Faculté de médecine vétérinaire

Université de Liège

Proceedings of the 1st FARAH-Day

Faculty of Veterinary Medicine
(University of Liege - Belgium)

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FARAH

One Health

L'Animal et l'Homme, une même santé

Proceedings of the 1st FARAH-Day

Faculty of Veterinary Medicine
(University of Liege - Belgium)

October 17, 2014

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

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

Welcome to the 1st 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 100 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 1ère 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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BELGIUM

Program

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

09:00 - Opening and Welcome Speech

Dr Catherine DELGUSTE, *President of the Scientist Staff*

Prof Laurent GILLET, *President of the FARAH*

09:15 Invited speaker:

Dr Fabien ECTORS

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

Chairmen: Prof Frederic FARNIR *and* **Valeria BUSONI**

09:45 Protective impact of murid herpesvirus 4 infection on the development of an anti-pneumovirus vaccine-induced immunopathology
Michaël Dourcy, Immunology-Vaccinology, FARAH

10:00 Cyprinid herpesvirus 3 encodes a soluble homologue of the mammalian TNFRSF14 receptor: roles in the biology of the infection in vitro and in vivo
Joanna Jazowiecka-Rakus, Immunology-Vaccinology, FARAH

10:15 TNF-α induces behavioral fever in common carp (Cyprinus carpio)
Krzysztof Rakus, Immunology-Vaccinology, FARAH

10:30 The envelope glycoprotein gp150 promotes sexual transmission of Murid Herpesvirus-4
Caroline Zeippen, Immunology-Vaccinology, FARAH

10:45 Rational design of a safe and efficacious attenuated recombinant vaccine against cyprinid Herpesvirus 3 using prokaryotic mutagenesis and In Vivo Imaging System
Maxime Boutier, Immunology-Vaccinology, FARAH

11:00 - Coffee break and poster session 1 (Room P, building B45)

11:30 - Oral session 2: Companion animal research (lecture hall C, building B45)

Chairmen: Prof Stefan DELEUZE *and* **Stephanie CLAEYS**

11:30 Assessment of pulmonary hypertension in dogs with chronic pulmonary diseases by thoracic CT- angiography
Thierry Couvreur, Medical Imaging Department, CHC

11:45 Usefulness of ultrasonography of deep cervical lymphnodes in diagnosis of neoplastic disease in horses
Laurence Evrard, Diagnostic Imaging Section, FARAH

12:00 Detection of Angiostrongylus vasorum in the bronchoalveolar lavage fluid or faeces of coughing and healthy dogs in Belgium

Morgane Canonne, Department of Clinical Sciences, FARAH

12:15 Modified maquet technique for treatment of canine cranial cruciate ligament injury: early results, complications and risk factors in 109 dogs

Nicolas Barthelemy, Department of Clinical Sciences, FARAH

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

14:00 Invited speaker

Prof Véronique DELCENSERIE

14:30 - Oral session 3: Animal models for human diseases (lecture hall C, building B45)

Chairmen: Prof Laurent GILLET *and* **Vinciane TOPPETS**

14:30 Exploring the role of BLV and HTLV1-encoded micro- and long noncoding RNAs: lessons from RNA-Seq

Keith Durkin, Unit of Animal Genomics, GIGA-R

14:45 A murine model for co-infection using the human blood fluke Schistosoma mansoni and γ-herpesvirus

Annette Dougall, Immunology-Vaccinology, FARAH

15:00 Contribution to the study of the pathogenesis of dermatophytosis using a murine model of infection with Arthroderma benhamiae

Marie-Pierre Heinen, Veterinary Mycology, FARAH

15:15 Hepatoprotective effects of crude rhubarbe extract extend to the modulation of gut microbiota in a murine acute alcohol-induced steato-hepatitis model

Bernard Taminiau, Food Sciences Department, FARAH

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

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

Chairwomen: Dominique CASSART *and* **Dominique VOTION**

16:00 Comparison of blood and milk parameters in dairy cows managed with two different diets: a grass silage based diet or a diet with an extruded compound feedstuff

Emilie Knapp, Nutrition Unit, FARAH

16:15 The impact of Schmallenberg in Belgian cattle and sheep: a case-control study

Antoine Poskin, Epidemiology, FARAH

16:30 Experimental in vivo infection of pigs by a wild boar hepatitis E virus strain

Damien Thiry, Virology, FARAH

16:45 Evasion of cytotoxic T cell response by Alcelaphine herpesvirus 1 genome maintenance protein

Océane Sorel, Immunology-Vaccinology, FARAH

17:00 Selection in action: dissecting the molecular underpinnings of the increasing muscle mass of Belgian Blue Cattle

Tom Druet, Unit of Animal Genomics, GIGA-R

17:15 - Awards

Dominique VOTION, Board member of FARAH

17:30 - Closing session

Prof Albert CORHAY, *Rector of the University of Liège*

Prof Pascal LEROY, *Dean of the Faculty of Veterinary Medicine*

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

20:00 - Dinner (couscous) and **dancing party** (Room P, building B45)

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Posters

Oral presentations

Oral presentations

09:45 - Protective impact of murid herpesvirus 4 infection on the development of an anti-pneumovirus vaccine-induced immunopathology

Dourcy M.[1], Machiels B.[1], Zeippen C.[1], Dumoulin J.[1], Javaux J.[1], Desmecht D.[2], Vanderplasschen A.[1], Dewals B.[1], 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. Here we use Murid herpesvirus 4 (MuHV-4), a natural pathogen of wild rodents, to investigate the impact of gammaherpesvirus infections on subsequent immune responses. We decided to study specifically the impact of MuHV-4 infection on the development of an anti-pneumovirus vaccine-induced immunopathology. Briefly, this respiratory Th2-skewed pathology was induced in BALB/c mice by the subcutaneous inoculation of formalin-inactivated pneumonia virus of mice (FI PVM) antigens and a following intranasal infection with wild-type PVM. To date, we have interestingly demonstrated that MuHV-4 infection, both before and after the FI PVM vaccination, protects the mouse from the development of the PVM-induced immunopathology. Notably, levels of Th2 cytokines and eosinophils in broncho-alveolar lavage fluids were highly decreased in MuHV-4 infected mice. This protective impact is maintained over time. In addition, recent results suggest that replicative MuHV-4 passage in lungs during infection is necessary to confer the protection, since intraperitoneal MuHV-4 infection does not prevent the development of the Th2-skewed inflammation. In conclusion, we have demonstrated here a protective MuHV-4 imprinting of its host immune system. In the future, this study could induce reflection on how we vaccinate against pathogens as some could be revealed in the end as beneficial for their host.

10:00 - Cyprinid herpesvirus 3 encodes a soluble homologue of the mammalian TNFRSF14 receptor: roles in the biology of the infection *in vitro* and *in vivo*

Jazowiecka-Rakus J.[1], Rakus K.[1], Reschner A.[1], Boutier M.[1], Ronsmans M.[1], Vanderplasschen A.[1]

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TNFR (tumour necrosis factor receptor) homologues were reported in different viruses including herpesviruses and poxviruses and were shown to play a role in immune evasion mechanisms. Cyprinid herpesvirus 3 (CyHV-3) causes a lethal disease in common and koi carp. CyHV-3 ORF4 encodes a soluble homologue of the mammalian TNFRSF14 membrane receptor (also called HVEM). Proteomic analysis of CyHV-3 infected cells demonstrated absence of ORF4 expression product in the CyHV-3 secretome but suggested its presence in CyHV-3 virions. To investigate the roles of ORF4 in the biology of CyHV-3 infection two recombinant strains deleted for ORF4 were produced using BAC cloning technologies. Restriction profile, Southern blot and full genome sequencing confirmed the correct molecular structure of these recombinant strains. *In vitro*, the two deleted strains replicated comparably to the control strain expressing ORF4. *In vivo* studies showed a difference in kinetics of the infection between fish infected with ORF4 deleted strains and the control strain. The development of the disease (clinical signs) and the peak of virus load (measured by real-time TaqMan PCR in gills, heart and brain) were delayed in fish infected with ORF4 deleted strains. Nevertheless, the cumulative mortality rates were comparable for the three viruses tested. The results of the present study demonstrate that CyHV-3 ORF4 could play a role in pathogenesis at the early stage of the disease. Additional research is required to study the interaction between CyHV-3 ORF4 and the carp immune system.

10:15 - TNF-α induces behavioral fever in common carp (*Cyprinus carpio*)

Rakus K.[1], Ronsmans M.[1], Forlenza M.[2], Boutier M.[1], Jazowiecka-Rakus J.[1], Penaranda M.[1], Farnir F.[3], Becco C.[4], Wiegertjes G.[2], Vanderplasschen A.[1]

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Fever in response to infectious or inflammatory agents is an ancestral mechanism of innate immunity existing in all vertebrates including fish. Cyprinid herpesvirus 3 (CyHV-3) is the causative agent of a lethal disease in common and koi carp. To study the mechanisms of behavioral fever in carp during CyHV-3 infection, we developed a multi-chamber aquaria with temperature gradient (24°C - 28°C - 34°C) and informatics system to monitor the position of the fish. The results of our study demonstrated that: 1) CyHV-3 infection induces behavioral fever in carp. Infected fish migrated to the compartment with the highest temperature (34°C) and stayed there until controlling the disease. 2) Behavioral fever induced by CyHV-3 is salutary. Migration of infected fish to 34°C resulted in the absence of lethality while mortality rates of 100% and 80% were observed when the fish were maintained in control tanks at 24°C and 28°C, respectively. 3) CyHV-3 ORF12 encodes a soluble TNF-α receptor that delays the onset of behavioural fever induced during the disease. *In vitro* studies demonstrated that CyHV-3 ORF12 encodes a soluble abundantly secreted protein able to bind to both carp TNF-α1 and TNF-α2. CyHV-3 recombinant strain deleted for ORF12 induced behavioural fever earlier than the control revertant strain. 4) Blocking of TNF-α inhibited behavioral fever. Anti-carp TNF-α antibodies administrated passively blocked migration of infected fish to the 34°C compartment. 5) TNF-α1 is sufficient to induce behavioral fever in a dose dependent manner. Fish injected with expression plasmids encoding TNF-α1 migrated to 34°C in contrast to fish injected with plasmid encoding TNF-α2 or no transgene.

10:30 - The envelope glycoprotein gp150 promotes sexual transmission of Murid Herpesvirus-4

Zeippen C.[1], Vanderplasschen A.[1], Stevenson P.G.[2], Gillet L.[1]

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Gammaherpesviruses are important pathogens in human 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. Murid Herpesvirus-4 (MuHV-4) is used as a model for the human gammaherpesviruses. Recently, we observed that MuHV-4 could be sexually transmitted between mice. This model offers the opportunity to test the importance of immune evasion mechanisms for transmission. One of these mechanisms could rely on the glycoprotein 150 (gp150) which seems to be used as an immunogenic decoy that could limit virus neutralization. Indeed, while gp150 is the main target of antibodies raised against MuHV-4, anti-gp150 antibodies are not neutralizing but instead enhance Fc-receptor dependent infection *in vitro*. In this study, we tested therefore the importance of gp150 in the context of MuHV-4 sexual transmission. Briefly, female mice were infected with a strain deficient for the expression of gp150 (gp150-) or with a wild-type strain, both expressing luciferase. They were imaged with an *in vivo* imaging system to follow the infection. Infected females were mated with naïve males at the moment of lytic replication in genital tract to compare the capacity of transmission of the two viruses. Our results show that, while the gp150- strain has no deficit in infection or in genital excretion compared to the wild-type strain, sexual transmission is less efficient for the gp150- strain. Further experiments should investigate the molecular mechanism by which gp150 promotes sexual transmission.

10:45 - Rational design of a safe and efficacious attenuated recombinant vaccine against Cyprinid Herpesvirus 3 using prokaryotic mutagenesis and *in vivo* imaging system

Boutier M.[1], Ronsmans M.[1], Ouyang P.[1], Fournier G.[1], Rakus K.[1], Reschner A.[1], Davison A.[2], Farnir F.[3], Bayrou C.[4], Lieffrig F.[5], Desmecht D.[4], Vanderplasschen A.[1]

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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 vaccine. With this goal in mind, recombinant strains deleted for single gene were produced using prokaryotic mutagenesis. While producing a recombinant deleted for ORF134, we unexpectedly obtained a clone additionally deleted for ORF56 and ORF57. Interestingly, this triple deleted recombinant exhibited an attenuated profile and induced an immune protection against a lethal challenge *in vivo*. To determine the role of each deletion in the attenuated phenotype, a series of recombinant were produced and tested in vivo. These experiments revealed that the attenuation resulted mostly from the single deletion of ORF57. Complementary experiments were performed to investigate the potential of the ORF56-57 double deleted recombinant as a safe and efficacious vaccine. These experiments revealed that (i) *In vivo* imaging (IVIS) of vaccinated fish challenged with a wild type strain expressing luciferase suggested that vaccination induces an immune protection close to sterile immunity. (ii) Study of viral tropism by qPCR, IVIS, and histopathological analyses demonstrated that the replication of the vaccine strain is delayed, lower and of shorter duration. (iii) Transmission studies demonstrated that there is no detectable spread of the vaccine from freshly vaccinated fish to naive fish located immediately downstream (water sharing). Altogether, this study demonstrates the potential of CyHV-3 ORF56-57 double deleted recombinant for safe and efficacious mass vaccination of carp against CyHV-3.

11:30 - Assessment of pulmonary hypertension in dogs with chronic pulmonary diseases by thoracic CT-angiography

Couvreur T.[1], Roels E.[2], Merveille A.C.[2], Clercx C.[2], Mc Entee K.[2,3], Bolen G.[4]

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Chronic alveolar hypoxia and lung parenchymal infiltration observed in cases of canine idiopathic pulmonary fibrosis (CIPF) and chronic lower airway diseases (CLAD) may lead to pulmonary hypertension (PH). Non-invasive diagnosis of PH and its cardiac repercussions is challenging. This study was intended to investigate, in normal dogs and dogs with CIPF or CLAD, the reproducibility of PH CT angiography (CTA) parameters and their correlation with echocardiographic variables. CTA images obtained from 6 dogs with CIPF (A), 7 dogs with CLAD (B) and 9 controls (C) were reviewed. The diameter of the right (RV) and left ventricles (LV) was obtained from transverse images (T) and both from reformatted 4-chambers (4C) and short axis (SA) cardiac views. Diameters of pulmonary trunk (PT) and aorta (Ao) and of the pulmonary right vein (PV) and artery (PA) were measured in T. Measurements were performed twice blindly by two radiologists. Measurements and ratios (PV/PACT, PT/AoCT and three RV/LVCT) were compared between groups and correlated with same ratios in echocardiography (US). Statistical analyses were performed using XLStat® software ($P \leq 0.05$). Intra- and inter-observer agreements were good. PV/PACT was significantly lower in A compared to C. PV/PACT correlated with PV/PAUS measurements. PT/AoCT was significantly higher in A and B compared to C. RV/LVCT in all views were significantly increased in A compared to C. Correlations between RV/LVCT and RV/LVUS were found. PV/PACT, PT/AoCT and RV/LVCT ratios are reproducible and may assess PH and its cardiac repercussions. These CTA ratios correlate with echocardiographic ratios except for PT/Ao.

11:45 - Usefulness of ultrasonography of deep cervical lymphnodes in diagnosis of neoplastic disease in horses

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Horses with neoplastic disease often present non-specific clinical signs including dyspnea, weight-loss or chronic/recurrent colic. Diagnostic imaging is routinely performed. This study aims: 1. to describe ultrasonographic (US) technique and anatomy of deep cervical lymph nodes in horses, 2. to present imaging findings in a series of horses with deep cervical lymphadenomegaly and cytologically or histologically confirmed neoplasia. Deep cervical lymph nodes were explored using a 5-7.5MHz curvilinear transducer. US examination of the base of the neck and the thoracic inlet was performed in 5 normal horses to establish normalcy. Medical records were reviewed to select horses with deep cervical lymphadenomegaly at ultrasonography and a final diagnosis of neoplastic disease. In normal horses deep cervical lymph nodes appeared as well-defined hypoechoic ovoid structures between 5mm and 1cm thick, in the area of bifurcation of common carotid arteries. Seven horses responded to the selection criteria. All of them had undergone US examination of abdomen and thorax and a radiographic examination of the thorax. A transrectal ultrasonography was done in 1 horse. Abdominal abnormalities were detected in all horses and included abdominal lymphadenopathy (4 horses), peritoneal effusion (3 horses), hepatic (3 horses) and splenic (2 horses) abnormalities. Intrathoracic abnormalities were detected in all horses and included mediastinal masses (7 horses), lung parenchymal abnormalities (5 horses) and pleural effusion (4 horses). Of the 7 horses, 4 were diagnosed to have lymphoma, 1 had an adenocarcinoma, 1 had an undifferentiated round cell tumor, one had macroscopic neoplastic findings at necropsy but final classification is pending. Lymphadenomegaly of deep cervical lymphnodes can be detected in horses with diffuse neoplastic process. Deep cervical lymphnodes are well visualized when enlarged and accessible for FNA. Their US assessment can be easily added to routine imaging assessment of thorax and abdomen in case of suspected neoplasia in horses.

12:00 - Detection of *Angiostrongylus vasorum* in the bronchoalveolar lavage fluid or faeces of coughing and healthy dogs in Belgium

Canonne A.M.[1], Roels E.[1], Caron Y.[1], Losson B.[1], Peeters D.[1], Peters I.[2], Billen F.[1], Clercx C.[1]

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Canine Angiostrongylosis is a disease increasingly reported in many european countries other than Belgium, where occurrence and prevalence are currently unknown. Although detection of the first-stage larvae using the Baermann technique remains the gold standard, recently developed quantitative polymerase chain reaction (qPCR) is now available. The aims of this study were to describe a clinical series of recent autochtonous cases and to retrospectively assess *Angiostrongylus vasorum* qPCR in bronchoalveolar lavage fluid (BALF) samples from a larger series of dogs, healthy or with other respiratory conditions. Seven dogs were recently diagnosed as having angiostrongylosis. They all presented with respiratory signs of variable severity. In 5 dogs, BALF was obtained and qPCR was positive in all, while larvae were detected in the faeces of only 2 of them. In the remaining two dogs, no BALF was obtained, but coproscopy was positive. BALF samples were collected over a 7 year-period (2007-2014) from 10 asymptomatic dogs and 55 dogs with chronic cough, related various respiratory conditions, including bordetellosis, eosinophilic bronchopneumopathy (EBP), chronic bronchitis and bacterial bronchopneumonia). Amongst those 65 dogs, only one BALF (a dog with EBP), yielded a positive qPCR result. In this dog, faecal analysis was negative. In conclusion, presence of *Angiostrongylus* in healthy and coughing dogs can be estimated to have been negligible in Belgium until the last 12 months. However, it should now be included in the differential diagnosis in coughing dogs. Moreover, qPCR from BALF is an adequate and reliable detection technique.

12:15 - Modified Maquet Technique for treatment of canine cranial cruciate ligament injury: early results, complications and risk factors in 109 dogs

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The purpose of this retrospective study was to describe the complications, risk factors and owner satisfaction associated with the modified Maquet technique (MMT). Medical records and radiographs of 109 dogs (117 stifles) were reviewed. A major complication was defined as complication requiring a second surgery. Risk factors analyzed for intra-and post-operative complication were age, drill hole at the distal part of the osteotomy, angle of opening, thickness of bony attachment, and post-operative tibio-patellar angle. Long-term follow-up was obtained by telephone interview. Complications occurred in 27% of the dogs (9% major and 18 % minor). Subsequent meniscal tear was the most common major complication and was not associated with a high tibio-patellar tendon angle. Fracture of the bony attachment of the tibial crest was the most common intra-operative and minor post-operative complication. Risk factors for intra-operative crest fracture were a high angle of opening, and a drill hole distal to the osteotomy. Risk factor for post-operative crest fracture was a thin thickness of bony attachment. Overall outcome was rated as excellent or good by respectively 82% and 13.1% of owners. Outcome of MMT was comparable with other techniques used for treatment of cranial cruciate ligament injury. The osteotomy should be extended distally, no drill hole should be performed, and the thickness of bony attachment of the tibial crest should be calculated with a maximum load equivalent to 6 times dog's bodyweight.

