Université de Liège

175e anniversaire de la Faculté de Médecine Vétérinaire

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Proceedings of the 1st Scientific Meeting of the Faculty of Veterinary Medicine (Liège – Belgium)

December 9, 2011

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

Proceedings of the 1st Scientific Meeting of the Faculty of Veterinary Medicine (Liège – Belgium)

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Edited by J.F. Cabaraux, C. Delguste, T. Frippiat, T. Jauniaux and A. Sartelet

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4000 Liège – Belgique

WELCOME TO THE FIRST FVM SCIENTIFIC MEETING

Each year, the Staff Scientists (SSc) of the Faculty of Veterinary Medicine (FVM) stimulates the meeting of new and older members of the SSc at its annual general meeting. As part of the 175th anniversary of our Faculty, the SSc have decided to organize a scientific afternoon.

The purpose of this meeting is to promote the social life and scientific research within the Faculty and to enhance the interactions within the Faculty regarding the different research topics. It is also an opportunity for the members of the SSc to practice writing an abstract submitted for selection, preparation of a poster and/or oral presentation as well as of their defense. Finally, this event can show to the students the career opportunities in the Faculty (clinics and research).

Submission of abstract for the 1st FVM Scientific Meeting was reserved for members of the SSc and the neo-graduates (as part of a award-winning study). The first edition is already a success with a total of **115 abstracts submitted**. Of these, 14 were selected for oral presentation by a scientific committee composed of PhD members of the SSc. For this first edition, the English and French were accepted.

Jean-François CABARAUX
President of the Staff Scientists

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Department of Clinical Sciences

ACKNOWLEDGMENTS

PROGRAMME

14.00: WELCOME SESSION

Welcome session

Jean-François CABARAUX, President of Staff Scientists

Prof. Freddy COIGNOUL, Vice-Rector of the University of Liege

14.30: SESSION 1 (CHAIRMAN : PROF. TATIANA ART & DOMINIQUE VOTION)

Description of a perilimbal pocket technique for surgical replacement of prolapsed nictitans gland in the dog

Johanna PREMONT, Department of Clinical Sciences

Atypical Myopathy Alert Group: what did we learn from the last European outbreaks?

Dominique VOTION, Department of Clinical Sciences

Méningo-encéphalite granulomateuse due à Toxoplasma gondii *chez trois taureaux, une explication possible à la méningo-encéphalite sporadique bovine*

Léonard THERON, Clinical Department for Production Animals

Investigation of the innate immunity in the lower respiratory tract in exercising horses

Linda FRELLSTEDT, Department of Functionnal Sciences

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Marie TOUSSAINT, Department of Functionnal Sciences

Serial translocation via circular intermediates underlies color-sidedness in cattle

Keith DURKIN, Departement of Animal Production

A splice site mutation in the bovine RNF11 gene is responsible of growth retardation and increased susceptibility to inflammatory diseases

Arnaud SARTELET, Departement of Animal Production

Evaluation comparative des statuts de races de poules locales : Algérie, Vietnam, Congo (RDC)

Nassim MOULA, Departement of Animal Production

16.30: COFFEE BREAK – POSTER SESSION 1

17.00: Session 2 (Chairman : Prof. Daniel Desmecht & Laurent Gillet)

Proteomic characterization of murid Herpesvirus *4 extracellular virions*
Sarah Vidick, Department of Infectious and Parasitic Diseases

Genital re-excretion of Murid gammaherpesvirus *4 following intranasal infection*
Sylvie François, Department of Infectious and Parasitic Diseases

Deletion of ORF73 renders Alcelaphine herpesvirus *1 unable to induce malignant catarrhal fever*
Benjamin Dewals, Department of Infectious and Parasitic Diseases

Comparison of bovine and human O26 EHEC strains by the Whole Genome PCR scanning
Marjorie Bardiau, Department of Infectious and Parasitic Diseases

Le résidu 100 de la nucléoprotéine virale contrôle la sensibilité des virus influenza A aux protéines Mx
Mutien-Marie Garigliany, Department of Morphology and Pathology

La métagénomique au service de la microbiologie alimentaire : étude de l'évolution des populations microbiennes lors du vieillissement de deux matrices alimentaires
Bernard Taminiau, Food Science Department

18.30: Closing session

Closing speech
Prof. Pascal Leroy, Dean of the Faculty of Veterinary Medicine

Award presentation
Catherine Delguste, Chairman of the Scientific Committee

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ORAL PRESENTATIONS

14.30 – Description of a perilimbal pocket technique for surgical replacement of prolapsed nictitans gland in the dog.

Premont J.E.[1*], Monclin S.J.[2] and Grauwels M.[1]

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Objective*:* To investigate the success rate, practicality and complications of a new perilimbal pocket technique for replacement of the prolapsed nictitans gland in dogs. To evaluate breed, sex and age distribution of the population, prevalence of unilateral versus bilateral gland prolapse, and age of onset of the initial prolapse. To assess tear production pre- and post-operatively. **Procedure***:* 30 dogs (44 eyes) which presented with nictitans gland prolapse were included. The perilimbal pocket technique consisted in making two conjunctival incisions, the first one, in the bulbar conjunctiva, 2 mm behind and parallel to the ventro-nasal limbus; the other 2 mm parallel to the free edge, on the bulbar aspect of the third eyelid. The gland was replaced by suturing the third eyelid subconjunctival tissue to the episcleral tissues, using 4 interrupted horizontal mattress sutures with absorbable Vicryl 6.0, taking care to bury the suture knots. Extended follow-up for most cases was undertaken by direct examination of dogs whenever possible, or by telephone contact to owners. **Results***:* The most common breeds represented included the English Bulldog, Neapolitan Mastiff, Great Dane, and American Cocker Spaniel. 83.3% of dogs were under one year of age when affected, with 53.3% of unilateral gland prolapse. The procedure had a 90.9% success rate. There was a statistically significant effect of age at presentation and duration of nictitans gland prolapse prior to surgery on the rate of recurrence. For the length of the follow-up period, there was a statistically significant increase in STT-1 between pre- and post-operative measurements.

14.45 – Atypical Myopathy Alert Group: what did we learn from the last European outbreaks?

Van Galen G.[1], Patarin F.[2], Saegerman C.[3], Serteyn D.[1], Marcillaud-Pitel C.[4], members of AMAG[5] and Votion D.M.[1,6*]

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Atypical myopathy (AM) is an acute, severe rhabdomyolysis occurring in grazing horses. When the first cases appeared in Belgium in 2000, little was known about this frequently fatal condition. In 2004, the University of Liege established the atypical myopathy alert group (AMAG). This network aims at collecting cases of AM from all over the world in order to identify (1) clinical signs, (2) diagnostic tools, (3) pathophysiological process, (4) symptomatic treatment, (5) risk factors, (6) preventive measures and (7) hopefully, the aetiological agent. The study of about 1000 cases originating from 15 countries has led to new knowledge into the diagnosis, pathophysiology, prevention and management of the condition. The main clinical features and specific signs of AM have been defined. From the biochemical defect recently elucidated, a new diagnostic test based on profile of acylcarnitines in serum has been validated. Monitoring of survivors has identified several prognosis factors useful to decide when horses should be treated. Therapeutic measures are now dictated by results of retrospective studies. At present, investigations are carried to challenge the recent aetiological hypotheses *i.e.* a role of mycotoxins or *Clostridium* spp. From this permanent survey, we try to improve continuously our understanding of the condition and our recommendations to prevent the disease. The AMAG acts as a platform across countries to promote exchange of knowledge between research units and also, it collects samples (from horses and from the environment) to provide the necessary material for the ongoing research performed in Europe but also in the USA.

15.00 – Méningo-encéphalite granulomateuse due à *Toxoplasma gondii* chez trois taureaux, une explication possible à la méningo-encéphalite sporadique bovine.

Theron L.[1], Tabaran F.[2], Cassart D.[2], Guyot H.[1], Dahmani K.[1], Frisée V.[1], Lamain G.[1], Touati K.[1], Desmecht D.[2] and Rollin F.[1]

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Toxoplasma gondii est un protozoaire responsable d'avortements et d'anomalies congénitales, on estime à vingt pourcent la séropositivité dans le cheptel bovin européen. C'est une cause d'encéphalite chez les animaux domestiques, mais aucun cas n'est décrit dans l'espèce bovine. Trois taureaux blonde d'Aquitaine élevés dans la même ferme et référés à la clinique entre 2009 et 2011 ont présenté une encéphalite normotherme sans autre modification de l'état général. L'examen neurologique permit de mettre en évidence une mydriase marquée malgré une vision conservée, sans autre atteinte fonctionnelle des nerfs moteurs, sensitifs et crâniens explorables. Les trois animaux présentaient un syndrome inflammatoire chronique sévère avec monocytose, neutrophilie et hypergammaglobulinémie. Seul un animal a survécu, malgré les nombreux traitements anti-inflammatoires et antibiotiques. L'analyse histopathologique a permis de mettre en évidence une encéphalite granulomateuse subaigue. En outre, des kystes ont été observés dans différents tissus. La recherche de protozoaires en sérologie *T. gondii* par immunofluorescence indirecte a montré une séroconversion IgG sur le dernier animal référé. Des tachyzoïtes nombreux ont pu être mis en évidence par immuno-histochimie dans tous les organes, y compris l'encéphale. Le diagnostic histologique de routine n'est pas évident car les kystes peuvent être aisément confondus avec d'autres structures et il semble que ces cas soient les premiers cas recensés d'encéphalite à *T. gondii* dans l'espèce bovine. La mydriase sévère semble être un signe clinique très conservé et suffisamment rare pour constituer une suspicion clinique. Cette découverte clinique pourrait constituer une étiologie possible pour le syndrome rare de méningo-encéphalomyélite granulomateuse sporadique bovine.

15.15 – Investigation of the innate immunity in the lower respiratory tract in exercising horses.

Frellstedt L.[*], Gosset P., Desmet C.J., Pirottin D., Bureau F., Lekeux P. and Art T.

Département de Sciences Fonctionnelles, FMV, ULg.

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Respiratory diseases, including inflammatory airway disease (IAD), viral and bacterial infections, are common problems in exercising horses. They represent the most commonly encountered ailments of failure to race, besides problems of the musculoskeletal apparatus, and cause huge economic losses in the racing industry. The etiology, diagnosis and treatment of these diseases have been investigated but the underlying innate and acquired immune mechanisms remain poorly documented. The airway epithelium constitutes the first line of defense and is an important part of the innate immune response. This first barrier determines the downstream cascade for elimination of the imposed pathogen. The objectives of this study are 1) to examine the expression of toll-like receptors (TLRs) in equine bronchial epithelial cells (EBEC) and peripheral blood monocytes at rest and after exercise as well as after a 3-month period of training; 2) to stimulate EBEC and monocytes *in vitro* with specific TLR ligands, in order to resemble bacterial/viral infections; 3) to describe the cytokine expression of EBEC and monocytes at rest and after exercise; 4) to apply this established experimental protocol in horses suffering from IAD/viral/bacterial infections. Bronchial biopsies will be taken during lower airway endoscopy at rest, after exercise and after a period of training. Bronchial epithelial cells will be grown *in vitro* and activated with TLR ligands. Blood will be collected from horses at rest and after exercise. Monocytes will be isolated via density gradient centrifugation and adherence. RNA will be extracted from EBEC and monocytes. The cytokine expression will be evaluated via real-time PCR and ELISA.

15.30 – Myeloid HIF-1α prevents airway allergy in mice by promoting macrophage-mediated immunosuppression.

Toussaint M.[1-3*], Fievez L.[1,3], Lekeux P.[1,2], Bureau F.[1,3] and Desmet C.J.[1,3]

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Hypoxia Inducible Factor (HIF) has major roles in promoting pro-inflammatory and bactericidal functions in myeloid cells. Conditional genetic ablation of its major subunit Hif1α in the myeloid lineage consequently results in decreased inflammatory responses in classical models of acute inflammation in mice. In contrast, we report here that mice conditionally deficient for Hif1α in myeloid cells display enhanced sensitivity to the development of allergic airway inflammation. Indeed, we support that upon allergen exposure MyD88-dependent upregulation of Hif1α boosts the expression of the immunosuppressive cytokine Interleukin-10 by lung interstitial macrophages. HIF activity in lung interstitial macrophages thereby counteracts the development of type 2 T helper responses to inhaled allergens by preventing the activation of lung dendritic cells. Thus, this study supports that in addition to its known pro-inflammatory activities, myeloid Hif1α also possesses immunosuppressive functions and is a regulator of lung mucosal immune homeostasis that helps preventing airway allergy.

15.45 – Serial translocation via circular intermediates underlies color-sidedness in cattle.

Durkin K.[1], Coppieters W.[1], Drögemüller C.[2], Ahariz N.[1], Cambisano N.[1], Fasquelle C.[1], Haile A.[3], Horin P.[4], Huang L.[5], Moser S.[6], Oldenbroek K.[7], Rieder S.[6], Sartelet A.[1], Sölkner J.[8], Stålhammar H.[9], Leeb T.[2], Georges M.[1*] and Charlier C.[1]

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Color-sidedness is a dominantly inherited phenotype of cattle characterized by the polarization of pigmented sectors on the flanks, snout and ear tips. It is also referred to as “lineback” or “witrik” (*litt.*: white back) as color-sided animals typically display a white band along their spine. Color-sidedness is documented at least since the Middle Ages and is presently segregating in several cattle breeds around the globe including the Belgian Blue and Brown Swiss. We have employed a number of techniques in the investigation of this phenotype, including, association analysis, array comparative genome hybridization, fluorescent in situ hybridization and next generation sequencing. The results obtained lead us to the remarkable observation that color-sidedness is determined by (i) a first allele (Cs_{29}) that results from the translocation of a 480Kb chromosome 6 segment encompassing *KIT* to chromosome 29, and (ii) a second allele (Cs_6) derived from the first by repatriation of a fused ~600Kb chromosome 6 and 29 sequence segment integrated adjacent to the *KIT* locus. Additionally, evidence suggests that both translocation events involved circular intermediates.

16.00 – A splice site mutation in the bovine *RNF11* gene is responsible of growth retardation and increased susceptibility to inflammatory diseases.

Sartelet A.[1], Druet T.[1], Michaux C.[2], Fasquelle C.[1], Géron S.[1], Tamma N.[1], Zhang Z.[1], Coppieters W.[1], Georges M.[1] and Charlier C.[1*]

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Belgian blue cattle breed (BBCB) is a specialized meat breed, famous for its extreme muscular development. Hence, growth and height are key components of its economical value. In order to dissect the complex determinants of growth retardation, a cohort of 143 BBCB calves, older than five months and presenting an obvious developmental delay without known etiology, was collected. Genome-wide genotyping was performed for 33 selected cases and 275 healthy controls sires. Genome-wide association study mapped the corresponding locus on bovine chromosome 3. In this region, a 3.2 Mb homozygous segment was shared by 14 cases, indicating a recessive mode of inheritance for a subset of them. Coding regions of positional candidates genes were amplified and sequenced from cases and controls. During this process, an A to G transition in the intron 1 splice acceptor site of the RING finger protein 11 gene (*RNF11*) was revealed. Recently, Rnf11 was recognized as a member of the A20 (Tnfaip3) ubiquitin-editing complex. As the A20 complex is required to limit the duration of NF-kB activation, Rnf11 is thought to be essential to control inflammatory responses. None of the 500 artificial insemination sires was homozygous mutant, despite a carrier frequency reaching 30%. However, we noticed an intriguing discrepancy between this high carrier frequency and a deficit in acknowledged growth-retarded mutated cases. A prospective study was designed to quantify and characterize this observed deviation from expected mendelian ratios. It has required the blind watch over, from birth to at least six months of age, of a cohort of 105 calves, born from carrier-carrier mating. The study main results were (i) no significant effect of the mutation at birth, but (ii) a highly significant difference at six months with 40% of homozygous cases dead from inflammatory disorders, (iii) a severe growth retardation of all remaining mutants resulting in their subsequent economic culling. In conclusion, our study highlights the major impact of a single recessive mutation on traits like growth and resistance to diseases, traits usually considered as complex traits.

16.15 – Evaluation comparative des statuts de races de poules locales : Algérie, Vietnam, Congo (RDC).

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Les races de poules locales contribuent significativement à la production mondiale de viande et d'œufs. Les races indigènes représentent plus de 80% de la population de volaille mondiale. Cependant, la majorité de ces races n'ont pas été décrites et sont mal connues. Environ 40% des races aviaires ont un statut de risque inconnu. Ainsi, des efforts considérables sont nécessaires pour évaluer ces races. La gestion des ressources génétiques animales en général et aviaires en particulier nécessite une identification préalable des phénotypes concernés, des effectifs, de leur distribution géographique voire de la diversité génétique intra- et interraciale par les méthodes de biologie moléculaire. Néanmoins, sans une compréhension des systèmes d'élevage les abritant, aucune stratégie de gestion durable de ces ressources ne peut être élaborée. Dans le cadre de nos travaux, la caractérisation des populations de poules locales et de leurs systèmes d'élevage a été menée en Kabylie (Algérie), au Nord-Vietnam et au Bas-Congo (RDC). Une grande diversité phénotypique est mise en évidence dans chaque région d'étude, apportant des éclairages différents sur le concept de race locale. Les systèmes d'élevage présentent en commun la multiplicité des objectifs - alimentaires, financiers (épargne, vente) et socioculturels -, le rôle important des femmes et des enfants, ainsi que le faible niveau d'intrants et l'exposition à une pollution génétique par les souches industrielles. Ces deux derniers éléments sont néanmoins sujets à variation entre les régions d'étude et au sein de celles-ci, ayant des conséquences quant aux propositions formulées dans l'optique d'une conservation des types génétiques locaux.

17.00 – Proteomic Characterization of Murid Herpesvirus 4 Extracellular Virions.

Vidick S.[1*], Francois S.[1], Leroy B.[2], Wattiez R.[2], Vanderplasschen A.[1] and Gillet L.[1]

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Gammaherpesviruses are archetypes of persistent viruses that have been identified in a range of animals from mice to man. They are host-range specific and establish lifelong latency of immunocompetent hosts. Most of the gammaherpesvirinae members are associated with neoplastic disease. For example, the best studied gammaherpesviruses are Human herpesvirus 4 and 8 that are respectively associated with Burkitt's lymphoma and Kaposi's sarcoma. By opposition to its human counterparts, Murid herpesvirus-4 (MuHV-4) is able to replicate to high titers in cultured cells and is therefore an excellent candidate for assessing gammaherpesvirinae virion composition. The MuHV-4 genome encodes 73 potential protein-coding open reading frames with extensive similarities to human gammaherpesviruses, as well as unique genes and cellular homologues. In this study, MuHV-4 extracellular virions were isolated and structural proteins were identified using liquid chromatography tandem mass spectrometry-based proteomic approaches. These analyses resulted in the identification of 43 structural proteins, which were classified as capsid (7), envelope (11), tegument (19) and unclassified (6) structural proteins. In addition, exponentially modified protein abundance index analyses were used to estimate the relative abundance of the identified proteins in MuHV-4 virions. Finally, a search for host proteins in purified MuHV-4 virions indicated the potential incorporation of several distinct cellular proteins among which some members of the annexin family. This analysis extends previous work aimed at determining the composition of gammaherpesvirus virions and provides novel insights critical for understanding MuHV-4 biology.

17.15 – Genital re-excretion of Murid gammaherpesvirus 4 following intranasal infection.

François S.[1*], Vidick S.[1], Sarlet M.[2], Desmecht D.[2], Stevenson P.G.[3], Drion P.V.[4] and Gillet L.[1]

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Gammaherpesviruses are the archetypes of persistent viruses that have been identified in a range of animals from mice to man. As the human gammaviruses have no well-established *in vivo* infection model, related animal gammaherpesviruses are an important source of information. We are studying Murid herpesvirus 4 (MuHV-4) in inbred laboratory mouse strains which are commonly accepted as a good model for studying gammaherpesviruses *in vivo*. To date, it has however never been possible to monitor viral reexcretion and virus transmission in this species. In order to identify potential re-excretion sites, intranasally infected mice were followed through global luciferase imaging for up to six months after infection. Surprisingly, we detected transient viral replication in mice genital tract at various times after latency establishment. *Ex vivo* imaging, quantitative PCR and immunohistochemistry revealed that virus genomes were present in high quantity in the vaginal tissue and that viral replication occurred mainly at the vaginal external border. Moreover, we highlighted the presence of free infectious viruses in the vaginal cavity at the moment of the observation of viral replication. As this ephemeral viral reexcretion could reveal a link with reproductive cycle, we compared reexcretion in normal and ovariectomized mice. Interestingly, no viral reactivation was observed in absence of hormonal cycle. In conclusion, we experimentally indentified for the first time a reexcretion site for MuHV-4 in mice that had been intranasaly infected. In the future, these results could help us to better understand the biology of gammaherpesviruses but should also allow us to develop strategies that could prevent the spread of these viruses in natural populations.

17.30 – Deletion of ORF73 renders *Alcelaphine herpesvirus 1* unable to induce malignant catarrhal fever.

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Alcelaphine herpesvirus 1 (AlHV-1) is a gamma-herpesvirus carried by wildebeest (*Connochaetes taurinus*) asymptomatically and is responsible for the induction of malignant catarrhal fever (WD-MCF) when cross-species transmitted to a variety of susceptible species of the *Artiodactyla* order, including cattle. In East-Africa, WD-MCF mainly occurs during the wildebeest calving period and has a high impact on the Maasai people whose cattle are essential to their economic and social welfare. Our recent results showed that the expansion of the $CD8^+$ T cell population occurring during WD-MCF is associated with a predominant latent mode of infection with high ORF73 RNA expression. ORF73 encodes the viral genome maintenance homolog protein in members of the *rhadinovirus* genera, allowing the latent episomes to persist lifelong in dividing mononuclear cells. In the present study, we first produced anti-ORF73 antibodies and demonstrated that the majority of the expanding cells in the tissues during WD-MCF are infected. Secondly, we investigated whether deletion of ORF73 would affect the induction of WD-MCF and viral persistence. Using an AlHV-1 BAC clone we produced an AlHV-1 ORF73 deleted ($ORF73^{null}$) and a revertant (RevORF73) strain. Although deletion of ORF73 did not affect viral replication *in vitro*, it completely impeded the induction of WD-MCF and viral persistence *in vivo*. The $ORF73^{null}$ recombinant strain could however induce an effective immune response that was protective for a subsequent intra-nasal challenge with the wild-type strain of AlHV-1.

