Faculté de médecine étérinaire

Université de Liège

Proceedings of the 2nd Scientific Meeting of the Faculty of Veterinary Medicine

(University of Liège – Belgium)

October 19, 2012

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One Health

L'Animal et l'Homme, une même santé

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

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

WELCOME TO THE SECOND SCIENTIFIC MEETING OF THE FACULTY OF VETERINARY MEDICINE

Each year, the Scientific Staff of the Faculty of Veterinary Medicine (FVM) stimulates the meeting of new and old members of the Scientific Staff at its Annual General Meeting.

The purpose of this meeting is to promote the social life and scientific research within the Faculty as well as to enhance the interactions within the Faculty regarding the different research topics. This year, the University of Ghent has been invited to join the meeting. This reunion is an opportunity for the members of the Scientific Staff to practice writing an abstract submitted for selection, and preparing a poster and/or an oral presentation as well as their defense. Finally, this event can show to the students the career opportunities in the Faculty (clinics and research).

Submission of abstracts for the 2nd Scientific Meeting was dedicated to members of the Scientific Staff of the FVM, and to the neo-graduates (as part of an award-winning study) of both Liege and Ghent Faculties. The second edition is a success again, with a total of 103 abstracts submitted, out of which 16 are from Ghent University. Of these, 20 were selected for an oral presentation by a scientific committee composed of PhD members of the Scientific Staff.

Jean-François CABARAUX

President of the Scientific Staff

BIENVENUE A LA 2EME EDITION DE LA REUNION SCIENTIFIQUE DE LA FACULTE DE MEDECINE VETERINAIRE

Chaque année, le Personnel Scientifique (PSc) de la Faculté de Médecine vétérinaire (FMV) stimule la rencontre des nouveaux et anciens membres du PSc lors de son assemblée générale. L'objectif de cette journée est de promouvoir la vie sociale et la recherche scientifique au sein de la Faculté et de favoriser la rencontre et les interactions des membres du personnel académique et scientifique en faisant connaître les différents sujets et programmes de recherche de la FMV.

Cette année, nos confrères de la FMV de l'Université de Gand ont été invités à se joindre à cette rencontre. C'est l'occasion pour les membres du PSc de s'exercer à la rédaction d'un abstract, à la préparation d'un poster et/ou d'une présentation orale, ainsi qu'à leur défense. Enfin, cet événement permet de montrer aux étudiants les possibilités de carrières à la Faculté (cliniques et recherches).

La soumission d'abstract pour le 2ème réunion scientifique de la FMV était réservée aux membres du PSc ainsi qu'aux néo-diplômés (dans le cadre d'un travail de fin d'études primé) de l'Université de Liège, mais également de l'Université de Gand. Cette seconde édition est à nouveau un succès avec un total de 103 abstracts soumis, dont 16 par nos confrères de l'Université de Gand. Parmi ces abstracts, 20 ont été sélectionnés pour une présentation orale par un comité scientifique composé exclusivement par des membres du PSc titulaires d'une Thèse de Doctorat.

Jean-François CABARAUX

Président du Personnel Scientifique

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ACKNOWLEDGMENTS

PROGRAMME

09.00: REGISTRATION

09.30: OPENING SESSION

Jean-François CABARAUX, President of the Scientific Staff, ULg

09.45: SESSION 1: FUNDAMENTAL RESEARCH

CHAIRWOMEN: Vincianne Toppets & Sandrine Vandenput, ULg

9h45 : INVITED SPEAKER: Benjamin Dewals, ULg, *An Essential Role of Gammaherpesvirus Latency in an Acute Lymphoproliferative Disease of Cattle.*

10.15: Cyprinid herpesvirus 3 of homology IL10 is secreted and it has functional in the viral infection.

Ping OUYANG, Immunology-Vaccinology, ULg

10.30: Macroscopic and microscopic lesions associated to in utero infection by Schmallenberg virus in calves.

Calixte BAYROU, Pathology, ULg

10.45: The mycotoxin deoxynivalenol predisposes for the development of necrotic enteritis in broilers.

Gunther ANTONISSEN, Pathology, UGent

11.00: Post-translational modification of the Myxoma virus chemokine-binding protein M-T7 by a virally encoded α-2,3-sialyltransferase.

Bérengère BOUTARD, Immunology-Vaccinology, ULg

11.15: Gammaherpesvirus latency impacts the host immune response and induces protection against asthma development.

Bénédicte MACHIELS, Immunology-Vaccinology, ULg

11.30: Study of the microbial flora of freshwater and seawater fish filets in different packaging conditions by metagenomic analysis targeted on the 16S ribosomal DNA.

Laurent DELHALLE, Quality Partners S.A.

11.45 : Participatory assessment of HPAI surveillance network at the community level in Vietnam.

Nicolas ANTOINE-MOUSSIAUX, Tropical Veterinary Institute, ULg

12:00: Microscopic and ultrastructural characteristics of acute gill lesions in koi carp Cyprinus carpio following immersion challenge using Flavobacterium columnare.

Annelies DECLERCQ, Morphology, UGent

12:15: A missense mutation in the ClC-7 chloride channel causes hamartomas with osteopetrosis in cattle.

Arnaud SARTELET, Animal Genomics, ULg

12:30: Hepatitis E virus infection in domestic swine, wild boar and human in Belgium.

Damien THIRY, Virology, ULg

12.45: LUNCH – POSTER SESSION

14.00: SESSION 2: Clinical research

CHAIRWOMEN: Prof. Tania Art & Dominique Cassart, ULg

INVITED SPEAKER: Prof. Annemie Decostere, UGent, *Aquatic veterinary medicine at Ghent University: an intriguing research domain.*

14:30: Antimicrobial and anti-inflammatory drug use in Belgian white veal calves.

Bart PARDON, Large Animal Internal Medecine, UGent

14.45: Equine neutrophil elastase in plasma, laminar tissue, and skin of horses administered black walnut heartwood extract.

Geoffroy DE LA REBIERE DE POUYADE, Equine Clinic, ULg

15.00: Clinical efficacy and safety of levobupivacaine and morphine by lumbosacral epidural route for elective hindlimb surgery in dogs.

Ilaria CERASOLI, presented by **Tutunaru A.**, Department of Companion Animals, ULg

15.15: Altered global and regional left ventricular function in horses with aortic valve insufficiency measured by tissue Doppler imaging and 2D speckle tracking.

Annelies DECLOEDT, Large Animal Internal Medecine, Ugent

15.30: Evaluation of the ultrasound-guided procedures to puncture the CSF in dogs: a cadaveric study.

Anne-Laure ETIENNE, Diagnostic Imaging Section, ULg

15.45 : Study of TLR-7 and TLR-8 in equine pulmonary alveolar macrophages at rest and after exercise.

Irène TOSI, Center of Sports Medicine, ULg

16.00: COFFEE BREAK – POSTER SESSION

16.30: SESSION 2: Clinical research

CHAIRWOMEN: Prof. Tania Art & Dominique Cassart, ULg

16.30: Toe-heel and medio-lateral hoof balance in sound horses with toed-in conformation.
Maarten OOSTERLINCK, presented by **Van der Aa R.**, Surgery of Domestic Animals, UGent

16.45: Surgical treatment of open joint injuries: a retrospective study of 22 horses.
Alexandra SALCICCIA, Equine Clinic, ULg

17.00: The three-dimensional reconstruction of the innervation pattern in the lymphoid compartments of the ovine pharyngeal tonsil highlighted a possible way of neuro-invasion by the scrapie agent.
Vinciane TOPPETS, Histology, ULg

17.15: Atypical myopathy: a big enigma finally resolved... and you will be the first one to know!
Dominique-M. VOTION, Equine Pole, Clinical Sciences, ULg

17.30: AWARDS AND CLOSING SESSION

Prof. Albert CORHAY, First Vice-Rector, ULg

Prof. Pascal LEROY, Dean of the Faculty, ULg

Catherine DELGUSTE, President of the Scientific Committee, ULg

18.00: COCKTAIL – POSTER SESSION

20.00: DINNER & DANCING PARTY

TABLE OF CONTENTS

ORAL PRESENTATIONS

POSTERS

ADDENDUM

Oral Presentations

10.15 - *Cyprinid herpesvirus 3* of homology IL10 is secreted and it has functional in the viral infection.

Ouyang P.[1], Rakus K.[1], Reschner A.[1], Fournier G.[1], Ronsmans M.[1] and Vanderplasschen A.[1]
[1] *Immunology-Vaccinology (B43b), Department of Infectious and Parasitic Diseases, Faculty of Veterinary Medicine, University of Liège, B-4000 Liège, Belgium.*
Corresponding author: a.vdplasschen@ulg.ac.be

IL-10 homologues encoded by viruses (v-IL-10) have been described in members of the *Herpesvirales* order and the *Poxviridae* family. Sequencing of CyHV-3 genome revealed that it encodes a viral homolog of IL-10 (vIL-10). Phylogenetic analysis of CyHV-3 ORF134 demonstrated that it is distant from all vIL-10 studied so far and that its sequence was probably acquired from an ancestral ancestor of cyprinid fish. Our goal was to determine whether the ORF134 give rise to an expression product (pORF134) secreted by infected cells in the extracellular medium and to production of the 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). The MS analysis 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 CyHV-3 ORF134 did not affect the capacity of the virus to replicate *in vitro* and to induce CyHV-3 disease in carp comparably to the wild-type parental and revertant strain. All strains tested conferred a protective immune response against an homologous lethal challenge. Further studies are required to reveal the functional of ORF134 in the infection 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 infection of an *Alloherpesvirus.*

10.30 - Macroscopic and microscopic lesions associated to *in utero* infection by Schmallenberg virus in calves.

Bayrou C.[1], Garigliany M-M.[1] and Desmecht D.[1*]
[1.] *Service de Pathologie et Autopsies, Département de Morphologie et Pathologie, FMV, ULg, Boulevard de Colonster 20, Liège, Belgium.*
**Corresponding author: Daniel.Desmecht@ulg.ac.be*

At the end of 2011, a new *Orthobunyavirus* belonging to the Simbu serogroup was identified in Germany and provisionally named Schmallenberg virus (SBV). Viruses belonging to the Simbu serogroup are known to cause fetus malformations mainly in ruminants. The beginning of 2012 showed, in Belgium, the first Schmallenberg positive abnormal calves. This study presents the macroscopic and microscopic lesions of the first 15 positive calves collected at the pathology service of the University of Liège (Wallonia, Belgium). In most cases, SBV *in utero* infection results in a hydranencephaly-arthrogryposis syndrome linked to a clear neurotropism of the virus.

10.45 - The mycotoxin deoxynivalenol predisposes for the development of necrotic enteritis in broilers.