14:30 - Exploring the role of BLV and HTLV1-encoded micro- and long noncoding RNAs: lessons from RNA-Seq

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More than 20 million people are infected by HTLV1 worldwide and this will cause T-cell leukemia in ~5% of them. Yet the molecular mechanisms that underlie the oncogenic potential of this virus remain largely unknown. BLV is closely related to HTLV1 and causes a similar B-cell leukemia in the bovine or -when experimentally infected- in sheep. As for HTLV1, the oncogenic mechanisms underlying BLV-induced leukemia remain poorly understood. In both diseases, leukemic cells harbor an integrated provirus, yet the integration sites are variable. As a consequence, it is generally assumed that the oncogenic effect of the provirus is largely mediated by virally-encoded proteins, essentially Tax. Paradoxically, however, both HTLV1 and BLV proviruses are found to be epigenetically silenced in tumor cells. We have recently made two observations that might dramatically change the prevalent dogma of HTLV1 and BLV-induced leukemia. The first is that BLV is not silent at all in tumor cells. A cluster of BLV-encoded miRNAs is abundantly expressed, accounting for 40% of the miRNAs present in leukemic cells. They are exceedingly conserved across BLV isolates, strongly supporting an essential yet still unknown function. The second is that BLV strongly expresses non-coding antisense transcripts from the 3'LTR. To further explore the tumor transcriptome, we carried out deep sequencing of RNA libraries prepared from primary tumors. Our strategy allows an unbiased view of the complete transcriptome and the identification of overlapping sense and antisense-oriented transcripts at both virus- and genome-wide level. Insights drawn from tumor RNA-Seq datasets will be presented.

14:45 - A murine model for co-infection using the human blood fluke *Schistosoma mansoni* and γ-herpesvirus

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Schistosomiasis is caused by numerous species of trematode from the genus *Schistosoma* and affects some of the world's poorest populations. More than 200 million people are infected worldwide, with most occurring in sub-Saharan Africa. Geographically, *S. mansoni* overlaps with the human γ-herpesviruses such as the Kaposi's sarcoma-associated herpesvirus, responsible for severe malignancies. The strongly regulated Th2-type immune response generated during infection by *S. mansoni* may jeopardize or improve the host's ability to generate effective immunity against co-infecting pathogens, such as viruses. Here, we have trialled two approaches using the murine γ-herpesvirus (MuHV-4) model. The first used a *S. mansoni* egg model, whereby eggs are injected intravenously which circulate to the lungs to induce granulomas and Th2-type responses. This is followed by intranasal infection of a MuHV-4-Luc virus (luciferase reporter). Alternatively, we naturally infected mice with *S. mansoni* cercariae which migrate and pair as adults in the mesentery venules (6-7 weeks post-infection). Eggs produced are caught within the liver inducing granulomas and strong Th2-type responses. Initial studies show a significant reduction of MuHV-4 replication in the lungs at day 5 and 7 post-infection as confirmed by *in vivo* imaging and virus titration. Additionally, weight loss caused by MuHV-4 infection was significantly reduced at day 8 post-infection for the co-infected group in the model of natural infection. Further examination of viral latency, antibody isotypes, cytokine responses and cell populations within the lung are also presented. This project provides insights into the complexity of viral co-infection with helminths and may uncover undescribed immunological pathways that could inhibit or protect against viral infection.

15:00 - Contribution to the study of the pathogenesis of dermatophytosis using a murine model of infection with *Arthroderma benhamiae*

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Dermatophytoses are common zoonotic skin diseases whose immunology remains largely unknown, which could explain the failure of most vaccination assays against them. Despite their confinement in keratinized structures of the skin and its annexes, dermatophytes can induce a specific immune response that can lead to total or partial protection against reinfection. It is commonly accepted that the protective immune response is of Th1 type but the involvement of the Th17 pathway has so far not been evaluated, although its role is increasingly recognized as being instrumental in the evolution of many other fungal and microbial infections. The aim of this study was to evaluate the potential involvement of the Th17 pathway in the immune response against dermatophytes using a new mouse model of infection. C57BL/6J mice were cutaneously inoculated with spores of *Arthroderma benhamiae*. The clinical, histopathological and mycological follow-ups were performed during primary and secondary infections and showed that the secondary infection was less severe with a smaller fungal burden, a more infiltrative pattern and a higher myeloperoxidase activity. Then, lymphoproliferative assays with the cells of the draining lymph nodes were performed and the orientation of T lymphocytes was determined by flow cytometry, ELISA quantification of cytokines and qRT –PCR analysis of transcription factors. Results showed that the immune response was predominantly Th17. Altogether our results show that the Th17 pathway could be predominant for protective immunity against *A. benhamiae* in mice. Theses results will be validated using knockout mice and neutralizing antibodies for certain components of the immune response.

Oral presentations

15:15 - Hepatoprotective effects of crude rhubarbe extract extend to the modulation of gut microbiota in a murine acute alcohol-induced steato-hepatitis model

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Binge drinking is emerging and alarming social and health problem, leading to alcoholic liver disease (ALD). It is characterized by hepatic steatosis coupled with inflammation. A way to limit the evolution of ALD would be the use of bioactive compounds to reduce fat accumulation and inflammation. A specific crude rhubarb extract has been tested in a murine model of acute alcohol-induced steato-hepatitis. 18 male C57BL/6J mice has been fed with a standard diet, enriched with (OH-EV1 group) or without the rhubarb extract (CT and OH groups) for 17 days. Groups OH and OH-EV1 were then submitted to binge drinking and euthanazed. Physiological parameters have been measured. Moreover, bacterial ceacal contents were analyzed by pyrosequencing and 16S rDNA v1-V3 targeted metagenomic analysis. Inflammatory markers levels in liver and total hepatic cholesterol level show that the standardized extract limits inflammation and lipid accumulation. Moreover, metagenomic analysis revealed the composition of major bacterial populations in caecum. Several populations were statistically influenced by the rhubarb enriched diet compared normal diet as shown after binge drinking. Among these populations, few were strongly correlated with the levels of inflammatory markers in the liver. We targeted 3 populations for further analysis: two *Alistipes* populations and *Parabacteroides goldsteinii*. The *Alistipes* belong to yet undescribed species. Based upon the only nucleic trace from pyrosequecing, 3 quantitative RT-PCR tests were created to target these populations in gut samples. Quantitative determination of these populations confirmed the statistical correlation with inflammatory markers and results from metagenomic study.

16:00 - Comparison of blood and milk parameters in dairy cows managed with two different diets: a grass silage based diet or a diet with an extruded compound feedstuff

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Specific fat supplementations are used in dairy cow diet in order to increase the n-3 fatty acids content and to reduce the extent of the NEB, but with no monitoring at farm level. A survey was carried out on 5 commercial dairy farms. Three farms were characterized by a feeding management in which more than 50% of the dry matter intake was from grass silage. The cows from the 2 other farms received less grass silage but 1.5kg/c/d of a linseed extruded compound feedstuff. Twelve cows in each group were examined and milk along with blood samples were obtained monthly during the first 4 months of lactation. The feeding of the extruded compound significantly reduced both the fat and the protein contents in milk (3.5 vs 4.3%, $P<0.001$ and 3.1 vs 3.2%, $P<0.01$). With the extruded supplement, the short chains fatty acids (FA) in milk were reduced and the unsaturated FA proportions significantly increased, but with no significant differences for the unsaturated FA content due to the fat content drop. The body condition score losses were lower in the extruded group. However, they had higher ketone bodies and insulin concentrations and lower glycemia. The NEFA concentrations were similar in the 2 groups, indicating a similar fat mobilisation. The feeding of the extruded supplement did not affect either the days open or IGF1 and progesterone concentrations corroborating clearly the lack of effects on the reproduction. No improvements on health, production or reproduction were observed when comparing farms with good management practices.

16:15 - The impact of Schmallenberg in Belgian cattle and sheep: a case-control study

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Schmallenberg virus (SBV) emerged during summer 2011. SBV causes an acute unspecific syndrome in cattle and reproductive diseases in domestic ruminants. To date, the real impact of SBV in Belgium and the risk factors for this disease remain unknown. Therefore a case-control study, via a telephone questionnaire, was conducted for cattle and sheep farms. Questions related to reproduction parameters and clinical signs observed in newborn and adult animals were designed. Several management parameters were also investigated (insecticide treatments, surrounding area, vaccination protocols). Comparisons between the case and control farms were implemented to identify potential risk factors. In cattle, the rate of malformed calves born in the case herds represented 3.37% of gestations. In addition 23% of the case cattle farmers observed a decrease in fertility (more inseminations per succeeded gestation were needed). In this study, abortions and still-births were not specific signs of SBV infection in cattle whereas the observed malformations were typical signs of SBV. The impact of SBV was higher in sheep holdings. This was reflected in the case herds by a high proportion of lambs aborted (3.26%), stillborn (10.49%) and malformed (8.26%). In this study, no significant risk factor for SBV was identified in cattle and sheep. In conclusion, this study showed that the impact of SBV was particularly high in Belgian cattle and sheep farms with relation to reproductive disorders encountered during the epidemic. For the farmers, the birth of a malformed animal was the key event to suspect the presence of SBV in the farm.

16:30 - Experimental in vivo infection of pigs by a wild boar hepatitis E virus strain

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Hepatitis E virus (HEV) possesses four genotypes. In Europe, genotype 3 mainly circulates and its route of transmission is highly suspected to be zoonotic. The aim of this study is to investigate the potential transmission of a wild boar (Wb) HEV strain from Wb to swine. Seven pigs were divided in 3 groups (Gp) and inoculated by intravenous route with 0.45µm filtrates of livers from pig and Wb. The Gp1 contained 3 pigs inoculated with WbHEV strain; the Gp2 (negative controls) contained 2 pigs inoculated with a HEV negative swine liver and the Gp3 (positive controls) contained 2 pigs inoculated with a HEV swine strain. All the inocula belonged to genotype 3 subtype F. The pigs were euthanized after 8, 9 and 10 days of infection and necropsy were done. Samples were analysed in qRT-PCR, ELISA and histopathology. As expected, no clinical signs were observed during the infection. The hepatic enzymes (ALT and AST) were not increased in each group. The virological results showed that bile and liver were positive in Gp1 and Gp3 pigs and sera were positive in Gp1 pigs from the 4th until the euthanasia. The serological results showed that all groups were negative in ELISA. In this study, we developed a swine model of in vivo infection with a WbHEV strain and we showed for the first time that a WbHEV strain could replicate in swine. These results provide experimental data about the likelihood of the crossing of the Wb–swine barrier in the context of outdoor breeding of pigs.

Research supported by the Belgium Federal Public Service, Health, Food Chain Safety.

16:45 - Evasion of cytotoxic T cell response by Alcelaphine herpesvirus 1 genome maintenance protein

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Alcelaphine herpesvirus 1 (AlHV-1) is a gammaherpesvirus carried by wildebeest (*Connochaetes taurinus*) asymptomatically. After transmission to a variety of ruminant susceptible species, AlHV-1 is responsible for the development after a long incubation period of an acute and fatal disease, namely malignant catarrhal fever (MCF). MCF is characterized by the infiltration and proliferation of infected CD8+ T cells in many organs. Recent studies have demonstrated that viral latency is essential during the disease. Among the rare transcripts detected during MCF, ORF73 is highly expressed. The ORF73 encodes AlHV-LANA (for *latency-associated nuclear antigen*), an orthologous protein of the genome maintenance protein conserved in γ-herpesviruses. The high protein expression of AlHV-LANA should therefore induce an adaptive immune response. Even though such a response could exist, it is not protective since infected animals develop MCF. Genome maintenance proteins are responsible for the maintenance of viral episomes in latency and have cis-acting immune evasion properties. Here, we tested the capability of the AlHV-LANA to escape the cytotoxic T cell response by inhibiting its own presentation by the major histocompatibility complex class-I (MHC-I). We observed that AlHV-LANA was able to inhibit the MHC-I-restricted presentation to the cell surface of an OVA peptide fused to it, a mechanism involving canonic proteasomal pathways. Using targeted mutagenesis, we further demonstrated that the deletion of the GE-rich domain of AlHV-LANA restored the peptide presentation. The GE-rich domain did not affect the stability of AlHV-LANA but its presence was associated with reduced steady-state expression of the protein, an observation that could be correlated with reduced mRNA transcription levels. In conclusion, we demonstrated that the GE-rich domain of AlHV-LANA mediates *cis*-acting immune evasion properties, putatively through a mechanism inhibiting mRNA transcription. Future work should determine whether this mechanism enables latently infected cells to evade the immune system of the infected host.

17:00 - Selection in action: dissecting the molecular underpinnings of the increasing muscle mass of Belgian Blue Cattle

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Belgian Blue cattle are famous for their exceptional muscular development or "double-muscling". This defining feature emerged following the fixation of a loss-of-function variant in the myostatin gene in the eighties. Since then, sustained selection has further increased muscle mass of Belgian Blue animals to a comparable extent. In the present paper, we study the genetic determinants of this second wave of muscle growth. A scan for selective sweeps did not reveal the recent fixation of another allele with major effect on muscularity. However, a genome-wide association study identified two genome-wide significant and three suggestive QTLs affecting specific muscle groups and jointly explaining 8-21% of the heritability. The top two QTL are caused by presumably recent mutations on unique haplotypes that have rapidly risen in frequency in the population. While one appears on its way to fixation, the ascent of the other is compromised as the underlying *MRC2* mutation causes crooked tail syndrome in homozygotes. Genomic prediction models indicate that the residual additive variance is largely polygenic. Contrary to complex traits in humans which have a near-exclusively polygenic architecture, muscle mass in beef cattle (as other production traits under directional selection), appears to be controlled by (i) a handful of recent mutations with large effect that rapidly sweep through the population, and (ii) a large number of presumably older variants with very small effects that rise slowly in the population (polygenic adaptation).

Posters

Posters

1. Supplementation with Azawak bovine colostrum during the first two weeks postpartum improves performance, and survival in Red kids during the first year of life

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The aim of this work was to measure the effect of supplementation with bovine colostrum Azawak among newborn Red goats breastfed in breeding conditions for semi-intensive type of Niger. The study was conducted in Secondary Goat Breeding Center of Maradi (SGBCM). Forty newborn kids, regardless of sexe, were divided randomly into two groups: a "T-Control " group receiving only breast milk per feeding (n = 20) and a "C-Colostrum" group having the same diet but supplemented with bovine colostrum thawed (50 ml the first day of life, then 25 ml in 2 meals/ day between the ages of 2 and 15d, n = 20). The results indicate that supplementation with bovine colostrum in the first 15 days of life, increases the growth of kids until weaning, modifie some barymetric changes some settings improves health status and reduces the mortality rate. To our knowledge, in the studied environment, this work is original and seems to bring new knowledges likely to have practical applications in areas with farming conditions quite difficult.

Keywords: Colostrum; Bovine Azawak; Performance; Survival; Goats.

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

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These last years have seen the emergence of a wealth of biological information. The availability of ever more extensive genetic information has revolutionized intense research on the search for the genetic factors that influence common complex traits. In GWAS, hundreds of thousands of Single Nucleotide Polymorphisms (SNPs) are analyzed to determine whether they are associated with the disease or conditions of interest. Given the weak part of the genetic variance explained by single genes in many (so-called complex) traits, interactions of multiple loci, and consequently of single nucleotide polymorphisms (SNPs), are highly hypothesized to affect an individual's susceptibility to complex diseases. Although many works have been done to identify and quantify the importance of multi-SNP interactions, few of them could handle the genome wide data due to the combinatorial explosive search space and the difficulty to statistically evaluate the high-order interactions given limited samples. So we propose a novel KNN-MDR-BOOST approach using K-Nearest Neighbors (KNN), Multi Dimensional Reduction (MDR) and boosting (BOOST) for detecting gene-gene interaction as a possible alternative, especially when the number of involved determinants is potentially high. The idea behind our method is to take case-control genotype data as input and produce a ranked list of multi-SNP interactions; KNN-MDR-BOOST builds a boosting KNN classifier based on multi-SNP interactions and measures the importance of SNPs on the basis of their contributions in the classifier. Software has been developed allowing testing various scenarios of interactions, and the method will also be used to reexamine real datasets in order to potentially illuminate new regions of interest.

Key words: gene mapping, interaction, genome wide association study.

3. Phenotyping Belgian Blue cattle for their susceptibility to psoroptic mange

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The Belgian Blue cattle breed is extremely sensitive to psoroptic mange, a severe dermatitis caused by the mite *Psoroptes ovis*. This marked breed predisposition indicates that the susceptibility to psoroptic mange is partly heritable. Determination of the genetic factors underlying this high sensitivity of the Belgian Blue breed requires the definition of a clear phenotype. The mange phenotype was defined based on lesion extent, lesion appearance and mite counts. "Lesion extent" reflects the percentage of clinically affected body surface, calculated from a sketch of the lesions. The second parameter differentiates active, inactive (healing) and chronic lesions and is called "lesion appearance". Mites were counted in skinscrapings of each animal to establish the third parameter. Disease evolution (time span≈14 days) and reaction to acaricide treatment (1 month after treatment) were evaluated at 3 consecutive farm visits. Finally, the 3 quantitative phenotypic parameters of all 3 visits were mean centered, weighed and combined to a final score for each animal. In this approach, psoroptic mange in cattle is considered to be a quantitative trait rather than a class variable, so phenotyping cattle is sorting them based on their sensitivity score rather than dividing the population in a sensitive and resistant class. This finding is of great importance for the future genome wide association study of the susceptibility to psoroptic mange in cattle.

Keywords: Cattle -Belgian Blue – Mange- Psoroptes ovis – Genetics

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4. Approche participative de la situation sanitaire des élevages ovins dans la région de Ksar el Boukhari (Algérie)

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La situation sanitaire de l'élevage ovin de la région de Ksar el Boukhari (Algérie) a été décrite, utilisant des outils de l'épidémiologie participative. L'enquête a abordé les principaux problèmes des éleveurs, les principales maladies ovines rencontrées et leur importance économique. Selon la perception de quatre-vingt dix éleveurs, interrogés séparément ou en petits groupes, en termes d'importance relative (%) obtenue par empilement proportionnel, les maladies les plus fréquentes durant la dernière année écoulée sont respectivement les pneumonies (19%), l'avortement (15%), le piétin (10%), la gale (10%), l'ecthyma contagieux (8 %), l'arthrogrypose (7 %) et l'entérotoxémie (6%). Selon les mêmes méthodes, les maladies les plus coûteuses dans la perception des éleveurs étaient l'avortement (24%), les pneumonies (16%) et l'arthrogrypose (16%), le piétin (4%), la gale (4%), l'ecthyma contagieux (5%) et l'entérotoxémie (7%). Contrairement aux données de la littérature qui précisent l'importance des maladies de façon fragmentaire, la méthode d'approche participative a permis de décrire de façon globale les principales contraintes sanitaires des éleveurs, servant de base à une hiérarchisation des priorités de lutte sanitaire dans la région.

5. Prediction of bending stiffness of a canine long bone (humerus) by finite element analysis and validation by ex vivo mechanical tests.