17.45 – Comparison of bovine and human O26 EHEC strains by the Whole Genome PCR Scanning.

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In many industrialized countries, enterohaemorragic *Escherichia coli* (EHEC) strains are responsible for food poisoning in humans quite often after consumption of foodstuffs contaminated by ruminant faeces. In humans, the clinical conditions vary from undifferentiated diarrhoea to haemorrhagic colitis with, in 10 % of the cases, renal sequelae that can lead to death. The "reference" EHEC serotype is O157:H7, but EHEC can belong to hundreds of O:H serotypes whose virulence and host-range may differ. In veterinary field, EHEC strains belonging to O26, O111, O118 serogroups f.i. are also responsible for undifferentiated diarrhoea in young calves. The aim of this study is to compare the genomes of ten O26 EHEC strains (5 bovine and 5 human) by the Whole Genome PCR Scanning (WGPS) method to identify regions of the chromosome that are different between bovine and human strains and may therefore carry host-specific virulence-associated genes. This technique is based on the amplification of the whole bacterial genome using 579 long range PCRs. After comparison of the WGPS profiles of the 10 EHEC strains, their genomic diversity was studied focusing on regions that give different amplification profiles and may therefore help to differentiate between bovine and human strains. Seven such regions out of the 579 amplified fragments have been so far identified. Therefore, 55 additional strains (O26 EHEC and EPEC strains isolated from bovines or humans) were tested by PCR for these regions to confirm their host specific character. Statistical analyses are currently ongoing.

18.00 – Le résidu 100 de la nucléoprotéine virale contrôle la sensibilité des virus influenza A aux protéines Mx.

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Le virus influenza est un pathogène majeur, tant en médecine humaine qu'en médecine vétérinaire. Dans le contexte d'un pourcentage croissant de souches humaines multi-résistantes, l'étude de l'effet inhibiteur des protéines Mx apparaît comme un moyen prometteur d'identifier de nouvelles cibles thérapeutiques. Les protéines Mx comptent parmi les plus puissants inhibiteurs de la réplication du virus influenza, tant *in vitro* qu'*in vivo*, conférant une protection clinique efficace. Peu de choses sont connues quant au mécanisme d'action de ces protéines, mais il a été récemment démontré que la nucléoprotéine virale déterminait le degré de sensibilité des virus influenza à l'effet de la protéine MxA humaine. Pour apporter de nouveaux éléments de réponse, nous avons entrepris l'isolement *in vitro* de souches de virus résistantes à l'effet de la protéine Mx1 bovine. Nous avons ainsi pu identifier la substitution I100T dans la nucléoprotéine comme étant suffisante pour conférer un phénotype de résistance complète. De plus, cette substitution rend également le virus résistant à d'autres Mx, cytoplasmiques (les Mx humaine et porcine) ou nucléaires (les Mx des rongeurs). Par ailleurs, elle modifie drastiquement le niveau d'activation de la voie NF-κB par le virus. Ces résultats confirment le rôle prépondérant de la nucléoprotéine virale dans le mécanisme d'action des protéines Mx et ouvrent de nouvelles perspectives quant au mécanisme d'action précis des protéines Mx.

18.15 – La métagénomique au service de la microbiologie alimentaire : étude de l'évolution des populations microbiennes lors du vieillissement de deux matrices alimentaires.

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L'optimisation de la conservation de la viande, importante tant du point de vue économique que de santé publique passe par une meilleure connaissance de ces biotopes et des processus microbiens de détérioration de leurs qualités. Les microbiologistes ont depuis longtemps abordé ce problème en utilisant différentes approches. Ainsi, les études basées sur les méthodes de culture ont été complétées par des stratégies axées sur des approches indépendantes de la culture microbiologique. Notre propos est de démontrer que l'analyse métagénomique d'un grand nombre d'individus au sein d'une population microbienne mixte peut être appliquée avec succès à l'étude des flores microbiennes des denrées alimentaires et pourra être adaptée, à court terme, aux exigences spécifiques de la microbiologie alimentaire. Dans cette étude, un produit de viande crue et une préparation de viande cuite ont subi un test de vieillissement dans différentes conditions de conservation. L'ADN ribosomal 16S a été extrait des produits originaux et des échantillons à la date limite de consommation et, après normalisation, les régions hypervariables V5 et V6 de l'ADNr 16S ont été séquencées. L'analyse subséquente des populations microbiennes via la métagénomique permet de mettre en lumière la biodiversité et les évolutions des bactéries présentes tout au long du vieillissement de la viande. Elle identifie également les groupes plus difficiles à détecter par d'autres méthodes (culture-dépendante et indépendante confondues). Enfin, la sensibilité de cette technologie rend possible l'analyse des denrées alimentaires présentant un taux microbien très faible et permet donc l'identification des contaminants microbiens avant leur développement.

Posters

1. Contribution aux méthodes de cartographie génétique utilisant la statistique non-paramétrique.

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Il y a un intérêt croissant et soutenu dans l'identification de marqueurs génétiques (comme les polymorphismes nucléotidiques simples (SNP)) et dans la recherche de leur association avec des phénotypes de maladie ou de production. Pour les maladies, un des buts de la plupart de ces études est d'aider à découvrir les mécanismes biologiques, menant ainsi à une meilleure compréhension de la pathogénèse et conduisant à des stratégies améliorées pour la prévention de la maladie, voire son traitement. Une autre approche est d'utiliser l'information des marqueurs pour obtenir une prédiction des valeurs génétiques liées au caractère d'intérêt (susceptibilité à une maladie, p.e.). Pendant de nombreuses décennies, de telles prévisions ont été accomplies avec succès en utilisant les informations phénotypiques uniquement. Aujourd'hui, l'existence de cartes denses de marqueurs moléculaires et l'incorporation de données denses de marqueurs moléculaires dans des modèles pose de nombreux défis statistiques et informatiques. En particulier, les modèles doivent s'adapter à la complexité génétique des traits multifactoriels, tout en s'affranchissant de la course à la dimensionnalité quand le nombre de marqueurs dépasse le nombre d'observations. La régression non-paramétrique peut être utilisée pour résoudre certains de ces défis. Cette méthodologie, qui permet d'effectuer des régressions sur presque tout type de variables, va être utilisée dans ce travail pour tester son applicabilité et son potentiel dans les problèmes de la génomique actuelle, en particulier les problèmes de cartographie génétique et les problèmes de calculs de la valeur génétique des individus.

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2. Effects of gender, breed, age and body weight on echocardiographic measurements in the equine species.

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Little is known concerning the effect of various physiological variables on echocardiographic reference values in the equine species. The objective of this study was to evaluate the effect of gender, breed, age and body weight (BW) on echocardiographic measurements in the equine species. Echocardiography was performed in 163 equids of various breeds, 2 months to 37 years-old, BW 45-705 kg, and free of cardiac disease. Within this group, 50 animals (age 2 months to 37 years-old; BW 77-662 kg) were also examined using the Doppler mode. Standard bidimensional and time-motion mode echocardiography was performed on all animals. Standard pulsed-wave Doppler examination of each cardiac valve was performed in 50 of the examined animals. Data were analyzed using a general linear model including the effect of animal, gender, age, breed and BW. Most of the morphological echocardiographic measurements were significantly affected by breed (lower values in donkeys, ponies and halfbreds than in Standardbreds and Thoroughbreds) and BW but not by gender. Only interventricular septal thickness, aortic diameter and left atrial diameter were significantly affected by age. Left ventricular fractional shortening was significantly affected by breed (higher in donkeys than in the other breeds) and BW. None of the Doppler measurements were significantly affected by the tested variables. In conclusion, the results of this study suggest that the equine species, echocardiographic reference values should be established using regression equations as a function of BW within each breed. This could increase the diagnostic value of this leader technique in equine cardiology.

3. Les top-modèles.

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La modélisation mathématique permet de comprendre les systèmes et phénomènes complexes. Elle permet d'en dégager les concepts ou principes, en faisant abstraction des bruits de fond. Les modèles ne remplacent pas mais complémentent les découvertes scientifiques. Ils sont devenus un outil indispensable d'appui aux investigations scientifiques dans tous les domaines de recherche. Parmi nos « top-modèles », citons la modélisation de la réponse immunitaire qui a permis l'identification d'un seuil pré-inflammatoire critique nécessaire pour combattre l'infection mammaire; la modélisation des relations présumées entre populations sanguines lors de l'infection par *Trypanosoma evansi* ; la quantification de l'effet sur les performances de la sélection pour la résistance ou/et tolérance aux maladies contagieuses dans un troupeau infecté; la compréhension de l'impact d'une contrainte d'accès au crédit sur le choix de l'éleveur pastoral en matière d'orientation de la génétique du troupeau vers la productivité ou la gestion du risque ; ou encore la prédiction de maladie sur base de tests de substitution et l'évaluation de leur valeur pronostique.

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4. Identification and functional analysis of a new splicing variant of bovine CD46, the (co)-receptor for Bovine Viral Diarrhea virus.

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Bovine Viral Diarrhoea (BVD) virus causes major economic losses for cattle farms. The transmembrane molecule CD46 belongs to the complement regulatory receptors. It has a cofactor activity contributing to the cleavage of complement component C3b by serine protease factor I. It is also a cellular receptor for BVD. The virus adsorption onto the CD46 leads to cell invasion by endocytosis. Inventory of bovine splicing variants in our laboratory revealed a shorted form of the protein (neither transmembrane domain nor cytoplasmic tail) that we expect to be soluble. This study goal was to run a functional test in order to analyse the effect of an early interaction between the virus and its soluble receptor (sCD46). Indeed, sCD46 should be able to interact with the virus before this one reaches targeted cells. Our results demonstrate a lower virus infectivity after incubation with recombinant sCD46. These results highlight a possible relation between bovines with high sCD46 levels and a higher resistance to the virus.

5. Comparaison de souches de Salmonelles isolées de pintades dans les élevages traditionnels au Nord-est Bénin.

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En Afrique de l'Ouest, l'élevage des pintades est toujours réalisé selon le système traditionnel extensif, avec la couvaison des œufs assurée par les poules locales, les jeunes pintadeaux divaguant librement et des taux de mortalités avoisinant 70% parmi les jeunes pintadeaux (0-2 mois). Le but de ce travail était d'évaluer l'importance de *Salmonella enterica* dans ces mortalités dans les élevages du département du Borgou, au Nord-Est du Bénin. Après isolement et identification à l'espèce par galerie API20E, les souches ont été sérotypées et pulsotypées. De 2007 à 2009, 52 souches de *Salmonella enterica* ont été isolées à partir de 24 foies et/ou contenus coecaux de pintadeaux morts, 3 œufs non éclos, 25 matières fécales de pintades adultes (8), de poules couveuses (14), d'éleveurs (2) et d'une souris domestique. Douze sérotypes ont été identifiés, dont 5 plus fréquemment : 19 souches Oakland, 7 souches Farakan, 5 souches Sangalkam, 4 souches Legon, et 4 souches Kingston. Il n'y avait pas de relation absolue entre l'origine de la souche, le biotype et le sérotype. Toutefois, certaines souches appartenant au même sérotype ont été isolées des pintadeaux morts, des poules couveuses et/ou des pintades adultes. La comparaison des souches par PFGE montre que les profils sont identiques à l'intérieur d'un même sérotyppe, sauf dans le sérotype Oakland avec trois profils différents. Le typage par PCR pour différents îlots de pathogénicité et la réalisation d'antibiogrammes compléteront l'identification et la comparaison des souches et permetront de mieux appréhender leur rôle dans les mortalités des pintadeaux.

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6. Considerations on the Pathophysiology of Canine Condylar Fractures by Finite Element Analysis.

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Introduction: Condylar humeral fractures are classified as medial, lateral or bicondylar (Y-T) fractures and are believed to be associated with minor, indirect trauma, predominantly in immature dogs. **Objective of the study:** The objective of this three-dimensional finite element analysis was to confirm the pathogenesis of condylar fractures, to determine the influence of bone positioning on fracture type and to evaluate the intraosseus stress distribution before fracturing. **Materials and Methods:** Based on computer tomographic scans (n=6; two right forelimbs, Beagle, 4 months of age, male, 7-7,5 kg, scans in -10°,0° and +10° of endo-/exorotation; 1mm sections) finite element analysis was performed[2,3] (n=3) to create a three-dimensional model of the canine elbow, simplified by considering the unit of radius and ulna as a rigid body. **Results:** In contrast to the hypothesis that the radial bone would be the interacting structure, the humeroulnar interaction is clearly dominant, less often radius and ulna are both interacting. Abduction/ Adduction of the elbow at the time of trauma seems to be an important influence on fracture type. **Conclusion:** Condylar fracture pathogenesis is more complex than described in the literature. They may not only occur in elbow extension as previously reported. The ulna may even play a more important role in condylar fracture pathogenesis then the radius. Additionally fracture type may be sensitive to bone positioning during trauma. The suspected fracture type is sensitive to abduction-adduction as well as radioulnar endo-/exorotation.

7. Les agents de zoonose des oiseaux de compagnie : revue de littérature.

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Les oiseaux de compagnie, parmi lesquels figurent les passériformes (canaris, mandarins…) et les psittacidés (perruches, perroquets…), sont une frange de la clientèle vétérinaire encore assez méconnue. Pourtant, ces oiseaux représentent un commerce et un savoir-faire en matière d'élevage assez important, qu'il s'agisse de la perruche familiale achetée au marché aux canaris de concours, comme le Bossu Belge, en passant par les aras et autres perroquets de grande valeur. Les oiseaux de compagnie peuvent être affectés par une large variété de maladies, parmi lesquelles plusieurs zoonoses. On peut notamment citer les affections digestives à Salmonella typhimirium, Campylobacter jejuni et Yersinia pseudotuberculosis, les maladies systémiques comme la chlamidiose et la tuberculose. L'impact en matière de santé publique est encore fortement méconnu à ce jour ; or ces animaux se retrouvent à peu près dans tous les foyers et donc en contact direct avec la population humaine. Ce travail a pour objectif de faire l'état des lieux des connaissances en matière de maladies des oiseaux de compagnie, avec un focus particulier sur les cas décrits de transmission à l'homme et des risques que représentent les réservoirs aviaires de pathogènes potentiellement zoonotiques.

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8. The α-2,3-sialyltransferase encoded by Myxoma Virus is not essential for virus replication *in vitro* but contributes to virulence *in vivo*.

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Myxoma virus is a pathogenic Poxvirus that induces a lethal disease called myxomatosis in European rabbits. Myxomatosis is characterized by fulminating lesions at the primary site of inoculation, followed by rapid dissemination to internal organs, production of external secondary lesions and bacterial superinfection. Myxoma virus is one of the very rare viruses that encodes an α-2,3-sialyltransferase that transfers sialic acid from CMP-Sia to glycoproteins and glycolipids. Very little information is available about the role played by this glycosyltransferase in the biology of the infection and in the pathogenesis. Here, we report the construction and *in vivo* characterization of two recombinant Myxoma virus strains deleted for the M138L gene encoding the α-2,3-sialyltransferase and a derived revertant strain. After a classical *in vitro* characterization that did not reveal major differences between the strains, the virulence of the recombinant strains was compared to the parental strain by intradermal inoculation of rabbits. Our data show that the M138L deleted strains are highly attenuated *in vivo*, in comparison with the revertant and parental strains. A two-dimensional differential gel electrophoresis identified the chemokine-binding protein encoded by the M-T7 gene as a target of the viral α-2,3-sialyltransferase. Comparison of M-T7 proteins from wild-type virus and deleted strains confirmed this information. The difference of M-T7 sialylation could therefore be responsible of the *in vivo* attenuation observed. This hypothesis was reinforced by the similarity of the *in vivo* phenotypes of M138L and M-T7 deleted strains. All together, these results demonstrate that, although non essential, the α-2,3-sialyltransferase is a virulence factor for Myxoma virus pathogenesis in the European rabbit.

9. Mechanical testing of a new designed osteotomy for tibial tuberosity advancement using the modified Maquet technique.

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Objectives: To evaluate patellar tendon tension load to failure and maximal advancement of tibial tuberosity using a new designed osteotomy for modified Maquet procedure. To evaluate the tension at failure and tibial tuberosity advancement for a given distal tibial tuberosity thickness and dog's body weight. **Materials and methods:** Length of the osteotomy was 150% of tibial crest's length. The distal 50% of the curved osteotomy was parallel to the cranial cortex. To evaluate patellar tendon tension load to failure, samples (n=100) were placed in a mechanical testing system with a custom jig and the patellar tendon was loaded perpendicular to the tibial plateau axis. To evaluate the maximal advancement of tibial tuberosity, samples (n=100) were placed in a mechanical testing system with a custom jig. Tibial tuberosity was advanced cranially up to 25mm or failure. In both tests, dog's bodyweight and technical mistakes were recorded, thickness of distal tibial crest was measured on radiographs and area of tibial crest attachment was measured after transverse osteotomy. Clinical requirements were reached if the load at failure was higher than 3 times dog's bodyweight, and if the advancement was higher than 6, 9, 12, 15 mm for dog's bodyweight less than 15, 25, 30, 50 kg respectively. Statistical analysis is pending. **Preliminary results:** Forty-four out of 47 (94%) samples reached the clinical requirements for the loading test. Sixty-six out of 75 (88%) samples reached the required advancement. Optimal thickness of distal crest attachment will be determined to obtain both required advancement and tension resistance.

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10. Urodynamic findings following Hansen type I intervertebral disc extrusion and surgical decompression in dogs – A Pilot Study.

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Objectives: To characterize the urodynamic findings occurring with acute suprasacral spinal cord compression, their relationship with the neurological status as well as the urodynamic changes occurring after surgical decompression. **Animals:** Twenty dogs with upper motor neuron lesions due to Hansen type I intervertebral disc extrusion (IVDE) and with abnormal urodynamic findings at admission. **Methods:** Prospective study. Urethral pressure profiles and cystometry as well as neurological examinations were performed preoperatively (Day 1) and following surgical decompression (Day 3 and Day 7). **Results:** Preoperatively, urethral pressure spikes were present in 2 dogs with grade 3, 4 dogs with grade 4, 8 dogs with grade 5 and 6 dogs with grade 6 IVDE, even in patients with voluntary urination (n=9). No abnormality in bladder threshold pressure, threshold volume or compliance was detected. After surgical decompression, a significant decrease in urethral pressure and integrated pressure was observed, even in dogs remaining incontinent. No change in cystometric measurements was found. Postoperatively, the return of voluntary urination was always associated with the return of motor function and all patients without nociception were incontinent. **Conclusions and clinical importance:** UMN bladder following Hansen type I IVDE is characterized by urethral sphincter hypertonicity. However, no bladder function abnormality could be observed in this study. As urodynamic findings after surgical decompression failed to be prognostic of the recovery of voluntary micturition, urodynamic examination cannot be recommended as useful for bladder management in patients with acute spinal cord trauma.

11. Diarrhea and weight loss in gilts infected with *Trichuris suis.*

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Trichuris suis, also called whipworm, is a parasite of caecum and colon distributed widely and considered as a fairly common problem in swine. It is the causal pathogen of porcine trichurosis characterised by diarrhea, anorexia, growth retardation, dehydration, emaciation and anemia. This poster reports a case of trichurosis diagnosed in a Belgian pig herd. The infection was associated with severe and persistent diarrhea, growth retardation and even emaciation and anemia in 10 gilts. Less obvious clinical signs in dry sows (pasty faeces) and fattening pigs (diarrhea and growth retardation) were detected. There was a good clinical response to medication with levamisole.

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12. Treatment of a case of trichurosis (*Trichuris suis*).

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A case of trichurosis was diagnosed in a farrow-to-finish Belgian pig herd. Except in the farrowing room, all pig production stages were kept on or have access to sawdust deep litter. The infection was associated with severe and persistent diarrhea, growth retardation and even emaciation and anaemia in 10 recently purchased gilts. As there was a good clinical response to levamisole (8 mg/kg BW) given individually per os to gilts, it was decided to treat affected fattening pigs (persistent diarrhea and growth retardation) with the same medication. This poster reports the effect of consecutive anthelminthic treatments on the evolution of eggs of *Trichuris suis* per gram of faeces (EPG) collected in 9 batches of fattening pigs, between Augustus 2009 and December 2010. Treatments were based on levamisole in drinking water, febantel in feed or on flubendazole in drinking water and were administrated at 3 to 5 weeks intervals. At first, it was difficult to reduce EPG with levamisole. This situation could be explained by the short administration period of levamisole (4 to 6 hours) compared to flubendazole (5 days) More, drug ingestion could have been impaired by the course of the disease. Afterwards, flubendazole and levamisole seemed effective to maintain a low EPG.

13. An optimized DNA extraction and multiplex PCR for the detection of *Fasciola* sp. in lymnaeid snails.

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This study deals with the development and validation of an original PCR protocol to assess the presence of *Fasciola hepatica* in *Galba truncatula* its main intermediate host in Western Europe. In the present study two DNA extraction techniques are compared and a new multiplex PCR is described. The Chelex® DNA extraction technique showed to be more appropriate than the classical Phenol/Chloroform/Proteinase K based method because of the absence of toxic organic solvent, shorter duration and lower cost, and a higher reproducibility regarding DNA concentrations and wavelength ratios. The multiplex PCR was set up to amplify the lymnaeid internal transcribed spacer 2 sequence (500-600 bp) that act as an internal control and a 124 bp *Fasciola* sp. sequence that is repeated more than 300,000 times in fluke whole genome. Ninety six snails were pooled and 6 snails (6.25%) found positive for *Fasciola* sp. The limit of detection is lower than the minimal biological infestation unit (one miracidium). DNA extracts from *Paramphistomum daubneyi*, *Dicrocoelium lanceolatum*, and *Fascioloides magna* didn't cross react.