Antonissen G.[1,2*], Van Immerseel F.[1], Pasmans F.[1], Ducatelle R.[1], Haesebrouck F.[1], Timbermont L.[1], Verlinden M.[1], Dewulf J.[3], Eeckhout M.[4], De Saeger S.[5], Delezie E.[6], Martel A.[1] and Croubels S.[2]

[1.] Department of Pathology, Bacteriology and Poultry Diseases, F.V.M., Ghent University. [2.] Department of Pharmacology, Toxicology and Biochemistry, F.V.M., Ghent University. [3.] Department of Reproduction, Obstetrics and Herd Health, F.V.M., Ghent University. [4.] Department of Food Science and Technology, Faculty of Biosciences and Landscape Architecture, Ghent University, Schoonmeerstraat 52, 9000 Gent, Belgium. [5.] Department of Bio-analysis, Faculty of Pharmaceutical Sciences, Ghent University, Harelbekestraat 72, 9000 Gent, Belgium. [6.] Institute for Agricultural and Fisheries Research (ILVO) Animal Sciences Unit, Scheldeweg 68, 9090 Melle, Belgium.

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Clostridium perfringens induced subclinical necrotic enteritis (NE) causes important economic losses in the broiler industry. *Fusarium* mycotoxins, like deoxynivalenol (DON), may affect the intestinal epithelial integrity. The objective of this study was to examine whether DON at contamination levels below the maximum guidance level in poultry feed is a predisposing factor for NE in broilers. In this study we used a highly reproducible *in vivo* infection model mimicking subclinical NE (Gholamiandehkordi et al., 2007). A total of 360 one-day-old Ross 308 broilers were randomly divided into four groups of three replicates with 30 birds per replica. Throughout the entire experiment, groups 1 and 4 received a blank diet while groups 2 and 3 received a diet experimentally contaminated with DON. All birds in group 1 and 2 were challenged orally with *C. perfringens* strain 56 containing approximately 4 x 10^8 cfu/ml for four consecutive days starting at day 17. The remaining groups received sterile medium. At 1, 2 or 3 days after the final challenge with *C. perfringens*, chickens were euthanized and scored macroscopically for intestinal NE lesions. Chickens that received DON and *C. perfringens* had significantly (alpha=0.05, $P<0.001$) more lesions than chickens that received only *C. perfringens*, with 46.6% and 19.5% of chickens positive for NE lesions, respectively. In non-inoculated groups no NE lesions were present. In conclusion, the presence of DON in the feed in concentrations lower than the maximum guidance level of 5000 µg/kg is a predisposing factor for the development of NE in broilers.

11.00 - Post-translational modification of the Myxoma virus chemokine-binding protein M-T7 by a virally encoded α-2,3-sialyltransferase.

Boutard B.[1*], Vankerckhove S.[1], Markine-Goriaynoff N.[1], Bentaib A.[2], Sarlet M.[3], Wattiez R.[4], Leprince P.[2], Desmecht D.[3], McFadden G.[5], Vanderplasschen A.[1] and Gillet L.[1]

[1] Immunology-Vaccinology (B43b), Department of Infectious and Parasitic Diseases, FMV, ULg. [2] CNCM, GIGA Neurosciences, ULg. [3] Department of Pathology, FMV, ULg. [4] Department of Proteomics and Protein Biochemistry, University of Mons, Belgium. [5] Department of Molecular Genetics and Microbiology, College of Medicine, University of Florida, USA.

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Myxoma virus is a pathogenic Poxvirus that induces a lethal disease called myxomatosis in European rabbits. It is one of the very rare viruses that encodes an α-2,3-sialyltransferase that transfers sialic acid to glycoproteins and glycolipids. Very little information is available about the role played by this glycosyltransferase in the pathogenesis of the infection. Previous experiments showed that the enzyme, encoded by the M138L gene, is not essential for virus replication *in vitro* but is important in the *in vivo* pathogenesis of myxomatosis. The objective of this study was the identification of the viral and cellular proteins modified by the α-2,3-sialyltransferase. A two-dimensional differential gel electrophoresis revealed that a target of the enzyme is the viral chemokine-binding protein M-T7. This information was confirmed by western blots. Moreover, a mass spectrometry glycan analysis of purified M-T7 proteins revealed precisely the nature of the modifications introduced by the M138L gene product. As M-T7 is a known important virulence factor of the virus, the difference in M-T7 sialylation could therefore be responsible of the *in vivo* attenuation observed. These results demonstrate that, although non essential, the α-2,3-sialyltransferase is a virulence factor for Myxoma virus pathogenesis in European rabbit, that acts by post-translational modification of others viral proteins.

11.15 - Gammaherpesvirus latency impacts the host immune response and induces protection against asthma development.

Machiels B.[1], Sabatel C.[2], Dourcy M.[1], Sarlet M.[3], Guillaume A.[1], Massart F.[1], Desmecht D.[3], Vanderplasschen A.[1], Bureau F.[2], Gillet L[1].

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The immune system is continuously shaped by environmental antigens. However, the effect of the infection history is ignored in most animal models. Gammaherpesviruses are highly prevalent pathogens that establish lifelong latency in their host. While these viruses persist in lymphoid tissues, little is known about how they imprint the host immune response. In humans, although the Epstein-Barr virus (EBV) and the Kaposi's Sarcoma-associated Herpesvirus (KSHV) are associated with several malignancies, most of their infections remain asymptomatic. Studying how EBV and KSHV imprint the human immune response is difficult because they have no well-established *in vivo* infection model. Related animal gammaherpesviruses allow us to tackle the same fundamental questions in a more accessible form. In this study, we used Murid herpesvirus 4 (MuHV-4), a natural pathogen of rodents, to investigate the impact of gammaherpesvirus infection on subsequent allergic asthma development. Using a murine model of house dust mite (HDM)-induced allergic asthma, we compared the sensitization of mice mock infected or infected by MuHV-4 by: (i) broncho-alveolar lavage analysis, (ii) lung histology, (iii) determination of serum total level of IgE and (iv) restimulation of bronchial lymph node cells. These experiments showed that previous infection by MuHV-4 is associated with protection against HDM-induced airway allergy. As epidemiological studies suggest that similar phenomenon could happen with human gammaherpesviruses, these results could have important implications for researches on asthma development. Moreover, these results reinforce the view that lifelong persistence of herpesviruses is a symbiotic relationship that could be beneficial to the host in some circumstances.

11.30 - Study of the microbial flora of freshwater and seawater fish filets in different packaging conditions by metagenomic analysis targeted on the 16S ribosomal DNA.

Delhalle L.[1*], Taminiau B.[2], Nezer C.[1], Darcis A.[1] and Daube G.[2]

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**Corresponding author: l.delhalle@ulg.ac.be*

Metagenomics has appeared as a powerful tool to study bacterial composition of various environmental samples. This work describes the application of this technique to study the bacterial population of two fresh fish filets. The two fish species are from freshwater (pangasius) and seawater (haddock), respectively. Samples where directly analyzed the day of receipt. Others samples were analyzed at the end their shelf life after storage at 4°C (1/3 of their shelf life) and 8°C (2/3 of their shelf life). For these samples, packagings were made in plastic wrap for atmospheric air condition and in trays under modified atmosphere. Classical microbiological and 16S rDNA metagenomic analysis were carried out on all these samples. The composition and evolution of microbial populations of fish filet stored under different packaging conditions and temperatures of storage were investigated with identification of bacteria species. A total of 40 different species were identified for both fish types. Gram-negative bacteria are always predominated among the initial flora and at the end of the shelf life in all the trials. At the beginning of storage, the predominant Gram-negative microflora consisted of *Moraxellaceae* (*Acinetobacter* spp, *Psychrobacter* spp.), *Pseudomonadaceae* (*Pseudomonas* spp), and *Shewanella* spp and the Gram-positive flora was identified as *Lactobacillaceae* (*Carnobacterium* spp), *Brochothrix thermosphacta* and *Planococcus donghaensis* (only for pangasius). For the pangasius, *Planococcus donghaensis* is only present before the fish is packed and its dominant presence could provide an indication of the freshness of the fish. The metagenomic analysis is a useful tool to identify and to measure the relative proportions of bacterial species in fish filet samples.

11.45 - Participatory assessment of HPAI surveillance network at the community level in Vietnam.

Antoine-Moussiaux N.[1], Delabouglise A.[2], Binot A.[2], Ton V.D.[3], Khong N.V.[4], Goutard F.[2], Roger F.[2] and Peyre M.[2]

[1.] Tropical Veterinary Institute, FMV, ULg. [2.] AGIRs research unit, CIRAD, France. [3.] Faculty of Animal Science and Aquaculture, Hanoi University of Agriculture, Vietnam. [4.] National Institute of Veterinary Research, Hanoi, Vietnam.

The need for efficient and sustainable animal health surveillance networks is a major concern that must be continually placed at the heart of the global issue of development. In developing countries, the political priority to poverty alleviation renders it crucial to include social aspects in public health management decision-making. Animal health surveillance is deeply embedded in agents' everyday life. Hence, the flow of information about animal health responds to both economic and social drivers. Its assessment should, therefore, benefit from participatory approaches, due to their flexibility and semi-quantitative tools. This study considers the case of highly pathogenic avian influenza surveillance in Vietnam. It aims at establishing a protocol allowing for understanding and quantifying social and economic drivers of epidemiosurveillance at the community level. A survey was conducted in four villages of Câm Hoàng commune, Hai Duong province, from April to July 2012. Thirteen focus groups and fifty individual semi-structured interviews were conducted, being guided by checklists. Tools and concepts from anthropology, participatory epidemiology and experimental economics were combined. More particularly, social network analysis, participatory observation, companion modeling and stated preference surveys were applied for the thorough examination of constraints and costs of health information flows. A wide array of stakeholders were met (140 persons in total), including farmers, official and private veterinarians, veterinary authorities at district and provincial levels, local political authorities, drug and feed sellers, feed company representatives, chicken and pig traders. The major methodological lessons drawn from this ongoing project are presented.

12.00 - Microscopic and ultrastructural characteristics of acute gill lesions in koi carp *Cyprinus carpio* following immersion challenge using *Flavobacterium columnare*.

Declercq A.M.[1], Van den Broeck W.[1], Chiers K.[2], Dewulf J.[3], Bossier P.[4], Haesebrouck F.[2] and Decostere A.[1]

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Fingerling koi carp, Cyprinus carpio, were exposed by immersion challenge to seven different strains of Flavobacterium columnare, the causative agent of columnaris disease. Five strains were considered to be highly virulent, with moribund fish appearing as soon as nine hours post challenge and exhibiting marked gill lesions with ubiquitous white necrotic lesions. Two strains could be classified as low virulent, giving low or no morbidity/mortality. Light microscopic examination of hematoxylin-eosin stained paraffin sections of the gills of fingerlings exposed to highly virulent strains revealed extensive loss of branchial structures. Desquamation and necrosis of gill epithelium with fusion of gill filaments and lamellae were present. Large parts of the filaments had disappeared and the conserved areas were covered with necrotic debris. Massive clusters of F. columnare bacteria, enwrapped in an eosinophilic matrix, were inclosed in between necrotic areas. Scanning and transmission electron microscopic observations of the affected gill tissue pictured the presence of long slender bacterial cells of about 0.3 µm wide and up to 10 µm in length, attained in an extracellular matrix and in close contact with the destructed gill tissue. Microscopic examination of the gill tissue of the control fish and of the fingerlings inoculated with the low virulent strains uncovered intact gill filaments without the presence of bacteria. This is the first study in its kind to reveal such severe lesions of the gill tissue at macroscopic, light microscopic and ultrastructural level in koi carp.