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Internal osteosynthesis is a widely used stabilization technique for long bone fractures in dogs. Technical failures have been reported to contribute to up to 80% of fracture complications[1]. The objective of the present study was to create a bone model by finite element analysis (FE) including the heterogeneity of bone properties and to validate this model by experimental studies. This will enable us to evaluate the mechanical aspects of different types of osteosynthesis in the future. Based on computer tomographic scans (n=8, 17-39 kg, embedded in resin molds, 1mm sections), a finite element three-dimensional model of the canine humerus was created[2,3,4,5]. Trabecular and cortical bone were considered transversely isotropic elastoplastic5 (axial direction Ea=2065ρ3.06, transverse direction Et=2314ρ1.57) and heterogeneous. Embedded humeri were tested in 3-point bending and experimental data were compared to the FE simulations. Experimentally fracture onset appeared on the tensile surface of the humeral shaft, where tensile forces are maximal and parallel to the axis of the bone diaphysis. The experimental modelling of bone failure was set at a maximum principal strain6 with an arbitrary value of 3.5%[6]. Bending stiffness and yield load of the bone were satisfyingly predicted by the model (less than 20% error). This result is the first step to realize our long-term objective which is to introduce different osteosynthesis patterns in FE simulations to optimize plate length and size, screw placement and in the following the type and amount of implants to achieve an optimal balance between rigid and elastic fixation for optimal fracture healing.

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6. Changes in the numbers of lymnaeid snails and their infection with *Fasciola spp.* in the Red River Delta region of Vietnam

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Freshwater snails belong to Lymnaeidae family were reported as intermediate host of fasciolosis. The aim of this study was to investigate the fluctuation of lymaeid snail density and their Fasciola spp. infection among season. Lymnaeid snails were collected monthly during, 2010 and 2011, from selected habitats in three communes on the Red River delta of northern Vietnam. Only two lymnaeid species were found, *Radix auricularia* and *Austropeplea viridis*. The former was only found along the edges of water in the large channels. Numbers were highest in March to May with smaller numbers from November and December and none in months of highest rainfall, July to September. A. *viridis* were more plentiful at the edges of rice fields than in adjacent small channels and numbers were highest when growing rice crops were irrigated, February to April and July to September. Dry habitats after harvest had no snails. Cercariae of Fasciola spp. were not found in any R. auricularia and in less than 1% of *R. viridis snails*. Infections were higher in snails from the edges of rice fields compared to small channels and highest numbers were in March and April and from October to December.

Keywords: lymnaeid snail, *Fasciola spp.*, Red River Delta, Vietnam

7. Current status of fasciolosis in cattle from abattoirs and characteristics of *Fasciola* samples based on morphology and molecular approach

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The present study was conducted from January to December 2012 to determine the prevalence of *Fasciola spp*. infection in cattle slaughtered in abattoirs in Hai Boi commune, Dong Anh district and in Quang Minh commune, Me Linh district, Ha Noi. A sum of 360 gall bladders from cows and buffalos were examined for the presence of adult *Fasciola spp*.. The gall bladders' fluid were examined for the presence of *Fasciola spp*. eggs using the sedimentation method. *Fasciola* adult specimens were measured. The second internal transcribed spacer (ITS-2) of nuclear ribosomal DNA (rDNA) was amplified from 4 phenotype isolates by polymerase chain reaction (PCR), and the representative amplicons were sequenced. The result showed high prevalence of *Fasciola spp*. infection in cattle (62%; 224/360) with highest density of 38 adult fluke per gall bladders. Plenty of the gall bladders were not found *Fasciola* adult but the presence of eggs (38.3%; 138/360). Four sizes of *Fasciola* adult were determined, includes: long-large (35-53mm in length, 11.5-14mm in width), long-narrow (32-45mm in length, 7-10.5mm in width), medium-large (21-28mm in length, 8.5-12.6mm in width), and medium-narrow (29-31mm in length, 7-9mm in width). Among them, the medium-large fluke size was similar to *F. hepatica* while others were identified as *F. gigantica*. Based on ITS-2 sequence data, it was confirmed that the sequence of *Fasciola* medium-large size represented *Fasciola hepatica*, and the other is almost identified to that *F. gigantica*. It is the first demonstration of *F. hepatica* in cattle in Vietnam using a molecular approach.

Keywords: *Fasciola hepatica, Fasciola gigantica*, abattoirs, Vietnam

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8. Monophosphoryl lipid-A adjuvant induces partial protection in guinea pigs against *Microsporum canis* infections

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The aim of this work was to assess the immunogenicity and protective efficacy of secreted components from *Microsporum canis* adjuvanted with the monophosphoryl lipid-A (MPLA), in a vaccine study using the guinea pig as an experimental model. Animals were vaccinated subcutaneously with either the secreted components adjuvanted with the MPLA, the MPLA adjuvant alone or phosphate-buffered saline (PBS) three times at two-week intervals, until 42 days prior to *M. canis* infection challenge. A blind evaluation of dermatophytosis symptoms development and fungal persistence in skin was monitored weekly. The antibody response towards the secreted components was assessed by enzyme-linked immunosorbent assay every 14 days throughout the entire experimental study, while the levels of interferon (IFN)γ and interleukin (IL)-4 expressed in peripheral blood mononuclear cells were evaluated by quantitative RT-PCR at the end of the experiment. The animals that received MPLA had a significantly lower clinical score than those that received PBS. However, no significant difference was observed between the guinea pigs vaccinated with the secreted components adjuvanted with the MPLA and those having received MPLA alone. The results also showed that vaccination induced a strong antibody response towards the secreted components and a slight increase in IFNγ and IL-4 mRNA levels. Our results show that the MPLA adjuvant used in this vaccine study can induce per se a partial protection against a *M. canis* infection while the secreted components do not confer an additional protection under the present experimental conditions.

9. Detection of *Bordetella bronchiseptica*, *Mycoplasma canis* and *Mycoplasma cynos* by specific quantitative polymerase chain reaction assays in the bronchoalveolar lavage fluid of dogs with eosinophilic bronchopneumopathy

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Canine eosinophilic bronchopneumopathy (EBP) is a disease characterized by eosinophilic infiltration of the lung and bronchial mucosa. Aetiology remains unclear although immunologic hypersensitivity is suspected. In humans and cats, infections with *Mycoplasma spp.* seem to be involved in inflammatory bronchial disease. Potential role of *Bordetella bronchiseptica* (Bb), *Mycoplasma canis* and *M. cynos* in canine inflammatory bronchitis has not been investigated. Presence of these bacteria were therefore retrospectively assessed by quantitative polymerase chain reaction (qPCR) in bronchoalveolar lavage fluid (BALF) samples from 18 dogs with EBP and 8 dogs with chronic bronchitis (CB). Disease severity was assessed by determination of a severity index. All BALF culture were negative for Bb, while qPCR detected Bb more frequently in EBP dogs (5/18) than in CB dogs (0/8) (p = 0,009). Presence of Bb in EBP dogs was significantly associated with clinical severity (p = 0,041). Results of qPCR were positive for *M. canis* and *M. cynos* in 6 (33%) and 2 (11%) EBP dogs and in 1 (13%) and 1 (13%) CB dogs, respectively (no statistical difference). In conclusion, *M. canis* and *M. cynos* do not seem to be predominantly involved in the pathogenesis of canine EBP. However, Bb is more frequently detected in BALF from EBP dogs than from CB dogs and its presence is associated with clinical severity. Whether Bb is able to trigger eosinophilic inflammation or is a frequent secondary contaminant in EBP dogs is unknown. Anyway, presence of Bb should be assessed in canine EBP cases and treated accordingly.

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10. Detection of *Mycoplasma canis* and *Mycoplasma cynos* by specific quantitative polymerase chain reaction assays in the bronchoalveolar lavage fluid in dogs infected with *Bordetella*

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Bordetella bronchiseptica (Bb) is one of the primary causative agents of canine infectious respiratory disease (CIRD). Although *Mycoplasma cynos* (*M. cynos*) was recently identified as an emerging pathogen in CIRD, role of *M. canis* and *M. cynos* as primary pathogens still remains unclear. Detection of these bacteria is now improved by quantitative polymerase chain reaction (qPCR) identification. In dogs with CIRD due to Bb, the frequency of co-infection with *M. canis* and *M. cynos* and their possible role in the clinical severity are unknown. Therefore, Bb, *M. canis* and *M. cynos* were assessed by qPCR in the bronchoalveolar lavage fluid (BALF) sample from 16 dogs with Bb, and compared with results obtained in 10 dogs with bacterial bronchopneumonia (BBP) and 10 healthy dogs. Bordetellosis was confirmed based on BALF cytology together with positive qPCR on BALF. BBP was confirmed based on BALF cytology and culture. A clinical severity index (CSI 0 to 12) was assigned based on clinical signs, thoracic radiographic pattern and BALF score. *M. canis* was indifferently detected in healthy (5/10), BBP (4/10) and Bb dogs (3/16) while *M. cynos* tended to be more frequently detected in Bb group (8/16) than in other dogs. In Bb dogs, no correlation could be detected between CSI and presence of *M. cynos*. In conclusion, co-infection with Bb and *M. cynos* is frequent in CIRD but is not correlated with clinical severity. Further studies are required to investigate whether coinfection of Bb and *M. cynos* deserves specific therapeutic considerations.

11. *Crenosoma vulpis* infection in two young dogs in Belgium

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Crenosoma vulpis has an indirect life cycle requiring an intermediate host (terrestrial gastropod) and is causing chronic respiratory disease in dogs. An eighteen month old entire male Bouvier des Flandres and a fifteen month old spayed female Basset Hound were presented for chronic cough unresponsive to long term treatment with antibiotics and/or low dose corticosteroids. Thoracic radiography revealed diffuse bronchial pattern. Bronchoscopy revealed the presence of freely moving worms in the airway lumen, further identified as adults of *Crenosoma vulpi* Additionally bronchoalveolar lavage fluid analysis (both dogs) and Baermann faecal analysis (one dog) revealed the presence of larvae with a morphology compatible with *C. vulpis* first stage larvae. In the Bouvier des Flandres, a first treatment with oral fenbendazole at 50 mg/kg during 7 days failed to cure the infection. Therefore, a single topical application of 10% imidacloprid + 2.5% moxidectin at 0.1 ml/kg was given. The Basset Hound was successfully treated with two administrations of milbemycin oxime at one month interval. This lungworm infection might be underdiagnosed and should be excluded before initating a long term corticosteroid therapy. To the author's knowledge, this is the first time *C. vulpis* infection is reported in dogs in Belgium.

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12. Characterization of host factors involved in resistance/susceptibility to influenza A infection in Mx-negative mouse models

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DBA/2J mice and C57BL/6J are two extremes in terms of susceptibility to influenza A virus among Mx-negative mouse strains. Several research teams focused on the factors explaining this difference, mainly by genetic approaches using Recombinant Inbred Lines between those two strains. Several candidate-genes have been proposed, but it was not possible to determine their importance. We chose a phenotypic approach, by dissecting each stage of influenza A infection virus in mice of each line, aiming at identifying critical differences between C57BL/6J and DBA/2J. Preliminary observations suggest that either the viral infection of the airway epithelium of DBA/2J is more productive, either alveolar macrophages from C57BL/6J are more efficient in viral particles phagocytosis, or a combination of these two mechanisms. We isolated and cultured alveolar macrophages from both strains of mice to quantify and compare the cytokine expression induced by influenza A (H1N1) virus infection with real-time rt-PCR. We have demonstrated a greater expression of cytokines, chemokines and interferons on alveolar macrophages of C57BL/6J mice.

13. Effects of mare's milk fat globule on virulence genes expression of *Escherichia coli* O157:H7

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Escherichia coli O157:H7 is a human pathogen able to induce severe diseases such as bloody diarrhea or hemolytic uremic syndrome. The present study aims to explore the effects of equine milk fat globule (MFG) on *Escherichia coli* O157:H7 virulence factors. The globules are composed of a triglyceride core surrounded by three phospholipid layers in which are embedded and adsorbed proteins originating from mammary glands. The MFG were extracted from raw and heated equine milk in order to assess the potential effects of such commonly used treatments in dairy industries. Heat treatment was comparable to a « Low Temperature - Long Time » pasteurization by maintaining samples to 65°C during 30 minutes. In order to quantify and concentrate the MFG extracts, a freeze-drying treatment was applied. Thanks to quantitative reverse transcriptase polymerase chain reactions (RT-qPCR) we examined the modulation induced by the presence of MFG on several *Escherichia coli* O157:H7 virulence genes involved in motility (*fliC* gene), quorum sensing (*luxS* gene), shiga toxin production (*stx2b* gene) and enterocyte effacement regulator (*ler* gene). Our preliminary results showed a strong increase in expression of the gene *fliC* involved in motility in both heated and non-heated samples, and a slight decrease in the expression of *luxS* with raw MFG. More repetitions are needed to statically confirm this trend. To our knowledge, this study is the first centered on the potential of equine MFG in virulence modulation of pathogenic bacteria and can lead to a better knowledge of the role of milk against pathogenic bacteria.

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14. Assessment and spatial characterization of pastoral resources in northern Morocco

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Rangelands represent the most important feed sources for goats in the Northern Morocco. Land cover and land use change has become a central component in current strategies for managing natural resources and in environmental change monitoring. For a sustainable and integrated development of pastoral and forest resources, it is essential to establish a resource assessment system. The first step of this system will be to discover the trend of development of forest areas in Rif Mountain between 1984 and 2011 from satellite images and create a pastoral map of Chefchaouen region by using remote sensing and geographic information system (GIS).

15. Efficient aseptic and automatable vitrification of human pluripotent stem cells using bio-safe and chemically defined media

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Thanks to their pluripotent stem cells (PSCs) status, human embryonic stem cells and induced pluripotent stem cells are considered as a virtually unlimited source of cells for biomedical research purposes and cell-based therapies. However, their use requires efficient and bio-safe handling. Cryopreservation is an unavoidable key step for storage and transportation, during which the cells are submitted to extreme physical and chemical conditions prone to alter their viability as well as their biological properties. Human PSCs cryopreservation routinely consists in conventional slow-freezing, of which poor survival rates are mainly due to cell damages resulting from water crystallization. In order to prevent this, several groups have adapted vitrification protocols to hPSCs and have shown that they are more effective than slow freezing. However, they cannot ensure biological safety since cells are stored in containers that are prone to leakage when plunged into liquid nitrogen. This study presents our newly developed cryopreservation method, based on aseptic and automatable vitrification in sealed french straws. Only bio-safe and chemically defined cryopreservation media are used. We have shown that our aseptic vitrification method is as efficient as conventional slow freezing in terms of cell survival but more efficient to maintain the cells in an undifferentiated state. Moreover, we have shown that hPSCs keep their biological and cytogenetic properties after warming. Our technique appears as very promising for the storage and transportation of hPSCs or equivalent cells, particularly when intended for clinical applications that require high standards of efficiency and safety.

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16. High seroprevalence of Q fever in large animal veterinarians in Southern Belgium

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Q fever is a zoonosis occurring worldwide and caused by *Coxiella burnetii*. In Belgium Q fever is enzootic in the domestic ruminant population. The disease is submitted to a compulsory declaration in animals since the new Royal decree of February 2014, and in humans since 1971. The human infection results mostly from the inhalation of aerosols generated from contaminated animals and animal products. Human Q fever may range from subclinical infection to endocarditis and long term debilitation such as chronic fatigue syndrome. Q fever is a well-known occupational zoonosis, but so far no epidemiological studies have been conducted to establish its seroprevalence among the different risk categories in Belgium. The aim of this work was to investigate the seroprevalence and characterize the immunological response after Q fever infection in large animal veterinarians in Southern Belgium. The study was performed after approval of the Medical Ethical Commission of the University of Liège. Collected sera were initially screened using a commercial ELISA kit, allowing detecting phase II IgM and IgG antibodies. Results were confirmed using indirect immunofluorescence assay (IFA), which allowed the detection of phase I IgM and IgG antibodies, as well as to define the antibody titre of each immunoglobulin. The results obtained from the two serological tests seemed to display a good agreement and a high seroprevalence was measured within the tested population. Furthermore, high phase I antibodies characterized the immune response of the seropositive large animal veterinarians.

17. Reassortant viruses obtained after in vitro co-infection with African horse sickness virus serotypes 4 and 9

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African horse sickness (AHSV) is an infectious disease caused by a double stranded positive RNA virus which belongs to the family *Reoviridae*, genus *Orbivirus*. The virus has nine serotypes and is transmitted by *Culicoides* biting midges. The objective of this study was to investigate the occurrence of AHSV genetic reassortment *in vitro* and to determine which segments of the genome are involved. One-step viral growth curve was performed for serotypes 4 (AHSV-4) and 9 of AHSV (AHSV-9) to evaluate the dynamic of *in vitro* replication. Co-infection experiments were performed first by using equal multiplicity of infection (MOI) for both serotypes and later by using a higher MOI for AHSV-4. After co-infection, four successive passages of the infectious supernatants were performed and passages 1 and 4 were used for viral isolation through plaque assays. Duplex qRT-PCR specific for serotype 4 and 9 based on the detection of VP2 and VP5 proteins were developed to perform a first screening of each plaque purified virus. The reassortant viruses were then confirmed by RT-PCR and genome sequencing of serotype-specific target regions of the amplified viral segments (1, 2, 6 and 10). Even if the one-step viral growth curve was similar for AHSV-4 and 9, co-infection experiments performed with an equal MOI did not generate any reassortant virus, with AHSV-9 predominantly detected by qRT-PCR. Co-infection experiments with a higher MOI for AHSV-4 highlighted the presence of reassortant viruses. Based on preliminary results, segment 6 encoding VP5 outer capsid protein seems to be the segment most frequently involved in reassortment.

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18. Protected Designation of Origin Belgian cheese microbiota characterized using metagenomic analysis

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Herve cheese is a Belgian soft cheese having a washed rind and made from raw or pasteurized milk. Its specific microbiota has never been fully explored before and the use of raw or pasteurized milk in addition to the starters is supposed to impact the rind and the heart microbiota of this cheese. The aim of the present study was to analyse its bacterial microbiota using classical microbiology and metagenomic approach based on 16S rDNA pyrosequencing. ISO protocol were used to enumerate total mesophilic flora, *Enterobacteriaceae* and lactic acid bacteria.Using the metagenomic approach, 207 different phylotypes were identified. The rind of raw and pasteurized milk cheeses was very diversified. However, 96.30 % and 97.93% of microbiota in respectively raw milk or pasteurized cheese rind was composed of species present in both cheeses such as: *Corynebacterium casei*, *Psychrobacter*, *Lactococcus lactis subsp*. *cremoris*, *Staphylococcus equorum*. Interestingly, *Psychroflexus casei*, also described as giving a red color to raclette-type cheese was identified in little proportions in the rind composition of both raw and pasteurized milk cheeses (0.17% and 0.5% respectively). In the heart of the cheeses, the common species reached more than 99% of the microbiota. The main identified species were *Lactococcus lactis subsp. cremoris*, *Psychrobacter* and *Staphylococcus equorum subsp. equorum*. Interestingly, 93 phylotypes were present only in raw milk cheese and 29 in pasteurized milk cheeses. This study shows that the characterization of PDO cheeses's microbiota is useful to gain better knowledge of the bacteria responsible for the character of those cheeses.