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14. Morphological alterations in horse skeletal muscles associated with *Purpura Hemorrhagica.*

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Known as being responsible for strangles, *Streptococcus equi* can also give rise to 3 immune-mediated diseases affecting horses muscles: *Purpura Hemorrhagica,* rapid muscle atrophy and acute rhabdomyolysis. *Purpura Hemorrhagica,* the most common form, is a vasculitis associated with immune complexes composed of IgA and streptococcal M protein; it is a type III hypersensitivity reaction. *Purpura Hemorrhagica* is characterized by infarcts involving mainly skeletal muscles but sometimes other organs such as skin, lung and intestine. Here, the morphopathological changes in striated muscles of 2 poneys dead after presenting signs of acute myopathy, identified as *Purpura Hemorrhagica* at necropsy, are illustrated. Histological examination of affected muscles confirmed hemorrhagic infarcts with vasculitis and fibrinoid necrosis of vascular walls. The histopathological muscular picture was central ischemic necrosis, with fragmentation of muscle fibers, surrounded by many erythrocytes and some inflammatory mononuclear cells at the periphery of the lesion. *Streptococcus equi* was isolated from laryngeal exudate of P2 ; no conclusive result was obtained from bacteriological diagnosis on retropharyngeal lymph node of P1. *Purpura Hemorrhagica*, a complication of *Streptococcus equi* infection, should be included in the differential diagnosis of horses with clinical signs of acute myopathy if there is a history of clinical strangles or if horses live on farms where infection is endemic.

15. Modulating effect of the respiratory rate of cardiomyocytes (H9C2 cells) and mitochondrial swelling by diazoxide analogues: structure–function relationships.

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Objectives: Diazoxide (DIAZ), an opener of mitochondrial ATP-sensitive K(+) channels, is well known to protect against hypoxic/ischemic stress in vivo, and as well as on the mitochondrial swelling and calcium accumulation due to ischemia/ reperfusion. However, its mechanism of action is not fully elucidated. In this work we studied the modulating effect of DIAZ versus synthetic analogues (BPDZ-490 and BPDZ-711) on the respiratory rate of H9C2 cells and on the mitochondrial swelling. **Methodology**: cardiomyocytes (H9C2 cells) were from ATTC (USA) and grown till confluence. The basal cellular respiratory rate was measured with the high resolution oxygraphy (Oroboros, Austria). Each compound: 10 uM of DIAZ, or DMSO used as vehicle, or both analogues (BPDZ-490 or BPDZ-711) were added to cellular suspension about 12 min after the beginning of the cellular respiration. Mitochondrial swelling (Mito-swelling) assays were performed on mitochondria isolated from mice, according to the protocol previously described by Plyte's group (Milano-Italy). **Results**: The basal respiratory rate of H9C2 cells ($5x10^6$/mL) was strongly enhanced when 10 uM BPDZ-490 was added with the milieu, while 10 uM DIAZ had no effect on the curve slope. In contrast, the addition of 10 uM BPDZ-711, instead of DIAZ, displayed a strong decrease of respiratory rate. In parallel to oxygraphic studies, the Mito-swelling assays showed that, at similar or higher concentration, DIAZ exhibited no effect on the swelling induced by calcium (80 uM) uptake. The Mito- swelling profile was totally different for analogues BPDZ-490 and BPDZ-711 as a dose-dependent inhibiting effect on the Mito-swelling was observed, with pronounced effect for BPDZ-711. **Conclusions**: Overall, our findings indicate that both DIAZ analogues present various profile depending on the chemical structure and the studied model: BPDZ-490 enhances the respiratory rate while BPDZ-11 inhibits it. The lead compound, DIAZ, has no real effect on the cellular respiratory rate. On the Mito-swelling assay, BPDZ-490 and BPDZ-711 inhibit the swelling induced by calcium while DIAZ has no effect even at high concentration.

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16. A deletion in the bovine *FANCI* gene compromises fertility by causing fetal death and brachyspina.

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Fertility is one of the most important traits in dairy cattle, and has been steadily declining over the last decades. We herein use state-of-the-art genomic tools, including high-throughput SNP genotyping and next-generation sequencing, to identify a deletion in the *FANCI* gene causing the brachyspina syndrome (BS), a rare genetic defect in Holstein dairy cattle. We demonstrate that despite the very low incidence of BS, carrier frequency is as high as 7.4% in the Holstein breed. We demonstrate that this apparent discrepancy is due to the fact that a large proportion of affected calves die during pregnancy. The major economic impact of BS is thus through its effect on fertility. We postulate that several other embryonic lethals may segregate in livestock and significantly compromise fertility. We propose a genotype-driven screening strategy to detect the corresponding deleterious mutations.

17. Ticks and associated pathogens collected from dogs and cats in Belgium.

Claerebout E.[1], Lempereur L.[2], Cochez C.[3], Dalemans A.C.[4], De Cat A.[1], Casaert S.[1], Madder M.[5], Saegerman C.[6], Heyman P.[3] and Losson B.[2]

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Background: The most common ticks on dogs and cats in North-Western Europe are *Ixodes spp. Ixodes spp.* are vectors of babesiosis, borreliosis and anaplasmosis. Recent reports indicated an expanding geographical distribution of *Dermacentor reticulatus* in Western Europe. *D. reticulatus* is an important vector of canine and equine babesiosis. Until now it was uncertain whether *D. reticulatus* populations had also established in Belgium. **Methods:** A survey was conducted in 2008-2009 to investigate the presence of different tick species and their associated pathogens on dogs and cats in Belgium. Seventy-five veterinary practices were selected by stratified randomization to collect ticks from cats and dogs. All collected ticks were morphologically determined to stage and species level. Ticks were analysed for the presence of *Babesia spp., Borrelia spp.* and *Anaplasma phagocytophilum* DNA by PCR, targeting the 18S rRNA, flagellin and ankA genes, respectively. **Results:** 2363 ticks were collected from 647 dogs and 506 cats. *Ixodes ricinus* (76.4%) and *I. hexagonus* (22.6%) were the predominant species. *Rhipicephalus sanguineus* (0.8%) and *D. reticulatus* (0.3%) were found in low numbers on dogs only. All dogs infested with *R. sanguineus* had a recent travel history, but *D. reticulatus* were repeatedly collected from one dog without a history of traveling abroad. The presence of a population of *D. reticulatus* was confirmed by flagging. None of the *R. sanguineus* and *D. reticulatus* ticks were positive for *Babesia* DNA, but 1.3% of the *Ixodes* ticks were found positive for the potentially zoonotic *Babesia microti* or *Babesia venatorum*. In addition, 23.2% and 10.1% of the collected *Ixodes* ticks were positive for *A. phagocytophilum* and *Borrelia* spp., respectively. *Borrelia afzeli, B. garinii*, *B. burgdorferi s.s., B. lusitaniae, B. valaisiana* and *B. spielmanii* were detected. **Conclusions:** *D. reticulatus* was confirmed to be present as an indigenous tick in Belgium. Several *Babesia* species and *Borrelia* species were detected in ticks in Belgium for the first time.

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18. *Dermacentor reticulatus* populations in Belgium and preliminary investigations of *Babesia* spp. associated pathogen.

Cochez C.[1], Lempereur L.[2], Madder M.[3], Claerebout E.[4], Simons L.[1], De Wilde N.[4], Linden A.[5], Saegerman C.[6], Heyman P.[1] and Losson B.[2]

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Background: The occurrence of indigenous clinical cases of canine and equine babesiosis in Belgium during the last two decades suggested that the vector of the pathogens responsible for these diseases, *Dermacentor reticulatus,* could be present in Belgium. Recent reports indicated an expanding geographical distribution of *Dermacentor reticulatus* in Western Europe but until now it was uncertain that indigenous *Dermacentor* populations were established in Belgium. **Methods:** Four different locations throughout Belgium, identified as potential *Dermacentor reticulatus* sites, were monitored by flagging during 2010. Ticks were stored in 100% ethanol immediately after trapping and morphologically identified. Tick DNA extraction was performed according to the proteinase K protocol (20mg/ml) and to avoid false-negative results, an additional PCR targeting the tick 16S rRNA gene was performed. Only tick-DNA positive samples were further analyzed for the presence of *Babesia* spp. A *Babesia* spp. genus-specific PCR was applied based on the amplification of the 18S rRNA gene. **Results:** Two different tick species were identified, *Ixodes ricinus* and *Dermacentor reticulatus*. A total of 236 *D. reticulatus* adult ticks were collected from the 4 sites. Ticks were found mainly from early March until the end of May with a peak of activity in April. Four out of 188 tick extracts remained negative for the 16S rRNA gene PCR even after diluting the samples 10 and 100 X. The remaining 184 DNA extracts yielded negative results for *Babesia*. **Conclusions:** This is the first record of indigenous questing populations of *D. reticulatus* in several areas of Belgium. The low number of ticks examined for the presence of *Babesia* spp. specific DNA did not allow us to conclude about the carrier status of *B. canis canis* by this tick species. Additional studies should be carried out in order to define more accurately the distribution and vectorial capacity of this tick species in Belgium.

19. Original findings associated with two cases of bovine papular stomatitis.

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Twelve 7 month-old female Holstein calves were housed in an insect-secure zone of biosafety level 3 (A3). They originated from different farms with no history of animal transfer or contacts. One month after the animal introduction in the A3 facility, lesions localized in the inner side of the lips and gums were observed in two calves, the lesions appeared flat, characterized by brownish erosions in the form of a ring or a horseshoe, allowing formulating a suspicion of bovine papular stomatitis (BPS). This suspicion was supported by transmission electron microscopy (TEM) performed on scrapings of the lesions. A PCR was carried out on the DNA purified from the animal biopsies using the pan-parapoxvirus primer pair PPP-1 and PPP-4 and a unique and specific band corresponding to a partial sequence of the B2L gene, was obtained leading us to confirm a parapoxvirus infection. ClustalW alignment performed to compare the genomic sequences to those of other parapoxviruses showed that the highest nucleotide identity (97%) was found with bovine popular stomatitis virus, followed by parapoxvirus of red deer in New Zealand (85.9%), pseudocowpoxvirus (84.3-85.1%) and orf virus strains (83.8-83.9%). The hypotheses on the pathogenesis of the infection (acute or chronic subclinical infection with occasional clinical episodes), the interesting features of the partial amino acid sequences of the major envelope viral protein encoded by the analyzed partial B2L gene, the importance of diagnostic tools available for World Organisation for Animal Health (OIE) not listed animal diseases, have been discussed.

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20. Retrospective analysis of a Listeria monocytogenes contamination episode of cheese made from raw goat's milk using quantitative microbial risk assessment.

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In 2005, Belgian authorities reported a Listeria monocytogenes contamination episode of cheese made from raw goat's milk. The presence of an asymptomatic shedder goat in the herd in a farm caused this contamination. On the basis of data collected at the time of the episode, a retrospective study was performed using a quantitative microbial risk assessment (QMRA) model covering the production chain from the milking of goats up to cheese consumption. Predictive microbiology models were used in the exposure assessment step to simulate the growth of L. monocytogenes during the cheese manufacturing process in relation to dynamic environmental conditions including changes in temperature, pH and water activity. The model showed significant growth of L. monocytogenes during chilling and storage of the milk collected the day before the cheese production (median increase of 2.0 log cfu/ml) and during the step of starter and rennet adjunction to milk (median increase of 1.3 log cfu/ml). The model estimate expressed as the median of the final simulated L. monocytogenes concentration in the fresh cheese was equal to 3.7 log cfu/g. This result was similar compared with the number of L. monocytogenes in the fresh cheese (3.6 log cfu/g) reported during the cheese contamination episode. A dose response model completed the exposure assessment and the average number of expected cases of human listeriosis was estimated between 0 and 1. A variance-based method sensitivity analysis identified the most important factors impacting the cheese contamination, and a scenario analysis then evaluated several options for risk mitigation.

21. Mise au point d'une stratégie analytique pour le contrôle des résidus d'acide oxolinique dans la chair du Tilapia "*Oreochromis niloticus*".

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Dans le cadre de l'établissement d'un plan HACCP pour la tilapiculture en Algérie, une stratégie analytique pertinente a été développée basée sur deux étapes pour le contrôle des résidus d'acide oxolinique chez le tilapia (*Oreochromis niloticus*). Une première méthode microbiologique de screening a été améliorée et partiellement validée pour l'identification de deux familles d'antibiotiques généralement utilisées en aquaculture et plus particulièrement l'acide oxolinique communément sollicité en tilapiculture. Une seconde technique LC-UV-MS a été optimisée pour l'identification et la quantification de l'acide oxolinique dans la chair du Tilapia. Les performances des deux techniques sont satisfaisantes, la capacité de détection du test de screening est de 0,75 fois la LMR de l'acide oxolinique et la limite de la quantification chromatographique est de LMR/20. Cette stratégie en deux étapes a été testée sur deux lots de tilapia contaminés expérimentalement par de l'acide oxolinique. La stratégie s'est avérée efficace et les résultats obtenus par les deux méthodes sont cohérents. Les échantillons de chair de tilapia positifs lors du screening étaient bien supérieurs à la LMR tandis que les échantillons négatifs lors du screening étaient inferieurs à celle-ci.

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22. Le macrophage alvéolaire, chef d'orchestre de la résistance aux *pneumovirus.*

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Le virus respiratoire syncytial humain est la première cause d'hospitalisation chez les nouveau-nés et infecte au moins une fois chaque bébé avant l'âge de 3 ans. Il est impliqué dans le développement ultérieur d'asthme chez les individus infectés. Son pendant bovin est quant à lui à l'origine de pertes économiques conséquentes dans les exploitations agricoles. L'étude de la pathogénie des *pneumovirus* a dernièrement connu un progrès significatif depuis l'utilisation du *Pneumonia virus of mice* (PVM), homologue murin des *pneumovirus* humain et bovin reproduisant fidèlement plusieurs aspects des maladies de ces deux espèces. Afin de mieux appréhender les premiers instants de l'infection, un modèle d'étude comparative de deux lignées consanguines murines (SJL/J et 129/Sv) opposées quant à leur phénotype de sensibilité au PVM a été développé. L'étude du premier stade de l'infection virale au niveau cellulaire nous a permis d'écarter le rôle de l'épithélium respiratoire dans la résistance de SJL/J au PVM et de mettre en évidence l'importance du macrophage alvéolaire. Pour ce faire, des expériences de permissivité virale *in vitro* des 2 types cellulaires précités, ainsi que des tests *in vivo* de déplétion en macrophages alvéolaires suivis d'infections-tests ont été réalisés. Ces résultats ont été confirmés par transfert adoptif de macrophages alvéolaires. De plus, ces derniers ont présenté *in vitro* des différences significatives de sensibilité au PVM et des capacités de phagocytose opposées entre souches, confirmant le rôle capital du macrophage alvéolaire dans l'issue de la maladie à *pneumovirus*.

23. Development of a LC-UV-MS analytical method for aldehydes formed during fatty acids degradation.

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A lot of products containing large amounts of omega-3 fatty acids can be found on the market, but unfortunately, those essential polyunsaturated fatty acids are known to be easily oxidized by light, temperature, etc, during food storage or processing. At the consumer level, the problem is that it is not always known how much omega-3 fatty acid remains in some products from animal origin, such as meat or eggs, nor how much oxidation products have been formed after storage and cooking. Those compounds, mainly aldehydes, are usually detected by the TBARS (thiobarbituric acid reactive species) method. This method measures the total content of aldehydes, expressed in malondialdehyde content. There is a need of having a more specific analytical method to assess the content of each of those compounds. Therefore, a HPLC-UV-MS method has been developed to evalute the concentation of the more toxic aldehydes in food samples. Separation and detection of aldehydes as dinitrophenylhydrazone derivatives were performed using a ThermoFinnigan Spectra System P4000 HPLC system, a Spectra System UV6000LP and a ThermoFinnigan LCQ Deca ion trap mass spectrometer, equipped with an Electrospray source. Separation was achieved isocratically with 62% water/38% acetonitrile on a Polaris C18 column (150 x 2 mm, 3 µm, Varian). The solvent flow was 0.25 ml/min, column temperature was set to 40°C. The analysis with the mass spectrometer was performed in MS/MS mode, with positive ionisation. Malondialdehyde, acroleine, 4-hydroxynonenal, hexanal and crotonaldehyde were detected by following specific mass fragmentations. Isotopic dilution technique was used for quantification, using methylmalondialdehyde as internal standard.

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24. Grazing with an automatic milking system: effects of environmental factors on yield and milking number.

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A herd of 45 Holstein dairy cows was milked with an automatic milking system (AMS) located in permanent pasture. The cows grazed from 22/06 until 20/10 on a rotational system. They were fetched twice a day to the AMS but they could also reach it freely. The sward height was measured at the entry and exit of each paddock. The data about daily milk yield and milking number were analysed according to a GLM including the effect of animal, days in paddock, distance between AMS and paddock, rotation cycle number and complementation. The cows produced 19.6 kg daily in 2.1 milkings and 95% of the cows passed at least more than twice a day in the AMS. The milking number decreased when grass height decreased ($p<0.001$; $r^2= 0.53$). The models explained 76 and 28 % of the milk yield and milking number variations respectively. Amongst the parameters studied, the animal effect explained 77% of the milk yield and 53% of the milking number variations, respectively ($p<0.001$). The distance explained a weak but significant variation in milk yield and milking number (2.3 % and 3.8% respectively; $p<0.001$). There were no clear relationships between milk yield or milking number and distance. From these results, it appears that, in a context of milking by AMS on pasture environment, the differences in milk yield may be ascribed mainly to an animal effect. The determinism of milking number remains largely undefined although the animal effect too appears to be important.

25. Morphological and histological studies of sheep's brain.

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The study of normal structures of the sheep's brain is very important to understand pathological changes caused by the bluetongue virus (serotype 8) in the fetus's brain at various stages of the gestation. The aim of this work is to improve knowledge of anatomy and histology of the central nervous system of the sheep. Five heads of adult sheep, four heads of adult goats and five heads of sheep's fetus were used. All opened heads were immerged in formalin for 10 days. After a good fixation, the brains were extracted and sectioned. Transversal, frontal and sagittal sections were realized. Sections of brains were stained with Berlin-blue and treated to be embedded in methylmetacrylate for gross morphology. The different parts of the resting brains were then embedded in paraffin, cut and the histological sections were stained with haematoxylin/eosin, cresyl violet or by use of silver impregnation. Gross morphological examination of the brains embedded in methylmetacrylate showed the detailed anatomy of the different parts. The staining with haematoxylin/eosin permitted to differentiate the grey matter, the different nucleus and the layers of cerebral and cerebellum cortex. The cresyl violet technique permitted to visualize the Nissl bodies and the silver impregnation revealed nerve fibers. In the fetus brain, blood vessels were very numerous in the brainstem, the cerebellum and the cerebrum. The grey matter was less organized and looser. This work establishes an anatomical and histological approach allowing future studies in ovine fetuses with and without brain lesions potentially caused by the bluetongue virus.

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26. Evaluation of two bone substitutes as spacer for tibial tuberosity advancement in dogs.

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Objectives: To evaluate the fatigue life and patellar tendon tension load to failure of 2 bone substitutes as spacer for tibial tuberosity advancement in dogs. **Study design**: In vitro biomechanical study **Methods**: Modified Maquet technique with new osteotomy design was used for tibial tuberosity advancement. Patellar tension load to failure was tested using Eurocer® (n=10) and Vet-Inst Wedge® (n=10) bones substitutes. Samples were placed in a mechanical testing system with a custom jig and the patellar tendon was loaded perpendicular to the tibial plateau axis. Fatigue life was tested using Eurocer® (n=4) and Vet-Inst Wedge® (n=4) bone substitutes and compared with the Kyon® cage (n=4) and Orthofoam wedge® (n=4) spacers. Samples were placed in a mechanical testing system with a custom jig and the patellar tendon was loaded perpendicular to the tibial plateau axis for 2.10^6 cycles at 4Hz or until failure at 100-500N. **Preliminary results**: Mean load at failure was 975N (n=2) and 1225N (n=2) for Vet-Inst Wedge® and Eurocer® respectively. None of the implants failed during the fatigue life test (Kyon® cage n=4, Orthofoam wedge® n=4, Eurocer® n=1) **Conclusion and clinical relevance**: If remaining tests confirm the preliminary results, Vet-Inst Wedge® and Eurocer® bone substitutes may be used as spacers for dog up to 25kg and 30kg respectively.

27. Radiography and computed tomography of snakes lungs.

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Introduction: Lung disease occurs commonly in captive snakes. The assessment of the snake's lungs by computed tomography (CT) has already been reported[1-3] but not compared with radiographic examination. The purpose of this study is to describe and compare pulmonary radiographic and CT findings in normal snakes and in snakes with bacterial pneumonia. **Methods:** Six healthy snakes (4 common boa constrictor -*Boa constrictor*-, a brown water python -*Liasis fuscus*- and an african rock python -*Python sebae*-), and four snakes (a carpet python -*Morelia spilota*- and three indian python -*Python molurus*-) suspected of respiratory disease. Radiographic examination included a lateral view of the pulmonary area. CT was performed using a high resolution CT (Somatom 16, Siemens, Germany, Erlangen) and obtaining transverse reformatted images of 1 mm thickness. **Results:** Radiographically, the normal lungs appeared as a radiolucent, cylindrical shadow with a "honeycomb" weft. On CT images, the healthy respiratory alveolar parenchyma was a circular hypoattenuating, homogeneous area with a "honeycomb" weft around a central lumen. Radiographic findings in the diseased lungs were the following: one nodule, several small nodules and 2 thick lines of soft tissue opacity. On CT images the following findings were recorded: the respiratory parenchyma was more heterogeneous than in healthy snakes with several focal or more extended hyperattenuating areas. **Discussion/Conclusion:** Pulmonary changes were seen in most snakes with suspected respiratory disease by radiographic examination and in all suspected snakes by CT. However radiographic findings were less obvious than CT findings and CT allowed a better evaluation of the extent of the damage.

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28. Metallic foreign body in a horse: Case report.

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Case Report. A young mare was presented with dyspnea, bilateral nasal mucohaemorrhagic discharge, dysphagia and fever. She had a parotid mass and regional lymph nodes were severely enlarged. Crackles were audible at auscultation. Blood results revealed neutrophilic leucocytosis and high levels of haptoglobin and fibrinogen. Tracheotomy was performed because of the dyspnea. Endoscopy revealed pharyngeal oedema and transitory displacement of the soft palate. A ventral necrotic mass displaced dorsally the epiglottis and the floor of the right guttural pouch. A deep ulceration was visible in the proximal oesophagal mucosa. Radiographs revealed a metallic linear image dorsal to the tracheotomy tube. A heterogenous mass containing gaz (abcess, haematoma with gas) was visible ventrally to the guttural pouches. It reduced larynx opening and displaced the epiglottis caudally. Ultrasonography confirmed the presence of a pharyngeal abcess. A fistula from the wall of the oesophagus was followed to the right subcutaneous soft tissues of the neck, dorsally to the tracheotomy tube, where a 4cm linear hyperechoic image causing acoustic shadowing was visible, between the carotid artery and the jugular vein. ***Conclusion.*** The final diagnosis of a metallic ingested foreign body was made. Necropsy confirmed the diagnosis: lesions were associated to the presence of a 5cm piece of wire. Entrance was on the right posterior wall of the soft palate, laterally to the epiglottis. An abcess was visible dorsally to the trachea in the cranial third of the cervical region, extending to a chronic perforated ulcer in the oesophagal wall.

