Evaluation of the Serum Amyloid A in horses with respiratory diseases

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Serum Amyloid A (SAA) is frequently used in human and small animal medicine as acute inflammatory marker for numerous pathologies. In horses, SAA has been used in neonatal critically ill foals and in horses with colic. However its diagnostic and prognostic value has received little study in adult horses with respiratory diseases. The aim of this study was to investigate the SAA plasmatic concentration in horses with respiratory disease and to compare its kinetic with those of fibrinogen and haptoglobin, two other inflammatory markers commonly used in horses. Fourteen horses admitted to the Equine Clinic of the Faculty of Veterinary Medicine of Liege for respiratory problem and 6 healthy control horses were included in this study. Plasmatic concentration of SAA, fibrinogen and haptoglobin were measured at admission (day 1), then at day 3 and day 7 after admission. SAA, fibrinogen and haptoglobin concentrations were compared between sick and control horses and between day 1, day 3 and day 7 ($p<0.05$ significant). SAA, fibrinogen and haptoglobin concentrations were higher in sick horses than in control horses. However SAA, fibrinogen and haptoglobin concentrations were not significantly different between day 1, day 3 and day 7. All 3 markers showed a tendency to decrease simultaneously with recovery and followed kinetics previously described in the literature for other species. In conclusion, SAA, fibrinogen and haptoglobin may have a good diagnostic and prognostic value in horses with respiratory diseases but a study involving larger number of horses should be considered to confirm it.

50. Tracheal diameter in puppies

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Tracheal diameter (TD) is assessed radiographically by TD on thoracic inlet distance ratio (TD/TI). The goals of this study were to (1) determine if there is a significant difference of the TD/TI between non-brachycephalic (NB), brachycephalic non-bulldogs (BNB) and bulldogs (B) breeds depending on age; (2) investigate if there is a positive correlation between TD/TI and age; (3) assess if there is an age limit to diagnose tracheal hypoplasia in dogs.

Seventy-seven puppies were prospectively recruited and classified in 3 categories (NB, NBB, B). Thoracic radiographs were obtained at 1, 2, 3, 6, 9, 12 months (M) and, for NBBB, also at 15M, and measurements of TD/TI were performed. Statistical analysis tests were performed.

Only 26 dogs completed the study. A significant difference of TD/TI was observed between the 3 breed categories for all age categories. In all dogs, a significant difference of TD/TI was obtained between all age categories except between 1M and 3M, 2M and 6M, and between 9M, 12M and 15M. No significant difference of TD/TI was obtained between French Bulldogs and English Bulldogs. A positive correlation was observed between TD/TI values and ages.

TD/TI was significantly different between the 3 categories and slightly different from previous reported values. TD/TI significantly changed with age up to 9M which seems to be the lower age limit to evaluate the definitive TD in dogs. A positive correlation between TD/TI values and ages indicated that dogs with small trachea at 1M are proned to have a small trachea at 12M.

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51. Prediction of sport performance in endurance horses by the study of muscle mitochondrial function with high resolution respirometry

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High-resolution respirometry (HRR) is a minimally invasive technique used to measure oxygen consumption in tissue samples following multiple substrate-uncoupler-inhibitor titration (SUIT) protocols. Substrates and inhibitors sequentially reconstitute the tricarboxylic acid cycle function leading to the measurement of maximal muscle oxidative phosphorylation ($\mathrm{OXPHOS}_{\mathrm{Max}}$) and electron transport system (*ETS*) capacities. As aerobic capacity is a primary determinant of athletic abilities in human endurance athletes, it was hypothesised that HRR contributes to sport performance in endurance horses. Two SUIT protocols were applied on muscle microbiopsies obtained from the *m. triceps brachii* of ten trained endurance horses belonging to the national endurance team of France. One month following this respirometric study, all horses participated to a 160 km endurance race (CEIO *** Compiegne, France). All horses completed the 160 km ride but one horse was eliminated after the race for lameness. Out of 47 participants, ranking of the team was the 1st, 2nd, 3rd, 4th, 7th, 9th, 11th, 16th and 19th place. Linear regression analyses were used to identify association between respirometric measurements and finishing place or racing average speed. Significance was set at $P < 0.05$. All respiratory $\mathrm{OXPHOS}_{\mathrm{Max}}$ and ETS capacities were correlated to finishing place and speed. Furthermore, $\mathrm{OXPHOS}_{\mathrm{Max}}$ was strongly predictive of the ranking for the four first places. Interestingly, the highest $\mathrm{OXPHOS}_{\mathrm{Max}}$ and ETS capacities were found in the horse who won the price of the best conditioned horse the next year at the World Equestrian Games (Kentucky, endurance athletes).

52. Effect of a CpG-ODN on the innate immune system of the horse: an *in-vivo* trial

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Oligodeoxynucleotides containing cytosine-phosphate-guanosine motifs (CpG-ODN) represent a class of agonists of Toll-like Receptor 9 (TLR9). TLR9 activation induces the secretion of cytokines and the maturation of immune cells, thus initiating both innate and adaptive immune responses. Therefore, CpG-ODN has been investigated in different species as a potential immune-modulator targeting infectious, allergic and neoplastic diseases. It has been administered by nebulisation to RAO-affected horses with promising results. Nonetheless, there is no *in-vivo* study on the effect of CpG administered systemically to the horse. Therefore, we tested the effect of CpG, given by intramuscular injection, on the equine immune response. Eight horses were used for this study. Five mg/horse were injected to 4 horses at D0 and D7; the other horses received a placebo (PBS). Blood was collected 2 days prior to each injection, then regularly up to D21. A clinical exam was realised daily. Laboratory analyses included haematology, ELISA tests for IFN-alpha, IFN-gamma, TNF-alpha and IL-10 and cytometry analyses for MCHII and CD86 expressions on B-lymphocytes. A cross-over of the 2 groups was realised after 2 months of washout. CpG was well tolerated. Significant transient eosinopenia, monocytosis and leukopenia were observed after CpG injection, while ELISA and cytometry analyses did not reveal any significant modification. This trial represents the first *in-vivo* study where CpG is administered systemically to healthy horses. Further studies are needed to adjust the dose, the formulation and the sampling schedule and to fully investigate this molecule as potentiel modulator of the equine immune system.

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