19. Evaluation of the microbiota of "steak tartare" with metagenomic analysis targeting 16S ribosomal DNA

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"Steak tartare" is highly sensitive to bacterial spoilage. A better understanding of the bacterial content of this product by metagenomic analysis would thus be insightful in controlling the risk of spoilage. A total of 58 samples were analyzed from 7 butcheries, 6 restaurants, 6 sandwich shops, 8 supermarkets without an internal butchery and 8 supermarkets with an internal butchery. Samples were analyzed on the day of receipt (day 0) and 2 days later (day 2), except in the case of the restaurants and the sandwich shops. Samples were kept in chilled storage – at 4 °C for the first day and 8 °C for the second day. Metagenomic analysis targeting the V1-V3 region of the 16S rDNA gene was performed using the Roche GS Junior System. Raw sequences were analyzed using bioinformatics pipelines. Metagenomic analysis identified up to 180 bacterial species and 90 genera in some samples, depending on their origin. Among all the samples, seven major bacterial species were found to be predominant: *Brochothrix thermosphacta, Lactobacillus algidus, Lactococcus piscium, Leuconostoc gelidum, Photobacterium kishitani, Pseudomonas spp* and *Xanthomonas oryzae*. Our results demonstrate that the bacterial flora of steak tartare is more diverse than previous studies have shown. Our dissimilarity analyses showed only little difference between the results obtained from pre-packed samples taken at D0 and D2 from supermarkets. This may be due to the additives and the environmental conditions used in industrial preparation. Compared to culture-based methods, metagenomic analysis combined provides more valuable information.

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20. Mediation analysis of milk losses associated with clinical mastitis

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Milk losses associated with mastitis can be attributed to pathogens (direct losses) or to the immune response triggered by infection (indirect losses). The level of infection is often unknown making it difficult to estimate direct losses, whereas indirect losses can be approximated by measuring the association between SCC and milk. An alternative is to perform a mediation analysis. We applied this method on data collected during a survey of clinical mastitis which included milk and SCC, bacteriological cultures, and cow characteristics. Inversely to direct changes in milk, losses mediated by SCC were significantly different from zero for all bacterial species. Therefore, we inferred that products to boost the immune system would not sustain milk production and that, preventive measures, such as genetic selection for cattle resistant to infection, able to clear an infection with a low SCC, or both would be more appropriate.

21. A massive open online course on genetic counseling for animals

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A connectivist massive open online course (MOOC) is being created to introduce concepts of genetic counseling. Genetic counseling provides information on methods to improve the performances, health and well-being of future animal generations. The course is organized in two key modules: (1) fundamental principles of genetics, epidemiology, statistics, evidence-base medicine and communication and (2) application of the principles to case studies. The MOOC learning environment is based on four major types of activity (eg., course reading, data collection, creation and work sharing) and included several tools (eg., forum discussions and automated feedback).

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22. In vitro study of coinfection/superinfection parameters which can influence recombination events in noroviruses

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Noroviruses (NoVs) are non-enveloped, single-stranded, positive-sense RNA viruses. They are important causes of acute non-bacterial gastroenteritis in humans worldwide but their study is currently yet hampered by the lack of a cell culture system. NoVs genetically evolve by both point mutations and recombination and the murine norovirus (MuNoV) is considered as the best model for human NoVs. The aim of this study was to develop an experimental model based on the MuNoV in order to investigate coinfection/superinfection parameters that could impact recombination events. Monolayers of RAW264.7 cells were coinfected or superinfected with two MuNoV strains (CW1 and WU20) using different multiplicity of infection (0.1/1; 1/1 and 10/1 for CW1 and Wu20 , respectively) and time delays (0h; 0.5h; 1h; 2h; 4h; 8h; 12h and 24h) for infection. Supernatants were collected at 24 and 48 hours post-infection. Genomic copies of both viruses were first quantified by RT-QPCR. Then, viruses from the supernatants were plaque purified (36 clones per condition) and their recombinant status was checked by a real-time PCR discriminating method using primers targeting both extremity of the MuNoV genome. Results of quantitative and plaque picking assays are compared. Together, the results confirm that recombination does not frequently occur, at least *in vitro* and raise the issue on why these events are however so usual with *in silico* detection methods. The data also showed that superinfection exclusion seems to be triggered from 4h post infection with the first MuNoV. The mechanisms of the later should be still studied.

23. Development of an analytical method for the simultaneous measurement of eight biogenic amines in food.

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The formation of biogenic amines is essentially the result of enzymatic decarboxylation of specific amino acids, due to microbial enzymes. Knowing that biogenic amines, particularly histamine and tyramine, can cause health problems in predisposed consumers, their presence in food is not desired. What is more, quantification of biogenic amines in meat is suitable for detecting the stage of deterioration of meat and their concentration may be associated with the freshness and the level of degradation of proteins of the product. Therefore, a UPLC-PDA-Fluorescence method has been developed to evaluate the concentration of eight biogenic amines in food: 2-phenylethylamine, putrescine, tyramine, histamine, spermine, cadaverine, spermidine and tryptamine. Amines were analyzed on a UPLC Acquity system integrated autosampler, solvent delivery system and column heater coupled to a Acquity PDA detector and an Acquity Fluorescence detector (Waters Corporation). The column used was a Acquity UPLC BEH C18 (2.1 x 100 mm, 1.7 µm), with a UPLC BEH C18 VanGuard precolumn (2.1 x 5 mm, 1.7 µm) (Waters Corporation). The oven temperature was set at 40°C and the injection volume was 5 µL. The flow rate was 0.6 ml/min. The peaks were identified by comparing their retention times with those of the corresponding standards. The detection wavelength for PDA was 217 nm and fluorimetric detection at 300 nm for excitation and 480 nm for emission was applied. The developed method was applied to quantify biogenic amines in meat and meat products, on the day just after production and at the end of shelf-life.

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24. Minimal inhibitory concentrations of basil essential oil (*Ocinum basilicum*) against *Salmonella enterica* from livestock and application of the agar diffusion method

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Despite the difficulty to obtain antibiotics in some African countries, the emergence of antimicrobial resistance in some farms can be dramatic. Therefore aromatic and medicinal plant extracts are frequently the last resort as therapeutic option for the breeders. Even though basil plant has been empirically used for decades in animal drinking water in different African countries, basil essential oil can represent a promising and economical alternative due to a bactericidal effect on different bacterial species. The first aim of this study is the determination of the minimal inhibitory concentrations (MIC) against *Salmonella enterica* isolated from poultry and guinea fowl. 66 strains were collected in traditionnal breeding farms in a study on the prevalence of these bacteria implicated in mortality of chick. 51 (and 3 control strains) have been tested in the liquid medium micromethod. The second objective of this work is to develop a basil essential oil sensitivity test by an adaptation of the agar diffusion method through a determination of different parameters like the diffusion curve, the basil essential oil load on the disk and the two critical values.

25. Range of O-serogroups of Shiga toxin-producing (STEC) and enteropathogenic (EPEC) *Escherichia coli* in cattle in Wallonia

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Escherichia coli producing Shiga toxins (STEC or ShigaToxigenic E. coli) and/or a histological lesion called "attachment-effacement (AE)" (EPEC or EnteroPathogenic E. coli), cause enteritis with (bloody) diarrhoea in humans and young calves and the haemolytic uremic syndrome (HUS) in humans. Infection of humans through consumption of foodstuffs (in)directly contaminated by cattle faeces (which are healthy intestinal carriers) is proved for STEC and suspected for EPEC. Besides the O157:H7 serotype, STEC and EPEC can belong to more than sixty O serogroups. Of them, 10 are regularly identified in humans and/or calves worldwide: O5, O26, O45, O103, O104, O111, O118, O121, O145 and O165. This study aims to identify the O serogroups of STEC and EPEC collected from healthy bovines at slaughterhouses in the Walloon region. Large intestine contents were grown at 37°C overnight in Lauryl sulfate broth (selective for *Enterobacteriaceae*). Bacterial DNA was extracted by boiling from an aliquot of the broth culture to perform a triplex PCR targeting the stx1, stx2 and eae genes, while another aliquot was frozen at -80°C. The 53 of the 184 samples that were PCR-positive will be inoculated onto *E. coli* CHROMagar® ES agar plates (Chromagar) to identify STEC and/or EPEC isolates. In parallel 234 enterohaemolysin-positive E. coli isolated from young diarrhoeic calves at necropsy after growth on EHLY Medium® (Oxoid) (ARSIA, Ciney) are being tested with the triplex PCR. All STEC and EPEC isolates will be further assayed with a multiplex PCR targeting the specific genes coding for the O serogroups listed above.

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26. Prevalence of mycobacteria and *Rhodococcus equi* and the commensal flora in sub-mandibular lymphadenitis in pigs

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The aim of this study was to determine the prevalence of mycobacteria, *Rhodococcus equi* (*R. equi*) and other bacteria that could induce sub-mandibular lymphadenitis in pigs. The inspection of 126000 pigs' carcasses randomly selected was done and all the submandibular lymph nodes with lymphadenitis were sampled. We focused on a random selection of 266 samples for the isolation of mycobacteria, *R. equi* and other bacteria. The bacteria were identified by PCR, 16S rDNA sequencing or MALDI-TOF. We identified *Mycobacterium avium* subsp. *hominissuis* in 17 samples (6,39%; 95% CI: 3,77-10,04), *Mycobacterium bohemicum* in five samples (1,88%; 95% CI: 0,61-4,33). Three mycobacterial isolates have still to be identified. *R. equi* was isolated in 195 samples (73,3%; 95% CI: 67,56-78,53). On a subset of 132 carcasses, the collateral lymph node (CLN) was also sampled. We isolated R. equi in 13 of the 27 CLN with gross lesions (48,14%; 95% CI: 28,67-68,05) and 26 of the 105 CLN without gross lesions (26,00%; 95% CI: 17,74-35,73). Concerning the other bacteria isolated, we isolated mostly bacteria of the normal flora of pigs. We isolated bacteria that could be responsible of infections in pigs like *Trueperella pyogenes*. Moreover the isolation of *Lactococcus garvieae* seems to be related to mycobacterial infection. In conclusion, we isolated zoonotic (myco)bacteria in sub-mandibular lymph nodes of pigs with lymphadenitis. The evaluation of the risk for public health of such infections in pigs must be investigated.

27. Characterization of olive cake production in northern Morocco

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In northern Morocco, olive trees occupy an area of 147400 ha with an annual production of 265320 t of olives. In this region, there are 90 modern oil mills with a capacity of 160000 t/year and 2168 traditional ones with a capacity of 76500 t/year. These units generate large amounts of olive cake which can be used in animal feed. The objective of this work was to characterize these oil mills and quantify the production and the availability period of olive cake. An investigation was conducted at 13 olive oil mills. According to the results, 61.54% of these units worked for 3 months a year (November to January); 15.38% for 2 months and 15.38% for 4 months. The availability of fresh olive cake is therefore limited in time. For extracting oil, 38.5% of mills used mechanical pressure, 38.5% used centrifugation and 23.1% used these two methods. 46.2% of units processed more than 1000 t/year (with a maximum of 7489.41 t/year). On average, olive cake represented 45.13% of olives. In this region, olive cake quantities can be estimated at about 106732 t/year (data obtained by extrapolation) and primarily intended for sale. Olive cakes obtained from mechanical pressure were sold at a price of 0.5dh/kg (0.05 euro/kg) to factories for combustion, while those obtained by centrifugation were sold to extract olive cake oil with a price about 0.2dh/kg (0.02 euro/kg). In conclusion, due to its large available quantities, olive cake produced in the region could be valued in animal feed but there is still a need to assess its dietary quality.

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28. CT features of a lacrimal gland tumor in a dog

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In the dog, neoplasms of the lacrimal gland are rare. Lacrimal cysts were reported. Computed tomography (CT) scan findings of these lacrimal gland abnormalities have not been described in dogs.

The present report describes clinical and CT features of a lacrimal gland tumor in a dog. A 3,5 year old female Leonberger was presented with a progressive swelling at the level of the supero-temporal area of the left upper eyelid and of the bulbar conjunctiva since 3 weeks. A slight enophthalmia and a ventronasal strabismus were observed with a firm mass in the upper eyelid extending posteriorly between the globe and the orbital ligament. A CT scan of the head was performed. A 3 cm hypoattenuating (+/- 10 HU) ovoid mass was observed dorsolateral to the indented left globe. This mass was well circumscribed by an unevenly thick irregular soft tissue-attenuating wall (50HU), which was strongly contrast-enhanced (150HU) and measured about 2 to 10mm in thickness. The left globe was displaced caudoventromedially. These findings were suggestive of an abscess or an orbital tumor with a necrotic/cystic center; a lacrimal cyst was considered less likely because of the unevenness and the thickness of the wall. Complete surgical ablation was done by a modified lateral orbitotomy. Histopathology revealed a mixed benign tumor of the lacrimal gland and a lymphocytic necrotic adenitis. Six months postoperatively no recurrence is noted. In conclusion, lacrimal gland tumor should be included in the differential diagnosis of a firm ovoid cystic/necrotic mass dorsolateral to the orbit.

29. The Application of Bayesian multi-QTL methods for the identification of causative variants within GWAS identified risk loci for common complex diseases

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The Application of genome-wide association studies (GWAS) resulted in the identification of hundreds of loci significantly affecting complex diseases (e.g., more than 160 loci for Inflammatory Bowel Disease). However, for most of these loci, the underlying genetic variants have not been discovered yet. Most of these loci might harbor several causative variants. Therefore, we study the properties of Bayesian multiple QTL methods to fine-map these loci. In addition, we propose to integrate biological information in our mapping approach. Our method is adapted from Bayesian Lasso. The method is applied to categorical traits such as disease (binary traits) by extension to a so-called threshold model. That model assumes an underlying liability function with threshold above which the observed phenotype switches from healthy to affected. Before running the model, SNPs presenting high linkage disequilibrium are clustered. The method are applied to IBD including 102 significant locus. Totally 164 CD affected locus and 125 UC affected locus are detected with new approach.

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30. Wound management of a pregnant Belgian blue cow with severe toxic cutaneous necrosis affecting the limbs

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We herein report the wound management of a pregnant Belgian blue cow, which presented severe skin necrosis of the four limbs secondary to a prolonged stay in a river. A four-year-old pregnant BBBCB cow was presented to the clinic for Ruminants for lameness and edema affecting the four limbs. The day before, the cow got stuck in a river for about 10 hours. The initial clinical examination revealed shock with a severe edema of the four limbs and reluctance to move. A combination of opioids and NSAIDs was administered for analgesia as well as broad-spectrum antibiotics. Hydrotherapy alternating was initiated. At day 9, the necrotic skin started to slough spontaneously. Lavage was performed twice a day using warm 0.05 % diluted chlorhexidine solution. Devitalized tissue was manually removed during each lavage and wet-to-dry bandages were applied during the debridement process. Granulation tissue was not healthy and Debrisoft® sponges were used to eliminate superficial necrotic tissue. Silver sulfadiazine dressings were then applied once a day. At day 22, healthy granulation tissue was covering the entire wound surface and the epithelialization process was progressing. Vaseline dressings containing 10 % diluted povidone iodine were applied on the wounds until complete epithelialization occurred at day 135. During hospitalization, a high-protein diet was administered to optimize healing and to maintain pregnancy. After five months, a hairless completely epithelialized tissue was present. She calved of a healthy male calf weighting 43 kg, 22 days after the expected calving date. The most likely etiology of these lesions seems to be a prolonged contact with chemically contaminated water of the polluted river. The favourable outcome reported in this pregnant cow shows that extensive wounds may be successfully managed in cattle using available products.

31. Growth of microorganisms on white pudding in different conditions of temperature followed by classical microbiology and metagenomic analysis

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While the temperature instructions are generally respected during process and distribution of food products, it is not always the case with the consumers. Indeed, a few persons reach the right temperature level required for a safe storage of foodstuffs in their refrigerator. Besides, the food can sometimes spend a few hours in ambient temperature between the buying in the supermarket and the storage in cold temperature. In this study, we propose to model the growth of microorganisms on white pudding, stored in different conditions of temperature that reflect the situations described above (constant 4°C, constant 8°C, constant 12°C, 1/3 4°C – 2/3 8°C, 1/3 4°C – breach during 4h at 20°C – 2/3 4°C and 1/3 4°C – breach during 4h at 20°C – 2/3 8°C). The product was surface inoculated with potential spoilage and biopreservative strains (*Raoultella terrigena*, *Serratia quinivorans*, *Carnobacterium maltaromaticum*, *Lactobacillus oligofermentans*, *Lactobacillus nenjiangensis*, *Lactobacillus fuchuensis*, *Leuconostoc mesenteroides*, *Lactococcus lactis* and *Lactobacillus graminis*). Analyses by classical microbiology and V1-V3 16S rDNA metagenomics were done each day until the out of date of the food matrix. The transition from 4 to 8°C and the breach at 20°C during 4h have clearly boosted the growth of the microorganisms. The metagenomic analysis was a powerful tool to follow separately each population in each condition of storage. The results of this communication show the importance of keeping the foodstuffs in 4°C or lower in the refrigerator with the goal to avoid the spoilage or the development of pathogens and the potential of metagenomics for selection of biopreservative strains.

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32. Growth of microorganisms on precooked pasta in different conditions of temperature followed by classical microbiology and metagenomic analysis

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While the temperature instructions are generally respected during process and distribution of food products, it is not always the case with the consumers. Indeed, a few persons reach the right temperature level required for a safe storage of foodstuffs in their refrigerator. Besides, the food can sometimes spend a few hours in ambient temperature between the buying in the supermarket and the storage in cold temperature. In this study, we propose to model the growth of microorganisms on precooked pasta, stored in different conditions of temperature that reflect the situations described above (constant 4°C, constant 8°C, constant 12°C, 1/3 4°C – 2/3 8°C, 1/3 4°C – breach during 4h at 20°C – 2/3 8°C and 1/3 8°C – breach during 4h at 20°C – 2/3 8°C). The product was surface inoculated with potential spoilage and biopreservative strains (*Lactococcus piscium, Leuconostoc mesenteroides* and *Leuconostoc citreum*). Analyses by classical microbiology and V1-V3 16S rDNA metagenomics were done each day until the out of date of the food matrix. The transition from 4 to 8°C and the breach at 20°C during 4h have clearly boosted the growth of the microorganisms. The metagenomic analysis was a powerful tool to follow separately each population in each condition of storage. The results of this communication show the importance of keeping the foodstuffs in 4°C or lower in the refrigerator with the goal to avoid the spoilage or the development of pathogens and the potential of metagenomics for selection of biopreservative strains.

33. Plantigrady due to a MRSI localized myopathy in a newborn Belgian Blue calf

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We herein report two cases of gastrocnemius dysfunction leading to "plantigrady", in a newborn calf due to a localized severe myopathy. The first case is a seven-day-old Belgian blue male calf. He was referred because he was not able to stand up since he was 2 days old. He had previously been treated with NSAIDs and vitamins without success. The initial clinical examination revealed weakness, hyperthermia and tachypnea. The calf was not able to stand-up on his own and was not bearing weight on his right hind limb which was swollen, hot and painful from the hip to the claws. Deep pain sensation of the right hind limb was present but motor function was completely absent. Hematological analysis highlighted a severe neutrophilia and the blood biochemistry revealed a severe increased of the CPK. Radiographies of the right hind limb revealed a heterogeneous soft tissue swelling with radioluminescent spots caudally to the tibia without bone abnormalities. Ultrasonography of the right thigh showed a hyperechoic spots in gastrocnemius and long digit extensor muscles, without evidence of fluid collection and an enlarged right popliteal lymph node. A muscular biopsy of the gastrocnemius muscle was performed under general anesthesia. Histopathological examination identified a very severe acute and chronic necrotic myositis. Bacterial culture identified a MRSI *Staphylococcus pseudointermedius* sensitive to florfenicol and tetracycline. Despite the poor prognosis, the breeder decided to try a treatment using tetracycline and NSAIDs. After two months, the calf is still alive with a good condition. Lameness of the right hind limb is still present and the growth is slightly delayed. Plantigrady is a rare disease affecting calves. We herein report a severe MRSI myopathy of which the origin was not determined.