29. Carp pharyngeal periodontal mucosa: an unexpected portal of entry of Cyprinid herpesvirus 3.

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Cyprinid herpesvirus 3 (CyHV-3), also known as Koi herpesvirus (KHV), is the etiological agent of a mortal disease in both common (*Cyprinus carpio carpio*) and koi carp (*Cyprinus carpio koi*). Recently, we investigated the entry of cyprinid herpesvirus 3 (CyHV-3) in carp. Using bioluminescence imaging and a CyHV-3 recombinant strain expressing luciferase (LUC), we demonstrated that the skin is the major portal of entry after inoculation of carp by immersion in infectious water. While this model of infection mimics some natural conditions in which infection takes place, other conditions could favor entry of virus through the digestive tract. Indeed, naïve fishes could ingest infectious materials such as droppings from infected fish, food contaminated by infectious droppings, tissues from infected dead carp or invertebrates which concentrated the virus by filtration. Here, we investigated whether ingestion of infectious materials mediates CyHV-3 entry through the digestive tract. Carp were feed with materials contaminated with the CyHV-3 LUC recombinant. At different time points after infection, carp were submitted to bioluminescent imaging, histology and electron microscopy analyses. All together, the results obtained demonstrated that pharyngeal periodontal mucosa act as a portal of entry after CyHV-3 oral contamination. Finally, we compared the spread of CyHV-3 in carp according to two modes of inoculation: immersion versus oral. While we observed differences between the two modes of inoculation in term of viral tropism at the portal of entry and spreading to secondary sites of infection, the two modes of inoculation led to comparable disease and mortality rate.

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30. Analysis of mRNA expression in equine skeletal muscle highlights the existence of oxidative stress, inflammation, modified immune response and increased oxidative metabolism induced by a 120 km endurance race.

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Endurance racing is one of the most challenging competitions of equestrian disciplines. Horses invest an extreme effort and appropriate energy metabolism in their skeletal muscles is paramount for good racing performances. Besides, these horses can be subject to oxidative and inflammatory stress induced by the race, and the leucocytes responses to exercise can be involved in aseptic muscle inflammation associated with muscle injury. In order to understand the molecular mechanisms underlying muscular adaptation to endurance exercise, microarray gene expression profiling was used to identify genes that were differentially expressed in skeletal muscles after a 120km race. Muscular microbiopsies were performed before and 3 hours after the completion of the race in the *Gluteus Medius* of four endurance horses from a single training centre. RNA was hybridized on equine specific microarrays, and control RT-qPCR were realised on a panel of genes. Ingenuity Pathway Analysis was used for signalling and metabolic pathways analysis, and molecular network analysis. The race induced an increase in the expression of 220 genes and a decrease in the expression of 68 genes. These genes were mainly involved in energetic metabolism, showing an increase of mitochondrial biogenesis, fatty acids uptake and oxidation by muscular cells. The changes in gene expression highlighted also muscular tissue remodelling, increases of inflammatory response, oxidative stress and DNA damages handling. This study allows a better understanding of the adaptations occurring in response to endurance exercise and could serve as a "normal" reference for further studies assessing endurance horses exhibiting muscular or metabolic problems.

31. Sub-clinical diseases underlying poor performance in endurance horses: diagnostic methods and predictive tests.

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Endurance racing is a very challenging equestrian discipline. Medical problems occurring during rides are quite well documented. On the contrary, sub-clinical diseases likely to affect endurance horses, that could induce poor performance and play a role in the development of metabolic disorders during races, have not been screened yet. This study's aims were: determining the prevalence of different sub-clinical diseases in a population of endurance horses; comparing the physiological response to exercise on a treadmill and in the field between poorly- and well-performing horses, and determining which tests would help to discriminate between well-performing horses and horses with decreased performance.Thirty-eight endurance horses underwent clinical and ancillary examinations, including haematological and biochemical evaluation, standardised exercise tests (SETs) both on a treadmill and in the field, Doppler echocardiography, impulse oscillometry, video-endoscopy and collection of respiratory fluids. All of the examined poorly-performing horses were affected by sub-clinical diseases, and most of them had multiple concomitant disorders. On the contrary, the well-performing horses were free of any sub-clinical disease. The most frequently diagnosed diseases were respiratory disorders, followed by musculoskeletal and cardiac problems. Poor-performers exhibited lower speeds at blood lactate concentration of 4 mmol/L and at heart rates (HR) of 160 and 200 bpm on the treadmill and in the field, as well as slower recovery HR. The most useful tests to reveal sub-clinical diseases appear to be SETs with concurrent HR recordings, measurements of blood lactate concentrations and muscular enzymes, in combination with airway endoscopy and cytology of the collected respiratory samples.

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32. Comparison of overground and high-speed treadmill endoscopy for the diagnosis of upper airway dynamic obstructions in saddle horses.

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Up to now, high-speed treadmill exercise (HSTE) has been the gold standard for performing upper airway endoscopy in exercising horses. However HSTE does not reproduce normal exercise conditions and the effect of the rider cannot be reproduced. Recently, overground endoscopes have been developed to examine the horse in field conditions, ridden or lunged, as well as in order to overcome the commonly encountered unavailability of HSTE. The aim of this study was to determine if endoscopic observations during track and treadmill exercises are comparable and if riding influences upper airway function. Endoscopy was performed in 11 saddle horses (showjumping, eventing, dressage or leisure riding). For the track exercise, horses were equipped with a telemetric endoscope (DRS®, Optomed, France). The horses were examined in both conditions, on separate days. The duration and intensity of exercise was standardized, as well as the head position. The endoscopic examinations of the upper airways were similar in 8/11 cases. However, riding often caused a reduction in nasopharyngeal diameter, even at a walk and promoted further dynamic collapse of the pharyngeal walls at higher speeds. Three horses developed more obvious or different clinical signs when ridden in comparison to treadmill examination (1 nasopharyngeal collapse, 1 epiglottal retroversion). These results show that similar observations can be obtained from treadmill and overground endoscopy but there could be some differences according to the type of discipline. Riding may markedly influence upper airway dynamics.

33. Methacholine bronchoprovocation test in horses: relationship with lower airway inflammation.

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The clinical examination and ancillary tests used in clinical routine may be poor diagnostics in horses suffering from subclinical inflammatory airway disease or in clinical remission of recurrent airway obstruction (RAO). Methacholine bronchoprovocation test, which is used for diagnosis of human asthma, has been used in some studies in horses, but none have examined its potential diagnostic interest in this species. The objective of this study was to determine whether this test may be used as an applicable and repeatable method for the diagnosis of an increased susceptibility to bronchospasm in horses, and whether there is a relationship between the results of this test and some markers of inflammation in the bronchoalveolar lavage fluid (BALF). Eight horses (6 with RAO history and 2 controls) were used. They underwent 2 bronchoprovocation tests at a 24 hours interval. They were then tested prior to and after an environmental challenge of 7 days duration. Simultaneously, bronchoalveolar lavages were performed for the measurement of cytokine mRNA expression in leukocytes and the presence of their proteins in the BALF. The results showed that the test has a good feasibility and a significant repeatability. Dust exposure significantly increased the bronchoreactivity, while it did not affect other clinical and functional parameters or inflammatory markers. In conclusion, the bronchoprovocation test could find its place as a diagnostic tool for the detection of asymptomatic horses that are susceptible to develop bronchospasm in poor environmental conditions.

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34. Causes of mortality in roe deer (*Capreolus capreolus*) in Southern Belgium: results of the passive surveillance 2010.

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Through a passive surveillance program, 69 roe deer (found dead or selectively culled for illness) were collected in 2010 in Wallonia. Necropsy was performed at the Veterinary Faculty of Liège according to a systematic protocol. No massive mortality in roe deer was recorded this year although several hunting districts deplored a decrease in their roe deer population. Regarding the found dead animals transported at the Faculty (n = 52), the distribution of causes of death was as follows: parasitic 24/52, traumatic 15/52, infectious 3/52, miscellaneous 4/52 and undetermined causes 6/52. Infectious causes included paratuberculosis, enterotoxemia and acute meningitis; miscellaneous causes were rumenal acidosis and tumours. Animals that didn't show any significant gross lesions or cases for which putrefaction didn't allow a post-mortem diagnosis were listed as undetermined causes. Among culled animals, various causes of morbidity were diagnosed: heavy parasitism (n = 6), traumatic injuries, ocular lesions, acute polyarthritis, chronic abscess, rumenal acidosis, and undetermined etiology in two cases. The roe deer showing heavy parasitic loads were mostly female adults and juveniles. All except one showed severe body alteration. Nine roe deer showed diarrhoea. Overall, after road injuries, parasitic diseases are the major causes of mortality in roe deer in southern Belgium. Whether or not parasitic diseases are secondary to other pathogens is difficult to determine. More attention should be given to undetermined cases and passive surveillance has to be continuously stimulated to maintain a sufficient caseload.

35. Cardiac troponin and natriuretic peptide in canine emergencies with a systemic inflammatory response syndrome.

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B-type natriuretic peptide and cardiac troponins are prognostic in human systemic inflammatory response syndrome (SIRS) patients. In veterinary emergencies only lactateis commonly analyzed. Our study in canine emergency SIRS patients hypothetized that lactate, cardiac tropinin T (cTnT) and the N-terminal fragment of pro-BNP (NTpro-BNP) are (1) increased, (2) prognostic biomarkers and (3) influenced by the underlying etiology. Dogs with SIRS were prospectively included. Patients with renal insufficiency were excluded. Samples were obtained at presentation and at regular intervals until discharge or death and at a control visit (T1m) over one month after discharge. Lactate, NTpro-BNP and cTnT were measured with a validated handheld device, a commercially available canine ELISA-kit and a human validated automated device, respectively. Statistical analysis was performed with SAS. Univariate analysis was used to assess normal distribution. A correlation procedure, mixed procedure on a linear model and a logistic procedure were performed ($p < 0.05$). Sixty nine dogs (infection (n=12), neoplasia (n=13), trauma (n=6), GDV (n=11), other GI (n=5) and miscellaneous disease (n=19)) were included. Forty-four patients survived, 34 of which had control visits, while 25 deceased. Lactate was increased at presentation (3.61mmol/L±0.34) but normalized by T24. cTnT was undetectable at T1m but was detectable in 28 dogs during hospitalization. NT-proBNP at T72 (1079.33848pmol/L ±183.504845) was higher than T1m (449.35pmol/L±83.58). Increased lactate, cTnT and NT-proBNP concentrations were negatively correlated with survival, irrespective of disease category. Lactate and NT-proBNP concentrations increase while cTnT becomes detectable in canine SIRS, regardless of the underlying disease process. These biomarkers have prognostic information.

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36. Fine-mapping and functional analysis of the 5p13.1 risk locus for Crohn's disease.

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In a GWAS conducted in 2006, Libioulle et al. (2007) identified a new risk locus for Crohn's disease on 5p13.1. The strongest association with disease was observed for SNPs mapping to three adjacent haplotype blocks spanning ~200Kb and located in the midst of a ~1.2Mb gene desert. eQTL analyses performed with the Dixon et al. (2007) data (lymphoblastoid cell lines) indicated that the disease-associated region affected the expression of *PTGER4*, the nearest gene located at ~250Kb. *PTGER4* is a suitable candidate gene as KO-mice exhibit increased susceptibility to DSS-induced colitis, IL23 secretion by dendritic cells requires functional PTGER4 signalling, and IL23 induced proliferation of Th17 cells is potentiated by PTGER4 signaling. However, the causative variants of both the effect on disease predisposition and eQTL effect remain unknown and the causality of other genes in the region (including complement factors and *CARD6*) cannot be excluded. The objectives of my PhD project are (i) to perform fine-mapping of the 5p13.1 risk locus using a very large CD case-control cohort assembled by the IIBDC, genotyped with the Illumina Immunochip and in which I will perform imputation from the 1,000 Genomes Project, and (ii) to perform eQTL analysis focusing on the corresponding region in the nine cell types that are being assembled as part of Study 1.1.4 described above. Latest results will be presented.

37. Identifying recessive lethals from local autozygous depletion.

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Livestock populations typically have very low effective population size. This causes deleterious mutations to rise to high frequencies embedded in long haplotypes. The presence of a recessive lethal at a given chromosome location is predicted to cause a local depletion in autozygosity for the corresponding haplotype. We are mining our database of > 10,000 Dutch Holstein-Friesian animals genotyped with 50K SNP arrays to identify corresponding signatures. The SNP genotypes were first phased using Phasebook. We then scanned the genome for local depletions in homozygosity for hidden haplotype states. To verify whether the observed depletions in haplotype autozygosity are due to recessive lethals, we will test the effect of the corresponding haplotypes on fertility traits. Latest results will be presented.

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38. Contribution à l'identification des domaines impliqués dans l'activité anti- influenza des protéines « Mx ».

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Les protéines Mx sont des GTPases formant une classe distincte dans la superfamille des dynamines. Elles sont induites par des interférons et sont le résultat du produit de deux à trois gènes distincts selon l'espèce. Structurellement, on peut les subdiviser en trois domaines majeurs, le domaine GTPase N-terminal (G domain), un domaine intermédiaire (MD) et le domaine effecteur GTPase C-terminal (GED). Face à l'influenza, certaines isoformes (*Homo sapiens, Mus musculus, Rattus norvegicus, Sus scrofa* et *Bos taurus*) exercent une activité anti-virale significative alors que, malgré une identité de séquence, d'autres isoformes (*Gallus gallus, Anas platyrhynchos*) n'ont pas d'activité anti-virale reconnue à ce jour. Bien que la connaissance des mécanismes impliqués dans leurs effets anti-viraux reste très limitée, nous savons que la liaison au GTP est indispensable (G Domain) et qu'une partie du potentiel antiviral résulte de leur capacité à se lier avec des protéines virales ou cellulaires entraînant l'inhibition du cycle viral (MD et GED). D'ailleurs, des mutations ponctuelles et des délétions dans ces derniers domaines sont connues pour abolir l'activité antivirale de la protéine. Le projet de recherche entamé ici consiste à produire un jeu de chimères entre une protéine Mx dépourvue d'activité anti-influenza (la protéine aviaire) et une protéine Mx très active contre les virus influenza (la protéine bovine) dans le but d'identifier le support structural minimum sous-jacent à l'activité anti-influenza des protéines Mx en général. Le projet en cours consiste à produire un jeu de cellules véro inductible capable de produire conditionnellement, les chimères précitées.

39. Assessment methodology of the intra-dermal tuberculosis skin test performed in cattle by field practitioners.

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Bovine tuberculosis remains of great concern in the European Union despite the implementation of eradication programs. The present survey aimed at developing an original and useful methodology to compare skin test practices between different regions/countries using an anonymous postal questionnaire dispatched to Belgian veterinary bovine practitioners involved in skin testing (*N*=859). The questionnaire included items focusing on the skin test performance. International experts in the field of bovine tuberculosis (*N*=5) were asked to fill the questionnaire and a scoring scale was built as follows: 0 = 'ideal' answer, 1 = acceptable answer, whereas 2 = unacceptable answer. Furthermore, experts (*N*=11) were asked to rank the questionnaire's items according to their possible impact on the risk of not detecting reactors. A global score was further calculated for each participant and a comparison of practices between the two regions of the country, i.e. Wallonia and Flanders, was performed. The participation rate was 18.3% (*N*=157 veterinary practitioners) and representative for the different provinces of the country (Pearson's correlation coefficient of 0.96, *P*<0.0001). Significant differences were observed between both regions for three categories of criteria: materials, reading of the response and others epidemiological aspects. A harmonisation at the country level is thus essential. No veterinarian summed a null score (ideal skin test procedure), which suggests that skin-testing is far from being performed correctly. Field practitioners need to be sensitised to the importance of correctly performing the test. The authors recommend the questionnaire is suitable for application in other countries or regions.

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40. Caractérisation des viandes bovines à très longue durée de conservation sous vide.

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Le but de cette étude a été d'évaluer la conservabilité de viandes bovines de différentes origines (Royaume-Uni et Irlande, Australie et Brésil) et l'influence sur celle-ci de la température de conservation (–1 °C *vs*. +4 °C). Des paramètres physico-chimiques (pH, couleur, proportion des différentes formes redox de la myoglobine (FRMb), indice TBARS et acides organiques) et microbiologiques (flore aérobie totale, flore lactique, *Enterobacteriaceae*, *Pseudomonas* spp. et *Brochothrix thermosphacta*) ont été mesurés sur sept lots de contre-filet conditionnés sous vide : aux ⅔ de la DLC et à la fin de la DLC. La diversité bactérienne a été évaluée par galeries API50 CHL et par métagénomique. Le pH a diminué au cours de la conservation dans deux lots. La couleur et la proportion des FRMb sont restées stables. Une augmentation de l'indice TBARS, plus prononcée à +4 °C, a été observée. Les viandes australiennes et brésilienne ont présenté des taux en acides acétique et citrique plus élevés. Tous les lots conservés à –1 °C ont présenté une qualité microbiologique satisfaisante à la fin de leur DLC (viandes britanniques et irlandaises = 35 ~ 45 jours; australiennes = 140 jours et brésilienne = 120 jours). La conservation à +4 °C a favorisé la croissance d'entérobactéries, facteur limitant de la conservation de plusieurs lots. L'identification bactérienne a révélé la présence de bactéries connues pour leur effet bioprotecteur. La phase ultérieure de ce travail consistera à étudier la dynamique de la flore microbienne endogène en fonction des conditions environnementales appliquées (température, atmosphère).

41. Espèces bactériennes associées aux mammites dans la race zébu Azawak à la station expérimentale de Toukounous (Niger): caractérisation phénotypique et génotypique des *Staphylococcus aureus*.

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Les mammites dues à *Staphylococcus aureus* sont considérées comme l'un des troubles sanitaires majeurs dans les élevages bovins laitiers, caractérisés par une évolution chronique et de fortes répercussions économiques. L'objectif de ce travail consiste à identifier les bactéries associées aux mammites chez la race zébu Azawak élevée à la station sahélienne expérimentale de Toukounous au Niger et à caractériser phénotypiquement et génotypiquement les souches de *S. aureus*. Deux cent soixante cinq vaches issues des trois troupeaux de la station ont été testées au *California Mastitis Test* et les quartiers positifs ont été prélevés. Cinquante neuf souches bactériennes ont été isolées à partir de 112 prélèvements de lait. L'identification des souches a été réalisée par galeries Api et a révélé une prédominance de *S. aureus* (43%). Le phénotype des *S. aureus* a ensuite été étudié. La majorité des souches se sont révélées appartenir au biotype bovin, se sont montrées capables de former des *small-colony variants* et de produire du biofilm, et ont montré une résistance élevée à la gentamicine. Les propriétés de virulence ont également été déterminées à travers un virulotypage par *PCR* axé sur la recherche de gènes de virulence. Trois virulotypes prédominants se sont dégagés. La comparaison de l'ensemble des propriétés étudiées ne nous a cependant pas permis d'établir de lien avec l'évolution sub-clinique des mammites, mais a confirmé le caractère contagieux de l'infection. L'augmentation du nombre de souches et du nombre de gènes caractérisés nous permettra davantage d'appréhender le lien entre la clinique et les propriétés des souches.

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42. Causes of death and PCB's in harbour porpoises (Phocoena phocoena) stranded on the coast of Belgium and northern France between 1990 and 2009.

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Since 1990, the Marine Animals Research & Intervention Network (MARIN) investigated the cause of death of marine mammals stranded on the coastline of Belgium and northern France. The aim of the study is to compare various parameters (biology, pathology, toxicology, cause of death) of harbour porpoises (*Phocoena phocoena*) collected between 1990 and 2009. Two main causes of death appears: infectious diseases (severe parasitosis and pneumonia) and trauma (by-catch or capture in fishing gear). Most strandings occurred in winter with predominance of by-catches in February and March. There were more males (57%) than females (42%) and more juveniles (77%) than adults (22%). The majority of stranded by-caught animals were juveniles. Compared to by-caught porpoises, animals that had died of an infectious process had a thinner blubber layer (emaciation) and the histological investigation showed a marked lymphoid depletion (spleen, thymus and lymph nodes). The total PCBs concentration was determined in the blubber of selected individuals. The concentration was higher in males (61,73 ± 44,09 µg/g lip) than females (15,24 ± 12,64 µg/g lip) and higher in adults (66,20 ± 44,02 µg/g lip) than juveniles (15,46 ± 10,37 µg/g lip). The age variation is explained by the process of biomagnification while the sex variation is explained by the mother-calf PCBs transfer during the gestation and the lactation. Finally, the animals that had died of infectious disease (49,91 ± 43,16 µg/g lip) were more contaminated than by-caught porpoises. (12,35 ± 4,12 µg/g lip).

43. Holstein cows in early lactation: milk and plasma fatty acids contents along with plasma metabolites and hormones as influenced by days in milk, parity and yield.

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Physiological factors such as days in milk (DIM), lactation number and yield can affect the energy metabolism in dairy cattle. Blood and milk samples were obtained on 32 cows on 4 occasions on a monthly basis starting from calving. When the data were grouped according to 3 DIM periods (0-50, 51-99 and ≥ 100d), there were increases in the C18 fatty acids contents in milk ($P<0.001$) and decreases in the C4-14 contents ($P<0.001$) with no changes in fat content, in period 1. Plasma cholesterol, triglycerides and IGF_1 concentrations increased with time while β-hydroxybutyrate (BHB) and non esterified fatty acids (NEFA) decreased with DIM ($P<0.01$). The C18:1 in the NEFA fraction could be considered as a reliable marker of fat mobilization because of its great decrease ($P<0.001$) with DIM. When primiparous cows were compared with multiparous cows, the C4-C14 concentration were lower and the long chains fatty acids higher in milk ($P<0.05$, 0.01 or 0.001) indicating large mobilization with the younger animals even when multiparous cows had a higher yield. In the plasma, pluriparous cows were characterized by lower triglycerides ($P<0.05$) and IGF_1 ($P<0.001$) concentration and higher BHB ($P<0.001$) concentration. This could be associated to a faster adaptation of the liver to the NEB with older cows. When milk yield classes were compared (<29, 29-39 and >39kg) there were no significant changes between classes for most of the measured parameters. This suggested that cows with either a very high or with a lower yield were able to adapt their energy metabolism.