12.15 - A missense mutation in the ClC-7 chloride channel causes hamartomas with osteopetrosis in cattle.

Sartelet A.[1*], Stauber T.[2*], Coppieters W.[1], Fasquelle C.[1], Druet T.[1], Zhang Z.[1], Ahariz N.[1], Cambisano N.[1], Jentsch T.[2], Georges M.[1] and Charlier C.[1]

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We recently identified a new congenital defect in Belgian Blue Cattle with gingival hamartomas and peri-natal lethality as most prominent symptoms. We mapped the causative gene by autozygosity mapping in a 1.3 Mb interval encompassing more than 100 annotated genes on the centromeric end of bovine chromosome 25. To accelerate the identification of the culprit gene, we used genome-wide sequencing of cases. Obtained reads were mapped on the bovine reference genome sequence and analyzed for DNA sequence variants (DSV) within the shared region. After filtration, only a handful of candidate variants remained, including a cluster of three private SNPs causing the substitution of the ultra-conserved tyrosine 750 into glutamine in the cystathionine β-synthase 2 domain (CBS2) of the *Chloride Channel 7 gene (CLCN7)*. Deleterious mutations in this domain cause osteopetrosis in human and mice. Accordingly, a more thorough examination of affected calves revealed a severe osteopetrotic phenotype, hence strengthening ClC-7 causality. Functional studies showed that the Y750Q mutation neither inhibits ClC-7/Ostm1 interaction nor correct co-trafficking to lysosomes. However, it drastically affects channel behavior, changing its slow activation and relaxation kinetics into a so-called "fast mutant" with accelerated gating.

12.30 - Hepatitis E virus infection in domestic swine, wild boar and human in Belgium.

Thiry D.[1], Mauroy A.[1], Linden A.[2], Thomas I.[3], Miry C.[4], Saegerman C.[5], Czaplicki G.[6], Brochier B.[3] and Thiry E.[1]

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Hepatitis E virus belong to the *Hepeviridae* family where four genotypes are described. In Europe, genotype 3 strains mainly circulate and are suspected to be zoonotic. The aims of this study were to obtain data on the apparent viroprevalence and seroprevalence in pigs and wild boars (WB) in Belgium and to genetically compare the different strains identified in these species with those detected in human. A nested RT-PCR and, commercial ELISA and Western blot (adapted when used in animal), were used. A sampling of 840 pig's sera was carried out. A sampling on WB population was also conducted: 383 adult sera were dedicated to serology and 69 serum and 61 liver samples from young WB were dedicated to virology. Human sera were obtained from samples sent to the Belgium Institute of Public Health for HEV diagnosis. Apparent seroprevalences of 33%(±4.6 ; 125/383) and 73%(±4 ; 308/420) were obtained in WB and pigs. Five out of 61 livers and 4/69 sera of young WB were detected viropositive, 4/420 pig sera were also viropositive. All sequences were phylogenetically related to genotype 3. In humans, 25/340 sera in 2010 and 32/437 in 2011 were IgM positive and, from these, 10 and 24 respectively, were viropositive. Four sequences were phylogenetically related to genotype 1, 7 to genotype 3 and 1 to genotype 4. The high HEV seroprevalence in swine and WB in Belgium raises zoonotic concern about HEV transmission from pigs or wild boars to human and the role of pig herds as reservoir.

14.30 - Antimicrobial and anti-inflammatory drug use in Belgian white veal calves.

Pardon B.[1], Catry B.[2], Dewulf J.[3], Persoons D.[4], Hostens M.[3], De Bleecker K.[5], Deprez P.[1]

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The white veal industry is an intensive livestock production system where multidrug resistance is abundantly present. The objective of the present study was to describe and quantify drug use in white veal calves. Drug consumption data were prospectively collected on 15 white veal production cohorts (n= 5853 calves) in Belgium (2007-2009). Treatment incidences (TI) based on (animal) defined daily dose (ADD), prescribed daily dose (PDD) and used daily dose (UDD) were calculated. Drug use mainly consisted of oral group treatments (95.8%). The mean treatment incidence (TI_{DDD}) of antimicrobial treatments was 416.8 ADD per 1000 animals at risk per day, meaning that calves received an amount of antimicrobials enough to treat them for 40% of the production cycle. In 33.3% of the oral antimicrobial group treatments a combination of two antimicrobial preparations was used. Therefore, calves received antimicrobials for 26% of the production cycle in reality. The main indication for group and individual drug use was respiratory disease. The most frequently used antimicrobials (group treatments) were oxytetracycline (23.7%), amoxicillin (18.5%), tylosin (17.2%) and colistin (15.2%). Deviations from the leaflet dosage recommendations were frequently encountered, with 43.7% of the group treatments underdosed (often oxytetracycline and tylosin to treat dysbacteriosis). Smaller integrations used more antimicrobials in group treatments than larger ones ($P<0.05$). Anti-inflammatory drugs were far less frequently used (TI_{UDD}= 5.94) and markedly overdosed when individually injected. The present study demonstrates highly intensive antimicrobial use in the white veal industry. Reduction can only be achieved by reducing the number of oral group treatments.

14.45 - Equine neutrophil elastase in plasma, laminar tissue, and skin of horses administered black walnut heartwood extract.

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Laminitis is characterized by neutrophil activation and movement into the laminar tissues. We measured concentrations of neutrophil elastase in plasma, skin and laminar tissues obtained from control horses and horses given black walnut heartwood extract (BWHE) to induce laminitis. Healthy horses were assigned to 4 groups: 3 groups given BWHE via nasogastric tube, and a control group given an equal volume of water. The experimental groups consisted of horses euthanized 1.5h (n=5), 3h (n=6) or 12h (n=10) after BWHE administration. Control horses (n=7) were euthanized 12h after administration of water. Plasma samples were collected in horses of the control and 12h BWHE groups at 0, 1, 2, 3, 4, 6, 8, 10, and 12h after treatment, and laminar tissue and skin from the neck were harvested at the time of euthanasia in horses from the 4 groups. Plasma and tissue concentrations of neutrophil elastase were determined using an equine specific ELISA, and statistical significance was set at $p<0.05$. Plasma concentrations of neutrophil elastase in the BWHE group were higher at 6 and 8h compared to the control group and at 8 and 10h compared to time 0. Concentrations of neutrophil elastase in skin and laminar tissue were higher in the 3 and 12h BWHE groups compared to the control group. Concentrations of neutrophil elastase were higher in the skin than in the lamina in the 12h BWHE horses. These results confirm a role for neutrophils in the developmental phase of laminitis, and the systemic nature of the inflammatory process.

15.00 - Clinical efficacy and safety of levobupivacaine and morphine by lumbosacral epidural route for elective hindlimb surgery in dogs.

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In dogs, epidural anaesthesia with local anaesthetic and preservative free morphine is a widely accepted technique to provide analgesia with minimized side effects and lowered systemic anaesthetics' concentrations. Levobupivacaine, routinely used in man, is considered less cardiotoxic than bupivacaine, but its efficacy and safety has not been investigated in dogs in clinical settings. The aim of our clinical study was to evaluate the efficacy and safety of the combination of levobupivacaine and morphine via epidural route in dogs. Ten ASA II dogs [aged 51±21(mean±SD) months and weighing 31±17(mean±SD)] undergoing elective hindlimb surgery were involved in the study. All dogs received methadone (0.2 mg Kg^{-1}) and diazepam (0.2 mg Kg^{-1}) as premedication; anaesthesia was induced with propofol (2-8 mg Kg^{-1}) and maintained with isoflurane in air/oxygen (1:1). Epidural anaesthesia consisted of levobupivacaine 0.5% (1 mg Kg^{-1}) and morphine (0.1 mg Kg^{-1}) up to a volume of 0.2 ml Kg^{-1}. Clinical parameters, VAS scale, Modified Glasgow Pain Score, simple descriptive scale, need for rescue analgesia, degree of sedation, neurological deficit and motor ability was assessed every 6 hours after the epidural injection for 24 hours. Results suggest a satisfactory pain management in dogs in the first 24 hours after elective hind limb surgery. Only one dog showed motor deficit that disappeared after 6 hours. Hypotension, intra-operatively, and urinary retention, post-operatively, were found side effects. No respiratory depression, vomiting or pruritus was observed. In conclusion, the combination of levobupivacaine and morphine is suitable for epidural anesthesia in dogs undergoing elective hind limb surgery.

15.15 - Altered global and regional left ventricular function in horses with aortic valve insufficiency measured by tissue Doppler imaging and 2D speckle tracking.

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Aims: Significant aortic regurgitation (AR) may cause left ventricular (LV) dilatation and heart failure. The aim was to quantify LV function in AR horses by tissue Doppler imaging (TDI) and two-dimensional speckle tracking (2DST). **Methods**: Echocardiographic examinations were performed on ten healthy horses (10±4 years;509±58 kg) and fourteen horses with significant AR (17±4 years;497±93 kg). By 2DST, global radial (SR) and longitudinal (SL) strain were measured. Regional systolic radial displacement (DR_S) by 2DST and velocity (V_S) by TDI were measured in the interventricular septum and LV free wall. LV end-diastolic internal diameter (LVIDd) and fractional shortening (FS) were measured from a short-axis M-mode at chordal level. **Results**: Seven horses showed moderate AR (LVIDd range 11.0-12.7 cm), seven severe AR (LVIDd 13.3-16.9 cm). FS, SR and SL showed no significant differences. However, SL was significantly correlated with LVIDd in healthy horses and moderate AR ($r=-0.72$; $P<0.01$) but inversely correlated in severe AR ($r=0.82$, $p<0.05$), suggesting LV contractile dysfunction. Septal DR_S was correlated with LVIDd ($r=0.667$; $P<0.001$). Compared to healthy horses (17.4±3.1 mm), DR_S was increased in horses with moderate (21.1±4.2 mm) and severe (27.1±3.8 mm) AR ($P<0.05$). Similarly, septal V_S was correlated with LVIDd (-0.778; $P<0.001$) and increased in horses with moderate (-8.3±1.2 cm/s) and severe (-10.8±1.9 cm/s) AR compared to healthy horses (4.4±1.2 cm/s). This indicates increased septal motion in AR horses both with and without LV dilatation. **Conclusion**: TDI and 2DST allow quantification of altered LV function due to AR. The prognostic value of these measurements requires further investigation.