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34. Validation of HPTCL method for quantitative determination of Hypoglycin A in methanol extracts of Maple (*Acer*) samples

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Hypoglycin A (HA) variably contained in *Acer pseudoplatanus* and *A. negundo* seeds, is a highly suspected cause of atypical myopathy (AM) in Europe and seasonal pasture myopathy (SPM) in US. Curiously, incidence of AM increased over the last decade. In the fall 2013, AM Alert Group (AMAG) registered 415 European cases. As no curative treatment of these fatal myopathies exists, prevention remains important. The link of environmental factors (e.g. climatic condition) with toxicity of seeds deserves further investigations to improve preventive measures. This study aims to validate a reliable HPTLC method for determination and quantification of HA in plants. Method-Standard stock of 200µg/ml HA from TRC chemical© were prepared. Separation was performed on silica 60 Å F254 HPTLC plates as stationary phase and achieved with mobile phase of the following composition: methanol, acetic acid, water 70/ 20/ 10 (v/v/v). Determination and quantification were performed on a CAMAG scaner 3 at 490nm in absorbance mode. In respect of ICH guidelines, we observed a repeatable Retardation Factor value of 0,59 with a valuable selectivity and specificity. Linearity was also demonstrated. Precision is still to be improved but is under 6%. Limit of detection reached 11.58±0.47ng, limit of quantification 65.18±2.66ng and accuracy of the method were tested in a complete extraction, determination and quantification process. These results confirm suitability of HPTLC method for detection of HA in plant extracts. This methodology will be used to determine HA in autumnal samples of Acer trees collected on pastures where AM occurred in 2013 and 2014.

35. Thoracic fistula secondary to intra-thoracic foreign body penetrating the vertebral canal in a dog

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A 6-year-old neutered male mixed breed dog was presented with a thoracic fistula located in the right axillary region. He had been unsuccessfully treated by the referring veterinarian for a year with antibiotics, non-steroidal anti-inflammatories, local surgery and penrose drains. At presentation, clinical examination was normal except for the presence of a 2 cm purulent wound in the right axillary region. Thoracic radiographs demonstrated a subcutaneous fistula, bridging new bone formation from the second to the seventh thoracic vertebrae (T) and new bone formation on several ribs. CT scan revealed also these findings and the presence of a 17 cm long intra-thoracic foreign body surrounded by an inflammatory granuloma located in the dorsal mediastinum from T12 to T7. It eroded the right lateral aspect of T6 to T4 vertebral bodies and entered the vertebral canal from T4 to T2, causing a medullary compression of 25% of its diameter. Cranial mediastinal abscesses were also observed. A right ninth intercostal thoracotomy was performed to reach the intra-thoracic portion of the foreign body. The foreign body, a wooden skewer, penetrated the dorsal part of the right caudal lung lobe. Lung lobectomy using a stapler was therefore performed. The foreign body was then gently removed from the vertebral canal. A chest tube was placed at the end of surgery and removed 7 days postoperatively when production was minimal. The fistula was left to heal by second intention and was completely closed 14 days after surgery. No intra- or post-operative complications occured.

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36. Impact of aging technique and muscle on oxidative stability of beef packaged under high-oxygen atmosphere

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Two common approaches for beef aging are wet-aging and carcass-aging. Wet-aging refers to meat aged in a sealed vacuum package at refrigerated temperatures, while carcass aged at controlled temperatures and humidity is defined as carcass-aging. Carcass-aging is an ancient process used nowadays to produce beef characterized by its superior quality. The meat conservability is influenced by its sensitivity to oxidative process which can vary from one muscle to another. The aim of this study was to compare the effect of aging technique (wet-aging vs. carcass-aging), muscle (*longissimus dorsi vs. rectus femoris*) and previous vacuum storage time on colour and lipid stability of beef packaged in high-oxygen atmosphere. After a seven-day wet- or carcass-aging step, *longissimus dorsi* and *rectus femoris* muscle cuts from 4 Belgian Blue cows were vacuum packaged and stored at −1 °C for up to 28 days. At different times, part of these samples was repackaged under modified atmosphere – 70 % O2:30 % CO2 –, and stored during 7 days at +4 °C in order to simulate retail conditions. The following parameters were evaluated: colour (CIE L*a*b*), metmyoglobin %, lipid oxidation (TBARS), antioxidant enzyme activities (catalase, glutathione peroxidase and superoxide dismutase), a-tocopherol and fat content. The sensitivity of high-oxygen atmosphere repacked meat cuts to oxidation was influenced by the aging technique (wet > carcass conditions), muscle (*rectus femoris* > *longissimus dorsi*) and length of the vacuum storage. Oxidation stability could be associated with muscle catalase activity, and no association could be established with the a-tocopherol content.

37. Evaluation of morphological and functional characteristics of *Carnobacterium maltaromaticum* isolated from vacuum-packaged beef with long shelf life

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Carnobacterium maltaromaticum is a lactic acid bacterium, and many lactic acid bacteria associated with meat are known for their bactericidal or bacteriostatic activity against other strains, species or genera of bacteria. The presence of certain lactic acid bacteria adapted to a low temperature in fresh meat could extend the shelf life and improve the microbial stability and safety of this product. The aim of this study was to perform a morphological and functional characterization of a *C. maltaromaticum* strain with a potential bioprotective effect isolated from vacuum packaged beef with very long shelf life. The morphological, biochemical and enzymatic profiles, the influence of different temperatures and atmospheres, and the microbial stability of fresh beef inoculated with the *C. maltaromaticum* strain were evaluated. The isolated *C. maltaromaticum* strain presented similar morphological, biochemical and enzymatic profiles as those of two reference strains (LMG 11393 and LMG 22902). Among the studied conditions, a temperature of +12 °C and an atmosphere poor in oxygen were optimal for the growth of *C. maltaromaticum*. Vacuum packing is therefore suitable for this bacterium. An antimicrobial effect against *Enterobacteriaceae* was highlighted on inoculated fresh meat stored under N2. The functional characterization of this isolate will be further pursued by a genotypic characterization. Special attention will be taken to study its bioprotective properties.

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38. First report of autochthonous canine *Angiostrongylus vasorum* infection in Belgium

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Canine angiostrongylosis is considered as an emergent disease in Europe and Canada. A fatal case of *Angiostrongylus vasorum* infection is described in a four month old puppy born in Belgium. The dog was submitted for marked neurological disorders, body weight loss, a profound weakness and mild respiratory signs. The dog was given antibiotics and mucolytic compounds with very little improvement and consequently was referred to a specialist for additional examinations. As the general condition of the dog was rapidly declining, the animal was euthanized shortly after on its owners'request and a necropsy was carried out. Extensive gross pulmonary lesions were observed and histopathological examination revealed the presence of numerous larvae with morphology compatible with *A. vasorum*. Larvae were also found in the product of a bronchoalveolar lavage but faecal material was not examined. The presence of *A. vasorum* circulating antigen was demonstrated through ELISA; additionally an *A. vasorum* specific PCR was performed on brain material and yielded a positive result. This case confirms that the clinical diagnosis of canine angiostrongylosis can be very challenging especially when respiratory signs are absent or very mild such in the present case. This is the first reported case of canine angiostrongylosis in Belgium.

39. A 660-kb deletion with antagonistic effects on fertility and milk production segregates at high frequency in nordic red cattle: additional evidence for the common occurrence of balancing selection in livestock

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In dairy cattle, the widespread use of artificial insemination has resulted in increased selection intensity, which has led to spectacular increase in productivity. However, cow fertility has concomitantly severely declined. It is generally assumed that this reduction is primarily due to the negative energy balance of high-producing cows at the peak of lactation. We herein describe the fine-mapping of a major fertility QTL in Nordic Red cattle, and identify a 660-kb deletion encompassing four genes as the causative variant. We show that the deletion is a recessive embryonically lethal mutation. This probably results from the loss of *RNASEH2B*, which is known to cause embryonic death in mice. Despite its dramatic effect on fertility, 13%, 23% and 32% of the animals carry the deletion in Danish, Swedish and Finnish Red Cattle, respectively. To explain this, we searched for favorable effects on other traits and found that the deletion has strong positive effects on milk yield. This study demonstrates that embryonic lethal mutations account for a non-negligible fraction of the decline in fertility of domestic cattle, and that associated positive effects on milk yield may account for part of the negative genetic correlation. Our study adds to the evidence that structural variants contribute to animal phenotypic variation, and that balancing selection might be more common in livestock species than previously appreciated.

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40. Caractérisation morphobiométrique des populations caprines locales de Laghouat, Algérie

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Cette étude caractérise la population caprine dans la région de Laghouat, Algérie. Un total de 114 chèvres, dont 82 de race Arabia et 32 Makatia, et 9 boucs Arabia, tous âgés d'un an et plus, ont fait l'objet d'une caractérisation morphobiométrique (15 caractères qualitatifs et 20 quantitatifs). 87,5% des femelles Makatia et 92,68% Arabia ont le profil facial rectiligne. Il est convexe chez 55,56% des mâles. La ligne du dos est concave ou droite, avec une croupe inclinée. 81,25% des femelles Makatia, et 75,61% Arabia présentent des cornes courbées et dirigées en arrière, chez 50% des mâles elles sont spiralées. La crinière et les pendeloques sont absentes chez 78% des animaux. Dans la race Arabia, la barbiche est généralement présente et les oreilles pendantes. Ces caractères sont trouvé chez environ la moitié des femelles Makatia. La robe est généralement polychrome (prédominance marron, noir et blanc) dans les deux races. Les deux races présentent des différences significatives ($p<0,05$) pour la longueur de la tête, des cornes, des oreilles, du cou, des poils, la largeur de la tête, du bassin, le tour de canon antérieur, les écarts entre les pointes de l'épaule et la largeur aux ischions. Suite à cette caractérisation morphologique, une caractérisation génétique des deux races étudiées serait nécessaire pour déterminer leur degré de ressemblance et, de manière plus générale, permettrait d'objectiver les classifications raciales des caprins en Algérie et de diriger les efforts de conservation éventuels.

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

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A recent study showed that the tegument protein encoded by open reading frame 63 (ORF63) of the Kaposi's sarcoma Associated Herpesvirus (KSHV) could block inflammasome activation. However, because KSHV has no well-established *in vivo* infection model, the significance of this finding remains unknown. Related animal viruses such as Murid herpesvirus-4 (MuHV-4) allow us to tackle the same fundamental question in a more accessible form. Interestingly, the proteins encoded by MuHV-4 and KSHV ORF63 share potential structural folds suggesting that the both proteins could have similar functions. In this study, we showed that a lack of the ORF63 in MuHV-4 was associated with a severe viral growth deficit both *in vitro* and *in vivo*. The latter deficit was mainly associated with a defect during the viral lytic cycle 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 NLRP3 inflammasome did not restore the growth of the ORF63 deficient mutant. This growth deficit was also not associated with a defect in virion egress from the infected cells. In contrast, it appeared that the entry process of MuHV-4 virions was deeply affected by the absence of ORF63. Indeed, MuHV-4 ORF63 deficient mutants failed to address most of their capsids to the nucleus, suggesting that ORF63 plays a role in capsid movement along the microtubule network. Altogether, our results could help us to decipher further how gammaherpesviruses enter into some of their target cells.

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42. Bilateral pheochromocytoma treated by unilateral complete and contralateral partial adrenalectomy in a Shih Tzu

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A 12-year-old female Shih Tzu was presented for hypertension of unknown origin inducing ocular lesions. Abdominal ultrasonography showed a right adrenal mass compressing the caudal vena cava and an asymmetric left adrenal gland. Abdominal CT scan confirmed the presence of a 3 cm right adrenal mass associated with a thrombus in the corresponding phrenico-abdominal vein. A 1.5 cm nodule was also identified in the cranial pole of the left adrenal gland. CT scan also revealed the presence of a small splenic nodule but no pulmonary metastases were identified. The urinary metanephrine-to-creatinine ratio and urinary normetanephrine-to-creatinine ratio were significantly increased. A pheochromocytoma was therefore suspected. Hypertension was treated with prazosin and benazepril for 3 weeks before surgery. Complete right and partial left adrenalectomy with partial splenectomy were performed. Transfusion of packed red blood cells and plasma was required during surgery due to blood losses. Systolic blood pressure reached 330 mmHg intraoperatively during manipulation of the adrenal masses. Histopathological analysis revealed bilateral adrenal pheochromocytoma and splenic lymphoid nodular hyperplasia. Blood pressure and ionograms were closely monitored postoperatively and were within normal limits. Prednisolone was administered postoperatively and tapered gradually. Nine months after surgery, blood pressure, urinary catecholamine metabolites concentrations and locoregional and chest assessment of extension are still within normal limits. In conclusion, bilateral adrenalectomy (unilateral complete and contralateral partial) allowed complete removal of 2 pheochromocytomas in our patient. Using this surgical technique, it was possible to maintain a functional adrenal gland without recurrence at this point (nine months post-surgery).

43. Innovative method for the detection of bovine blood products in feedingstuffs

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The use of animal by-products in feedingstuffs depends on their nature defined by the cell type and the species of origin and on its destination. Currently, the detection of unauthorised ingredients is based on light microscopy and Polymerase Chain Reaction (PCR) methods. The light microscopy method identifies particles on the basis of typical and morphologically features such as muscle fibres, cartilages, bones, hairs, horns, bristles or feathers; while the PCR method is able to detect and identify the presence of specific animal DNA in feed. Nevertheless, some cases require additional analyses to determine the by-products nature. The aim of this study was the identification of specific peptide biomarkers using tandem mass spectrometry for the detection of bovine blood products in animal feed. Bone meals and blood products of porcine and bovine origin as well as milk products were analysed using a Q-TOF mass spectrometer. A vegetal feed adulterated with bovine plasma powder was also analysed to evaluate the applicability of the method. Eleven peptides sequences were identified as present in bovine blood products as well as in adulterated feed, but were not detected in the other meals. These preliminary results are promising. Efforts are now focused to enlarge the blood products and blood meal database and to evaluate the protocol on several matrices.

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44. Congenital venous aneurysm of the right external jugular vein in a Great Dane

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An eleven-month-old intact female Great Dane was presented for a painless swelling on the ventral right side of her neck. The mass appeared one month prior to presentation without history of trauma. The size of the mass was variable, increasing when the head was in a down position and disappearing when the head was up. Fine needle aspiration of the mass revealed the presence of blood. Ultrasonographic examination of the ventral cervical region revealed an anechoic subcutaneous tubular structure, limited by a wall and containing some valves, measuring 2 cm in diameter and 4 cm in length at the right ventral aspect of the cervical region, just cranial to the thoracic inlet. A diagnosis of jugular aneurysm was made. Surgery consisted in ligature of the right external jugular vein cranial and caudal to the aneurysm and removal of the aneurysm. Ligation of the external jugular vein is possible without any consequence in dogs due to the presence of an internal jugular vein, which will take over venous drainage. No intraoperative complication occurred. The dog developed a seroma postoperatively, which was successfully treated conservatively. Histopathologic examination of the abnormal vessel was consistent with the diagnosis of aneurysm. Jugular aneurysm has been rarely reported in dogs. Venous aneurysms may be the result of several processes including tumors, inflammation, trauma, or may appear spontaneously or congenitaly, when no etiologic cause can be identified. Surgical excision is the treatment of choice due to the risk of thrombosis, possible rupture, or for cosmetic reasons.

45. A retrospective serological survey on human babesiosis in Belgium

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Babesiosis is a tick-borne disease caused by protozoa of the genus *Babesia*. In humans, clinical symptoms that develop early or during a mild *Babesia* sp. infection can be confused with malaria, showing high fever, headaches and myalgia. In acute clinical infection, anaemia, icterus and haemoglobinuria are conspicuous and this condition can be fatal. In Europe, most clinical babesiosis cases have been attributed to *Babesia divergens* and *Babesia* sp. EU1. *Babesia microti* occurs mainly in the USA although a case of autochthonous *B. microti* infection and serological evidence of human infections in different European countries have been reported. Because of the recent first report of potentially zoonotic *Babesia* species in the *Ixodes ricinus* tick together with an older report of a human clinical case in Belgium, a retrospective serological survey was performed. IFAT was used to screen 200 sera from anonymous Belgian patients with history of tick bite and clinical symptoms compatible with a tick-borne disease. The serological screening detected positive reactivity in 9% (18), 38.5% (77) and 42% (84) of the samples against *B. microti, B. divergens or Babesia* sp. EU1, respectively. Presence of the three potentially zoonotic species of *Babesia* has been confirmed together with evidence of contact with human reported in Belgium. Preventive actions, such as improved awareness of physicians and development of better diagnostic tools should be helpful to prevent clinical cases and assess the true risk to public health.

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46. Effects of concentrates' levels on milk production and traffic of cows milked by an mobile automatic milking system on pasture

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The use of an automatic milking system (AMS) implies to stimulate cows 'traffic to the AMS. Several studies showed discrepancies about the effects on cows' traffic of concentrates distributed at milking, some of them finding no effect on milking frequency (Jago *et al.*, 2007, Bach, 2007) while in more recent studies (Lyons, 2013) returns to the robot seem to be improved by supplement distribution. The aim of this study was to analyse the influence of concentrates' distribution on milk yield and returns of grazing cows milked by an AMS in pasture. Cows milked by an automatic milking system in pastures were assigned in 2 groups receiving different amounts of concentrates (2.1 kg vs 4.1 kg). The effect of concentrates' level on milk yield (MY) and returns to the robot was assessed. Concentrates' level had a positive influence on daily milk production over the grazing period as cows of low concentrates group produced 21.43 ± 0.62 kg compared with 24.33 ± 0.62 kg in high concentrates group. However this effect was modulated subsequently to grass quality and availability. Regarding daily voluntary returns to the robot, high concentrates group showed higher frequency (3.66 ± 0.05, compared with 3.22 ± 0.04 in low concentrates group) demonstrating positive impact of complement distribution on cows' traffic.

47. Effects of mild heat stress periods on milk production, milking frequency and rumination time of grazing dairy cows milked by a mobile automatic system

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Cows milked by automatic milking systems (AMS) are most often confined indoors or have a limited access to pasture in summer. However, grazing allows decreasing feeding costs and improves animal health and welfare. During HS periods, it is known that feed intake can be reduced especially with temperature above 25 or 26°C (Rhoads *et al.*, 2013). Little information exists on effects of heat stress on grazing dairy cows milked by an AMS. The aim of this study was to determine the effects of HS periods on the milk yield, milking frequency, fat to protein ratio in milk (F/P) and rumination time (RT) of grazing dairy cows milked by an AMS located on pasture. Grazing dairy cows milked by an automatic system (AMS) experienced mild heat stress (HS) periods, twice during the summer 2012. The daily temperature humidity index (THI) during these periods were higher than 72. Milk production, as well as milking frequency, rumination time and milk fat to protein ratio (F/P) during these periods were compared to adjacent periods with mean THI of 61. The daily milking frequency, the total number of visits to AMS and the milk production were significantly higher in HS periods (2.12 vs 1.97, 2.99 vs 2.69, and 19.7 vs 18.5 kg milk per cow, respectively). There were significant interactions between times and periods for milking frequency and number of visits, while the daily rumination time was significantly lower (339 vs 419 min) and the F/P in milk tended to be decreased (1.17 vs 1.23). These results could be explained by changes in cow behaviour during HS periods.