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44. Development of a methodology to analyse reproduction problems linked to environmental factors in cattle herds in Wallonia with key indicators and decision making trees.

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The impacts of decreased of the reproduction performance in cattle herds on the profitability are large. There are many environmental factors which can influence reproduction performance. The clinical signs are almost unspecific. At farm level, the farmers and their partners are faced with two major difficulties: definition and quantification of the problem and diagnosis of the environmental factor(s) implicated. Two tools were developed to answer these questions. The first one is a computer program available on every veterinarian-inseminator PDA of the Association Wallonne de l'Elevage. This program calculates online relevant retrospective and prospective key indicators. So, at any time, the real reproduction performances of the herd can be compared with targets fixed by the farmer and the inseminator. Watch lists (oestrus, calving, infertility…) can be printed in the farm after every visit for the monitoring of the reproduction. When the targets are not obtained, a methodology of diagnosis of environmental problems linked to reproduction is available. This is the second tool. Three decision-making trees (one for each reproduction problem: heat detection, infertility or abortion) organised in four levels allow the user to progress with logic and method to adjust the diagnosis. The first level indicates the clinical signs to observe, the second one proposes the complementary exams, the third one shows the different possible diagnosis and the fourth one explains the physiological links to the reproduction problem. The user can highlight specific reproduction problems, prioritize the major causes and propose relevant solutions to the farmer.

45. Can Biomarkers Help to Differentiate Idiopathic Pulmonary Fibrosis and Chronic Bronchitis in Dogs?

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Idiopathic pulmonary fibrosis (IPF) is a chronic interstitial lung disease affecting mainly the West Highland white terrier (WHWT). The diagnosis is based on clinical examination, HRCT findings, and exclusion of other respiratory diseases, mainly chronic bronchitis (CB). The purpose of our study was to investigate three different biomarkers, procollagen type III aminoterminal propeptide (PIIINP), endothelin-1 (ET1), and transforming growth factor ß-1 (TGFß1) in IPF, CB and healthy dogs. Dogs with IPF (14 WHWTs, one Scottish terrier), 19 dogs with CB (various breeds) and 13 healthy WHWTs were included in the study. There was no difference between groups in serum PIIINP concentration. However, bronchoalveolar lavage fluid (BALF) PIIINP concentration was significantly elevated in dogs with IPF compared with dogs with CB, $p<0.001$, and healthy WHWTs, $p<0.05$. Serum ET1 concentration was elevated in dogs with IPF compared with dogs with CB, $p<0.005$, and healthy WHWTs, $p<0.001$. BALF ET1 concentration was below the detection limit in dogs with CB (n=5) and healthy WHWTs (n=5) but it was detectable in all tested WHWTs with IPF (n=6). Serum TGFß1 concentration was significantly higher in dogs with IPF compared with dogs with CB, $p<0.05$. However, there was no difference in serum TGFß1 concentration between WHWTs with IPF and healthy WHWTs. The area under the ROC-curve was 0.833 for BALF PIIINP and 0.858 for serum ET1. Our results show that PIIINP and ET1, but not TGFß1, are biomarkers that could help to differentiate IPF from CB in dogs.

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46. Wild cervids are host for tick vectors of Babesia spp. with zoonotic capability in Belgium.

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Background: Molecular evidence for the presence of *Babesia* sp. EU1 and *Babesia microti* in *Ixodes ricinus* ticks collected on dogs and cats was recently described for the first time in Belgium. *Babesia* sp. EU1, renamed *B. venatorum,* was first described in two asplenic patients and in roe deer in Europe. There is little information regarding the prevalence of the different species of *Babesia* and their potential zoonotic impact on this continent. This study was designed to investigate the presence of specific *Babesia* DNA sequences in ticks collected from cervids in Belgium. **Methods:** In 2008, ticks were collected from wild cervids (*Cervus elaphus* and *Capreolus capreolus*) found dead, hunted or killed for sanitary reasons in the context of the activities of the WildScreen Network in Southern Belgium. Ticks were kept in ethanol 70 % and identified through the use of morphological keys. DNA extraction was performed for individual ticks with the NucleoSpin tissue kit. Each extracted sample was evaluated for DNA quality and absence of inhibition with a PCR based on the 16S rRNA gene. Only positive samples were further analyzed for identification of *Babesia* spp. A *Babesia* spp. genus-specific PCR was applied; it is based on the amplification of a part of the 18S rRNA gene. Sequencing and blast analysis were performed to identify *Babesia* species. **Results:** A total of 1044 adult ticks were collected and 1023 were validated and screened for *Babesia*. Twenty eight samples were found to be positive for *Babesia divergens* (8), *B. venatorum* (14), *B. capreoli* (2) and *Babesia* spp. (4) respectively. The infection rate was evaluated to be 2.7% (95% CI: 1.8 – 3.9). **Conclusions:** This study confirms the presence of *Babesia capreoli* together with two potentially zoonotic *Babesia* species in Belgium, most notably *Babesia venatorum*, in ticks collected from wild ruminants. The estimated *Babesia* spp infection rate (2.7%) is in agreement with other previous publications *B. venatorum* and *B. capreoli* are known to have cervids as a host, but the question remains for the role of deer as a reservoir for *B. divergens*. Therefore, knowledge of the most common reservoir source for transmission of zoonotic *Babesia* sp. will be useful for modelling the risk potential of this infection to humans.

47. Two-Dimensional, M-Mode and Pulsed Wave Doppler Echocardiographic Reference Values in Healthy Adult Saanen Goats.

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Echocardiography has become a routine non invasive cardiac diagnostic tool in most species. Accurate measurement of cardiac dimensions requires reference values, which are poorly documented in goats. Goats are animals easy to handle with a body and heart size comparable to humans'. This makes goats an attractive candidate for the development of animal models for human cardiology research. The aim of this study was to test the repeatability and to establish the reference values of bi-dimensional (2D-), time-motion (M-) mode and pulsed-wave (PW) Doppler echocardiographic variables in adult goats. Six healthy female adult Saanen goats were investigated three times by the same observer at one day interval using a standardized 2D-, M-mode and PW Doppler echocardiographic protocol. Calculation of the coefficient of variation for each variable measured within day and between day allowed to evaluate their degree of variability. A single echocardiographic examination was performed in 6 other goats by the same observer, and the obtained values were added to these obtained on the third day of the 6 first goats. Then the observed mean, the standard deviation and the range of these measurements were calculated to establish the reference values of echocardiographic parameters in unsedated adult healthy female Saanen goats. Statistical analysis revealed a good inter-day repeatability of the 2D- and M-mode echocardiographic cardiac measurements, but PW Doppler parameters presented moderate to high variability, as documented in other species. Echocardiographic reference values obtained in healthy adult Saanen goats were similar to those reported in healthy adult sheep and humans.

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48. Bovine herpesvirus 4 glycoprotein L is non-essential for infectivity.

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The core entry machinery of mammalian herpesviruses comprises glycoproteins B, H and L (gB, gH and gL). gH and gL form a heterodimer with a central role in viral membrane fusion. When archetypal alpha- or beta-herpesviruses lack gL, gH misfolds and progeny virions are non-infectious. However, the gL of the rhadinovirus Murid herpesvirus 4 (MuHV-4) is non-essential for infection. In order to define more generally what role gL plays in rhadinovirus infections, we disrupted its coding sequence in Bovine herpesvirus-4 (BoHV-4). BoHV-4 lacking gL showed altered gH glycosylation and incorporated somewhat less gH into virions but remained infectious. However, gL- virions showed poor growth and cell penetration. Moreover, a major part of their entry defect appeared to reflect impaired endocytosis, which occurs upstream of membrane fusion itself. Thus the rhadinovirus gL may be more important for driving virion endocytosis than for incorporating gH into virions, and is non-essential for membrane fusion.

Sweet itch in the horse and the impact of biting midges on the potential introduction of horse sickness in Europe.

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Sweet itch is considered as being the most frequent hypersensitivity among horses. Prurit is the main clinical sign of this seasonal disease that spreads worldwide. Intradermal skin tests combined with histopathology results and skin biopsy allows us to confirm the diagnosis. It is true that so far, no real curing treatment for hypersensitivity of sweet itch has been efficient and disease prevention is thus particularly important, although it has not been developed so far. The main etiology of sweet itch lies in an insect that belongs to the biting midges family. In Belgium, this insect is particularly active from End of April to End of October. With more than 1000 different species, the biting midges represent the group of haematophagous insects that are most frequently observed. They can carry lots of pathogenic agents in particular in Equidae. About horse sickness, the death rate of horses is close to 90%. There are 4 clinical signs of disease ranging from mild fever to heart failure or dyspnea. In Africa, horse sickness and sweet itch have a common vehicle: *C. imicola.* Although horse sickness is endemic in this continent, it spreads in Europe in recent years. Vaccination and fight against biting midges are the only efficient protection against this disease. Field experience in Belgium, near a riding school, allowed showing the large amount and the diversity of the biting midges. *C. obsoletus s.l* is the most frequent, but the evolution of species is constant. Indeed, from May 2010, several new species have been identified in Belgium. A link between involved species in sweet itch and horse sickness is not established, but the potential arrival of new species in different European countries increases the risk of horse sickness emergence in Europe.

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49. Antibody Evasion by a Gammaherpesvirus O-Glycan Shield.

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Herpesvirus transmission between immune hosts implies some kind of antibody evasion. In particular, gammaherpesviruses that represent a significant cause of disease both in human and animal populations have evolved to coexist with antibody and to resist neutralization. Nevertheless, the underlying molecular mechanisms remain largely unknown. All gammaherpesviruses encode a major glycoprotein homologous to the Epstein-Barr virus gp350. These glycoproteins are often involved in cell binding, and some provide neutralization targets. However, the capacity of gammaherpesviruses for long-term transmission from immune hosts implies that in vivo neutralization is incomplete. In this study, we used Bovine Herpesvirus 4 (BoHV-4) to determine how its gp350 homolog -gp180 - contributes to virus replication and neutralization. A lack of gp180 had no impact on the establishment and maintenance of BoHV-4 latency, but markedly sensitized virions to neutralization by immune sera. Antibody had greater access to gB, gH and gL on gp180-deficient virions, including neutralization epitopes. Gp180 appears to be highly O-glycosylated, and removing O-linked glycans from virions also sensitized them to neutralization. It therefore appeared that gp180 provides part of a glycan shield for otherwise vulnerable viral epitopes. Interestingly, this O-glycan shield could be exploited for neutralization by lectins and carbohydrate-specific antibody. The conservation of O-glycosylation sites in all gp350 homologs suggests that this is a general evasion mechanism that may also provide a therapeutic target.

50. Développement d'une technique d'empreinte de l'IS621 des *Escherichia coli* entérohémorragiques et entéropathogènes O26:H11 isolées d'humains et de bovins.

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La surveillance des toxi-infections alimentaires collectives (TIAC) par des *Escherichia coli* entérohémorragiques (EHEC) demande des méthodes rapides de comparaison des souches, à côté de la méthode d'électrophorèse en champ pulsé (PFGE). L'empreinte IS*629* est basée sur une PCR multiplexe détectant les différences en nombres de copies et en sites d'insertion de l'IS6*29* entre EHEC O157:H7 (Ooka et al., 2009. *J Clin Microbiol* **47,** 2888-2894). Le but de ce travail était de développer une méthode similaire de détection des différences en nombres de copies et en sites d'insertion de l'IS*621* dans les EHEC O26:H11. Après standardisation, deux PCR multiplexes ciblant les jonctions IS*621*/chromosome 5' ou 3' de 12 copies et une PCR détectant la présence d'une copie supplémentaire ont été appliquées sur 69 EHEC et EPEC (*E. coli* entéropathogènes) bovines et humaines de collection, isolées dans différents pays. Trente-six empreintes IS*621* différentes ont été observées alors qu'aucune amplification n'était obtenue pour 9 non-EHEC/-non-EPEC. La comparaison des résultats d'empreinte IS*621*, de profils PFGE et des données anamnestiques suggère que les EHEC/EPEC avec des empreintes IS*621* différentes sont indépendantes épidémiologiquement. Inversement, de nombreuses EHEC/EPEC, mais pas toutes, avec des empreintes IS*621* identiques sont reliées épidémiologiquement et montrent des profils PFGE très proches, voire identiques. En conclusion, l'empreinte IS*621* représente une méthode rapide (<48 heures) de première ligne de typage des EHEC et EPEC O26:H11, complémentaire, bien que moins discriminante que le PFGE. Il sera intéressant dans le futur d'appliquer les empreintes IS*629* et IS*621* lors de TIAC à EHEC O157:H7 et O26:H11 respectivement.

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51. Release and innate immune detection of host cell DNA mediate the adjuvant activity of aluminum salts.

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Much remains to be elucidated of the immunological mechanisms that govern the responses to aluminum-based adjuvants (alum), the most widely used vaccine adjuvants. It was recently proposed that alum might boost adaptive immune responses through the activation of innate immune signaling pathways. We observed that, in mice, alum causes the release of host cell DNA, which acts as an endogenous innate immunostimulatory signal mediating the adjuvant activity of alum. Indeed, purified mouse DNA in quantities similar to those detected at alum injections sites was as potent as alum in boosting T and B cell responses. Furthermore, digestion of extracellular DNA at sites of alum injection decreased both cellular and humoral responses. We further obtained evidence supporting that host DNA signaling differentially regulates IgE and IgG1 production upon alum immunization. Indeed, we observed that, following alum immunization, host DNA activates inflammatory dendritic cells (iDCs) through Interferon response factor (Irf)3-dependent mechanisms. These iDCs in turn stimulate 'canonical' T helper type 2 responses, associated with IgE isotype switching and peripheral effector responses, but unable to promote IgG1 responses. On the other hand, we propose that host DNA release also boosts IgG1 production through the induction of T follicular helper responses via iDC- and Irf3-independent mechanisms. The finding of a link between innate immune responses to an endogenous danger signal, extracellular host DNA, and the boosting of adaptive responses upon alum-adjuvanted immunization may increase our understanding of the mechanisms of action of current vaccines and help in the design of new adjuvants.

52. Epreuves virulentes successives avec le virus de la fièvre catarrhale ovine sérotype 8 avec ou sans vaccination sur génisses gestantes.

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Objectifs : La fièvre catarrhale ovine (FCO) est une maladie des ruminants causée par le virus de la bluetongue (BTV). L'introduction du sérotype 8 (BTV-8) en Europe Occidentale a été caractérisée par une sévérité inattendue chez les bovins, avec un possible passage transplacentaire. Ce travail évalue les degrés de protection conférés par un vaccin commercial inactivé contre le BTV-8. **Méthodes** : Douze génisses gestantes et leurs veaux ont été répartis dans trois groupes : A (vaccinés/infectés), B (non vaccinés/infectés) et C (témoins). Les groupes A et B ont été infectés à 120 (J0) et 240 (J121) jours de gestation. Les anticorps spécifiques du sérogroupe ont été quantifiés dans le sang et le lait par ELISA, et la virémie évaluée par RTqPCR. Veaux et génisses ont été abattus et autopsiés 10 mois après J0. **Résultats et conclusions** : Les signes cliniques reproduits sont comparables à ceux obtenus lors d'infections naturelles ou expérimentales. Le vaccin s'est révélé efficace dans la prévention de la virémie et des signes cliniques. Dans le lait des génisses vaccinées et dans le sang de leur veau, la quantité d'anticorps circulants diminue rapidement alors que chez les animaux uniquement infectés, ces anticorps se maintiennent de manière prolongée. Les différences de profils sérologiques (sang, lait), ainsi que la présence d'une hémorragie intramurale de l'artère pulmonaire, lésion considérée comme quasiment pathognomonique chez un veau né de mère vaccinée, soulèvent certaines questions quant à la durée de l'immunité et l'étendue de la protection fœtale conférées par le vaccin.

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53. Cas de démodécie chez deux veaux de race Holstein.

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Demodex bovis est l'agent de la démodécie des bovins, qui est assez répandue en zones tropicales, mais plus rare et aussi vraisemblablement sous-diagnostiquée en régions tempérées. Les parasites se multiplient dans les follicules pileux, qui se dilatent et forment des nodules cutanés contenant un matériel épais et blanchâtre, qui constituent en général la seule manifestation clinique. Dans certaines circonstances, on peut observer une démodécie généralisée avec alopécies. Un cas de démodécie chez deux veaux de race Holstein, de 6-6,5 mois, détenus dans un cadre expérimental, est décrit. Cette observation est rare en zone tempérée et n'a, à la connaissance des auteurs, jamais été décrite chez des veaux de cet âge en Europe. Le suivi clinique, l'évolution des lésions jusqu'à disparition de la dermatose en l'absence de traitement, les hypothèses diagnostiques et les voies de transmission de cette maladie sont discutées.

54. Characterization of a new potential virulence factor of *Microsporum canis*, the secreted subtilisin Sub6.

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Microsporum canis is a dermatophyte responsible for cutaneous superficial mycoses in domestic carnivores and humans. The pathogenesis of dermatophytoses, including *M. canis* infections, remains poorly understood. Secreted proteases including members of the subtilisin family are thought to be involved in the infection process. In particular the subtilisin Sub6 could represent a major virulence factor. Genomic *SUB6* was amplified by PCR using specific primers and *M. canis* IHEM 21239 DNA as a target. The *SUB6* cDNA was obtained by reverse transcriptase (RT)-PCR using total RNA extracted from the same *M. canis* strain grown in liquid medium containing feline keratin as unique nitrogen source. Both *SUB6* cDNA and genomic DNA were sequenced. The *SUB6* cDNA was cloned in pPICZA to produce recombinant Sub6 (rSub6) in *Pichia pastoris* KM71. This protease rSub6 was produced in methanol medium at a yield of 30 mg/ml and purified by anion exchange chromatography using a DEAE-sepharose column. Polyclonal antibodies against purified rSub6 were produced in a rabbit using a standard immunization procedure with saponin as the adjuvant. Seventy days after the first immunization, serum was collected and IgG were purified by affinity chromatography. We have characterized for the first time Sub6 from a dermatophyte species as a recombinant secreted active enzyme and purified it until homogeneity. Active rSub6 and Sub6 specific antiserum will be used to further study the role of *M. canis* Sub6 protease in pathogenesis, notably the pattern of *in vivo* Sub6 secretion in different host species.

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55. Profils de liaison et mécanismes d'internalisation des norovirus bovins de génotype 2.

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Les norovirus (NoV), famille *Caliciviridae* genre *Norovirus*, infectent l'homme et les animaux (bovins, porcins, murins). Les NoV humains et animaux sont relativement proches génétiquement et coexistent parfois de manière très étroite en Europe du Nord, ceci impliquant logiquement un risque zoonotique lié aux NoV animaux. Les objectifs du travail étaient d'étudier les types de structures pouvant être impliquées dans la liaison de pseudoparticules de BoNoV (BoNoVLP) aux cellules, ainsi que les mécanismes entrant en jeu pour leur internalisation. Dans une première étude, la fixation de VLP d'une souche BoNoV sur des lignées cellulaires bovines d'origines tissulaires différentes a été mise en évidence par immunofluorescence indirecte et la fluorescence diminuait d'intensité après traitement par le periodate de sodium. Dans une deuxième étude, les structures permettant l'attachement des BoNoV de génotype 2 ont été investiguées quantitativement en cytométrie en flux avec les VLP. Le periodate de sodium, l'α-galactosidase, la trypsine, la chymotrypsine et la phospholipase C ont très significativement diminué l'intensité du signal tandis que la neuraminidase a également permis de le réduire modérément. Les sulfates d'héparane ou de chondroïtine n'étaient par contre pas impliqués. Une troisième étude, en cytométrie en flux, a permis d'évaluer les voies d'internalisation des VLP (voies liées aux radeaux lipidiques et à la macropinocytose). La structure impliquée dans la liaison des BoNoV de génotype 2 est un saccharide présent sur de nombreux types cellulaires bovins ; cette structure comprend un résidu α-galactose ; un résidu acide neuraminique peut être aussi impliqué dans cette liaison ou peut la faciliter ; les sulfates d'héparane et de chondroïtine ne le sont pas ; des voies alternatives peuvent être utilisées pour l'internalisation de la VLP. Les différents résultats peuvent être intégrés dans le profil de risque zoonotique associé aux BoNoV.

56. Detection of *Alternaria* and *Cladosporium* DNA in nasal mucosa from dogs with idiopathic lymphoplasmacytic rhinitis.

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Idiopathic lymphoplasmacytic rhinitis (LPR) is a common cause of nasal discharge in dogs, but its aetiopathogenesis remains unknown. In people with chronic rhinosinusitis, *Alternaria* is the suspected trigger for local inflammatory reaction. In addition, *Cladosporium*, another common fungus part of the human nasal flora, has been implicated in respiratory allergy. Therefore, the aim of the present study was to compare the amount of *Alternaria* and *Cladosporium* fungal DNA found in nasal mucosal biopsies from dogs with LPR with that in biopsies from dogs with nasal neoplasia and without nasal disease. Quantitative real-time PCR (qPCR) assays detecting DNA from *Alternaria* and *Cladosporium* fungi were applied to nasal mucosal biopsies collected from dogs with LPR (n=8), nasal neoplasia (NEO; n=10) and controls (CTL; n=10). A copy number for each sample was calculated using a standard curve of known copy number and differences amongst the groups were assessed using the Kruskal-Wallis test. A low level of *Alternaria* DNA (<10 copies/qPCR) was detected in 7 samples (4 CTL, 1 NEO, 2 LPR) and 21 samples (6 CTL, 9 NEO, 6 LPR) were negative. High levels of *Cladosporium* DNA (10-100 copies/qPCR) were detected in 2 samples (2 CTL); a low level of DNA (<10 copies/qPCR) in 18 samples (5 CTL, 5 NEO and 8 LPR), and 8 samples (3 CTL, 5 NEO) were negative. Present results show that *Alternaria* and *Cladosporium* are part of the normal canine nasal flora, and that these fungi are probably not implicated in the pathogenesis of canine LPR.