15.30 - Evaluation of the ultrasound-guided procedures to puncture the CSF in dogs: a cadaveric study.

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The introduction of spinal needle in the cisterna magna (CM) and in the lumbar subarachnoid space is commonly carried out blindly. US-guidance is considered to reduce the risks of the procedure and to increase operator's confidence. A direct US-guided technique for lumbar subarachnoid space puncture and an indirect US-guided technique for cisternal tap have been described in dogs. These image-guided procedures have a potential use but the ability to transfer these techniques to less experienced operators has not been studied. Eighty-three veterinarians and veterinary students with different experience in CSF punctures and ultrasonography have undergone a theoretical teaching on the four CSF punctures techniques: blind and US-guided techniques for the cisternal and lumbar puncture. Then, each operator carried out each technique on different cadavers (41 dogs, mean weight 14Kg, range 4-32Kg). For each procedure the operator had maximum 4 attempts and 10 minutes to do it. After each puncture, the volunteer filled in a questionnaire about previous experience, performance and self –confidence. Descriptive and statistical analysis of data was performed. For the cisternal puncture, success rates were 78% and 95% with the blind and ultrasonographic techniques respectively, for the lumbar puncture 46% and 78% respectively. Several parameters were significant on the success rate ($P<0.05$): ultrasonographic experience, number of attempts, body scoring, time used, self-confidence, technique used (blind or ultrasound-guided) and puncture location (cisternal versus lumbar). The use of an ultrasound-guided technique to puncture CSF in dogs improved the success rate on cadavers.

15.45 - Study of TLR-7 and TLR-8 in equine pulmonary alveolar macrophages at rest and after exercise.

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16.30 - Toe-heel and medio-lateral hoof balance in sound horses with toed-in conformation.

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Empirically, toed-in hoof conformation has been associated with an increased risk of lameness and reduced longevity of equine athletes, presumably linked with changes in hoof balance. Therefore, toe-heel and medio-lateral hoof balance were objectively and quantitatively explored in 5 visually sound, toed-in warmblood horses (556 ± 94 kg) using a 2m-pressure plate (RSscan International). Five measurements of each forelimb were recorded at the walk. Forelimb hoof prints were divided in a toe and heel region and in a medial and lateral zone. Toe-heel and medio-lateral hoof balance of the vertical ground reaction force were calculated throughout stance (126 Hz). Toe-heel balance in 4 of the 5 toed-in horses presented higher loading of the toe zone at impact, whereas in normal horses, the heel zone receives higher loading than the toe zone early in the stance phase. Unexpectedly, all horses in the current study presented similar medio-lateral balance as reported in normal, sound horses, with higher loading of the lateral zone at impact, equivalent loading of the medial and lateral zone at mid-stance, and increased lateral loading at the end of the stance phase. These results indicate distinct differences in toe-heel hoof balance between toed-in and normal horses. This may be linked with distal limb lameness, as increased loading of the toe early in the stance phase has been observed in horses with navicular disease. In conclusion, pressure plate analysis opens perspectives for the evidence-based evaluation of horses with conformational deficits and the effect of therapeutic farriery.

16.45 - Surgical treatment of open joint injuries: a retrospective study of 22 horses.

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Aims: To evaluate the prognosis of horses treated surgically for open joint injuries. **Methods**: The medical records of horses admitted for surgical treatment of a wound in communication with a joint were reviewed. A telephone questionnaire was used for the long term evaluation. Fisher's exact tests were used for the statistical analyses. **Results**: Twenty two horses were included in the study with the following distribution of lesions: 6 carpi, 6 fetlocks, 4 tibiotarsal joints, 2 proximal and 3 distal interphalangeal joints, 1 elbow. The duration of the injury before referral ranged from 3 hours to 10 days. Surgical treatment consisted of 1-3 joint lavages. Of the 22 horses, 4 were euthanatized during hospitalization and 18 were discharged. After discharge, 3 horses died due to colic, 2 were lost and 13 were still alive. The survival was not influenced by the duration of the wound. All horses with cutaneous defects less than 5 cm and all horses affected in the lower limb (below the level of the canon) were discharged. Having an affected joint proximal to the canon was significantly associated to the need of multiple surgeries (OR: 17.5; p= 0.024). **Conclusions**: Even if the prognosis remains guarded for open joint injuries, a long delay between injury and treatment should not be systematically associated with a bad prognosis for survival. Open joint injuries of the lower limb were associated with survival. They required less often multiple articular lavages than open joint injuries of the upper limb, warranting thus a better prognosis.

17.00 - The three-dimensional reconstruction of the innervation pattern in the lymphoid compartments of the ovine pharyngeal tonsil highlighted a possible way of neuro-invasion by the scrapie agent.

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Scrapie is a neurodegenerative disease affecting sheep and goats, caused by an unconventional transmissible agent: the pathogen prion protein (PrPd). During a first silent phase of amplification inside lymph follicles, the pathogen reaches the peripheral nervous system and spread retrogradely to the central nervous system. Recently, it has been demonstrated that the respiratory system and more specifically the pharyngeal tonsil could serve as natural portal of entry for scrapie. In this context, we realised a three-dimensional reconstruction of the innervation pattern in the lymphoid compartments of the ovine pharyngeal tonsil. The AMIRA 4.0.1 Mercury system software allowed the 3D reconstruction of a chain of 3 follicles surrounded by the nervous pattern revealed by the immunolabelling of unmyelinated nerve fibres on serial cryosections. The nervous network seemed to follow the vasculature and was denser on the follicle side close to the connective central axis of the pharyngeal tonsil. This density diminished as the fibres walked along the follicle in direction to the respiratory epithelium covering the tonsil surface. Inside the entire reconstruction, only two nerve fibre extensions invaded a lymphoid follicle. The computing 3D reconstruction ensures a representation closer to the reality than an analysis on histological slides and allowed to evaluate the frequency and distribution of the nerve fibres surrounding lymphoid follicles of the pharyngeal tonsil. Because some nerve fibres were detected inside the lymphoid follicles, neuro-immune connections between nerve endings and immune cells responsible for prions amplification could be one of the link between lympho- and neuro-invasion.

17.15 - Atypical myopathy: a big enigma finally resolved... and you will be the first one to know!

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Posters

1. Effect of bovine Azawak colostrum administration on plasma protein profile in red kid.

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This study evaluated the impact of heterologous Azawak colostrum administration on plasma protein profile in red kids from Niger. Forty (40) newborn kids were affected to one of two treatments: control group (access to water and the mother) and colostrum group (access to the mother, water and additional 50 ml of colostrum Azawak/animal the day of birth, then 25ml/animal/day from the 2nd to 15th day). Blood samples (10ml/animal) were obtained at the age of 10 and 30 days by jugular puncture into EDTA vacutainer tubes. The quantification of total protein was performed by the Biuret method. The agarose gel electrophoresis was used to determine serum levels of albumin, γ-globulin, β_1-globulin, β_2-globulin, γ-globulin and finally the albumin/globulin ratio. In general, the average concentrations of these proteins obtained at both ages reached higher values in colostrum than in control group. At day 10, the colostrum group tended to show ($P < 0.07$) higher levels for γ-globulin and showed higher values ($P < 0.04$) for β_1-globulin. At day 30, total protein and β_2-globulins were higher in colostrum group. The administration of heterologous colostrum in kid seems to have positive effects on some plasma parameters. It would be worth to discriminate the plasma proteins derived from bovine and maternal colostra.

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2. Contribution to genetic methods of mapping using non-parametric statistical.

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These last years have seen the emergence of a wealth of genetic information. Some of the main recent breakthroughs in biology originate from this new knowledge at the molecular level.At the cell scale, abundant genome, transcriptome and proteome data is nowadays routinely available to dissect traits of interest not only in the human species, but also in many other species, including main livestock animals. In the latter, concerned traits are production traits, disease traits and many others, such as disease resistance, behavior or external aspect.In parallel to these technical improvements, methodological advances are needed to address the various questions of scientific interest. One such question concerns the exact relationship between genomic configuration and phenotypic expression. The main idea is to identify variants at the molecular level and to try to associate these variations at the molecular level to observed variations at the macroscopic level.To study this relationship between the studied phenotype and the underlying genetics, we will propose a semi-parametric model. The method is based on regressing the quantitative trait of interest on a nonparametric smooth function of single nucleotide polymorphisms (SNP) genotypes in the region, while taking into account the parametric effects of all covariates of interest.

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3. Placental and gastric arpartic proteinases: new insights from bovine species.

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Fetal pepsinogen (PgnF) constitutes a new class of gastric molecules intermediate between placental aspartic proteinases (e.g. pregnancy associated glycoprotein, PAG) and gastric-expressed molecules (e.g. prochymosin, pepsinogen A and progastricsin). The first characterized PgnF was identified as a prenatal specific protein expressed in rabbit stomach. Similar molecules were identified in rat stomach, yolk sac and stomach of neonatal mouse as well as in equine and feline placenta. In general, PgnF identified in gastric mucosa more closely resemble placental PAG family members than other gastric aspartic proteinases. The present research aimed to investigate the expression of new molecules from aspartic proteinases family in gastric mucosa of bovine fetuses. Bovine fundic and pyloric mucosa were dissected immediately after slaughter of the cow-foetus unity. Purification procedure included extraction of proteins at neutral pH, ammonium sulfate precipitations, Sephadex G-100 and ion exchange chromatographies (DEAE and Mono Q). The most immunoreactive fractions were initially analysed by SDS-PAGE and Western blotting, before transfer to polyvinylidene difluoride (PVDF) membrane for NH2-microsequence determination. Our investigations allowed identifying a PAG-like immunoreactivity in extracts of fundic mucosa removed from fetuses in early pregnancy. Prochymosin immunoreactivity was more abundant in pyloric area removed late in pregnancy. Our data support the theory of switching of gene expression for aspartic proteinases during fetal, neonatal and adult phases.

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4. Specification of data collection on animal diseases to increase the preparedness of the Animal Health and Animal Welfare Panel (AHAW) of the European Food and Safety Agency (EFSA) to address questions from risk managers.

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Risk assessment is a core part of EFSA Animal Health and Welfare (AHAW) panel methodology used to assess the relationship among entities and risk. The lack of accurate quantitative data often hampers quantitative and evidence-based risk assessments. Therefore, we propose a methodological framework stating how to specify the data needs in accordance with the type of risk questions and the risk assessment modelling approaches. A retrospective analysis of AHAW panel opinions, published from 2004 to 2010, was first carried out to identify and categorize their most recurrent risk questions, the related list of data needs and the suitable risk assessment modelling approaches. Based on four case studies (*Echinococcus multilocularis* and *E. granulosus*, Porcine Reproductive and Respiratory Syndrome and Venezuelan Equine Encephalitis), we performed an inventory of the possible sources of data needed, assessing their availability and accessibility. A methodology for AHAW Panel data collection, including the definition of metadata standards for outcomes values to support the data validation and quality assessment, was developed. Different risk assessment model approaches were assessed with regards to the types of risk questions. Within the 38 opinions selected for the review, twelve main categories of risk questions were identified. Eight main types of data sources, made of 471 sites and references, mainly in PDF and HTML forms, were gathered. The DATASPEC conceptual model was based on Dublin Core Metadata Element Set, EFSA and international data standards, including 358 types of data and fifty related metadata.