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

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The Bo17 gene of Bovine herpesvirus 4 (BoHV-4) is the only virus gene known to date that encodes a homologue of the cellular core 2 β-1,6-N-acetylglucosaminyltransferase-mucine type (C2GnT-M). The nucleotide sequence of the Bo17 gene has 95% identity with the cellular sequence from which it was acquired. However, by opposition to what is observed for the cellular gene, we showed in this study that two different messenger RNAs are encoded by the Bo17 gene. The first one corresponds to the entire coding sequence of the Bo17 gene. Surprisingly, the second results from the splicing of a 138 pb intron. Analysis of different homologous sequences showed that, compared to cellular sequences, only Bo17 gene presents the consensus sites for this splicing and that these sites are conserved in all the BoHV-4 strains identified to date. This splicing does not change the reading frame of the protein and antibodies generated against Bo17 C-terminus showed that the two forms of Bo17 are expressed in BoHV-4 infected cells. By using an *in vitro* assay, we showed that the spliced form of Bo17 is not anymore active and could therefore regulate enzymatic activity. Finally, recombinant strains expressing only the long or the short form of pBo17 showed that BoHV-4 could use alternative splicing to modulate the cellular C2GnT-M activity. We postulate that the relative abundance of active/inactive forms of pBo17 in Golgi oligomeric complexes may define the level of enzymatic activity in the cell. This new regulatory mechanism could have implication in viral immune evasion but also more generally in cellular biology.

49. Preliminary results of IGF2-in7-G162C genotyping on four pig breeds in Vietnam

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The objective of this study is to identify IGF2-in7-G162C genotypes (GG, GC and CC) of 4 pig breeds, namely stress negative Piétrain (Pi), Duroc (Du), Landrace (L) and Large White (LW), the main breeds raised under industrial conditions in Northern Vietnam. A total of 87 tissue samples of 25 boars (21 Pi and 4 L) and 62 sows (11 Pi, 12 Du, 25 L and 14 LW) was collected from Experimental farm, Hanoi University of Agriculture (Hanoi) and Dong Hiep Hai Phong farm (Haiphong) from March to June 2014. Pi and Du pigs were from Hanoi, while L and LW came from Haiphong. IGF2 polymorphism was genotyped by PCR-RFLP method in the laboratory of Department of Animal Breeding and Genetics, Hanoi University of Agriculture. Overall, 96.55% (84/87) of the pigs carried either a genotype CC (60.92%) or GC (35.63%), while only 3.45% (3/87) of pigs were GG. Genotype GG was absent for all Piétrain and Large White pigs, while CC genotypes for Piétrain and Large White were 84.38% (27/32) and 64.29% (9/14) respectively. For Duroc and Landrace pigs, three genotypes (GG, GC and CC) were observed. The frequencies of GG were low for Duroc (8.33%) and Landrace (6.90%); on the contrary, GC and CC were 50% and 41.67% respectively for Duroc and 51.72% and 41.38% respectively for Landrace. These preliminary results suggest further research on the study of these genotypes on reproduction and production traits and on breeding strategies to exploit these potential effects.

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50. A gammaherpesvirus infection imprints the lung immunity and protects against HDM-induced asthma development.

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Allergic asthma is the result of complex interactions between genetic and environmental factors. While some respiratory viruses trigger asthma onset or exacerbation, the "hygiene hypothesis" proposes that the dramatic increase in the occurrence of allergic diseases in developed countries could be linked to a reduced exposure to infections during childhood. Moreover, some epidemiological studies have highlighted a correlation between the increasing occurrence of asthma and the increased age of seroconversion to the Epstein Barr virus. However, the potential protective role of herpesvirus infection against allergy development has never been addressed directly. In this study, we used the Murid herpesvirus 4 (MuHV-4) to study the impact of a persistent gammaherpesvirus infection on the development of House Dust Mites (HDM)-induced allergic asthma. Our results revealed that the MuHV-4 intranasal infection of mice affected both the sensitization and the challenging phases of HDM-induced airway allergy, resulting in a significant reduction of the pathogenesis. In particular, we highlighted that MuHV-4 infection strongly impacts the lung innate immune response. Indeed, while the dendritic cells remain competent to uptake antigens and to migrate to the draining lymph nodes, MuHV-4 infection impaired their ability to trigger HDM sensitization. Intriguingly, we observed that MuHV-4 infection also induces some phenotypic changes on alveolar macrophages. As these cells represent the most abundant leukocytes in the distal conducting airways, they could participate to the establishment of respiratory tolerogenic responses in the context of MuHV-4 infection.

51. Identification of molecular components of the host-microbiota-connectome by using "Omics approaches"

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The host immune system plays an critical role in maintaining homeostasis with resident microbial communities, therefore ensuring that the complex symbiotic relationship is maintained. At the same time, resident microbiota contribute to host nutrition and energy balance and to the development or maintenance of a robust immune system. Dysbiosis of the microbiota is associated with various immunological disorders, including inflammatory bowel diseases (IBD). Both genetic and environmental factors are implicated in this disturbance; however, the relative contributions of these two factors, and the mechanism by which they interact remain unclear. Recently, we started a project that aims to identify molecular components of the host-microbiota-connectome by taking advantage of common variation in – on the one hand – the genome, transcriptome and metabolome of the host, and – on the other hand – the composition of its gut microbiota. We will take advantage of the already established CEDAR cohort that provides integrated genetic (SNP genotypes) and transcriptome data (circulating immune cells subset, as well as samples from various anatomical locations in the intestine). We will further enrich the dataset in this cohort with metabolome (plasma), and gut microbiota data (16srRNA sampled at the ileum, colon, and rectum). We anticipate to reveal novel connections between the microbiota and IBD by this integrative "omics" approach, thereby shedding new light on the pathogenesis of IBD. Latest results will be presented with respect to the microbiota composition of from different anatomical locations in the intestine using the V2 and V5-6 regions of the bacterial 16S rRNA.

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52. Experimental infection of sheep with Schmallenberg virus at 45 and 60 days of pregnancy

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Main impact of Schmallenberg virus (SBV) on livestock consists in reproductive disorders, with teratogenic effects, abortions and stillbirths. Characteristics of placental crossing remain currently poorly understood. Therefore, we implemented an experimental infection of ewes, inoculated with SBV at 45 or 60 days of pregnancy. "Mourerous" breed ewes, about 1-year old, were separated in three groups: eight and nine ewes were subcutaneously inoculated with 1 ml of SBV infectious serum at 45 and 60 days of pregnancy, respectively (G45 and G60). Six other ewes were inoculated with sterile PBS and constituted a control group. All inoculated ewes showed RNAemia consistent with previously published studies, they seroconverted and no clinical signs were reported. Lambs were born at term via caesarian-section, and right after birth they were blood sampled and clinically examined. Then both lambs and ewes were euthanatized and necropsied. No lambs showed any malformation suggestive of SBV infection and none of them had RNAemia or anti-SBV antibodies prior to colostrum uptake. However viral RNA was found in umbilical cord and placenta of two different ewes in G45. In G60, viral RNA was found in the placenta of 4 ewes, the cotyledon of 3 ewes and the umbilical cord of 3 ewes, along with the prescapular lymph node and cartilage of one lamb and the brainstem of another one. Overall, no teratogenic effect could be reproduce; however the significant highest detection rate of viral RNA in G60 suggests a time dependency for successful tansplacental transmission and persistence of the virus until birth.

53. High throughput sequencing analysis reveals drift and selection pressure in different murine norovirus genomic regions during in vitro replication

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Murine norovirus (MuNoV), a single stranded positive sense RNA virus belonging to the *Caliciviridae* family, is considered as a representative model for human norovirus infections, one of the most important etiological cause of both epidemic and sporadic gastroenteritis cases worldwide. Four open reading frames are described in its genome: ORF1 encodes the non-structural (NS) proteins; ORF 2 encodes the single capsid protein (VP1); ORF3 encodes a minor structural protein; and ORF4, currently only found in viruses genetically related to MuNoV encodes a virulence factor. In this study, we demonstrated by high throughput sequencing that, during serial passages of MuNoV in cell culture, the nucleotide mutation rates, estimated by Bayesian inference, did not significantly differ across the five targeted genomic regions except one. These rates were similar in the four genomic regions encompassing partial non-structural 1-2 protein (NS1-2)-, NS5-, NS6-, NS7 (RdRp)- and VP1-coding sequences (also including the ORF4 region). In the partial minor structural protein-coding region, this mutation rate was however estimated to be at least one log unit higher when expressed as mutation/site/day. The precise localisation of the detected nucleotide point mutations (base change, deletion and insertion) was reported as well as the quantitative increase or decrease of the sequences harbouring them over ten cell culture passages. The non-synonymous mutations were also depicted in 3D models for four out of the five studied regions. These results have important implications for diagnosis, classification methodology and genetic evolution of noroviruses.

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54. The effect of oregano (*Origanum heracleoticum*) essential oil and carvacrol on virulence gene transcription by *Escherichia coli* O157:H7

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Most outbreaks and sporadic cases of hemorrhagic colitis (HC) and haemolytic uremic syndrome (HUS) have been attributed to enterohaemorrhagic *Escherichia coli* (EHEC), in particular strains belonging to serotype *E. coli* O157:H7. The bacterium was first recognized in 1982 as a food pathogen with an outbreak related to the consumption of undercooked ground beef. Despite modern technology, still an urgent need to develop more potential antimicrobial agents is required to control the pathogen and virulence toxins both in food and pharmaceutical industries. Therefore, the present study aimed to determine the effect of essential oil of oregano (*Origanum heracleoticum*) and carvacrol on the expression of virulence-associated genes of *E. coli* O157:H7 ATCC 35150 strain by reverse transcription quantitative PCR (RT-qPCR) analysis. At sub-inhibitory concentrations (0.05 µL/mL and 0.08 µL/mL), both oregano essential oil and carvacrol showed similar efficacy in inhibiting the expression of gene *ler* involved in attaching and effacing lesions and in decreasing Shiga toxin and *fliC* virulence gene expression. Moreover, a strong inhibition of gene *luxS*, involved in the quorum sensing, was observed. Those results were dose-dependent and show, for the first time, a specific effect of oregano essential oil and carvacrol in downregulating virulence gene expression of *E. coli* O157:H7. Oregano oil and carvacrol are suggested to have the potential to mitigate the health adverse effects caused by *E. coli* O157:H7 and their use as natural antibacterial in foods or as alternative to antibiotics should be further investigated.

55. Local goat in Kabylie (Chemini and Bouzeguene)

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The livestock sector plays a dynamic role in the economic development of rural areas in Algeria. Due to its adaptation to harsh environments, goat breeding is practiced in many areas of the country. To the north, it is confined to mountainous areas, but the bulk of the workforce is left in the steppe and semi-desert areas. The goat population in Algeria reached 3.8 million heads, ranking second after sheep. The present work contributes to a better characterisation of local goat in Kabylie for production and reproduction traits. The study was conducted in the regions of Chemini (Bejaia province) and Bouzeguene (Tizi-Ouzou province). A survey was conducted in 29 herds. The results revealed that the overall mean of goats per households was 6.69 ± 2.97. The purposes of keeping goat in Chemini and Bouzguene was private consumption of milk (82.76%) and meat (68.97%) as well as for commercial exchange (62.07%). The Kabyle goat is small (male: 62.23 cm and female: 58.41 cm) with long hair. Its ears are drooping, its convex profile has a slightly pronounced nasal split and its coat colour ranges from dark brown to black. However, the crossbreeding with exotic breeds (mainly with Saanen goat), controlled or uncontrolled, increased the frequency of white coat. The average age at puberty in males was 6.93 ± 1.04 and 7.38 ± 0.94 months in females. The reported age at first kidding and kidding interval were 13.03 ± 0.87 months and 7.83 ± 1.29 months, respectively.

Keywords: Algeria, Goat, Production, Reproduction, Socio-economic, Biodiversity

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56. The effect of the inclusion of *Urtica dioica* leaves meal in diets on growth performances and carcass characteristics of broiler chicken

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The objective of this study was to evaluate the effects of the inclusion of *Urtica dioica* leaves meal in diets on growth performances and carcass characteristics of broiler chicken. The study was conducted from May to June 2014 in the area of Chemini (Algeria). In a completely randomized design, a total of 100 one-day-old broilers (industrial strain) were divided into 2 groups and 2 repetitions with 25 chickens each. Group 1 was the control group fed a standard commercial diet based on corn and soybean purchased on the local market, whereas the birds in group 2 were fed the nettle diet (control diet with addition of 2% of nettle). The animals were housed inside in experimental pens and fed *ad libitum*. The results showed that nettle inclusion significantly promotes growth performance of broilers at 42 days of age (1644.8 ± 45.5 vs. 1565.1 ± 45.5g; $P<0.05$). The broilers fed the nettle diet had higher thigh yield (26.0 ± 0.3 vs. 25.2 ± 0.3%; $P<0.05$) but lower abdominal fat yield (4.39 ± 0.16 vs. 3.90 ± 0.16%; $P<0.05$). No significant effect of the diet was observed for carcass yield (around 71.1%) and breast percentage (around 32.40%). The overall mortality was not significantly different ($P>0.05$) for any of the dietary regimens (around 20%). Feed conversion ratio of the two groups were very close (around 2.17). This experiment showed that dietary inclusion of *Urtica dioica* has positive effects on growth performance and carcass quality of broilers.

Keywords: Broilers, carcass, commercial strain, Urtica dioica

57. Mechanoreceptors in the anterior horn of the equine medial meniscus: an immunohistochemical approach

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Mechanoreceptors are "encapsulated sensory end-organs" involved in proprioceptive function. Given the high incidence of meniscal injuries in horses, the clinical interest in these mechanoreceptors, particularly in the meniscus, and the lack of information concerning them in equine menisci, our objective was to study these corpuscles in the anterior horn of the equine medial meniscus, which is the most common localization reported for equine meniscal injuries. An immunohistochemical approach to detect Schwann cells and nerve fibres allowed us to localize and identify these corpuscles within the meniscus. Three types of mechanoreceptors were identified and localized between the abaxial quarter and the abaxial third of the meniscus: the Ruffini, Pacini and Golgi corpuscles. In conclusion, from a purely fundamental point of view, our work highlights for the first time the presence of MCR at the level of the anterior horn of the equine medial meniscus and proposes a classification based on specific immunocytochemical techniques. This morphological approach could serve as a basis for clinical studies, in order to evaluate the impact of these corpuscles on the poor sportive prognosis in equine meniscal tears.

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58. Nutrient digestibility in broiler chickens fed locally solvent-treated *Jatropha curcas* kernel, in Senegal

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Jatropha curcas is a drought-resistant shrub belonging to the *Euphorbiaceae* family. The kernel contains approximately 60% lipid in dry matter and meal obtained after oil extraction could be an exceptional source of protein for family poultry farming, in the absence of curcin – a lectin inhibiting protein synthesis – and, especially, some diterpene derivatives phorbol esters that are partially lipophilic. Nutrient digestibility of *J. curcas* kernel meal (JKM), obtained after partial physicochemical de-oiling was thus evaluated in broilers. Twenty broilers, 6 weeks old, were maintained in individual metabolic cages and divided into 4 groups of 5 animals, according to a 4x4 latin square design where de-oiled JKM was incorporated into grinded corn at 0, 4, 8 and 12% levels (diets 0J, 4J, 8J and 12J), allowing measurement of nutrient digestibility by the differential method. Dry and organic matter digestibility of the diets was affected to a low extent by JKM (85 and 86% in 0J up to 81 in 12J, respectively) in such a way that DM and OM digestibility of JKM was estimated to be close to 50%. Ether extract digestibility of JKM was quite variable but, on average, close to 60%. Crude Protein and CF digestibility of the diet was negatively impacted by jatropha, consequently with negative specific values for JKM.

59. Comparison of bacteriological, immunological and genetic identification approaches of mammo-pathogenic bacteria and typing of *Staphylococcus*

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The purpose of our study is to compare results obtained with genetic tests or immunological detection and classical bacteriological analysis, as regards the mammo-pathogenic bacteria. Then, the comparison of biochemical identification (biotyping) and genetic (PCR) methods of these bacteria, in parallel with the identification of antibiotic resistance (sensitivity). Finally, the demonstration of the association between the type of mastitis and intracellular survival of *Staphylococci* and coliforms will be established. This first phase of our study is therefore to carry out by conventional bacteriological culturing on selective media of milk samples from cows suffering from mastitis, in order to compare the results with those of genetic tests and immunological detections. Milk samples were collected between 30 January 2014 and 25 March 2014. A total of 120 samples were taken from seven different farms at the rate of one sample per quarter achieving mastitis (CMT> 300 000 SC after 3 controls + recurrence). Preliminary bacteriological results of our study show that samples are mostly infected with *Streptococci* / *Enterococci*. Our results also show that *Streptococci* / *Enterococci* and *Staphylococci* are the two main bacterial types present in the farms. The methods used do not detect *Mycoplasma*. From the foregoing, it can be said that most cows are suffering from contagious mastitis except those of only 2 farms where there would also be some cases of environmental mastitis. Particularly heavily infected cows will be investigated for the comparison of methods for detecting their germs.

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60. Surgical management of ectopic ureters: clinical outcome and prognostic factors for long-term continence.

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The objectives of this study were to evaluate clinical outcome after surgical correction of ectopic ureters (EU) and to determine prognostic factors for long-term continence. Medical records of 42 animals (38 dogs, 4 cats) that underwent surgical correction of EU were reviewed and long-term follow-up was obtained. Thirty-three females and 9 males were included. Twenty-nine animals were neutered before or during surgical correction. Colposuspension was performed at the time of surgical correction of EU in 11 dogs. A continence score, defined as : 1= incontinent, 2= continent with sporadic episodes of incontinence, 3= continent with additional treatment, 4= continent, was assessed at 4 time points: preoperatively, postoperatively at discharge, 1 month postoperatively, and at long-term follow-up (mean : 61 months ; range : 1-148). The continence score (mean±SD) was significantly lower at long-term follow-up (2.8±1.2), compared to the other postoperative time points (3.2±1.4 and 3.5±1 respectively). Incontinence recurred in 26% of animals after a mean period of 125 days (range : 15-365 days). At long-term follow-up, a score of 4 was observed in 43% of animals, a score of 3 in 22%, a score of 2 in 8%, and a score of 1 in 27%. Colposuspension during surgical correction of EU was predictive for long-term continence, while species, gender, uni- versus bilateral EU, extra- versus intramural EU, pelvic bladder and neutering were not predictive factors for postoperative incontinence. In this conclusion, recurrence of incontinence was observed at long-term follow-up. However, good to excellent long-term outcome could be achieved in 73% of animals.

61. Fitness evolution of a recombinant murine norovirus during serial passages in cell culture

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Human norovirus (NoV) infections are among the most important causes of gastroenteritis in both children and adults and often occur as outbreaks which may be foodborne. Recombination can dramatically change virulence properties of the viruses and has been often evidenced in silico for different NoV strains. Recently, after *in vitro* coinfection of RAW264.7 cells with parental murine norovirus (MuNoV) strains CW1 and Wu20, we obtained a recombinant Wu20/CW1 strain (Mathijs *et al.*, 2010). This recombinant strain showed reduced plaque size compared to the parental strains. The aim of the study was to observe and molecularly characterize the natural genetic evolution of the recombinant MuNoV strain across *in vitro* replications. The recombinant strain was serially replicated *in vitro* (up to 14 passages). Viral plaque diameters of early and late progenies were compared with the Image software. A significant difference was shown between them with the Mann and Whitney non parametric statistical test. The average size of plaques increased from the earlier to the later progenies (from 0.1 mm^2 to around 0.5 mm^2). Molecular investigations are currently performed in order to specify in which genetic region mutations occur and whether or not this could explain fitness modifications during *in vitro* evolution. In addition two other parameters of *in vitro* virulence modification will be investigated (i) virus production and (ii) one step growth kinetics. The data should provide interesting information about genetic evolution in the genus *Norovirus*, especially regarding recombination events and explain how a recombinant strain, first disadvantaged compared to its parental strains, could regain fitness by genetic evolution.