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57. Identifying genetic determinants of variation in cardiovascular physiology in healthy dogs.

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Cardiovascular diseases (CVD) are the main cause of death in Europe, counting for nearly half (49%) of all mortalities. CVD result from complex interactions between genetic and environmental factors. Identifying susceptibility genes is one of the major present biomedical targets as it is expected to lead to novel drug targets and diagnostic aids. However, and with the exception of Mendelian forms of hypertension - which have highlighted the importance of renal function - the quest for genetic risk factors has so far remained relatively unsuccessful. This is due in part to the complexity of the interplay of environmental conditions and genetic heterogeneity in human beings. The objective of this project is to provide an alternative way towards the identification of genes that influence blood pressure, glucose and lipid metabolism, i.e. the major physiological determinants of hypertension and metabolic syndrome. Healthy dogs show exceptional variation for a range of morphological traits. We speculated that similar levels of variation might exist between and possibly within breeds for a number of physiological parameters, including cardio-vascular. Identifying the genes underlying such variation might uncover novel pathways and hence targets for therapeutic intervention in human. Towards that goal, we collected 65 highly standardized phenotypes pertaining to cardiovascular physiology on 473 healthy dogs from eight breeds. All dogs were genotyped with the 180K Illumina SNP array. We are attempting to map QTL influencing the measured parameters using approaches that target within breed QTL segregation, yet allow signal combination across breeds.

58. Towards transgenic-based semen sexing.

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An alternative approach towards overcoming the limited economic value of dairy bulls is to change the sex ratio towards females. This can presently be achieved by using sexed semen that has been flow-sorted on the basis of DNA content. However, this approach remains tedious and expensive, and its use has remained relatively limited. We herein explore an alternative, transgenic-based approach in a mouse model. Our aim is to achieve exclusive expression of green fluorescent protein in the membrane of Y-bearing sperm. To that end, we plan to target constructs driving expression in post-meiotic elongated spermatids on the Y chromosome. Y targeting will be achieved by recombinase mediated cassette exchange (RMCE) using a previously developed embryonic stem cell line having a floxed exchange cassette on its Y chromosome. Two major limitations need to be overcome to achieve our objectives. The first is to block diffusion of the expressed gene product to adjacent X-bearing sperm via the cytoplasmic bridges that are connecting "sister spermatids". This is being attempted by generating fusion transcripts and proteins between *GFP* sequences and the *Tcr* gene encoding the antidote in the poison-antidote system underlying segregation distortion at the murine *T* locus (only gene product known not to diffuse between spermatids). The second is to overcome meiotic sex chromosome inactivation (MSCI) during spermatogenesis. We will initially check whether the protamine 1 promotor can overcome MSCI silencing when placed on the murine Y chromosome. Latest results will be presented.

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59. Les races de poules belges de grande taille.

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La diversité des populations animales domestiques, résultant de la sélection menée par l'homme dans des systèmes d'élevage variés, décroît rapidement du fait de l'intensification de l'élevage survenu durant la deuxième moitié du XX[e] siècle. La spécialisation extrême de certaines races domestiques et la dissémination mondiale de celles-ci ont en effet eu lieu au détriment de races locales moins productives, et donc de la biodiversité. En Europe où les lignées commerciales ont absorbé le marché des productions animales, les races anciennes figurent parmi les ressources génétiques animales les plus menacées au monde. Les ressources génétiques aviaires n'échappent pas à ce phénomène global et connaissent une situation très alarmante. La Belgique, avec une quarantaine de races et plusieurs centaines de variétés de poules ne fait pas exception à cette règle ; en effet, 95% de ces races sont en danger d'extinction. Dans le cadre de nos travaux, un suivi des évolutions des effectifs de races de poules belges de grande taille a été mené en Flandre et en Wallonie. Une vingtaine de races de grande taille ont été concernées par cet inventaire. Les races de poules belges étant majoritairement menacées d'extinction, du fait de leur faible effectif, l'objectif majeur est d'éviter leur disparition pure et simple. Il convient donc d'assurer la reproduction des animaux dans de bonnes conditions et de limiter l'augmentation de la consanguinité par l'utilisation de méthodes de gestion efficaces. Afin de préserver la biodiversité en général et avicole en particulier, il convient de protéger le patrimoine animal local par la conservation des races menacées d'extinction.

60. Identification of plasmid-mediated fluoroquinolone resistance-encoding genes among pathogenic and non-pathogenic *Escherichia coli* strains of human and animal origin in Belgium.

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Until the end of the 20th century, the identified mechanisms of resistance to (fluoro)quinolones were accumulation of mutations within chromosomal genes either encoding the DNA gyrase and/or the topoisomerase IV or regulating the expression of efflux pumps. However, a plasmid-mediated quinolone resistance (PMQR)-encoding gene was identified in a clinical isolate of *Enterobacteriaceae*: the so-called *qnr* gene. The interaction with the Qnr protein confers some level of protection of the DNA gyrase from the action of (fluoro)quinolones. Up-to-date, five major groups (A, B, C, D and S) of *qnr* genes have been described with several variants in some groups. The PMQR mechanisms confer sub-clinical level of resistance and also enhance the probability of emergence of clinically resistant isolates in the presence of fluoroquinolones at therapeutic levels. The purpose of this work was to study the presence of the *qnr* genes by using specific PCR in a collection of approximately 4000 strains of pathogenic and non-pathogenic *Escherichia coli* isolated from humans and from different animal species. A total of 142 strains tested positive with one of the PCRs. The *qnrB* gene was detected in 64 strains; the *qnrC* gene was identified in 6 isolates; the *qnrD* gene was found in 4 strains; 71 strains were positive for the presence of the *qnrS* gene. None of the isolates tested were positive with the PCR for the *qnrA* gene. Work is currently performed to compare the sequences of the amplicons obtained by PCR and to identify the plasmid carrying the different *qnr* genes identified.

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61. The A3 gene of *Alcelaphine herpesvirus 1* encodes a viral semaphorin that is non-essential for the induction of malignant catarrhal fever.

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Alcelaphine herpesvirus 1 (AlHV-1) is a γ-Herpesvirus carried by wildebeest asymptomatically. AlHV-1 is however responsible for the development of malignant catarrhal fever (MCF) when cross-species transmitted to a variety of ruminant susceptible species. Wildebeest-derived (WD)-MCF is a frequently fatal lymphoproliferative and degenerative disease of ruminants. Experimentally, WD-MCF can be reproduced in rabbits. The A3 open reading frame (ORF) of the AlHV-1 encodes a putative semaphorin homolog protein, thereafter named AlHV-sema. Semaphorins are secreted and membrane-associated proteins characterized by a conserved amino-terminal 'Sema' domain. Initially identified as neural guidance factors, semaphorins have been shown to have significant functions in various processes of immunoregulation. Bioinformatics analyses revealed that AlHV-sema has a high homology to the mammal Sema7A, a semaphorin notably involved in immune system. In order to investigate whether AlHV-Sema could play a role in the pathogenesis of WD-MCF, we used the AlHV-1 BAC clone and produced a strain deleted for A3 and a revertant strain. The strain deleted for A3 replicated comparably to the wild-type parental strain *in vitro*. *In vivo*, rabbits infected with the strain deleted for A3 developed WD-MCF similarly to that observed with the parental strain with both severely increased $CD8^+$ T cell frequencies and viral genomic charge over time in peripheral blood and in lymph nodes at time of death, as well as indistinguishable histopathological lesions in lymphoid organs and in liver, lung and kidney. In conclusion, this study demonstrates that AlHV-sema is not essential for the induction of WD-MCF in rabbits.

62. Towards positional cloning of a gene causing heterogeneous endocrine neoplasias in a large, AIP-negative family.

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As part of our study targeting familial isolated pituitary adenoma (FIPA), we identified a three-generation family of Northern European descent in which 11 of the 17 related individuals were either affected with acromegaly (two cases), prolactinoma (one case), Cushings disease (one case), thyroid cancer (five cases) or obliged but unaffected carrier (three individuals). The segregation pattern was compatible with simple autosomal dominant transmission. We genotyped 16 family members with genome-wide 300K SNP arrays, and identified one 15.4Mb chromosome 4 segment (57 annotated genes) that cosegregated perfectly with the condition (non-parametric nominal p-value = 0.0019). *SLIT2*, a strong candidate gene in the corresponding region, was resequenced and the methylation of its promoter examined in cases, but this did not reveal any suspicious DNA variants or methylation profile. We generated ~10GB of sequence from a paired-end library (400bp inserts), and ~2GB of sequence from a mate-pair library from an affected individual (Cushing) on an Illumina GIIx instrument. Mate-pair reads did not provide evidence for structural rearrangements in the linked chromosome segment. We detected 11,993 DNA Sequence Variants (DSV) of which 377 (including two coding SNP) were not reported in the 1,000 Genome Project database. Genotyping the remainder of the family excluded the two coding SNPs as being causative. Analysis of the non-coding SNPs is in progress. We obtained tissue from a thyroid tumor of one of the members of the family. SNP genotyping of tumor DNA did not reveal evidence for extensive loss-of-heterozygosity, particularly in the region of linkage.

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63. Combined urodynamic and morphometric study of the effects of the prepubertal period and the two first estrous cycles on the lower urogenital tract in littermate female Beagle dogs: a pilot study.

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Objective: To compare the values of the urodynamic and vaginourethral measurements obtained during the prepubertal period and during the two first estrous cycles in healthy dogs in order to determine possible functional or anatomical modifications of the lower portion of the urinary tract associated with those phases. **Materials and Methods:** Five littermate female Beagle dogs were included in the study. Urethral pressure profilometry, diuresis cystometry and vaginourethrography were performed at 3.5, 4.5, 5, 6, 7, 8, 8.5 and 9 months of age and during proestrus, estrus, early, mid and late diestrus, and early and late anestrus of each cycle. **Results:** At the end of the prepubertal period, the urodynamic and morphometric measurements increased significantly compared to the earlier times. Bladder capacity was reached at 9 months of age. In all dogs, the bladder was intermittently located in an intrapelvic position during the prepubertal period. Thereafter, the bladder regained an intraabdominal position from 9 months of age until the end of the study. During the two first estrous cycles, the urethral pressure decreased significantly during estrus and early diestrus. The bladder capacity increased significantly during the diestral phase. During proestrus and estrus, the urethral and vaginal lengths were significantly increased compared to the anestral phases. **Conclusions:** In immature and young adult Beagle dogs, the urodynamic and morphometric measurements of the lower urogenital tract are influenced by the growth and the estrous cycle. The age and phases of estrous cycle must be taken into account when interpreting urodynamics and vaginourethrography data.

64. Telemetric investigation of the vesico-urethral function in the bitch.

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Objective: Telemetric urodynamic investigation allows measurement of physiological values in awake and non-restrained animals. The main purposes of the study were the standardization of telemetric urodynamic recordings and the investigation of the effects of various molecules on the vesico-urethral function in dogs. **Materials and Methods:** In six Beagle dogs, diuresis cystometry was performed before surgical implantation of telemetric devices. Continuous telemetric recordings were performed during 1 week. Detrusor pressure (Det P) and the presence of detrusor instability (DI) were recorded. Threshold bladder volume (Vth) and compliance were measured. Thereafter, various molecules (phenylpropanolamine, ephedrine, oestriol, oxybutynine, bethanechol, duloxetine) were administrated during 14 days and 24h telemetric recordings were performed at D0, D1, D7 and D14. **Results:** Vth values recorded by telemetry were significantly lower than values obtained by diuresis cystometry. Repeatability of telemetric measurements was higher during the night than during the day. During the night, frequency of DI decreased and Vth increased. During micturition, the bladder capacity was increased after the administration of both phenylpropanolamine and ephedrine, but furthermore, a decrease in Det P was observed after administration of ephedrine. An increased Det P value was observed after oestriol treatment. Data collected after administration of the other molecules are currently analyzed. **Conclusions:** In dogs, repeatability of nocturnal telemetric recordings allows interpretable results. At this point of the study, it appears that telemetric urodynamic recording is a useful tool for the investigation of the effects of molecules affecting bladder function in dogs.

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65. Comparaison de souches de Staphylococcus aureus isolées de mammites bovines en région Wallonne (Belgique).

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Les mammites bovines à *Staphylococcus aureus* sont considérées comme l'une des pathologies majeures chez les vaches laitières, ayant tendance à évoluer de manière chronique et à engendrer de lourdes pertes économiques. L'objectif de ce projet était d'obtenir un test de prédiction du développement de mammites chroniques à *S. aureus*, via l'analyse des propriétés des souches impliquées. Vingt-trois souches de *S. aureus*, isolées de cas de mammites chez des vaches élevées en Région Wallonne, ont été virulotypées par PCR mettant en évidence 40 gènes de virulence. Différentes propriétés associées à la chronicité de l'infection ont aussi été étudiées. La capacité de survie intracellulaire a été déterminée par des expériences d'invasion de cellules MAC-T. La production de biofilm a été quantifiée par *tissue culture plate*. L'apparition du phénotype *small-colony variant* a été évaluée par comptage après culture en condition sub-inhibitrice de gentamicine. Six souches se sont avérées capable de survie intracellulaire jusqu'à 96 heures. Malgré l'influence du milieu de croissance, la majorité des souches se sont montrées capables de produire du biofilm. Une dizaine de souches se sont également révélées capable de former des SCVs en condition sub-inhibitrice de gentamicine. Cependant, aucune corrélation n'a pu être mise en évidence entre les virulotypes des souches et les différentes propriétés étudiées. Nous envisageons d'élargir le nombre de souches testées afin d'augmenter le nombre de virulotypes représentés. Nous allons également déterminer le sérotype de capsule des souches, ainsi que l'activité du facteur alternatif de transcription *sigB*, deux autres paramètres associés au développement de mammites chroniques.

66. Study of the roles of Cyprinid Herpesvirus 3 ORF134 in the biology of the infection.

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The koi herpesvirus (KHV), also known as cyprinid herpesvirus 3 (CyHV-3) is the aetiological agent of an emerging and lethal disease in common and koi carp. Sequencing of CyHV-3 genome revealed that it encodes a viral homolog of IL-10 (vIL-10). The present study has two goals. The first goal was to determine whether the ORF134 give rise to an expression product (pORF134) secreted by infected cells in the extracellular medium. The second goal consisted to produce CyHV-3 recombinants required to study the roles of ORF134 in the biology of the infection *in vitro* and *in vivo*. Concentrated supernatant of CyHV-3 infected CCB cultures was analyzed by mass spectrometry (MS). MS analyses demonstrated that the presence of pORF134 in the supernatant of CyHV-3 infected cells, this protein being the second most abundant protein (after pORF12). Using a CyHV-3 bacterial artificial chromosome clone, we produced a strain deleted for ORF134 and a revertant strain. Reconstituted recombinant viruses were compared to the wild-type parental strain *in vitro* and *in vivo*. Deletion of ORF134 did not affect the capacity of the virus to replicate *in vitro*, nor to induce CyHV-3 disease in carp comparably to the wild-type parental and the revertant recombinant strains. All strains tested conferred a protective immune response against an homologous lethal challenge. Further studies are required to unravel the roles of ORF134 in the biology of CyHV-3. The presence of ORF134 in the CyHV-3 genome provides an interesting model to investigate the roles of IL-10 homologues in the biology of the infection of an *Alloherpesvirus*.

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67. *Bos taurus* and *Alcelaphine herpesvirus 1* gene expression in malignant catarrhal fever-infected cattle.

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The Maasai people are a nomadic pastoral people from East Africa whose lifestyle centers around livestock herding, sharing their grazing land with wildlife species. During wildebeest (*Connochaetes taurinus*) calving period, nearby cattle can be subject to a fatal lymphoproliferative disease called malignant catarrhal fever (MCF) which has a high economical impact on pastoralists. The causative agent of wildebeest-associated MCF is *Alcelaphine herpesvirus 1* (AlHV-1), a gammaherpesvirus of the rhadinovirus genus. In East Africa, 100% of wildebeest are asymptomatically infected by AlHV-1. Viral agents causing this and other forms of MCF (*e.g. Ovine herpesvirus 2*) show an asymptomatic long-life infection in their natural host and a high virulence leading to high mortality when cross-species transmitted to susceptible hosts. In order to investigate the viral and cellular transcriptomes induced by AlHV-1 associated MCF, we developed a custom-designed microarray to simultaneously characterize viral and cellular transcripts using (*i*) 17,408 tiling probes targeting each strand of the AlHV-1 genome to identify all known and unknown transcripts and (*ii*) 43,603 bovine probes targeting each annotated transcript of the *Bos taurus* genome. This microarray design was first used to characterize the replicative viral transcriptome using Mock- and AlHV-1-infected MDBK cells. This allowed us to identify 73 viral transcripts and their boundaries of which 23 (31.5%) were previously undescribed. The same design was then used to characterize the lymphnodes of Mock- and AlHV-1-infected calves. We thus identified 19 MCF-related viral transcripts including ORF73, of which 8 were previously undescribed and will be further investigated, as well as 225 differentially expressed (DE) cellular genes. These DE genes show interesting patterns of up- and down-regulation which shed a new light on the current knowledge on MCF.

68. Comparison between blood and salivary cortisol levels in horses (Equus caballus) using an ACTH challenge.

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In horses, serum cortisol concentration is considered by searchers as an indirect measurement of stress. However, it includes the free and the transcortin-bounded fractions, and the sampling method is invasive. This is not the case for saliva cortisol, which represents a part of the free cortisol fraction, the biologically active form. The aim of this study was to compare salivary and serum cortisol assays in horses, on a large scale of concentrations using an ACTH stimulation test. In five horses, blood samples were drawn using intravenous catheter. Saliva sampling was made using swab. Cortisol was assayed by a radioimmunoassay. MDL were 0.2nM and 8nM respectively in saliva and serum. Precision and reproducibility of both RIAs were acceptable (CV<10%). Variables normality was assessed (Shapiro-Wilk test). Methods for pooling multiple subject data for linear analysis were applied (Chin-Sang Poon). At rest, cortisol concentrations were 188.81±51.46nM (mean±sd) in serum and 1.19±0.54nM in saliva. Peaks were reached after 96±16.7min in serum (356.98±55.29nM) and after 124±8.9min in saliva (21.79±7.74nM) (Student t test, $p<0.05$). Percents of discharge were also different (225% in serum and 2150% in saliva, Student t test, $p<0.05$). The Pearson correlation coefficient between serum and saliva cortisol concentrations was 0.90 (adjusted $R^2=0.80$; $p<0.001$). The strong link existing between serum and saliva cortisol levels has been estimated by a regression analysis: $F_{serum}=159+56.7\log_e(F_{saliva})$. Reliability of both RIAs and regression founded between serum and salivary cortisol levels permit the validation of saliva sampling as a non invasive technique for cortisol level assessment in horses.

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69. Effects of dietary fibres on greenhouse gas and ammonia emissions associated to gestating sows.

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Usually, gestating sows are restrictedly-fed to prevent excessive body weight gain and fat deposition. However, feed restriction causes sustained feeding motivation resulting in stereotypic behaviour and impairment of animal welfare. High fibre diets (HFD) are known to reduce feeding motivation without impairments of performance but the effects of HFD on emissions of pollutant gases are very few studied. Thus, a study was carried out to compare the effect of two fibre contents (standard diet (STD) with 22% of non-starch polysaccharides (NSP) *vs.* HFD with 44% of NSP) on emissions of ammonia (E-NH_3), nitrous oxide (E-N_2O), methane (E-CH_4) and CO_2-equivalents (E-Eq_{CO2}) associated to gestating sows. Three successive batches of 10 gestating sows were divided into 2 homogeneous groups and randomly allocated to one of two treatments: STD *vs.* HFD. The groups were kept separately in two identical rooms equipped with a pen divided into a lying area with slatted floor and five individual feeding stalls with permanent access. Emissions were measured by infrared photoacoustic detection. The HFD significantly decreased E-NH_3 (12.1 vs. 15.9 g/sow.day) but increased E-Eq_{CO2} (0.68 vs. 0.47 kg/sow.day) in relation with an increase of E-CH_4 (18.4 vs. 9.1 g/sow.day), E-N_2O being not impacted by the diet, with value around 0.60 g/sow.day. So, the effects of HFD offered to gestating sows on slatted floor on environment seem conflicting with a decrease of NH_3 emissions which mainly contribute to acidification of soils and waters and eutrophication, but an increase of CO_2-quivalents emissions which contribute to greenhouse effect and climate change.

70. Is neutrophil elastase associated with myeloperoxidase concentration and post-thawing parameters in equine frozen semen?

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A negative relation is described between post-thaw semen quality and myeloperoxidase, an enzyme released by neutrophils. Elastase, another neutrophil enzyme, is a genital inflammation marker in human. Experiments had three aims: measure elastase concentration in post-thaw semen; identify associations between non-sperm cells, MPO and elastase concentrations; associate these factors to post-thaw quality. Twenty good quality ejaculates were used. In fresh semen, non-sperm cells per spermatozoa ratio was determined on smears and non-sperm cells concentration was obtained by multiplying ratio by spermatozoa concentration. Semen was frozen according to usual protocol. Post-thaw progressive motility was determined by computer. Post-thaw MPO and Elastase concentrations were assayed with ELISA. Mean post-thaw progressive motility was 21.524±12.164%. Mean MPO and Elastase concentrations were 29.449±56.859µg/ml and 3.818±3.563ng/ml respectively. Unlike Elastase, MPO concentration was higher ($p=0.048$) in unfreezable semen (progressive motility <15%). Using progressive motility <25% as threshold, unfreezable semen had higher non-sperm cells concentration ($p=0.038$). Linear correlation ($p=0.004$; $R^2=0.369$) was found between fresh semen non-sperm cells and post-thaw MPO concentrations. No correlation between post-thaw MPO and elastase concentrations was observed. Relation between fresh semen non-sperm cells and post-thaw MPO concentrations is surprising as epithelial cells but no neutrophils were present. Post-thaw neutrophil elastase concentration in semen was lower than in healthy horses plasma. Although the same neutrophil granules normally release both enzymes, MPO and elastase post-thaw concentrations were not correlated. Fresh semen non-sperm cells and MPO concentrations were higher in unfreezable semen. Elastase is not associated with non-sperm cells and is not correlated to semen freezability.

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71. Multiple congenital ocular anomalies syndrome in a family of shetland ponies in Belgium.