5. Retrospective study of 44 cases of Equine Grass Sickness in Belgium (2008-2012).

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This study reports on 44 horses with equine grass sickness (EGS) presented to Ghent University. Cases were presented from January till October with a clear peak in spring. Fifty percent of affected horses were less than 5 years old and more than 80% were continuous on pasture. Thirty-one were diagnosed with acute EGS, with typical signs of colic, and 13 were chronic cases with anorexia, weight loss and muscle tremors. Histopathological confirmation after ileal biopsy was obtained in 27 acute cases. Other horses were diagnosed based on typical clinical signs and positive phenylephrine-eye-test. Thirteen acute cases were operated, 5 were immediately euthanized and in 13 conservative treatment was attempted. None of the acute cases survived. Mean survival time after surgery and conservative treatment was 3.6 and 2.5 days, respectively. Two operated cased survived the acute stage but progressed to chronic EGS and were euthanized several weeks later. Two horses with chronic EGS were immediately euthanized. Ten chronic EGS cases were discharged but only 1 survived. On 4 yards multiple EGS cases from the same premise were found. We noticed an increase in cases over the last 4 years (17 cases from 2008 till 2010 versus 27 cases over the last 18 months). Whether this reflects a true increase in the prevalence of EGS in Belgium or is caused by a change in the referral process from private practice remains to be examined. We concluded that EGS is relatively frequently encountered in Belgium and that it is often fatal.

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6. Feline polymorphonuclear neutrophils produce pro-inflammatory cytokines in response to *Microsporum canis.*

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Microsporum canis is responsible for most dermatophytoses in cats, its natural host, and other mammals including humans. Despite their superficial localization in skin and keratinized structures, dermatophytes can induce a cellular immune response which is correlated with clinical recovery and protection against reinfection. However, for *M. canis* infection as for other dermatophytoses, little is known about the mechanisms involved in the establishment of this specific immune response, including the potential role of immune cells encountered by the fungus during the early stages of infection. In some other superficial mycoses, polymorphonuclear neutrophils (PMNs) are the first leucocytes recruited to the site of infection and are responsible for elimination of the fungus by phagocytosis and production of superoxide anions. Additionally, they can interact with dendritic cells and are able to initiate the immune response by producing specific cytokines during microbial infection. The aim of this work was to assess the cytokine production by feline PMNs following stimulation with both *M. canis* arthroconidia and secreted or structural components of the fungus. The amount of specific cytokines secreted by PMNs during stimulation was measured by ELISA and quantitative RT-PCR (qRT-PCR). Results obtained by ELISA showed that PMNs produce high levels of TNFα, IL-1β and IL-8 following stimulation with *M. canis* arthroconidia and secreted as structural components. Only IL-8 mRNA level increased after stimulation with *M. canis* arthroconidia as measured by qRT-PCR. In conclusion, feline PMNs could be key actors involved in the initiation of the immune response against *M. canis* by producing pro-inflammatory cytokines.

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7. Effect of 120 minutes of high pressure-volume and low pressure-volume mechanical ventilation on plasmatic markers of systemic inflammation in horses during general anaesthesia

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Mechanical ventilation (MV) may activate an inflammatory response without preexisting lung disease (1). Conventional ventilation enhances the systemic inflammatory response after major surgical procedures (2). We investigated systemic changes of IL-6, TNF-α, neutrophil elastase (ELT) and myeloperoxidase (MPO) during MV in 34 ASA I-II horses (age 9.8 ± 5.4 years; weight 510 ± 88 kg) anesthetized for surgery. Horses were premedicated with acepromazine (0.1 mg kg^{-1} IM) and xylazine (0.6 mg kg^{-1} IV), induced with midazolam (0.06 mg kg^{-1} IV) and ketamine (2.2 mg kg^{-1} IV) and maintained with isoflurane in oxygen 70% plus ketamine-midazolam infusion (1 and 0.02 mg kg^{-1} h^{-1}, respectively). NSAIDs and antibiotics were administered before surgery. Horses randomly received either high pressure-volume (PIP 30 cmH_2O-VT > 10 ml/kg) or low pressure-volume (PIP 15 cmH_2O-VT ≤ 10 ml/kg) MV, in dorsal or lateral recumbency. I:E was set between 1:2-1:3 and RR at 4-15 breaths/minute to achieve the PIP and VT. Plasmatic pro-inflammatory mediator concentrations were measured by ELISA at the beginning and after 60 (T1) and 120 minutes (T2) of MV. Plasma MPO and ELT concentrations were significantly lower at T1. Plasma IL-6 did not vary and TNF-α concentration was significantly lower at T1 and T2. These changes were not linked to ventilation strategy or recumbency.None of the ventilation protocols enhanced systemic inflammatory response during surgery. The anti-inflammatory properties of drugs included in the anaesthesia protocol may have contributed to the overall decreased systemic inflammatory mediator concentration, despite MV. Systemically undetectable pulmonary inflammation cannot be excluded.

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8. Experimental co-infections of calves with bluetongue virus serotypes 1 and 8.

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Two independent experiments of co-infections were realized in calves with bluetongue virus serotypes 1 and 8 (BTV-1 and 8) with the objective to study their clinical, virological and immunological outcomes in comparison with BTV-1 and BTV-8 single infections. In the course of the first experiment, the incidental contamination of the BTV-1 inoculum with BTV serotype 15 (BTV-15) led to a triple co-infection. Prior to the second experiment, BTV-1 and BTV-8 inocula were tested to exclude other serotype contaminations. The use of a standardized clinical form for bluetongue disease allowed the registration and the attribution of clinical scores. EDTA blood and sera samples were collected daily to allow viral detection and seroneutralization assays. Approximately three weeks post-infection, calves were euthanized and necropsied. A typical clinical expression of bluetongue disease was observed. In both experiments, high levels of BTV-8 RNA were detected in the course of viraemia (high titres of viable BTV-8 were also measured). Interestingly, while during single infections BTV-1 induced high levels of viraemia and neutralizing antibodies, during co-infections only traces of BTV-1 RNA and low titres of anti-BTV-1 neutralizing antibodies were measured. The development of viraemia and high titres of neutralizing anti-BTV-1 antibodies during single infections proved that the inoculum was infectious and the detection protocols were efficient. Furthermore, *in vitro* growth kinetic experiments did not reveal any differences between BTV-1, 8 and 15. Our study suggests the occurrence of interference between serotypes in the course of multiple *in vivo* co-infections. The reasons of interference have to be investigated.

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9. Study of the virulence of serotypes 4 and 9 of African Horse Sickness Virus in two mouse models.

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African horse sickness is an infectious disease caused by a double stranded positive RNA virus which belongs to the family *Reoviridae*, genus *Orbivirus*. The virus has nine known antigenically distinct serotypes and is transmitted by *Culicoides* biting midge, principally *Culicoides imicola*. African horse sickness causes severe morbidity and mortality up to 95 % in horses with severe economic losses. The establishment of an experimental mouse model is needed for the investigation of the pathogenesis of this infection. Two mouse models, interferon-α receptor knock-out mice and immunocompetent mice, were tested. The used virus for mice inoculations belonged to the two serotypes which caused epidemics in Europe, serotypes 4 and 9. The virus was inoculated by subcutaneous route and/or by intra-nasal route. Samples of whole blood were taken for each infected and knock-out mice at regular intervals. The organs (liver, spleen, kidney, lung and brain) were taken at the end of experience of when the most affected mices were euthanasied. All these samples were tested by a qRT-PCR targeting African horse sickness genome segment 7. The results demonstrate the potential of the immunodeficient mouse model for both clinical and biological features. Both serotypes of African horse sickness were detected by qRT-PCR until three weeks post-infection (corresponding with the end of the experience) in blood. The setting up of this mouse model has developed a tool for efficient *in vivo* study to characterize the *in vivo* virulence of this virus, to monitor the evolution of viral populations during *in vivo* replication cycles and to test the competence or vectorial capacity of indigenous *Culicoides*.

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10. Bronchoalveolar lavage in calves: assessment of sampling place.

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Although bronchoalveolar lavage (BAL) is frequently used as a diagnostic tool for infectious bovine respiratory disease, no information is available about the specific sampled lung location in vivo. The aim of this study was to investigate if catheter intromission length could predict the sampling location. Repeated BAL catheter introductions were performed on 11 calves and location was verified by endoscopy and/or radiography. Influencing factors of the sampling place were analysed by logistic regression with calf as a random factor. A linear regression model based on intromission length was built to predict whether cranial or caudal lung parts were sampled. In 97% (80-100) of cases, the catheter was introduced in the dorsocaudal lung parts. The probability of sampling the accessory lobe or the dorsocranial lung parts was very low (respectively 1.8 (0-20) and 1.2% (0-7.7). The individual calf was the only factor which significantly influenced (P<0.001) the sampled lung part. A formula to predict the sampling location based on the measured intromission length of the catheter could be obtained from a linear mixed model. Both the models based on weight and wither height were useful, explaining respectively 77% and 87% of the variation of the intromission length between the calves. In conclusion we can state that the predominant sampling place by BAL is the dorsocaudal lung part which implies that BAL does not sample the most affected part of the lungs. Repeated catheter intromissions showed little variations in the sampled lung part within a calf but significant differences between calves.

11. The new potential of metagenomic tools in food microbiology: an example with microbiota of four cheeses.

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During the last three years, metagenomic studies were used essentially for environmental samples but it could be used also to analyse bacterial populations of food samples. This work describes the application of this technique to study the bacterial population of different types of soft cheeses. Among these, three of them are a typical Belgian soft cheese with washed rind (two with raw milk and the third with pasteurized milk). The fourth is a French creamy soft cheese made with raw milk. Classical microbiological and 16S rDNA metagenomic analysis were carried out in the core and on the rind of the four cheeses, giving a total of 8 samples. In total, 48 genus and 163 species were identified for all samples. As expected *Lactoccocus lactis* and/or *cremoris* are the most representative species in the core of the four cheeses. On the rind of cheeses, the predominant bacterial species are *Psychrobacter glacinola*, *Staphylococcus equorum*, *Corynebacterium casei* and *Marinilactibacillus psychrotolerans*. *Brevibacterium* spp and *Psychroflexus* spp are important for the rind of washed rind cheeses. The two Belgian soft cheese made with raw milk are composed of many more different bacterial species. While the cheese made from pasteurized milk contains less species mainly composed by *Lactococcus lactis* (97,6%) in the core. The subtleties of cheese character, as well as their shelf-life, are largely determined by the evolution of their microbiota. Among the culture-independent techniques, ultra-sequencing has contributed to place metagenomic analysis as the best alternative to study complex microbiota.