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62. Skin mucosal response of carp (*Cyprinus carpio*) against CyHV-3 infection and vaccination

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Fish skin serves as a first line of defense against a wide range of pathogens present in the aquatic environment. Secretion of mucus is the most prominent characteristics of fish skin. This mucus acts as a physical, chemical, and biological barrier that plays a critical role in protecting fish against infections. In contrast to mammals, little is still known about the components and mechanisms of skin mucosal immunity induced by natural infection or vaccination. The present study aims to investigate the role of skin mucus in the protection of carp against cyprinid herpesvirus 3 (CyHV-3), a pathogen that enters mainly through the skin and cause a lethal disease in koi and common carp. Preliminary results revealed a significant anti-CyHV-3 neutralizing activity in clarified mucus extracts (CME) of vaccinated carp that survived subsequent CyHV-3 challenge, suggesting induction of a skin mucosal adaptative immune response. To investigate this further, a time course study will be conducted for the neutralizing activity of carp CME following either CyHV-3 wild type infection or immunization with an attenuated CyHV-3 vaccine. These infected or vaccinated fish will then be challenged and the resulting protective immunity will be studied by monitoring the replication and spread of CyHV-3 at key time points via *in vivo* bioluminescence imaging and viral load quantification. Correlation between observed protection and anti-CyHV-3 activity in CME and/or fish sera will be determined. In addition, the specific carp immunoglobulin isotype(s) associated with skin mucosal response against CyHV-3 will be identified.

63. Effects of dietary fibre and floor type on greenhouse gas and ammonia emissions associated with gestating sows

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Gestating sows are usually restrictedly-fed to prevent excessive body weight gain and fat deposition that may impair reproductive performance. However, feed restriction may result in stereotypic behaviours and deleterious effect of animal welfare. High fibre diets (HFD) are known to reduce feeding motivation without deterioration of performance, but the effects of HFD on pollutant emissions are few studied. Therefore, a study was carried out to investigate the effect of dietary fibre content (23% of non-starch polysaccharides (NSP) with a standard diet based on cereals vs. 44% of NSP with HFD based on sugar beet pulp) and the floor type (slatted floor vs. straw-based deep litter) on emissions of ammonia (E-NH_3), nitrous oxide (E-N_2O), methane (E-CH_4) and CO_2-equivalents (E-Eq_{CO2}). Six successive batches of 10 gestating sows were divided into 2 groups kept in 2 experimental rooms differing by the floor type. The standard diet was administered to the sows of the first 3 batches, the fibrous diet to the sows of the next 3 batches. Emissions were measured by infra-red photoacoustic detection. With the slatted floor, HFD decreased E-NH_3 (12.0 vs. 15.5 g $sow^{-1}.d^{-1}$) but increased E-Eq_{CO2} (0.69 vs. 0.57 kg $sow^{-1}.d^{-1}$) in relation to the increase of E-CH_4 (18.4 vs. 12.8 g $sow^{-1}.d^{-1}$) while E-N_2O were not impacted by the diet (around 0.62 g $sow^{-1}.d^{-1}$). With the straw-bedded floor, HFD increased E-NH_3 (12.3 vs. 9.2 g $sow^{-1}.d^{-1}$) and E-CH4 (14.6 vs. 9.6 g $sow^{-1}.d^{-1}$) but decreased E-N2O (0.99 vs. 1.64 g $sow^{-1}.d^{-1}$) with consequently similar E-Eq_{CO2} for the two diets, around 0.74 kg $sow^{-1}.d^{-1}$.

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64. Generation and validation of a mouse model for conditional inactivation of Plagl1

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PLAGL1 (PLeomorphic Adenoma Gene-Like 1), also known as *ZAC* (Zinc finger protein that regulates Apoptosis and Cell cycle arrest), is a zinc finger transcription factor that has been shown to be implicated in diverse situations such as apoptosis induction, cell cycle arrest, tumor suppression or transient neonatal diabetes mellitus. Notably, *PLAGL1* undergoes genomic imprinting, the maternal allele being silenced. Constitutive inactivation of Plagl1 in the mouse induces mortality in 70% of the pups during the 3 first days after birth, presumably due to respiratory defects. The 30% surviving pups are smaller than their wild type littermates and some of them show bones and cartilage formation defects. While a role of Plagl1 in mouse embryonic development is clearly identified, little is known about its potential roles in adult stage. For instance, Plagl1 is strongly expressed in both mouse and human pituitary gland and it has been shown that this expression is lost in human Non Functioning Pituitary Adenomas (NFPAs), suggesting potential roles of Plagl1 in pituitary development and/or function, or as a tumor suppressor gene. In order to explore roles of Plagl1 in the adult mouse and/or in particular tissues, we decided to generate a conditional knock-out (cKO) mouse model using homologous recombination in embryonic stem cells. To validate the cKO model, we crossed it to an ubiquitously Cre-expressing mouse to generate a constitutively inactivated Plagl1 allele. Our phenotypic data are consistent with previously published data for constitutive inactivation of Plagl1, thus validating our Plagl1 cKO model for future use.

65. Glucose use and lactate production by equine fresh semen in human and equine extenders

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Aim of the study was to assay equine sperm metabolism in media containing different glucose and lactate concentrations. Five stallions were collected four times. Each ejaculate was divided in 4 samples. After centrifugation, pellets were diluted to 40 or $100x10^6$spz/ml in extenders (INRA96™ and Allgradwash™), with 25% of seminal plasma. Concentration and motilities were assayed in raw semen and after 1, 8 and 24 hours. Preservation of motility was calculated by dividing post-storage motility by raw semen motility. After 24h of storage, lactate and glucose concentrations in samples supernatant were assayed by Nuclear Magnetic Resonance. Differences were analyzed by *Kruskal-Wallis test*. INRA96 contained more glucose than Allgradwash; the opposite relation was observed for lactate. Preservation of TM was lower in Allgradwash after 24h at $40x10^6$spz/ml ($p<0.01$) and 8h at $100x10^6$spz/ml ($p<0.05$). Preservation of PM was lower in Allgradwash after 1h at both storage concentrations ($p<0.05$). Glucose and lactate concentrations differed between media after 24h of storage ($p<0.01$). Glucose and lactate concentrations after 24h in the same medium didn't differ between sperm storage concentrations. Median glucose concentration observed in INRA96 after 24h of storage (25.57mmol) was higher than in native medium (17.86mmol). This study shows that this human semen extender doesn't support equine semen preservation. Sperm cells' glucose consumption and lactate production seem to be negligible, as these parameters were not affected by sperm concentrations in our study. Our results suggest that spermatozoa are able to cleave complex carbohydrates as glucose concentration in INRA96 increased over time.

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66. Determination of cerebrospinal fluid albumin in healthy dogs

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Background: Measurement of CSF albumin aids diagnosis in human medicine but technical difficulties related to its low CSF concentration prohibit its routine use in veterinary medicine. High-resolution electrophoresis (HRE) has been described but often results in non-interpretable integration profiles preventing albumin determination. Fraction quantification using HRE may be more precise after concentration (cHRE) using a membrane microconcentrator technique but has not been evaluated in CSF with total protein levels below 20mg/dL. Immunoturbidimetry is routinely used for human CSF albumin measurement and was recently applied on canine samples with encouraging results. **Objective:** The purpose of this study was to compare HRE (including the use of a concentration step) and immunoturbidimetric assay for the measurement of albumin levels in normal canine CSF. **Methods:** 30 CSF specimens from 15 healthy dogs were evaluated. CSF total protein was measured by the pyrogallol red methoda and CSF albumin was determined by HREb (n=15), cHREc (n=30) and immunoturbidimetric assayd (n=30). Validation of the human immunoturbidimetric assay was performed using a purified canine albumin standarde. **Results:** Mean CSF total protein was 17.5 (range 7-39) mg/dL. HRE integration profiles were non-interpretable in all unconcentrated specimens. However, clear distinction of the major protein fractions was achieved for all cHRE specimens. CSF albumin levels were measureable in 29/30 specimens using immunoturbidimetry. Excellent correlation (Pearson $r=0.92$, $p<0.001$) was found between the two techniques. **Conclusion:** Immunoturbidimetry and cHRE may be used for routine measurement of CSF albumin.

67. Diversity of *Clostridium difficile* isolates from humans and animals

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*Clostridium difficil*e is an important cause of infectious diarrhea in hospitals. The major risk factors for the development of nosocomial *C. difficile* associated disease include antibiotic therapy and increasing age. In animals, as pigs, calves and horses, *C. difficile* also seems to be an important cause of enteric disease. The main objective of this study was to characterize and compare animal and human *C. difficile* strains with respect to the PCR-ribotype and the antibiotic resistance. Multilocus sequence typing (MLST) and multiple-Locus Variable number tandem repeat analysis (MLVA) were performed in order to study clonal relationships of the isolates. Human *C. difficile* isolates were obtained from care home residents and hospitalized patients. Animal isolates were collected from stool samples and carcasses of pigs and cattle at slaughter. An identification of the strains was performed by PCR-ribotyping. Further characterization was performed by antibiotic resistance, MLST and MLVA analysis. A neighbourd-joining phylogenetic three was constructed in order to determine the correlation between human and food isolates. A great variety of PCR ribotypes was found among the animal isolates, including PCR ribotypes 078 and 014. The most prevalent PCR-ribotypes in the nursing home were PCR-ribotypes 027 and 020. A high resistance to moxifloxacin, erythromycin, gentamicin and clindamycin was detected for some of the strains. Phylogenetic analysis showed that human and animal isolates with the same PCR-ribotype cluster in the same lineage, suggesting a potential risk of interspecies transmission.

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68. Right pulmonary vein to pulmonary artery ratio: a new echocardiographic index of pulmonary hypertension in West Highland white terriers with idiopathic pulmonary fibrosis

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Canine idiopathic pulmonary fibrosis (CIPF) is a progressive interstitial lung disease mainly affecting West Highland white terriers (WHWT). Pulmonary hypertension (PH) may develop secondary to CIPF and may be difficult to diagnose non-invasively. A new echocardiographic index, the right pulmonary vein to pulmonary artery ratio (PV/PA) has been described for the detection of pulmonary venous hypertension. The aim of this study was to investigate PV/PA in CIPF in order to determine its utility in the detection of PH. This study included 10 WHWT with CIPF (Group A), 9 healthy WHWT (Group B) and 25 healthy dogs from other breeds (Group C). Echocardiographic parameters measured were: PV/PA in both bi-dimensional (BD) and M-modes (MM), speed of tricuspid regurgitation (TR), acceleration time to ejection time ratio of the pulmonary flow (AT:ET) and pulmonary artery to aorta ratio (PA/Ao). In BD and MM mode, PV/PA was lower in group A (MM: 0.62±0.25, BD: 0.51±0.20) compared to group B (MM: 0.98±0.17, BD: 0.93±0.12, P≤0.01) and C (MM: 1.03±0.13, BD: 1.00±0.10, *P*≤0.0001). TR was found in 60% of dogs with CIPF; mean pressure gradient was 34.03±16.90 mmHg. AT:ET was lower in group A (0.42±0.08) compared to group C (0.50±0.04, *P*=0.002) and tended to be lower compared to group B (0.48±0.07, *P*=0.09). PA/Ao was not statistically different between groups. PV/PA was correlated with AT:ET and the speed of TR, but not with PA/Ao. In conclusion, in WHWT affected by CIPF, PV/PA could be a useful indicator of PH.

69. Vascular endothelial growth factor: a blood biomarker in canine idiopathic pulmonary fibrosis?

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Canine idiopathic pulmonary fibrosis (CIPF) is a progressive interstitial lung disease that mainly occurs in the West Highland white terrier (WHWT) breed. CIPF diagnosis is challenging. Identification of measurable markers of fibrosis might be helpful in this circumstance. VEGF is an angiogenic regulator involved in a variety of physiological and pathological processes. The aims of the present study were (1) to investigate the potential role of VEGF as a peripheral blood biomarker in CIPF; and (2) to investigate possible breed-related differences in basal VEGF concentration, that might explain the high predisposition of the WHWT breed for CIPF. Therefore, VEGF was determined by ELISA in the serum of 14 WHWT with CIPF, 18 healthy WHWT, and 85 healthy dogs of other breeds, including : 14 Scottish terrier (ST), 16 Jack Russell terrier (JRT), 15 Maltese, 14 King Charles Spaniel (KCS), 12 Labrador Retriever (LR) and 14 Malinois Belgian Shepherd. Eight CIPF WHWT (57%) have serum VEGF concentrations above the kit detection limit (39.1 pg/ml) compared to 1 WHWT (0.05%) in the group of healthy dogs (P=0.001). Concerning inter-breed differences in healthy dogs, most values obtained were below the kit detection limit with only 3 KCS (21%), 3 JRT (19%), 3 LR (25%) and 1 ST (7%) having VEGF serum levels above 39.1 pg/ml (P=0.147). Results of the present study show that (1) VEGF might be an interesting blood biomarker for CIPF; (2) canine VEGF Quantikine Elisa kit is not appropriate for measurement of serum VEGF levels in healthy canine populations.

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70. The soluble TNFR 2 homologue encoded by cyprinid herpesvirus 3 is essential neither for viral replication *in vitro* nor for virulence *in vivo*

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Cyprinid herpesvirus 3 (CyHV-3) is the causative agent of a lethal disease in common and koi carp. The present study was devoted to ORF12 encoding a putative soluble tumor necrosis factor receptor 2 (TNFR 2) homologue. Proteomic analyses of the supernatant of Cyprinus carpio brain cells (CCB) infected by CyHV-3 demonstrated that ORF12 expression product is the most abundant viral protein of CyHV-3 secretome. To investigate the role of ORF12 in viral replication *in vitro* and in virulence *in vivo*, an ORF12 deleted strain and an ORF12 revertant strain were produced using BAC cloning technologies. The recombinant ORF12 deleted strain replicated *in vitro* comparably to the parental and the revertant strains. Infection of fish by immersion in water containing the virus induced comparable CyHV-3 disease for the three virus genotypes tested (wild type, deleted and revertant). Quantification of viral DNA by real time TaqMan PCR (in the gills) and analysis of carp cytokine expression (in the spleen) by RT-qPCR at different times post-infection did not revealed any significant difference between the groups of fish infected with the three virus genotypes. Similarly, histological examination of the gills of infected fish revealed no significant differences between fish infected with ORF12 deleted virus versus fish infected with the control parental or revertant strains. All together, the results of the present study demonstrate that the TNFR 2 homologue encoded by CyHV-3 is essential neither for viral replication *in vitro* nor for virulence in common carp.

71. Hepatocholecystitis due to *Salmonella Dublin* in a crossbred calf

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We herein report a case of acute hepatocholecystitis in a calf due to *S. Dublin*. A two-month-old, female crossbred Belgian Blue calf was presented at the Clinic for Ruminants for anorexia for two days. The initial clinical examination revealed weariness, dehydration and a mucosal jaundice. A mild fluid splashing sound was audible on succussion of both sides, but the paracentesis was negative. The urine was dark-orange and the test strips were highly positive for blood and lightly positive for bilirubin. The haematology revealed a marked neutrophilia and monocytosis. Blood biochemistry revealed a slight hyponatremia, an important bilirubinemia, with an increase of bile salts and GGT. The transabdominal ultrasonography revealed a hyperechoic liver with a hypoechoic distended gallbladder and anechoic liquid between the two structures. Symptoms and results of analysis drew us to a suspicion of cholestasis with a severe inflammation. After the hydration status was restored, and after administration of antibiotics, NSAIDs and hepatoprotectors, a ventral midline exploratory laparotomy was performed, confirming our clinical suspicion. The calf died after 24 hours. The necropsy revealed generalized jaundice, and a distended gallbladder without obstruction. Unexpectedly, abomasal content was observed in the rumen. The histopathology identified a moderate necrotizing cholecystitis, a severe acute multifocal necrotizing hepatitis with a diffuse lymphoplasmacytic cholangitis, acute necrotizing lymphadenitis of hepatic lymph nodes and a mild lymphocytic interstitial nephritis, suggesting a Salmonellosis. At the bacteriological examination of the bile, *Salmonella enteriditis serovar Dublin* was isolated. *A posteriori*, breeder noticed that abortion due to salmonellosis occurred and that water contamination was suspected as well.

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72. Clinical sentinel surveillance of equine West Nile fever, Spain

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West Nile fever (WNF) is a viral zoonotic infection caused by a mosquito-borne flavivirus of the *Flaviviridae* family. According to a comparative study, the passive surveillance of horses by equine veterinarians appeared to be the most cost-effective system in the European context of WNF. Clinical data issued from a passive epidemiosurveillance network from September 2010 to December 2011 on horses in Spain were statistically compared and used to develop a predictive diagnostic decision tree, both with the aim to improve the early clinical detection of WNF in horses. Although clinical signs were variable in horses affected by WNF, four clinical signs and the month of occurrence were identified as useful indicators to distinguish between WNF related and unrelated cases. The signs that pointed out a presumptive diagnosis of WNF in horses were cranial nerves deficits, limb paralysis, photophobia and nasal discharge. Clinical examination of horses with neurological signs that are not vaccinated against WNV could provide important clues for the early clinical detection of WNF, and therefore, serve as an alert for possible human viral infections. The study of the clinical pattern of WNF in horses is of importance to enhance awareness and better understanding, and to optimize surveillance designs for clinical detection of WNF in horses in advance of epidemic activity affecting humans.

73. Effects of interaction between the energy content of the diet and parity on performance of local rabbit does

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Rabbit does are frequently lactating and gestating at the same time. These two functions are very costly in terms of energy and feed intake becomes the limiting factor in order to sustain production. In this context, sixty one (61) nulliparous local rabbit does were used in Algeria to study the effect of energy content in diet and its interaction with parity on milking and litter performances at the first and second lactations. Does received one of three isoproteic experimental diets (15% CP) with different digestible energy content, i.e., (2300, 2450 and 2600 kcal/kg for T, A and B diets respectively. Weight of does and their litters were controlled at parturition and each week post partum until weaning, done at 28 days old. Diets were supplied on an *ad libitum* basis. Rabbit does were submitted to mating at 10 days *post partum* for the second parturition. Experimental diets did not show effects on does live weight, live weight gain and milk yield, but diet with higher energy content decrease significantly ($p<0.0001$) feed intake at lactation and daily feed intake. There was no significant difference between diets for size and litter weight but interaction between diet and parity was significant ($p<0.05$). Mortality at partum and from birth to weaning did not differed significantly. There was significant differences between treatments on the main parameters of reproduction ($p<0.05$). In conclusion, an increase in dietary digestible energy allows to improve some performance of lactating local rabbit does in Algeria.

Keywords: Rabbit doe, diet, energy, reproductive performance.

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74. Effects of interaction between the energy content of the diet and parity on performance of local rabbit does

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The rabbit is known for its high production capacity according to his short reproduction cycle, his high prolificacy and his very easy manipulation. Reproductive function of this species is particularly sensitive to the nutritional status of the female because it can be pregnant and lactating simultaneously with defect in energy balance. In this context, the influence of diet protein content on reproductive performance, milk production and litters growth was studied in forty six (46) local rabbit does during the second lactation. Does were divided into three groups offered one of three isoenergetic diets (2600 kcal DE/kg) and different digestible protein (DP) content (15, 17 and 19 % DP for diet T, A and B respectively). Diets were supplied on an *ad libitum* basis. There was no diet effect on does weight at *partum* and between partum and weaning, as well as on weight gain during the second lactation. Does given B diet showed significantly higher daily protein intake (58.1g for group B vs 52.9g for group A and 44.3g for group T, $p<0.01$), but no difference was detected for the daily digestible energy intake and daily feed intake between partum and weaning (294.5g for group T vs 311.1g for group A vs 305.8g for group B). The litters size and weight at partum and at weaning were not affected by the diets but there was an effect of litter size on milk production $p<0.001$). There was no significant difference on milk production between the three diets. In conclusion, in Algeria, the local rabbit does is adapted to a digestible protein content of 15% to meet the requirements for reproduction and production.

Keywords: Rabbit doe, local population, diet, digestible protein, reproductive performance.