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Purpose: To describe the phenotypic and genotypic characteristics of the equine Multiple Congenital Ocular Anomalies (MCOA) syndrome in a family of Shetland ponies, including one stallion, one mare and a female offspring. The fourth pony descended from the same mare, but from a different sire. **Methods:** Complete ophthalmic examination, including tonometry, ocular ultrasonography (12 Mhz) and genotyping for the silver coat colour were performed. **Results:** The stallion had minor ocular abnormalities: thickened temporal iris, supero-temporal retinal atrophy and an iridociliary or peripheral retinal cyst. All three mares presented a wide range of more severe anomalies in both eyes: cornea globosa, peripheral temporal iridocorneal goniosynechiae, abnormal pectinate ligaments and poorly responsive pupils to light and to medically induced mydriasis. Pupils were miotic and dyscoric, with flattened, hypoplastic granula iridica encircling the pupillary ruff. Iris stromal hypoplasia and focal anterior cortical cataracts were detected in two mares. Bilateral lens luxation was present in the dam only. Temporal iridociliary and/or peripheral retinal cysts were confirmed ultrasonographically in all ponies. Two females also presented temporal peripheral retinal atrophy. Tonometry was within normal limits in all ponies. The stallion with black silver coat colour was heterozygous for the PMEL17 mutations (Zz). All females were homozygous (ZZ), and had a brown silver (two) and a pearl silver (one) coat colour. **Conclusions:** In this family of Shetland ponies bred for the Silver coat colour, both the heterozygous Cyst phenotype and the MCOA homozygous phenotype are described.

72. Pectinate ligament dyplasia and narrowing of the iridocorneal angle in the Jack Russell and Parson Russell Terrier breeds in Belgium

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Purpose: To describe the incidence of pectinate ligament dysplasia (PLD) and narrowing of the iridocorneal angle (NICA), and their relationship to glaucoma, in glaucomatous and clinically normal Jack Russell (JRT) and Parson Russell Terrier (PRT) dogs in Belgium. **Methods**: Complete ophthalmic examination, including tonometry and gonioscopy, was performed in 123 normal dogs (58 JRT, 65 PRT), and 17 dogs with glaucoma. The opening of the ICA was inspected by slit-lamp biomicroscopy, and the width was subjectively graded as open, narrow or closed. PLD was graded as follows: 0: normal, 1: < 25%, 2: 25 - 50%, 3: 50 - 75% and 4: > 75% of the examined circumference of the ICA presenting abnormal PL. **Results**: The 123 normal dogs included 65 females and 58 males with a mean age of 5.9 ± 3.4 years. PLD and/or NICA were detected in 43.9% of the normal dogs; this was 26/58 JRT and 28/65 PRT. The PLD and/or NICA dogs were affected bilaterally in 61.1% of cases. In dogs with a narrow ICA, fibrae latae and short laminae of pectinate ligaments were commonly found. Severe PLD and abnormal ICA were observed simultaneously in 14.7% of dogs (15 PRT, three JRT). Out of the 17 dogs with glaucoma, ten dogs (all JRT) were diagnosed with primary glaucoma associated with PLD and/or NICA. Their mean age was 5.9 ± 3.5 years. **Conclusions**: This study suggests that the incidence of NICA and PLD is high in JRT and PRT dogs in Belgium, possibly predisposing them to glaucoma.

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73. Cyprinid herpesvirus 3 induces behavioral fever in common carp (*Cyprinus carpio* L.).

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The roles of many factors that influence resistance of the host against pathogens are still largely unknown in fish. Temperature is recognized as one of the most important environmental factor controlling various life processes of fish, including immune functions. Since fish cannot rely on endogenous thermoregulatory mechanisms to control their body temperature, they mainly use behavioral thermoregulation. This process is based on active selection of a given temperature to optimize the metabolic processes in heterogeneous thermal habitats. Also, fever in fish is based on a behavior modification stimulating the individual to reside in a warmer environment, hence is named “behavioral fever”. Cyprinid herpesvirus 3 (CyHV-3) is the causative agent of a lethal disease in common and koi carp. We studied the effect of temperature on host-pathogen interactions using the carp - CyHV-3 model, both *in vitro* and *in vivo*. Our *in vitro* study indicates that CyHV-3 can penetrate CCB cells in the temperature range of 4°C-37°C and that virus can replicate at 15°C, 25°C, 30°C but not at 32°C. Next, using a special multi-chamber aquaria with temperature gradient and informatic system to monitor the position of the fish and water temperature we showed that CyHV-3-infected carps migrate during the course of infection to the compartment with higher temperature of water (34°C) which resulted in stopping of the disease. To the best of our knowledge, this is the first study that demonstrated the role of behavioral fever in fish for its ability to enhance the host resistance against a viral infection.

74. PTX3: A New Marker for Local Inflammation?

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Background: The long pentaxin 3 (PTX3) is a recently discovered soluble protein of the acute phase of inflammation. Contrarily to short pentraxins (*e.g.* CRP, SAP), it is produced at the site of inflammation. **Objective:** The purpose of this study was to evaluate PTX3 production in the respiratory tract, in response to an inflammatory challenge in horses. **Methods:** Two groups of six horses, healthy or affected by heaves, were submitted to a 10 days hay dust challenge. At days zero and ten, clinical and functional parameters were evaluated, broncho-alveolar lavages (BAL) were retrieved and BAL cell counts were performed. PTX3 released was evaluated by western blotting in the BAL supernatant. In addition, bronchial sections were obtained from two heaves-affected horses in crisis and two healthy horses. PTX3 production by the different cell types was evaluated by immuno-histochemistry. **Results:** In the healthy group, a slight inflammation occurred in the respiratory tract (assessed by functional tests and BAL cell counts) with no clinical signs. In the heaves-affected group, an important inflammatory response developed with the typical clinical signs of heaves. Following dust exposure, PTX3 was increased in both groups but the level of PTX3 detected in the supernatant of heaves-affected horses was markedly higher. The immuno-histochemistry revealed that macrophages and bronchial epithelial cells were the major cellular sources of PTX3 in the airways. Moreover, epithelial cells of heaves-affected horses contained higher level of PTX3 than those of healthy horses. **Conclusion:** PTX3 level in BAL supernatant could be a good sensor of airway inflammation, even without the apparition of clinical signs. Its level of excretion could help evaluating the severity of inflammation.

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75. Muscular forces affecting the outcome of CCL surgery.

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Introduction: To evaluate the effect of preload in a quadriceps (PLQ) tendon model in the stability of cranial cruciate ligament deficient stifles and the influence of the tibial plateau angle (TPA) on it. **Materials and methods:** Specimens: Six hind limbs from adult dogs, weighting 19-33 kg, euthanized for reasons unrelated to the study. The Quadriceps muscle was replaced by a tension band instrumented with a strain gage (Sensy, 150 DN) and a turn-buckle to adjust the tension in the cable. The limbs were placed in a press (Instron 3366, 10 KN) in a neutral position. Test 1: With a pre-tension between 2 and 5 N systematically put into the Quadriceps tension band, limbs were loaded at 30% of body weight (BW). Test 2: After transaction of the cranial cruciate ligament, measures were then repeated again at the same 30% of BW load. Upon loading, cranial displacement of the tibia was documented by radiographs. Test 3: Pre-tensions (10 - 90 N) to the Quadriceps band and loading process repeated as in test 2. Statistical analysis: A multinomial logistic model with cumulative logits was used to assess the effect of PLQ and TPA on the occurrence of a cranial drawer sign (CDS). **Results:** Preload (PLQ) was found to have a significant effect ($p=0.032$) on the occurrence of CDS, with higher levels of preload leaded to lower prevalence of CDS. TPA was also significantly ($p=0.0016$) associated to CDS, with higher values of TPA more likely to cause CDS.

76. Etude du rôle des facteurs de transcription c-Maf et MafB dans la différenciation et la fonction des macrophages régulateurs: impact sur l'homéostasie immune mucosale.

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Les macrophages sont des cellules hétérogènes aux rôles clés à la fois dans l'immunité innée et adaptative. Ainsi, notre laboratoire a récemment identifié un sous-type de macrophages pulmonaires, les macrophages interstitiels (MIs), impliqué dans la prévention des réponses inadaptées aux aéroantigènes environnementaux. Ces MIs présentent une analogie phénotypique et fonctionnelle frappante avec les macrophages de la lamina propria intestinale (MLPs), qui joueraient un rôle important dans l'homéostasie immune intestinale. Ceci suggère que ces « macrophages régulateurs » pourraient voir leur différenciation et fonctions régies par des mécanismes similaires. Nous avons identifié deux facteurs de transcription dont l'expression est spécifique des MIs et des MLPs: c-Maf et MafB. Plusieurs éléments suggèrent que ces facteurs pourraient être impliqués dans la différenciation terminale des macrophages en macrophages régulateurs. Comme actuellement aucun outil ne permet leur étude *in vivo*, nous avons entrepris de générer des souris conditionnellement déficientes en *maf*et/ou *mafb*dans la lignée myéloïde. Ces modèles murins nous permettront d'investiguer (1) les rôles de c-Maf et de MafB dans la différenciation et la fonction des « macrophages régulateurs » et (2) les conséquences d'une délétion de ces facteurs sur l'homéostasie immune pulmonaire et intestinale. Ce projet devrait permettre une meilleure compréhension des mécanismes de l'homéostasie immune mucosale dont la dérégulation conduit à des maladies humaines fréquentes telles que l'asthme et la maladie de Crohn.

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77. First clinical trial for the biological control of psoroptic mange in cattle using entomopathogenic fungi.

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Psoroptes ovis is responsible for a severe dermatitis in sheep, cattle, goats and rabbits. Entomopathogenic fungi could represent an alternative to chemical acaricides. In sheep *in vivo* trials were so far unsuccessful despite a high *in vitro* efficacy. The low density of mites, the presence of toxic components in the fleece, high local temperatures and inappropriate formulation could explain these failures. In this study a field trial was conducted with two hydrophilic formulations of *Metarhizium anisopliae* in *P. ovis* naturally infected cattle.

M. anisopliae IHEM 18027 was cultures on SDAY at 27°C. Mass production was performed according to Jenkins et al. (1998). Two hydrophilic excipients were used. Two groups of 8 naturally infected cattle were formed. Each group was sprayed with one litre of the excipient alone or the biopesticide (4 animals/formulation – 3 applications at 10 day intervals). Clinical and parasitological indices were monitored every 10 days from day 0 until day 40. Samples of hair were collected for fungal cultures. Body weights were evaluated on Days 10, 30 and 60 PT. Data were analysed with SAS.

There was a rapid worsening of the clinical condition of the control animals and because of welfare concern two had to be treated during the trial with injectable ivermectin. At trial end 5 control animals were still positive for *P. ovis* ($P<0.005$). In contrast, the condition of treated animals improved markedly and only one animal was still positive at trial end. Clinical and parasitological indices were significantly different ($P<0.001$). Most of the control animals lost weight whereas treated ones took weight. Live conidia of *M. anisopliae* could be isolated from hair up to 60 days PT.

M. anosopliae was successfully used in two hydrophilic formulations for the control of naturally acquired psoroptic mange in cattle.

78. Psychrotrophic and psycrophilic *Clostridium* responsible for meat spoilage.

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Worldwide meat exchanges led to a generalized application of conservation techniques such as chilling and vacuum-packaging. These two techniques, often combined, result in an increased shelf life as aerobic and mesophilic flora are inhibited. However, spoilage of chilled vacuum-packaged meat is an increasingly reported problem. This spoilage phenomenon, referred to as "blown pack spoilage" is caused by different species of psychrophilic and psycrothrophic *Clostridium*, namely *Cl. estertheticum* and *Cl. gasigenes*. Because these are environmental bacteria, meat contamination takes place at the slaughterhouse, due to cross contamination with faeces or hides. The aim of our study was to determine whether these bacteria are present in Belgian slaughterhouses and if so, to determine the prevalence. One hundred and seven faecal samples from cattle, collected at one Belgian slaughterhouse, were analysed. An enrichment step of 3 weeks at 8°C is necessary as a selection step, since there are no selective culture media. The enrichment is followed by isolation of *Clostridium*-like colonies. Selected colonies are incubated at 37°C to exclude psychrotolerant strains and at 22°C and 8°C to classify as psychrotrophic or psychrophilic. Finally, an identification of the isolated colonies is done by PCR. *Cl. estertheticum* and *Cl. gasigenes* were found in 13,1% of the faecal samples, 8,4% and 4,7% respectively. However, other psychrotrophic/psychrophilic *Clostridium* were isolated but did not belong to any of the two species. Further studies will be conducted in order to identify these colonies but also to compare strains identified as *Cl. estertheticum* or *Cl. gasigenes*.

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79. *Clostridium difficile* in farm and slaughterhouse animals: prevalence and typing.

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Clostridium difficile is an anaerobic gram-positive spore-forming bacillus identified as a severe pathogenic agent in animals and humans. Its recent isolation in foods opens up the occurrence of alimentary origin infections. The objective of the study is to establish if there is a reservoir of bacteria in animals engaged to human feed and consequent true risk of transmission to humans through the food chain. A total of 437 fecal samples were analyzed. Stools (202 from beef and 194 from pigs) were collected at the slaughterhouse and from breeding farms (18 from calves and 23 from piglets). Enrichment culture was carried out using a cycloserine cefoxitin fructose taurocholate broth. Isolates were identified by PCR detection of *tpi, tcdA*, *tcdB* and *cdtA* genes. Toxic activity was confirmed by a fecal cytotoxin inmmunoessay. Further characterization was performed by PCR ribotyping. *Clostridium difficile* was cultured from 8% (35/437) of the total samples: 14 from beef at slaughterhouse (6,9%), 17 from piglets (73,9%) and 4 from calves (22,2%) at breeding farms. Thirty-two of the total strains were toxin positive. Sixteen different PCR ribotypes were identified with type 078 predominant in breeding farms of calves (75%) and pigs (64,7%). Isolates from beef presented widest range in ribotypical variety. Present study proves the presence of *Clostridium difficile* in animals engaged to food in Belgium. The presence of the bacteria at the slaughterhouse and the high prevalence of pathogenic strains states a true risk of contamination to humans through the food chain.

80. Does feeding dairy cattle with different levels of condensed distillers solubles (Protiwanze®) increase the risk of SARA?

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Introduction: The objective of this study was to evaluate the effects of different levels of Protiwanze® (PW) supplementation, a highly acid (pH = 3.8 ± 0.8) and fermentescible CDS, on ruminal function of dairy cows. **Material and Methods**: PW supplementation was tested in 4 dairy herds (144 cows, DIM: 96 ± 61, daily milk production: 34.69 ± 8.22 L). In Herd 1, TMR was supplemented with 0% or 10% of PW on a dry matter basis for 4 weeks including a 7-day transition period. Each concentration was distributed twice during 2 periods alternatively with the other one, each cow being its own control. PW concentrations were 10 and 15% in Herd 2, 3 and 4. During every period, milk production was measured by the Dairy Herd Improvement and ruminal fluid sampled by a stomach tube (Ruminator®) on 5 cows. Samples were assessed for pH (portable pH meter), redox potential (Methylene blue test) and protozoa (optical microscopy). **Results and Conclusion**: Ruminal pH values ranged between 5.94 and 7.74. Even when a correction factor of 0.5 was applied to take into account possible saliva contamination, only 8 pH samples pleaded for SARA although protozoa and methylene blue tests were within norms and cows clinically normal. No significant correlation between pH value, milk production and fat content could be demonstrated. Ruminal pH did neither significantly differ between the different levels of PW supplementation. In conclusion, in this study, PW could be used in dairy cows TMR at a level as high as 15% without increasing the risk of SARA.

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81. Does increasing level of condensed distillers solubles (Protiwanze®) supplementation affect milk production in dairy cattle?

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Introduction: The objective of this study was to evaluate whether introducing the condensed distillers solubles Protiwanze® (PW) at different concentrations in dairy cows diet induces changes in milk production. **Material and Methods**: Three herds (72 Holstein and 25 Brown Swiss, DIM = 101 ± 57; daily milk production = 34.90 ± 8.40 L) received successively two RTM differing in PW content for 4 weeks, including a 7-day transition: 0% and 10% on a dry matter basis for Herd 1 and 10% and 15% for Herd 2 and 3. Both concentrations were distributed alternatively and repeated twice, each cow being its own control. Rations were formulated to meet energy and nitrogen requirements. During each testing period, animals were scored (Body Condition, Rumen Fill, Fecal Consistency, Undigested Fraction according to Zaaier (2001)) and their milk production and composition were measured by the Dairy Herd Improvement. **Results and Conclusion**: Increasing the level of PW did not significantly change milk production in any Herd: Values were for Herd 1 at PW0%: 33.08 ± 0.76 L, at 10%: 31.66 ± 0.68 L, for Herd 2 at PW10%: 32.85 ± 0.93 L and at 15%: 32.37 ± 0.89, for Herd 3 at PW10%: 28.71 ± 1.21 L and at 15%: 31.80 ± 1.16. In Herd 1, milk urea decreased from 243.80 ± 4.55 mg/L for PW0% to 218.05 ± 4.03 mg/L for PW10%, ($p<0.01$), while fat percentage increased from 3.38% ± 0.09 (PW0%) to 3.77% ± 0.08 (PW10%) ($p<0.01$). Although increasing level of PW in dairy cows diet did not change milk production or composition, it is profitable since PW is 30-40% cheaper than other protein supplements like soya and canola meals. Moreover, it allowed to lower milk cost price by 1,5 eurocent per liter, when used at higher concentration.

82. Susceptibility of *Cyprinus carpio* to Cyprinid Herpesvirus 3 at the early stages of life.

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Cyprinid herpesvirus 3 (CyHV-3) is the causative agent of a lethal disease in common and koï carps. The aim of the present study was to investigate the sensitivity and the permissivity of common carp (*Cyprinus carpio*) to the CyHV-3 infection from hatching to juvenile stage using in vivo imaging system. To reach this goal, we used a CyHV-3 recombinant strain expressing firefly luciferase (LUC) as reporter gene. Eight groups of 100 larvae were infected by immersion in water containing the virus every two days from hatching (day 0) to day 10. Two groups were infected during the juvenile period at day 21 and 35, respectively. 24 and 72 h after infection, 10 fish per group were injected with luciferin (the substrate for luciferase) under anesthesia, using a microinjector FemtoJet, then analyzed by bioluminescence imaging. For each group, the remaining 80 larvae were kept and the survival rate was measured at 15 and 30 days post-infection. The results demonstrated that independently of the day of infection, some larvae exhibited positive signals as soon as 24 h post-infection. Analyses performed 72 h post-infection revealed higher number of positive larvae than those performed at 24 h post-infection. Depending on the age of infection, we observed a progressive increase of fish sensitivity to CyHV-3 infection. Concerning mortalities, we observed different kinetics of mortality in larvae and juvenile stages. In conclusion, the present study demonstrates that carp larvae are susceptible to CyHV-3 infection immediately after hatching and that their sensitivity increase with the developmental stage.

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83. Time trends of blood leucocytes, neutrophils and plasmatic myeloperoxidase in the perioperative period of horses undergoing colic surgery.

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Reasons for performing the study: Neutrophilic activation and degranulation may play a key role in the complications of equine surgical colic cases. **Objective:** 1) To determine changes in blood leukocyte/ neutrophil counts and in plasmatic myeloperoxidase (MPO) concentrations. 2) To relate these changes to the location of the pathology, the degree (severity) of complication and to the outcome of surgical colic cases. **Methods**: Total leukocyte and neutrophil counts were performed in 52 horses undergoing colic surgery while plasmatic MPO levels were determined in 16 of them before and during surgery and then every 4 to 12 hours. A mixed model was used to analyse the time trends (TT) of the 3 parameters. **Results**: TT for leukocytes and neutrophils were similar and significantly different from the MPO TT, which increased during the first hours, while the neutrophil TT decreased immediately. The TT of neutrophils was higher in large than in small intestinal pathologies and the TT of MPO was lower in large than in small intestinal pathologies. The TT of neutrophils were significantly different between the degrees of complications. The TT of MPO was lower in survivors. The TT of neutrophils in survivors was higher during the first 4 days thereafter it became lower than in non survivors. **Conclusion:** These results confirm that neutrophil counts and MPO concentrations undergo timely changes and that they are related to the severity of the inflammatory reaction in surgical colic cases. Knowing the kinetics of these parameters will be helpful to determine predictive cut-off values.

84. Morphology of the suspensory ligament (interosseous muscle III) of the horse.

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Introduction: The injuries of the suspensory ligament (SL) are important causes of lameness and financial losses in the equine industry. Ultrasound permitted to examine some parts of the SL but "anomalies" are regularly observed. Their significance is not known. Until now, few studies described the relationship between the ultrasonographic appearance and the exact morphology in histological sections. The aim of this study is to develop good techniques for cutting and staining the SL and to improve knowledge about the normal morphology of the SL. **Methods**: In this study, the SL of eight <sound> horses were collected. The body of the SL was divided in 3 thirds and sampling was realised within each third and between the thirds. The samples were embedded in paraffin or in Tissue-Tek for cryosections. The sections were stained with hematoxylin/eosin or Masson's trichrome. For 3 SL, ultrasounds were performed before sampling. The digital tip was maintained in physiological position owing to a press. **Results**: Most of the paraffin sections were shredded because of the hardness of the tissue. Cryosection revealed a better preservation of tissues. Only some freezing artifacts (holes) appeared on a few sections. Muscles fibers surrounded by adipose tissue containing blood vessels were present mainly in the proximal and medium third of the SL whereas they were not found in the distal third. The remaining structure look like a tendon and was composed of collagen fibers, stained in green with the Masson's trichrome coloration. **Conclusions**: This study permitted to develop cutting and staining techniques for the SL and helped to map the adipose, muscular and tendinous parts within the SL. It lays down the bases of subsequent studies that will concern ultrasonographically examined digital tips of sound and pathological horses of different breeds and ages.

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85. Knocking the *CLPG* mutation in the mouse genome partially recapitulates the callipyge phenomenon.