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12. Analyse sensorielle, biochimique et microbiologique du Tilapia du Nil (*Oreochromis niloticus*) conservé à 30°C et à 4°C.

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La durée de conservation du Tilapia (*Oreochromis niloticus*) à +4°C et à 30°C a été étudiée à travers une évaluation de sa qualité sensorielle, biochimique et microbiologique. L'évaluation biochimique concerne l'évolution, au cours du stockage, des paramètres suivants : le pH, l'Azote Basique Volatile Total (ABVT), la triméthylamine (TMA) et les TBARS (produits secondaires de l'oxydation lipidique). L'extraction de l'ABVT et de la TMA a été réalisée par la méthode officielle du Règlement (CE) n° 2074/2005. Les TBARS ont été analysés par une méthode colorimétrique. L'analyse microbiologique concerne la flore totale mésophile et les entérobactéries en utilisant les méthodes des normes françaises NF V 08-051 et NF V 08-054, respectivement. Les résultats obtenus ont montré que les taux d'ABVT et de TMA augmentent significativement au cours du stockage. Le pH musculaire chute légèrement après la mort du poisson, puis reste inchangé tout au long de la durée de conservation dans les deux conditions de température. Les TBARS sont relativement stables et à faibles concentrations pendant les premières heures puis augmentent progressivement et continuellement à partir de 12h à 30°C et 3 jours à 4°C. Il existe une bonne corrélation entre les résultats des trois analyses réalisées indiquant une qualité acceptable pour une durée de conservation du Tilapia *(Oreochromis niloticus)* d'un maximum de 12 heures à 30°C et 5 jours à 4°C.

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13. Screening of methyltestosterone residues in Tilapia's flesh after a masculinization treatment of Tilapia fry.

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Nile tilapia (*Oreochromis niloticus*) is farmed in at least 100 countries, making it the second fish species widely cultured worldwide. Hormonal treatment with 17α-methyltestosterone (MT)-impregnated food is highly effective on tilapia fry. It is the most simple and reliable way to induce sex reversal and to produce all male-tilapia stocks, which consistently grow to a larger/more uniform size than mixed sex or all-female stocks. In this work, we try to assess the withdrawal of MT residues in tilapia's flesh after MT treatment (65 mg of MT/kg fry feed) for 28 days of tree batches of tilapia fry. An additional group was used as a control (hormone-free diet) in a second independent recycling system. Food was distributed over 8-h period by using automatic belt feeders. Two months after treatment, the sex ratio average in MT-treated groups were 97.78 % males and 2.22 % females which were significantly different from the control group (48.57 % and 51.43 % respectively). Flesh was sampled in both control and treated fish at 15 days intervals after cessation of treatment: from the first day until 3 months later. MT residues were analyzed using an ELISA method, after liquid/solid extraction of flesh. The LOD and LOQ of the ELISA test were 0.05 ppb and 0.1ppb respectively and the recovery of the extraction was 55%. The obtained results demonstrated that the concentration of MT is very low one day post treatment (1.59 ng/g), then continues to decrease and falls below the detection threshold (0.05 ppb) after 60 days post treatment. We can suggest that the withdrawal of MT is 2 months after treatment with a dose of MT ≤ 65mg/kg. The main conclusion is that MT treatment of tilapia carries no human health risks, provided it is applied only during the early fry stage (28 days) and the grow out period exceed 4 months to reach marketable size according to countries.

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14. Simple and Rapid High Performance Thin-layer chromatography method for monitoring of biogenic amines in Tilapia flesh (*Oreochromis niloticus*).

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A rapid and simple HPTLC method was developed for the monitoring of six biogenic amines from Tilapia (*Oreochromis niloticus*) conserved at +4°C and 30°C. The method was validated in terms of linearity, sensitivity, repeatability and recovery according to the recommendation of the Commission decision EC/2002/657. The coefficient of determination (R^2) was higher than 0.98 for all calibration curves. The limit of detection was 25 ppm and the recovery was more than 84%. The repeatability was below 8 %. The new method can be limited to a simple screening by comparing the intensity of the revealed spots from the analyzed samples with the intensity of the reference's spot (100 ppm witch is the regular limit of histamine in Europe). The method can be also used for quantification using a TLC scanner programmed by Win CATS software from CAMAG instrument. We found a good correlation between the screening results and the confirmation ones: spermidine, spermine and tyramine are constant at low concentrations (<100 ppm) throughout the shelf life for the two storage's mode. On the other side, putrescine, cadaverine and histamine appear from the 18th hour of storage only at 30°C. The concentration of putrescine and cadaverine exceeds the threshold of 100 ppm and the concentration of histamine is less than 50 ppm (± 34 ppm).

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15. Evaluation of the impact of electro shrimp trawl fishery.

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Bottom trawling, used for catching brown shrimp, is known to produce large amounts of discards and to disturb the seafloor habitat of benthic organisms. In order to consider ecological certification and increase the sustainability of these fisheries, technical adaptations are necessary to avoid these problems. Electric pulse fields have proven to be the most promising option for alternative stimulation in fishing gear, replacing the mechanical stimulation. Since 2008 the Belgian ILVO research institute has been successfully testing their Hovercran electro pulse trawl for brown shrimp fishery. In this device the bobbin rope is replaced by light weight electrodes creating a low-intensity electric field which selectively induces a startle response in the shrimps. Other benthic organisms are left untouched and can escape underneath the hovering trawl that collects the jumping shrimps without disturbing the seabed. Nevertheless, effects of suchlike electric pulse field on marine organisms are largely unknown. Preliminary exposure and survival experiments indicated that this low frequency pulse has no immediate significant effects on most adult fish and invertebrates. However, electro sensitive fish, like sharks and rays, and polychaete species were not included in these studies. Additionally, the influence on different life stages has never before been investigated, although electrofishing over active spawning grounds may affect survival of embryos, larvae or juveniles if exposed during their more sensitive stages. Further research to fill these gaps in knowledge hence is crucial to revalue pulse fishing and to provide information enabling to lift the standing ban on electric fishing in the EU.

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16. Conserving the skeleton from whale cadavers: a big challenge.

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Optimal preservation of skeletal parts for museological or research purposes primarily relies on proper cleaning and processing of the fresh specimen and its bones. The typical procedure as applied by our department involves 4 steps: manual removal and enzymatic maceration of soft tissues, a bleaching step using hydrogen peroxide and finally degreasing of the bones. The latter process in particular is important for long-term conservation, as disintegration of fat results in formation of fatty acids which erode the mineral substance of bone. In voluminous specimens with a high fat content such as whalebones, removal of this fat poses a major challenge. Degreasing typically involves toxic or polluting solvents which need to be applied in sealed systems with limited capacity. Currently, an optimal protocol for large cetacean skeletons is lacking, resulting in an incomplete removal and dripping of residual fat from exhibited specimens. Alternative procedures to preserve large skeletons such as burial are no longer allowed because of environmental and safety considerations. Recently, the Museum of Morphology (MuMo, Ghent University) has taken part in the dissection of two stranded whales. For the degreasing of the huge lower jaws, microbial dissolution of the fat and application of industrial 'cold' degreasers in an atomizing system are being tested. The exhibition of whale skeletons fits within the new acquisition policy of this museum, which seeks to diversify its collection, currently consisting mainly of domestic and laboratory animal species, since wildlife medicine and evolutionary biology are of increasing importance in the curriculum of veterinary students.

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17. Exploring an enigmatic organ: Surgery-oriented anatomy of the canine omentum majus.

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A wide variety of particular functions are currently attributed to the omentum, including fat storage, tissue fluid drainage, adhesion to sites of tissue damage and encapsulation of inflammatory processes. It also acts as a source of inflammatory cells and angiogenic factors. All these characteristics turn the greater omentum into a very intriguing organ, with beneficial properties in reconstructive surgery. Research into and application of new surgical techniques involving the omentum, such as creating pedicled flaps to reach extra-abdominal sites to improve wound healing, know a steady rise in small animal medicine. However, these surgical procedures are mostly based on knowledge derived from human anatomy since veterinary anatomical data and omental nomenclature is currently lacking or ambiguous. A thorough knowledge and univocal description of the omental anatomical configuration are a prerequisite for the successful development and optimal implementation of these surgical techniques. The omentum is characterized by a great plasticity, which demands creative solutions to define, describe and set out its borders. By applying polyurethane-based-foam injection into the omental bursa, we found a standardized solution to visualize and unequivocally describe the layout of the bursa and its enveloping walls. The studies on dog cadavers were performed both on freshly dissected specimens as well as in situ, by applying minimal invasive casting techniques. These techniques proved to be useful tools to study the canine omentum majus and bursa omentalis. The resulting casts showed fine anatomical details such as the compartmentalization of the omental bursa and its delineation by anatomical structures.

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18. Effect of water availability on milking frequency and yield of dairy cows milked in pastures by an automatic milking system.

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The milking frequency is decreased when grazing and automatic milking system (AMS) are combined compared to an indoor system. On pasture, cows tend to visit AMS by group and enter in close succession. An improved cow traffic would allow to increase milking frequency and avoid long waiting before milking. In this aim, the effect of water availability -by means of drinking bowl- in the grazed paddocks was tested. The trial was carried out during a period of 30 days from Augustus to September with a herd of 44 Holstein dairy cows milked with an AMS located on pasture. They had always access to a water trough located near the AMS. They grazed in a rotational system and were fetched twice a day (6:00am and 16:00pm) to the AMS. They were at 211 days in milk. The mean distance from paddock to AMS were 150 m. The mean temperatures were 16.9°C in paddocks with available water and 17.6°C in paddocks with no available water (NW). The treatment had not effect on daily milk yield (18.2 kg/d) but, in NW treatment, the milking time, the daily milking frequency and the milking refusal numbers were increased (37 vs 34%;$p< 0.05$, 2.4 vs 2.0;$p<0.001$, 33.5 vs 17.9;$p<0.05$ respectively). The number of voluntary returns, i.e., the sum of the milkings, the refused milkings and the failed milkings numbers minus the fetching numbers, was higher in NW treatment (1.21 vs 0.46;$p<0.001$). Offering cows drinking water near the AMS only can increase the milking frequency.

19. Characterization of T-helper lymphocytes in peripheral blood and bronchoalveolar lavage of healthy horses and in horses with recurrent airway obstruction.