75. Characterization of collagen fibrils after equine suspensory ligament injury: an ultrastructural and biochemical approach

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Suspensory ligament (SL) injuries are an important cause of lameness in horses. The mechanical properties of connective tissue in normal and pathological ligaments are mainly related to the fibril morphology, as well to the collagen content and types. The purpose of this study was to evaluate, using biochemical and ultrastructural approaches, the alterations in collagen fibrils after injury. Eight Warmblood horses with visible signs of injury in only one forelimb SL were selected and specimens were examined by transmission electron microscope (TEM). Collagen types I, III and V were purified by differential salt precipitation after collagen extraction with acetic acid containing pepsin. TEM revealed striking disorganization as well as alterations in the diameter and shape of fibrils after SL injury. The bands corresponding to types I, III and V collagen were assessed by densitometry after sodium dodecyl sulphate polyacrylamide gel electrophoresis (SDS-PAGE). Densitometric analysis indicated that the proportions of type III and type V collagen were significantly higher ($P < 0.001$) in damaged tissues compared to normal tissues with a mean increase of 20.9 and 17.3% respectively. Concurrently, a significant decrease ($P < 0.001$) in type I collagen within damaged tissues was recorded with a mean decrease of 15.2%. These alterations could be the molecular hallmark of a decrease in the tissue quality and mechanical properties of the ligament. This provides new insight for subsequent research on tissue regeneration that may lead to the development of future treatment strategies for SL injury.

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76. In vitro model to study the endocrine disrupting activity of migration products from plastic food contact materials

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During the last decades, the migration of Bisphenol A (BPA), used for the production of polycarbonate (PC), was documented to be a well- known source of food contamination. Some studies shoedn that BPA, an endocrine disrupting compound, may be associated to several health problems and diseases. Recently, the European Union banned the import and sale of PC baby bottles to reduce BPA exposure of infants. PC alternatives have massively appeared on Belgium market and there is very few information about consequences on human health of the chemicals possibly migranting from these PC alternatives. In a recent opinion (No. 8697, 11.03.2010), the Belgium Superior Health Council's asked to investigate the possible risks associated with the use of these PC alternatives. To know if they are safe to use, the activity of chemicals migrating from PC alternatives, identified by Simoneau & al, 20121, was evaluated on estrogen, androgen, progesterone and glucocorticoid receptor using a reporter gene assay. The activity of 38 pure compounds was measured. After the first screening, 4 substances clearly showed an activity on each receptor, other reacted on three, two or one receptor. Only 10 substances did not show any activity. For active substances only, the study will be continued and the dose response relationship will be characterized.

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77. 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 important animal disease which occurs in worldwide. The causative agent is a virus, member of the genus Aphthovirus belonging to the Family *Picornaviridae*. It exists seven serotypes, A, O, C, Asia-1, Southern African Territories (SAT)-1, SAT-2 and SAT-3 and there is no cross protection between them. FMD is endemic in Niger and cattle are mainly being involved. Reviews of submissions to WRL 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 FMDV 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 outbreak reported do not fully take into account neither the size of the population at risk nor the spatial distribution. Therefore, it is clear that, this approach does not assess the risk associated with the onset of disease. Therefore, there is need to analyze the relative risk of FMD and its spatial and temporal distribution.

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78. Concurrent hemangiosarcoma and squamous cell carcinoma in the cornea of a cat

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A 14-year-old spayed, domestic shorthair cat was presented for evaluation of a red mass occupying most of the left cornea. The owner had noticed a white coloration of the left eye since at least 5 years followed by reddening of the cornea since 1-2 years. The cat had a history of chronic respiratory disease and bilateral serous ocular discharge. Physical examination was within normal limits apart for a mild inspiratory stridor. Ophthalmic examination of the left eye revealed entropion of both eyelids and marked symblepharon. An intense red colored mass occupying about ¾ of the corneal surface and peripheral vascularization was noticed. Under general anesthesia two corneal biopsies were taken. Histopathologic examination revealed neoplastic cells with prominent and irregular nucleoli and anisokaryosis with keratin pearls and rare mitotic figures. Corneal squamous cell carcinoma was diagnosed. A transpalpebral enucleation of the left eye was performed subsequently. On histopathology the corneal stroma showed a proliferation of variably sized, anastomosing, vascular-like, blood-filled channels lined by a single layer of spindle cells. The central cornea showed markedly hyperplastic epithelium with moderate dysplasia of the epithelial layers at the periphery. Infiltration of dysplastic corneal epithelial cells into the superficial stroma was noticed. Based on these findings, a diagnosis of corneal hemangiosarcoma and incipient squamous cell carcinoma was made. A chronic, ulcerative, hyperplastic, and lymphoplasmacytic keratitis was diagnosed as well. To the best of our knowledge this is the first case of primary corneal hemangiosarcoma associated with a corneal squamous cell carcinoma described in a cat.

79. Unusual presentation of a mesenchymal eyelid hamartoma and an update on the incidence of periocular hamartomas

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Purpose: To describe a mesenchymal hamartoma in the dorsomedial eyelid of a dog and to document the incidence and pathologic features of 22 hamartoma cases in dogs. **Methods:** The clinical history, ocular examination, treatment, and pathology are described for a mesenchymal hamartoma located in the dorsomedial eyelid of a dog. The archives of the Comparative Ocular Pathology Laboratory of Wisconsin (COPLOW) were searched for canine mesenchymal periocular hamartoma. Signalment, clinical appearance, location, and histological findings are summarized for each case. **Results:** A well-circumscribed subcutaneous mass in the dorsomedial eyelid of a 10-yeard old Staffordshire bull terrier was composed histologically of fully differentiated collagen, adipose and skeletal muscle tissue with occasional nerves. Twenty-two mesenchymal hamartomas were reviewed in the COPLOW database, containing 14 different breeds (4-14 years old). Clinically, the proliferation was located subcutaneously, subconjunctivally, or in the orbit. In some cases the mass was well attached to the canthal ligament or orbital rim, whereas in others freely movable. Fifteen hamartomas were located at the lateral canthus, the remaining were located at different locations in the orbit and on the eyelids. Histologically, they consisted of fully differentiated fibrous tissue interspersed with adipose tissue and bundles of skeletal/smooth muscle in 10 cases, and peripheral nerves in two cases. In none of the cases mitotic figures were noted. **Conclusions:** Mesenchymal hamartomas may present as a subcutaneous, subconjunctival or orbital mass. Although they have a predisposition to occur at the lateral canthus, they can be located elsewhere on the eyelids or in the orbit.

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80. Normal bacterial conjunctival flora in the huacaya alpaca (*Vicugna pacos*).

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Purpose: To describe the bacterial flora of the normal conjunctiva of Huacaya alpacas (Vicugna pacos) and to determine the effect of age and gender on this flora. **Methods:** Fifty Huacaya alpacas underwent a complete ophthalmic examination. Conjunctival swabs were obtained from both eyes and cultured for aerobic and anaerobic bacteria. Logistic and Poisson regression analysis were used to evaluate the effect of age and gender on bacterial isolation. **Results:** Four animals were excluded because of signs of external ocular disease. Of the remaining 46 alpacas, bacteria were recovered from 96.7% (89/92) of the eyes. A total of 190 bacterial isolates were cultured with a mean of 2.1 bacterial isolates per eye. The majority of isolates (70%) were gram-positive. *Staphylococcus xylosus* (44/190: 23.2%) was the most commonly recovered organism, followed by viridans streptococci (32/190: 16.8%) and Pantoea agglomerans (24/190: 12.6%). Other frequently isolated bacteria included *Rothia mucilaginosa* (12/190: 6.3%), *Staphylococcus equorum* (12/190: 6.3%), *Bacillus* species (9/190: 4.7%), *Moraxella ovis* (9/190: 4.7%), and Moraxella catarrhalis (6/190: 3.2%). No anaerobic bacteria were cultured. Statistical analysis showed that alpacas harboring viridans streptococci and *Moraxella* species were significantly younger. Gender did not significantly affect type of bacterial isolation. There appeared to be no significant effect of age or gender on the bacterial isolation rate. **Conclusions:** Gram-positive aerobes were most commonly cultured, with *Staphylococcus xylosus* and viridans streptococci predominating. This is the first description of *Moraxella* species identified in the healthy conjunctival sac of alpacas. Alpacas harboring viridans streptococci and *Moraxella* species were significantly younger.

81. Comparison of gut microbiota highfat induced modifications in young and old mice models

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Gut health fitness is influenced by intrinsic and extrinsic factors such as age and genetic background as well as diet and living habits. Influence of ageing on the gut range from malabsorption to major risk as colorectal cancer and Inflammatory bowel disease. Western diet with its highfat content can aggravate these risks. The goal of this study was to compare microbiological perturbations induced by a highfat regime in young and old mice models. 36 male C57BL/6J mice - 18 young (three months old) and 18 old (24 months old) were fed a low-fat (10%) or high-fat (45%) diet for 8 weeks. Bacterial caecal content samples were recovered were subjected to pyrosequencing and 16S rDNA v1-V3 targeted metagenomic analysis. Young-old and normal/highfat diet groups were compared by ANOVA in order to underline bacterial populations whose abundance was statistically different. Several bacterial population proportions were statistically modified in old mice compared to young mice: important bacteria such *Akkermansia muciniphila* and several butyric producers belonging to *Lachnospiraceae* were more abundant in young mice. A new undescribed RC9 gut group population was more abundant in old mice.

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82. Analyzing bovine mastitis treatment efficacy in Belgium with an integrated udder health management file: Project LAECEA

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Though antibiotics and antibiograms are known to vets since the early fifties, our practices did not evolved a lot from empiric antibiotic therapy in bovine mastitis treatment. Our study was based on 1100 mastitis events from 30 Belgian farms collected between January 2011 and June 2012. We chose to compare tissular cure (TC) based on the threshold of 200.000 somatic cells/ml in milk at milk control at least 60 days after the clinical mastitis event. Severity, quarter, treatments were recorded. We assessed a chronicity status based on previous somatic cell count (SCC) of the cow. In our distribution, we see a seasonal rise of incidence between January and May. Overall TC reaches 46% of all mastitis events. Almost 49% of all mastitis was considered as chronic cases, which TC was 33% on average, whereas new cases reached 55,3% TC. 4th generation cephalosporins (C4G) were the most used in our cohort. One third of the cases were treated parenterally with antimicrobials, mostly macrolids, fluoroquinolones and penethacillin. Finally, 10% of mastitis was treated with anti-inflammatory drugs, mostly tolfenamic acid. Comparing mastitis, only PENA and C1G/AG reached more than 60% TC. Considering new cases, then C1G/AG, PENA/PENM and Prednisolone containing specialties were above 60% TC. Use of a parenteral injections increased TC only on new cases (+12%), but not on chronic cases. Severity, advanced days in milk, parity, rear quarters and chronicity negatively affected TC. Based on our data, we can modify routine management of mastitis, as some cases might not worth the antimicrobial treatment.

83. The effect of *Urtica dioica* supplements on egg quality

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Eggs are a rich source of lipids and proteins. Because of their low production cost, they represent a cheap source of animal proteins and lipids. recently, in Algeria, consumers' interest are oriented of healthiness and dietetic values food. The purpose of this study was to investigate the effects a dietary supplement of *Urtica dioica* on laying hen's performance and egg quality. The study was conducted from may to june 2014 in the area of Chemini (Algeria). Sixty, 25-wk-old Lohmann Brown layers were randomly divided into 3 groups with 20 hens in each group. Group 1 was the control group fed a standard commercial diet based on corn and soybean, whereas the groups 2 and 3 were fed the control diet supplemented with 1% and 1.5% of nettle respectively. The hens were housed in cages in the same local and fed ad libitum. Fifty five eggs were sampled 4 times in each group from 25 to 27 weeks. A total of 220 eggs were examined. A series of measurements were carried out on each egg, namely egg weight, form index (egg shape), yolk to albumen ratio (Y:A), Yolk color, egg shell thickness, Haugh's units. Egg weight, yolk color and Haugh Units (HU) were affected by age. Significant differences ($P<0.05$) were recorded between feeding type in total egg weight, yolk color and Haugh Units. The egg from group 2 proved to have a higher egg weight (57.67g; group 1: 55.41g; group 3: 56.70g; $P<0.05$), (33.4%), intermediate yolk color (8.53; group 1: 9.03 ; group 3: 9.38; $P<0.05$) and HU (79.81; group 1: 80.85; group 3: 76.92). The results showed that the use of 1% and 1.5% of *Urtica dioic*a, had positive effects on the color of yolk of laying hens. Using different levels of *Urtica dioica* could change the characteristics of egg and could meet the preferences of consumers for the intense color of the yolk.

Keywords: egg quality, laying hens, *Urtica dioica*, yolk color.

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84. The ORF25 gene family of Cyprinid herpesvirus 3: roles of the paralogues in the biology of the infection

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Cyprinid herpesvirus 3 (CyHV-3) is the aetiological agent of a lethal disease in common and koi carp. Sequencing of its genome revealed the ORF25 family, a family of type I membrane proteins. The ORF25 family is composed of 6 paralogous sequences: ORF25, ORF26, ORF27, ORF65, ORF148 and ORF149. ORF26 is a pseudogene. ORF27 was also described as a pseudogene due to deletion and/or stop codon observed in laboratory strains. However, sequencing of field strains revealed that they encode an intact ORF27. Four of the proteins identified as CyHV-3 structural envelope proteins belong to the ORF25 family: ORF25, ORF65, ORF148 and ORF149. ORF27 was not detected in the virion. However, it has to be noted that these analyses were performed on the laboratory strain FL BAC which encodes a disrupted ORF27. The present study was devoted to the study of the ORF25 family. First, to determine whether ORF27 encodes a structural protein, a FL BAC recombinant viral strain encoding the wild type ORF27 was produced. Mass spectrometry analyses of purified virions demonstrated that ORF27 encodes a structural protein, thereby extending the ORF25 family to 5 paralogous structural envelope proteins. Second, to allow future study of ORF25 family paralogues, polyclonal sera were produced against each paralogue using DNA vaccination. Immunostaining of cells expressing the paralogues with the sera produced revealed no cross-reaction between the members of the family.

85. Sinus carcinoma suspicion in a roe deer (*Capreolus capreolus*)

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The roe deer (*Capreolus capreolus*) is a common wildlife species in Europe. However, little is known about the type and the frequency of its naturally occuring noninfectious diseases, especially neoplasms. In this study, we report the case of one roe deer presenting an abnormal naso- frontal mass. In May 2014, a free-ranging, adult, female roe deer with a facial mass was killed for sanitary reason in northern Belgium (Fouron Saint-Martin). At necropsy, the carcass was in good body condition and no significant lesions other than the occurrence of nasal throat bot flies (Oestridae). The head was submitted for pathologic investigation of a grossly evident mass (15,5 cm length – 11 cm width - 33,5 cm circumference) located upon naso-frontal bones. Xrays of the head showed a soft tissue mass with zones of calcification and partial lysis of the chamfer. The cranium seemed not to be invaded. Gram and Diff- Quick-stained smear of the hemo-purulent fluid obtained by cytopunction showed neutrophils, cell debris and yeasts. No bacteria were isolated on aerobic culture. Examination of transverse sections of the muzzle at the level of the mass suggested thickening of the frontal sinus wall with necrotic tissue. Microscopically, the tumor was composed of irregular lobules with a tubulopapillary pattern and invasive cords of epithelial cells. The tissue had large areas of necrotic debris surrounded by irregular proliferating epithelial cells. Some exams are still in progress to determine the type of this tumor but all results suggest the evidence of a sino-nasal adenocarcinoma.

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86. Laparoscopic ovariectomy in 2 bitches with Von Willebrand disease

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Von Willebrand disease (vWD) is caused by a deficiency of vW factor, a key protein in platelet adhesion to damaged vascular endothelium. vWD causes a primary coagulopathy and patients present with a history of spontaneous bleeding. Dogs suffering from vWD are at lifelong risk of fatal hemorrhage requiring urgent treatment. Female dogs are particularly at risk during estrus. Therefore, despite the risks associated with surgery, sterilization is recommended. During hemorrhagic or (planned) traumatic events, several therapeutic options are available in veterinary medicine, such as whole blood transfusion, plasma transfusion, specific factor replacement (e.g., cryoprecipitate) and medications that release the available reserves of vW-factor of the patient (e.g., desmopressin acetate). This abstract describes a case series of two 1-year-old intact female dogs (Dobermann (#1) and Weimaraner (#2)) diagnosed with vWD that underwent laparoscopic ovariectomy following desmopressin and cryoprecipitate administration. Dog 1 was presented with a history of hematomas, while dog 2 had persistent and excessive bleeding during heat. A diagnosis of vWD was made, based on normal platelet counts and clotting times with abnormal von Willebrand factor activity. In order to minimize intra- and postoperative bleeding, cryoprecipitate and desmopressin acetate were administered and ovariectomy was performed laparoscopically using a two-port technique and Ligasure®. Both procedures were performed uneventfully. Postoperatively, dogs were screened for abdominal bleeding applying FAST-ultrasound. Recovery was uneventful and both dogs were discharged 12 hours after surgery. In conclusion, laparoscopic ovariectomy after treatment with desmopressin and cryoprecipitate is feasible and safe in dogs suffering from vWD.

87. Effect of biocides on murine norovirus and feline calicivirus, surrogates of human norovirus, in suspension, glove and stainless steel disc tests

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Human noroviruses (HuNoV) are one of the major agents of human gastroenteritis and transmission occurs mainly by the faecal-oral route. The purpose of this work was to test biocide products on surrogate viruses of HuNoV in order to get information on the residual viral infectivity and on the integrity of viral genomes after biocide treatment in various conditions. Murine norovirus (MNN) and feline calicivirus (FCV) have been chosen as HuNoV surrogates because presenting comparable structure and physico-chemical properties. Two biocide products have been chosen, Ethanol (70%) and Kenocid 2100® (Peracetic acid and hydrogen peroxide) and used in 3 different conditions (in suspension, on gloves and on stainless steel discs). The biocide product was tested according to Afnor norm EN 14476. The reduction of viral titre was inferred and RNA extraction followed by a 1 step RT-qPCR was performed. If biocides products tested are able to show a 4 log reduction of the viral infectious titre, they are considered as effective. Efficacy against HuNoV can be extrapolated from these results. MNV is sensitive to Ethanol and Kenocid 2100® with and without any effect on genomic copy numbers respectively. FCV is sensitive to Kenocid 2100® with an effect on genomic copy numbers but is resistant to EtOH. The absence of effect of Kenocid 2100® on genomic copy numbers of MNV indicates this biocide product does not interfere directly on the viral genome. It may likely act on the viral structure, the capsid for example.

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88. Development of an analytical strategy for the study of the contamination of fish products by pesticides used in cotton production in northern Benin

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Benin has a few water plans especially in the north, which are often sources of fish for the local population. Unfortunately these deductions are subject to strong pressures of high pesticide use in cotton, which is largely produced in these regions. This pollution affects the growth and production of fish. It is known that cotton production requires high quantity of pesticides and a first survey showed that Acetamiprid and Lambdacyhalothrin are the most used today in Benin. Previous studies has shown that these water plans become the ultimate repository of these contaminants. In addition, when it rains, fraction of the applied pesticide reaches the soil, or the surface water through streaming, or returns to underground water by infiltration. Finally, the physical and chemical properties of pollutants and topographic and soil characteristics of the land determine pesticide quantities carried to water plans. To improve the productivity of fish resources of water plans in northern Benin, it is essential to assess the level and the rate of contamination of this resource. This thesis project aims to develop an analytical strategy for rapid monitoring of contaminants (Acetamiprid and cyhalothrin) in water plan and in fish, using screening methods such as ELISA and confirmatory techniques such as GC-MS.

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