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In sheep, the *CLPG* mutation causes the callipyge muscular hypertrophy characterized by a unique mode of inheritance, known as polar overdominance, in which only heterozygous animals receiving the *CLPG* mutation from their father express the phenotype. To generate a more tractable system to study this unique biological phenomenon, we have used homologous recombination to introduce the *CLPG* A→G mutation, as well as a deletion of the affected dodecamer motif, in the orthologous position in the *Dlk1-Gtl2* domain of the mouse. We show that both mutations recapitulate part of the sheep phenomenology. More specifically, maternal inheritance of the mutations boosts the expression of the non-coding *Gtl2, Rian* and *Mirg* RNAs in postnatal skeletal muscle, and enhances intergenic transcription in the 90 Kb interval separating *Dlk1* and *Gtl2.* However, paternal inheritance of the mutations enhances expression of one of the paternally expressed genes, *Rtl1,* but not *Dlk1* nor intergenic transcripts in *cis*. Contrary to the situation in sheep, we observed no hypomethylating effect on the immediate vicinity of the *CLPG* mutation, neither on maternal nor on paternal transmission. While these results confirm the causality of the *CLPG* mutation and demonstrate that it invalidates a muscle-specific silencer element, they preclude the use of the mouse as a model to study polar overdominance.

86. The use of B-mode ultrasonography to investigate uterine involution in lactating sows.

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The present study investigated the uterine involution in 709 lactating sows during the first 4 weeks postpartum using trans-abdominal B-mode ultrasonography. The specific objectives were to detect possible uterine abnormalities and to assess possible associations between 3 uterine parameters (maximum uterine height, the diameter of the uterine horns and the presence of intraluminal uterine fluid) and the reproductive performance of the sows after weaning (weaning-to-first service interval, pregnancy rate and total number of piglets born). From D2 to D28 postpartum, the uterine height and diameter of the horns decreased from 11.8 ± 0.1 to 5.5 ± 0.3 cm, and from 3.2 ± 0.1 to 1.4 ± 0.0 cm ($p<0.0001$), respectively. Intraluminal fluid was observed in 81% (D2) and 16% (D28) of the sows ($p<0.0001$). The parity of the sows had a significant effect on uterine height ($p=0.03$), horns diameter ($p=0.005$) and intraluminal fluid ($p<0.0001$). The diameter of the uterine horns was smaller in sows showing oestrus within 5 days after weaning compared to sows not showing oestrus within that time (1.42 ± 0.01 *vs*. 1.50 ± 0.03 cm; $p=0.03$). No significant associations were found between the other uterine parameters and reproductive performance post weaning. Ultrasonography performed during lactation allowed to detect metritis and even foetoplacental retention in 2% of the sows. In conclusion, B-mode ultrasonography before weaning allows the detection of uterine infection, early treatment or culling decision and so potentially reduces non productive days.

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87. The use of trans-abdominal B-mode ultrasonography to assess uterine involution in sows: validation of the technique.

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The present study was performed on 20 cull sows. The day before slaughter, a trans-abdominal B-mode ultrasonography was performed to measure three uterine parameters: the maximum uterine height, the diameter of the uterine horns and the presence of intra-luminal uterine fluid. After slaughtering, uterine volume, uterine weight, and the length and width of horns were measured. The objective was to validate ultrasonography as a technique to assess uterine involution by comparing post-mortem measures and live animal ultrasonography measures recorded on-farm. The maximum uterine height (8.2 ± 1.3 cm) was positively correlated with uterine weight (1467 ± 696 g), uterine volume (1467 ± 688 ml), and width of horns (3.8 ± 1.1 cm) ($p<0.05$). The diameter of the uterine horns (1.5 ± 0.7 cm) was positively correlated with uterine weight and uterine volume ($p<0.01$) and with width of horns ($p<0.001$). These results, though performed on a small number of animals ($n=20$), suggest that ultrasonography may be a useful technique in assessing uterine involution in sows.

88. Expression of the cellular prion protein on follicular dendritic cells from ovine pharyngeal tonsils.

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Under natural conditions, ovine scrapie results from an oral contamination as suggested by a primary replication site of the infectious agent in the gut ileal segment. Experimental infections have shown the efficiency of other potential routes. Sheep and hamsters intra-nasally inoculated with scrapie did develop the disease and PrPd was mainly observed in pharyngeal tonsils, revealing this organ as a probable site of entry for the scrapie agent. Our study analysed a possible involvement of the follicular dendritic cell (FDC) network in replication of the scrapie agent in the ovine nasopharyngeal mucosa. Pharyngeal tonsils obtained from healthy sheep were cut in 1 mm thick slices. After dissection of the connective and epithelial tissues, FDC clusters were isolated from lymphoid follicles by enzymatic digestion and sedimentation gradient. Expression of the cellular prion protein (PrPc) on FDC was analysed with the mouse mAb Pri909, using first optical microscopy with an immunoperoxidase labelling. Data have been confirmed using electron microscopy on ultrathin sections with a mAb Pri909 immunogold staining. Clusters were composed of 1 or 2 FDC engulfing 8 to 22 lymphocytes. Following immunoperoxidase labelling, PrPc was located on membrane extensions wrapped around lymphocytes. At a higher magnification, electron microscopy showed that gold particles were truly localised on FDC dendritic evaginations. Our results demonstrate that FDC from ovine pharyngeal tonsils express PrPc on their cytoplasmic membrane, as FDC from other lymphoid organs permissive to PrPd accumulation during scrapie infection. These data support the hypothesis that pharyngeal tonsils might be involved in scrapie pathogenesis.

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89. Determination of trifluralin residues in cultured catfish and water.

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In the year 2010, many Vietnamese catfish and shrimp products exported to EU and Japan were found contaminated with trifluralin, an herbicide banned in EU since 2008. Trifluralin was then placed on the list of banned chemicals for aquaculture in Viet Nam, and the determination of trifluralin residues has become of more concern in seafood products. The study was carried out in order to optimize the method for determination of trifluralin residues by GC-ECD in fish muscle and water, and the withdraw period of trifluralin in Vietmanese catfish. The study contained two experiments. In the first experiment, extraction method and GC-ECD parameters were optimized. In the second experiment, trifluralin was added in fish tanks at two levels: 25 ng/mL and 50 ng/mL. Each treatment was repeated in triplicate in three tanks. Each tank (500L) contained 50 fish (20-25g per fish). The contamination was repeated one more time after 1 day. Merin (Trifluralin 48%, Thailand) was used as the spiking compound in experimental tanks. Sampling was performed in triplicate at different times during the contamination and decontamination periods for fish samples and water samples. Generally, the concentration of trifluralin in water rapidly decreased i.e. lower than 5% of the spiking concentration after 6 hours of the second application and lower than the LOD after 3 days of the second application. However, the decontamination of trifluralin in fish muscle was slower. In the period between 6 hours and 30 days after the second application, the concentration of trifluralin in catfish muscle dropped from 175±17 ng/g (25 ng/mL treatment) and 584±58 ng/g (50 ng/mL treatment) to 36±2 ng/g and 46±17 ng/g, respectively.

90. Increased HIF-1α activity in lung cells of horses with recurrent airway obstruction.

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Recurrent airway obstruction (RAO, also known as equine heaves) is an inflammatory condition with similarities to human asthma caused by exposure of susceptible horses to organic dusts in hay. The disease is characterized by neutrophilic airway inflammation, mucus hypersecretion and reversible bronchoconstriction. The immunological processes responsible for the development and the persistence of airway inflammation are still largely unknown. In recent years, most research focused on the role of type 1 and type 2 adaptive immunity in the pathogenesis of RAO. So far, few studies have addressed the role played by innate immunity, which is a central interface between external stimuli and the adaptive immune system. Hypoxia-inducible factor (HIF) is a major regulator of energy homeostasis and cellular adaptation to hypoxia. Recently, it has been shown that HIF also has major roles in promoting pro-inflammatory and bactericidal functions in innate immune cells. Conditional genetic ablation of its major subunit HIF-1α in the myeloid lineage notably results in decreased inflammatory responses in classical models of acute inflammation in mice. Here, we aimed at investigating the potential involvement of HIF-1α in myeloid cells in horse recurrent airway obstruction. We confirmed that HIF is present in equine myeloid cells and regulates similar genes as in human cells. We further showed that airway challenge with hay dust upregulated HIF-1α mRNA expression in myeloid cells from the bronchoalveolar lavage fluid (BALF) of healthy and RAO horses, with a more pronounced effect in cells from RAO horses. This increase in HIF-1α mRNA expression in myeloid cells positively correlated with mRNA expression of the main cytokines controlled by HIF, supporting its functional activation. Most interestingly, HIF-1α mRNA expression in BALF cells correlated positively with lung dysfunction. Taken together, these results indicate that activation of HIF-1α in myeloid cells could play a role in exacerbation episodes in RAO-affected horses.

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91. Gestion des ressources génétiques animales par les éleveurs de dromadaires de la région d'Ansongo (Mali).

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Une enquête a été réalisée auprès des éleveurs de dromadaires de la Région d'Ansongo (Mali) afin d'identifier les pratiques, contraintes et stratégies dans la gestion de leurs troupeaux. L'établissement d'une typologie sur base de ces résultats constituera la base d'un travail ultérieur d'évaluation de la productivité et des possibilités d'évolution de ces systèmes d'élevage. L'enquête a en outre porté sur la logique des éleveurs concernant la gestion des ressources génétiques animales en lien avec leur stratégie de mobilité. Les pratiques de gestion étaient variables au sein de la région, bien que généralement marquées par la marginalisation, avec un faible soutien alimentaire et vétérinaire des troupeaux. Différents types de mobilité étaient pratiqués, dont les motivations et les modes décisionnels au sein du ménage ont été abordés par l'enquête. Les nomenclatures des types de dromadaires différaient entre communes, les noms pouvant se référer à la couleur du pelage, la conformation ou certaines particularités. La sélection des mâles reproducteurs était pratiquée au sein du troupeau. Les critères les plus cités étaient la beauté et la production de lait. Ces résultats illustrent la difficulté de l'étude de la diversité génétique cameline ainsi que d'accorder les conceptions de cette variabilité dans l'optique de la mise en place de plans de sélection participatifs, tenant compte de la multifonctionnalité de l'animal, de son inclusion dans un portefeuille d'espèces et de la stratégie générale de mobilité. Afin de progresser sur cette voie, l'animation de groupes de discussion entre éleveurs au sein et entre communes est à envisager.

92. Measurement of thoracic lordosis in pigs using the centroid *vs.* the Cobb method.

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Lordosis with kyphosis (also known as "humpy-back" or "dipped shoulder") is sporadically observed in growing pigs. This condition is characterized by a thoracic spinal deformity with degeneration in the fibro-cartilage of the intervertebral discs. A significant effect of the sire on the incidence of lordosis leads us to suspect an inherited predisposition. To test this hypothesis we mated affected individuals, reasoning that this should increase the incidence and/or severity of the condition – if indeed heritable. To distinguish normal and affected pigs, lateral spinal radiographs were performed under general anaesthesia. In a previous study, thoracic vertebrae 5 (T5) and 8 (T8) were identified in order to measure the degree of spinal deformity using the Cobb method. Further data led us to prefer the centroid method for the measurement of thoracic lordosis.

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93. Evolution of the mammalian pseudoautosomal boundary (PAB).

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As part of the characterization of the bovine Y chromosome to identify suitable targeting sites we previously defined the bovine PAB (Van Laere et al., 2008). We showed that it potentially coincided with the limit between strata III and IV of the human X chromosome, suggesting that the organization of the bovine sex chromosome might reflect an ancestral intermediary state in the evolution of the human sex chromosomes. To gain additional understanding in the evolution of the mammalian sex chromosomes we have (i) positioned the PAB for armadillo, cat, dog, dolphin, hedgehog and pig (ii) determined the PAB sequence for cat, dog and pig and (iii) sequenced Y-specific BACs encompassing the PAB in cat, dog and pig.

94. High-resolution respirometry: a new method to perform bioenergetic studies in horses.

Votion D.M.[1,2*], Mouithys-Mickalad A.[3], Caudron I.[2], Niesten A.[3], Ceusters J.[3], Gnaiger E.[4,] Franck T.[1,3] and Serteyn D.[1,2,3]

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Oxidative phosphorylation (OXPHOS) is the dominant pathway for providing energy during aerobic exercise and for recovery after anaerobic exercise. Thus, mitochondria play a central role in muscles energetics in exercising horses and any mitochondrial dysfunction may induce exercise intolerance and/ or myopathies.
In search for a minimal-invasive procedure to investigate muscle bioenergetics in horses, we have introduced the microbiopsy technique to collect muscle samples in horses and designed specific protocols to study mitochondrial physiology in small (<2.5 mg) permeabilized muscle samples with high-resolution respirometry (HRR). Since the introduction of this technique in the equine field, we have measured mitochondrial respiratory control and capacity in muscle biopsies of healthy horses (*N* = 117) trained for different equestrian disciplines and myopathic horses (*N* = 25). Together with an increased OXPHOS capacity, training induced specific modifications at the level of mitochondrial complexes. Recently, top-trained horses from the French national team of endurance and trotters in track training were sampled by microbiopsy. Their OXPHOS capacities were compared to their subsequent performance results: horses with the highest capacities had the best results in their respective events. Likewise, low respiratory capacities predicted poor performance and even detected yet-unseen pathology in one trotter which experienced several episodes of rhabdomyolysis during the racing season. In myopathic horses, HRR revealed up- or down-regulation of several steps of the mitochondrial respiration. Our fundamental understanding of the pathophysiology of inherited and acquired myopathies might be improved by studying the mitochondrial function with HRR in horses but also in other animal species.

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95. Strong cytotoxic T lymphocyte responses to heat-killed *Staphylococcus aureus* are induced by CD40 triggering in mice: a new vaccine strategy for bovine staphylococcal mastitis.

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Staphylococcus (*S.*) *aureus* is a major pathogen involved in chronic bovine mastitis. Staphylococcal mastitis is difficult to control due to the ability of *S. aureus* to invade and survive within host cells. We therefore postulated that induction of $CD8^+$ cytotoxic T lymphocyte (CTL) responses leading to destruction of infected cells could help in the control of *S. aureus* mastitis. As CD40 signaling in antigen-presenting cells (APCs) is known to lead to the induction of robust CD4-independent CTL responses, we studied if CD40 triggering in mice during *S. aureus* immunization could protect mice from *S. aureus*-induced mastitis. We demonstrate in this work that immunization of mice with heat-killed *S. aureus* together with agonistic anti-CD40 monoclonal antibodies elicits strong CTL responses capable of protecting mice from subsequent staphylococcal mastitis. Our study shows promise for CTL-dependent vaccination against *S. aureus* mastitis.

96. Effect of preoperative meloxicam or tolfenamic acid administration on stress and pain induced by surgical castration in piglets.

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Sixty-six piglets (5-6 days of age) were allotted into 4 groups: castration (C); castration+tolfenamic acid (T); castration+meloxicam (M); simulation of castration (S). M-pigs spent more time during castration trying to escape than C- and T-pigs (41±9 *vs.* 34±1%; $P < 0.05$). T-pigs spent less time crying than C-pigs (52 ± 22 *vs.* 70 ± 20%; $P < 0.05$). Vocalization of M-pigs had a higher intensity (107 ± 4 *vs.* 103 ± 2 dB; $P < 0.05$). The mean heart rates in the four groups of piglets were not significantly different. Plasma cortisol concentration measured 30 minutes post-castration in T-pigs was higher than in S- and M-groups (20 ± 4 *vs.* 17 ± 0 µg/dl; $P < 0.05$). However concentrations measured in C- and S-groups were not significantly different. Post-castration, S- and T-pigs spent less time isolated than C-pigs ($P < 0.07$). More tremors and spasms were observed in M-group than in S-group ($P = 0.05$). Tail movements in C-pigs were observed more than in S- ($P < 0.001$) and T-pigs ($P = 0.02$). M-pigs showed more tail movements than T-pigs ($P = 0.06$). Pre-emptive analgesia seemed to reduce stress and pain induced by surgical castration in piglets. Tolfenamic acid seemed to be more efficient than meloxicam both during and post-castration.

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97. Etude comparative des spectres antiviraux de la Mx1 ovine et de la Mx1 bovine conduisant à une meilleure compréhension du mécanisme intracellulaire recruté par les protéines Mx.

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La protéine Mx1 bovine (boMx1) est connue comme étant une protéine Mx à forte activité antivirale notamment contre les virus de la stomatite vésiculaire (VSV) et Influenza A[1,2, article en soumission]. Il a été récemment mis en évidence qu'elle pouvait moduler la voie NF-κB par interaction avec certains facteurs TRAFs (Tumor necrosis Factor Receptor-Associated Factor) [article en soumission]. La protéine Mx1 ovine (oMx1) n'a jamais été étudiée dans un cadre antiviral. On sait que oMx1 a une identité très élevée avec son homologue bovin (93%)[3] et qu'elle possède un site d'interaction aux facteurs TRAFs intéressant. L'ADN complémentaire (ADNc) de oMx1 a été isolé à partir de cellules sanguines ovines préalablement stimulée au Poly I:C. Cet ADNc a été sous-cloné dans un vecteur d'expression lentiviral afin de produire des particules lentivirales recombinantes. En infectant des cellules Vero avec ces particules lentivirales, nous établirons une lignée stable et inductible pour l'expression de cette protéine. L'activité antivirale de oMx1 pourra dès lors être évaluée vis-à-vis des virus Influenza A et VSV et comparée à celle déjà connue de boMx1 afin de déterminer l'implication des différences de séquence entre ces deux protéines. En fonction des résultats obtenus, différents mutants seront réalisés afin de déterminer la signature antivirale de ces deux protéines et de confirmer les relations fonctions-séquences identifiées au cours du projet. La finalité de ce projet est double car elle permettra dans un premier temps de statuer une fois pour toute sur le statut antiviral de la protéine oMx1 et par la suite de mieux comprendre les mécanismes moléculaires recrutés par ces protéines Mx1.

Bibliographie : 1 Baise, E. *et al. J Interferon Cytokine Res* **24**, 513-521 (2004) ; 2 Garigliany, M. M. *et al. Transgenic Res* **18**, 719-732 (2009) ; 3 Ellinwood, N. M. *et al. J Interferon Cytokine Res* **18**, 745-755 (1998).

98. *Dermacentor reticulatus* as vector of *Anaplasma phagocytophilum* ?

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As the role of wildlife is recognized as major in emerging diseases in humans as well in domestic animals, tick borne diseases are in this context a subject of growing concern. In Belgium, the most health implicated tick is *Ixodes ricinus.* However, *Dermacentor reticulatus* seems to extend its geographical distribution more than previously thought, like in other parts of Europe. *D. reticulatus* can potentially or successfully transmit *Rickettsia sp, Francisella tularensis, Babesia canis, Coxiella burnetii* or TBEV, but was never related with *Anaplasma phagocytophilum*, the causative agent of granulocytic anaplasmosis. Indeed, in Europe, only *I. ricinus* was recognized to carry these bacteria. Here we deal with a new potential vector species. Within the WILDSCREEN network, we found on an adult red deer 35 ticks that were morphologically and genetically identified as *D. reticulatus*. We tested them for *Anaplasma phagocytophilum* by PCR and sequencing a part of the Msp2 gene and some of them were positive[1]. One of them was enhanced by sequencing a part of the 16S RNA gene. To extend our knowledge about the variants that may be found in *D. reticulatus,* 108 additional specimens were collected by flagging and some of them were tested positive for *A. phagocytophilum* by Real Time PCR on the Msp2 gene. The positive ticks will be sequenced regarding a part of the 16S RNA gene to compare them with *A. phagocytophilum* isolated from other sources. This approach will enable us to learn more about the variants newly isolated from *D. reticulatus.*

1. Wirtgen M et al. Detection of *Anaplasma phagocytophilum* in *Dermacentor reticulatus* ticks. Vet Rec. 2011, 168 : 248.

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99. Typing of Methicillin-Resistant *Staphylococcus aureus* (MRSA) strains isolated from bovine milk in Belgium.

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Staphylococcus (*S.*) *aureus* is a major human pathogen and a frequent cause of mastitis in ruminants, especially in cattle. Human and animal *S. aureus* strains can easily become resistant to antibiotics, especially □ lactams, by acquisition of new genes. One such □ lactam resistance-acquired gene (*mecA*) codes for a transpeptidase (PBP2a) resistant to several □ lactams including methicilline, oxacilline, nafcilline, and (di)cloxacilline. Animal MRSA are of great concern because they are suspected to have a zoonotic potential and/or to transfer their *mecA* gene to human strains. The purpose of this study was to compare the genomes of MRSA isolated from cow milk in Belgium between 2005 and 2009 by their virulence-associated gene (virulotypes), Pulsed Field Gel Electrophoresis (PFGE) and MultiLocus Sequence Typing (MLST) profiles. Nineteen *S. aureus* isolates (5.3 %) were phenotypically resistant to oxacillin and were positive with the PCR for the *mecA* gene. Their virulotypes were very similar, if not identical. Their PFGE profiles were compared after digestion with *Sma*1 and *Eag*1. Eighteen of the MRSA isolated in 2007 or later were not digested by *Sma*1 and had closely related *Eag*1 PFGE profiles. The last MRSA isolate was from 2005 and had a different *Eag*1 PFGE profile. In contrast, *Eag*1 PFGE profiles of several MSSA isolates were rather variable. According to these results the bovine MRSA isolates of our collection are closely related and maybe clonal isolates. They are now being compared by MLST. The next step will be the comparison of the *mecA* genes and cassettes.

100. Development of a method for haplotype-based association analysis of binary traits in structured populations.

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A hidden Markov model for haplotype reconstruction, QTL fine mapping and genomic selection has been developed previously (Druet & Georges, 2010). With that model, reconstructed haplotypes are assigned to hidden states that are shown to correspond to clusters of genealogically related chromosomes. In the original study, it was shown that these cluster states can directly be used to fine map QTL. We herein extend the approach to allow haplotype-based association mapping of binary traits. For continuous traits, association between inferred haplotypes and phenotypes is tested in the mixed model framework, which allows correction for polygenic effects or stratification (population structure). For binary traits, Generalized Linear Mixed Models (GLMM) were used while the score test was used to assess significance. We developed a method to solve GLMM and compute score tests with haplotypes. To study properties of the newly developed method we simulated data with several levels of stratification (population and/or polygenic effects) in addition to single SNP effects. Haplotypes presented high linkage disequilibrium with underlying SNPs and higher power was achieved if they were used instead of SNPs. It was shown that the method correctly accounted for stratification. Finally, the method was also tested on real data from the “Rilouke” project.

NOTES

With the support of :

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

4000 Liège (Belgique)

D/2011/0480/39
ISBN 978-2-87543-005-2

ISBN 978-2-87543-005-2

9 782875 430052