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Heaves, also known as recurrent airway obstruction (RAO), is a respiratory disease of horses that is characterized by lower airway neutrophilic inflammation and airway obstruction caused by hypersensitivity of susceptible horses to inhaled organic dusts. The immunological processes underlying RAO remain unclear . The objective of this project was to characterize the nature of the adaptive immune response in affected horses. We hypothesized that the immune response in affected horses is predominantly a Th17 type response with a Th17/Treg imbalance. We focused on three major types of Th responses (Th1, Th2 and Th17) by studying expression of transcription factors and cytokines associated with the polarization of T helper lymphocytes in peripheral blood and in bronchoalveolar lavages (BAL) by quantitative PCR. Four horses affected by heaves (neutrophilic lung inflammation) and three healthy horses were recruited and underwent an environmental challenge by exposure to hay and straw for 20 days. We observed that the expression of GATA3 was significantly upregulated in bronchoalveolar lavage cells from heaves-affected horses compared to healthy horses at the end of the environmental challenge. Furthermore, FOXP3 expression was significantly upregulated during the challenge in healthy horses but not in RAO-affected horses. Although our results allowed us neither to establish precisely the type of immune response implemented in equine heaves nor to prove an involvement of a predominant Th17 response in RAO-affected horses, these data suggest that a less pronounced regulatory immune response and a Th2 response may have an involvement in the immunopathologic mechanisms of heaves.

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20. Effect of chronic confinement stress on sperm quality in Eurasian perch.

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Stress plays a key role in the ability of fish to perform reproduction. Given the huge variability in fish reproductive strategies, large variability in the effects of stress on reproductive efficiency could be expected. Eurasian perch (*Perca fluviatilis*) is mainly represented in Europe and reared under intensive rearing conditions (e.i. high confinement), which are known to impair with many physiological function. In perch, stress induces an increase of cortisol, but little is known regarding the effect of stress on male reproductive capacity. The aim of the present study was to examine the impact of chronic confinement stress on sperm quality of perch during the final maturational period. Perch breeders were reared in 3 confinement conditions (0.70 m^3, 0.5 m^3 and 2 m^3) in duplicate (except for 0.70 L) at the same density (12 ind/m^3) from September 2011 at a 50:50 sex ratio. The 5th and 26th March, 3 males from each tank were randomly handled, anesthetized and sperm collected by stripping. 100 µL were dissolved in extender, sperm concentration assessed by counting spermatozoa with a Burker's cell and sperm motility determine by CASA system. Velocity parameters studied were % progressive (Prog) and motile (MOT) spermatozoa and VAP, VCL and VSL. Sperm concentration decreased with confinement from 70.5±17.2 x10^9 to 61.5±9.9 x x10^9. VAP (from 32.6 to 40.2 µm/sec), VSL (from 23.5 to 30.0 µm/sec), VCL (from 81.6 to 89.5 µm/sec), MOT (from 56.0 to 85.0 %) and PROG (from 21.5 to 28.7) were not significantly influenced by the confinement level.

21. Ultrasonographic percutaneous anatomy of caudal lumbar region in normal dogs and us-guided approach for lumbar subarachnoid puncture.

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The use of ultrasound (US) to identify lumbar landmarks is a technique commonly used in human medicine. In the dog US of the lumbar region has not been described. The aims of this poster are: 1. to present the percutaneous US anatomy of the caudal lumbar region in dogs, 2. to present the US landmarks that can be used for US guided subarachnoid puncture in dogs. Five normal dogs were used to illustrated normal US anatomy of the caudal lumbar region. Images were obtained by a percutaneous approach with several transducers depending on dog size and fatness. Adjust of the US-guide subarachnoid puncture was done on 3 cadavers and in 2 anesthetized dogs. The top of the dorsal spinous process is easily seen as a curved hyperechoic line very close to the transducer surface. Articular processes are seen in a parasagittal position. The ease in visualization of the articular processes greatly depends on the amount of fat present. A longitudinal parasagittal US view was obtained to visualize the intervertebral space between two contiguous articular facets, which used as a landmark. The vertebral canal was visible between the articular facets and the sagittal plan. The needle was directed US guided toward the space between the articular processes were the vertebral canal was visible to enter the subarachnoid space. Visibility of the anatomical structure of the lumbar region at percutaneous US mainly depend on the fatness of the dog. Parasagittal images can be used to localize by US the site for obtaining a lumbar subarachnoid puncture.

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22. Imaging findings in horses with pharyngeal Squamous Cell Carcinoma.

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Squamous cell carcinoma (SCC) has been occasionally reported in the equine pharyngeal region. The aim of this poster is to describe imaging findings in 4 cases of pharyngeal SCC. Four old horses, mean age 19.5, were referred for dyspnea (3/4) and/or dysphagia (3/4). Because of dyspnea radiographs were realized prior to endoscopy. Ultrasound (US) was performed in all cases. A post-mortem computed tomography (CT) of the head was performed in one case. Radiographic opacity of the pharyngeal region was increased in all cases. A soft tissue mass was also visible in the caudal maxillary sinus in 1 horse. The epiglottis and the soft palate were either not recognized or difficult to see with an abnormal shape. No bony damage was identified on radiographs. A hypoechoic heterogeneous mass was visualized at US in 2 cases and an enlargement of the mandibular lymph nodes was observed in 3 cases. Oral and pharyngeal endoscopic examination confirmed a pharyngeal mass in 2 cases but was unsuccessful or incomplete because of passage impairment in 2. CT revealed maxillary bone lysis in the horse with a mass in the maxillary sinus. Histopathological examination of local biopsies or necropsy revealed pharyngeal SCC invading epiglottis, pharyngeal wall and soft palate in the 4 horses and the maxillary sinus in one. Because endoscopy can be impaired by the size of the mass, radiology is helpful in estimating the extent and invasiveness of the process and US to confirm lymphadenopathy. However because of its relatively low sensitivity and the local increased opacity, radiographic examination may underestimate bone lysis.

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23. Ultrasonography of the collateral ligaments of the distal interphalangeal joint in horses: technique and reference images.

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This study evaluates the portion of the collateral ligaments (CLs) of the equine distal interphalangeal joint (DIPJ) assessable by ultrasound (US) and describes US technique and reference images of the CLs in a series of normal forelimbs. Transverse and longitudinal US images were obtained on 5 sound horses and on 25 equine cadaver forelimbs. On 6 limbs, a needle was placed under US-guidance at the distal limit of visualization of each CL and the portion of CL visible at US was evaluated on computed tomographic (CT) images. The proportion of CL visible at US examination was more than 50% of the total ligament length in 9 of the 12 CLs assessed by CT. The normal CLs appear as oval structures located abaxially to the fossae of the middle phalanx in the transverse sections, obtained at the level of the coronary band. A centro-dorsal hypoechoic image appears with increasing proximodistal probe inclination, demonstrating different fiber orientations within the ligament. Two main fascicles, a deep and a superficial, distally divergent, are visible on longitudinal images obtained in the central part of the ligament. Awareness of the estimated portion of DIPJ CLs visible at US and detailed knowledge of the US technique and CLs morphology are essential to efficiently use US examination on clinical cases.

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24. A study of the caeco-colic vessels and lymph nodes at equine transabdominal ultrasonography.

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The lateral caecal vessels and lymph nodes are visible adjacent to the abdominal wall while colonic vessels are located axially to the large colon. Ultrasound (US) visualization of additional mesenteric vessels has been described as a sign of right dorsal colon displacement. This study aims to describe transabdominal US features of equine caeco-colic vessels and lymph nodes in healthy horses and horses with digestive symptoms. Transabdominal US images of the right abdomen were obtained prospectively on 14 healthy horses. Mesenteric blood vessels were followed and topography and size recorded. Visiblity of caecal lymph nodes was evaluated. US images from horses with digestive symptoms and visible additional and/or abnormal mesenteric vessels (11) and/or visible caecal lymph nodes (10) were retrospectively reviewed. In healthy horses, caecal vessels were visualized adjacent to the body wall from right mid-paralumbar fossa extending to ventral midline. Caecal lymph nodes were difficult to see. One additional mesenteric vessel was seen in 2 healthy horses emerging from the caecal vein and running caudo-cranially on a short length. Only 1 of the sick horses with additional visible mesenteric vessels had right dorsal colon displacement confirmed at surgery. The 8 other cases resolved medically. Horses with visible caecal lymph nodes had US evidence of large (5) and/or small (2) intestinal wall thickening. Visualization of additional mesenteric vessels in the right abdomen at transabdominal US is not only associated to right dorsal colon displacement but may happen in medical cases. Easily visible caeco-colic lymph nodes are suggestive of infiltrative enteropathy.

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25. Ultrasound-guided brush cytology of bladder and urethral lesions in dogs: techniques and results.

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Bladder wall thickening is a common ultrasonographic finding in dogs with bladder neoplasia but also with severe chronic cystitis or mural hematomas. Histological diagnosis is therefore crucial for the prognosis and treatment planning. The aims of this study are to describe the procedure of US-guided brush cytology (USBC) of bladder and urethral lesions in dogs and to evaluate diagnostic quality of USBC sampling. Twenty-three dogs that underwent bladder or urethral USBC between 2004 and 2012 were included. The technical procedure and the results of the cytologic examination were reviewed. A urinary catheter was brought caudally to the lesion under US guidance to serve as protection for the passage of the sheath containing the brush. In small dogs, no catheter was used. The brush tip was positioned on the lesion and moved back and forth several times under US control. The couple brush-sheath was pulled out of the urinary catheter and the brush tip rolled on glass slides. The procedure was repeated at least 3 times. The bladder was reassessed for signs of haemorrhage. Bladder bleeding occurred in one patient. Based on cytology reports samplings were of good to high diagnostic quality in 20/23 cases, of moderate quality in 2/23 and inconclusive in the patient that showed bladder haemorrhage. Cellularity was high in 8/23 cases, variable with clusters in 7/23 and low to moderate in 7/23. USBC of bladder and urethral lesions in dogs can be easily performed and provides samples of good diagnostic quality. Complications remain rare.

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26. Investigation of the effect of exercise on the innate immunity in horses

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Introduction: Respiratory diseases, including inflammatory airway disease (IAD), viral and bacterial infections, are common problems in exercising horses. The underlying immune mechanisms remain poorly documented. In humans, exercise has been associated with down-regulated expression of Toll-like receptors (TLRs) on monocytes. The objectives of this study were 1) to examine the expression of TLRs in equine monocytes at rest and after strenuous exercise on the treadmill; 2) to stimulate monocytes *in vitro* with specific TLR ligands, in order to resemble bacterial/viral infections; 3) to describe the cytokine expression of monocytes at rest and after exercise. **Materials and Methods:** Blood was collected from 8 horses at rest and after strenuous exercise on the treadmill. Monocytes were isolated via density gradient centrifugation and adherence. Monocytes were stimulated with TLR ligands. RNA was extracted and cell culture supernatants were collected. The TLR expression was evaluated via real-time PCR. Cytokine production was measured with ELISA kits for equine TNF-alpha and IFN-beta. **Results:** The expression of TLR in monocytes at rest did not differ from that in monocytes post-exercise. In equine monocytes, TLR2 and TLR4 are the predominant TLRs. Stimulation of monocytes with synthetic lipoprotein (FSL) and bacterial lipopolysaccharide (LPS) resulted in significantly increased production of TNF-alpha at rest and after exercise. The production of TNF-alpha at rest and after exercise differed significantly for stimulation of equine monocytes with FSL and LPS. **Conclusion:** Equine monocytes are sensitive to bacterial endotoxin and a strong immune response is induced. Exercise provokes an increased production of inflammatory cytokines.

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27. Accuracy of different temperature reading techniques and associated stress response in hospitalized dogs.

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Introduction/Objectives: Though considered the gold standard for core temperature estimation, rectal thermometry (RT) can be stressful and is not recommended in patients with ano-rectal disease. We evaluated the accuracy and associated stress of auricular and axillary thermometry (OT and AT). **Methods:** We performed a prospective study including 250 hospitalized dogs. Dogs with diseases prohibiting investigation of one of the temperature sites were excluded. Signalment, analgesic therapy and length of hospitalization were recorded. We used a veterinary auricular infrared device for OT and an electronic predictive thermometer for RT and AT. All measurements were obtained by the same veterinarian in a randomized fashion. Heart rate was noted before and immediately after each measurement. Stress behaviors (vocalization, lip licking, shaking, panting, defensive behavior) were recorded and graded from 0 (lowest) to 4 (highest) for each measurement. **Results:** RT measurement was associated with the greatest increase in heart rate ($p<0.05$). Scores obtained for defensive behaviour, lip licking and vocalisation were lowest with AT and highest with RT measurements ($p<0.05$). Mean RT, AT and OT were respectively 38.00°C (SD: 0.8461°C), 36.95°C (SD: 0.9938°C), and 37.23°C (SD: 1.0382°C). AT and OT were moderately correlated with RT ($r=0.70$ and $r=0.64$ respectively). Gender ($p=0.0235$) and coat length ($p=0.0299$) had a significant influence on our results. No effect of dehydration, body condition, analgesia, age, reproductive status or operator experience was observed ($p>0.05$). **Conclusion:** AT and to a lesser extent OT, are valuable, less stressful alternatives to estimate RT in dogs.

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28. Qualitative bedside evaluation of the microcirculation via sidestream dark field imaging in dogs.

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Sidestream dark field imaging (SDF) is used to visualize the microcirculation. Current gold-standard, or 'consensus' analysis (CA) of SDF films takes 1 hour per film limiting the application of SDF in a clinical setting. We developed a subjective point of care scoring system (Bedside evaluation of the microcirculation, BEM) that could provide real-time intervention points and optimize care for the critical patient. The objective of this study is to evaluate whether the BEM-score correctly identifies films of sufficient and insufficient diagnostic quality as defined by CA. Three observers were trained using an instruction video to evaluate five quality parameters: stability, content, illumination, focus and pressure. BEM was performed by scoring each film four times in immediate succession. Quality parameters were scored (0 perfect, 1 sufficient and 2 insufficient) similar to CA. BEM pass-fail assessment matched CA 85.6% of the time with individual observer agreement of 84.4-87.8%. Agreement of BEM with CA did not change over the study period (84.4%, 87.8% and 84.4% on days 1,2 and 3 respectively) indicating accurate quality analysis after viewing the training video once. High levels of inter-observer agreement and the strong correlation with CA demonstrate that BEM quality evaluation can produce repeatable and reliable results. Variation in individual parameter scores may reflect systematic erroneous assignment of certain parameters or individual bias towards assessment of certain features. Nevertheless, this did not impact on the overall evaluation of film quality. This study provides a platform to investigate whether rapid semi-quantitative analysis of the microcirculation itself is feasible.

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29. Cardiac ultrasound in canine emergencies with a systemic inflammatory response syndrome.

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Cardiac dysfunction is a concern in human systemic inflammatory response syndrome (SIRS) patients. A previous canine SIRS study demonstrated increased cardiac biomarkers (NT-proBNP, cTnT and lactate) and their prognostic value. The present study evaluated basic echocardiographic parameters (Fractional shortening (FS) and left ventricular ejection fraction (LVEF), which reflect systolic function; and left atrium to aorta ratio (LA/Ao), which reflects preload) in canine SIRS. Our hypotheses were that (1) FS, LVEF and LA/Ao are altered in canine SIRS and (2) that these parameters carry prognostic information. Dogs presenting in emergency with SIRS, without primary cardiac disease, were prospectively included. Echocardiography was performed at presentation (T0), after 6 (T6), 12 (T12), 24 (T24), 72 (T72) hours of hospitalization and at control visits (T1m) over one month after discharge. Statistical analysis was performed with SAS. Univariate analysis was used to assess normal distribution. A mixed procedure and a logistic procedure was performed accordingly ($p < 0.05$). Thirty seven dogs were included, 9 of which died. FS and LA/Ao were significantly correlated with survival to discharge, however LVEF was not. Additionally, LVEF and FS did not change significantly during hospitalization; neither compared to T1m. LA/Ao did however increase significantly from 1.03 (0.76-1.74) (T0) to 1.12 (0.84-1.68) (T12), 1.20 (0.8-1.54) (T72) and 1.19 (1.05-1.59) (T1m). Unexpectedly, survivors had lower FS (35.7% (19-64) compared to 44.3% (33-53)). LA/Ao increased rapidly after hospitalization to values similar to T1m, which probably reflects efficient fluid therapy in emergencies. In this population, no echocardiographic evidence of cardiac dysfunction was demonstrated.

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30. Caractérisation du mouton Koundoum au Niger : description morphobiométrique et détermination d'une formule barymétrique.

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La race Koundoum, un des rares moutons à laine du Sahel, est victime des croisements avec d'autres races plus productives et de la dégradation de son biotope originel, le fleuve Niger. Cette race possède des qualités intéressantes d'adaptation au milieu et de production de laine, qui justifient la mise en œuvre d'un programme de conservation de ce patrimoine génétique original. Dans cette optique, notre étude vise à la caractérisation de la race Koundoum afin d'établir les fondements d'un plan pour sa conservation. Il a ainsi été procédé à la caractérisation morpho-biométrique de moutons de race Koundoum auprès de 26 éleveurs, principalement de la commune de Kourteye. Au total, l'étude a porté sur 147 sujets (45 mâles, 102 femelles), dont 56 de moins d'un an. La couleur de robe noire domine (62,6%), suivie de la couleur blanche (27,9%). Les pendeloques sont présentes chez 40,0% des mâles et 26,5% des femelles. Presque tous les béliers portent des cornes (95,6%) contre 3,9% des brebis. Le périmètre thoracique a été retenu pour l'estimation d'une équation barymétrique en raison de sa forte corrélation avec le poids (R^2 = 0,88). Des formules de prédiction du poids ont pu être proposées selon le sexe et l'âge. En dépit du manque de contrôle des croisements par la majorité des éleveurs inclus dans l'étude, l'échantillon a révélé des caractéristiques relativement homogènes. La détermination d'une formule barymétrique pourra venir en soutien d'une sélection incluant un objectif de croissance.

31. Multidisciplinary and Evidence-based Method for Prioritizing Diseases of Food-producing Animals and Zoonoses.

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The categorization of animal-related threats is one of the pillars of the new strategy for animal health recently adopted by the European Union. The method presented here is based on a multi-criteria decision making including multidisciplinary experts' opinion and evidence-based data. Hundred diseases were included in the process: 86 animal/zoonotic/common diseases of terrestrial warm-blooded animals reportable to the World Organization for Animal Health, 12 diseases reported in Europe between September 2009 and 2010, parafilariasis and salmonellosis (*Salmonella enteritidis*). Five categories of criteria (N=57) were considered: epidemiology-infectiology, prevention-control, economy-trade, public health and society. International experts performed intra-category (n = 40) and inter-category (n = 6) weighting of these criteria. Information corresponding to each criterion/disease, was collected through an evidence-based methodology. An overall weighted score was calculated for each disease based on a deterministic (mean of each weight) and probabilistic (distribution functions of weights by using Monte Carlo simulation) approaches. Consecutive ranking was established. Classification and regression tree analysis enabled classification of diseases into four subgroups according to relative roles. The present methodology is a generic and predictive tool applicable in a great variety of contexts. The standardization of criteria ensures the transparency and the reproducibility of the model in other context and for other diseases. It can be easily adapted if certain conditions are modified. The model could be applied to diseases affecting domestic or exotic pets (reptiles, etc.), but also to enzootic conditions in order to better re-target the surveillance system and re-adapt prevention and control measures.

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32. Validation of a method for measuring the colour and determining the proportions of myoglobin redox forms on beef.

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The aim of this study was to validate the use of the spectrophotometer Minolta CM-600d for measuring the colour and the proportions of different myoglobin redox forms (oxymyoglobin, deoxymyoglobin, and metmyoglobin) on the surface of meat. One vacuum-packaged (VP) striploin was supplied by a Belgian food wholesaler. It was cut in 3 cm thick steaks, repacked under vacuum and stored at –0.5 °C until analyses. The measurement of colour in the C.I.E. L*a*b* space and the determination of oxymyoglobin, deoxymyoglobin, and metmyoglobin were performed on VP and modified atmosphere-packed (70 % O_2/30 % CO_2 for 24 h) samples (n = 10). Results obtained were compared to two reference methods (colour measurement using a chromameter Minolta CR-400 and spectrophotometric determination of different myoglobin redox forms in aqueous meat extracts) by F-test for precision and t-test for accuracy. Statistic significance level was established at 5 %. The two colour measurement methods presented the same precision, when considering VP samples only, and different accuracies, probably because of the different detectors and observation angles used by both devices. The two methods for determining the different myoglobin forms presented also the same precision but different accuracies, probably due to the fact that oxygenation is favoured during some steps of the reference method (e.g. extraction, filtration). In conclusion, the results for colour measurement obtained by both devices cannot be compared. It is necessary to compare both methods for determining oxymyoglobin, deoxymyoglobin, and metmyoglobin in complete anaerobic conditions in order to eliminate the oxygenation bias.

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33. Brucellosis in a live stranded harbor porpoise (*Phocoena phocoena*).

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Brucella ceti infection in cetaceans is described in striped dolphins (*Stenella coeruleoalba*), Atlantic white-sided dolphins (*Lagenorhynchus acutus),* in bottlenose dolphins *(Tursiops truncatus),* harbor porpoises (*Phocoena phocoena*) and minke whale *(Balaenoptera acurostrata).* The present communication describes the first confirmed case of *B. ceti* infection and associated lesions in a live-stranded harbor porpoise along the Belgian coast (Marine Animals Research and Intervention Network program-MARIN). The animal was necropsied and histology, immunohistochemistry (IHC), transmission electron microscopy (TEM) as well as bacteriology were performed. The animal, a female of 41 kg and 152 cm was severely emaciated. Relevant lesions were skin ulcers, severe nematode infestation (airways and pulmonary blood vessels) and severe necrotizing pneumonia. The IHC for the detection of *Brucella spp.* revealed intracytoplasmic positive staining in mononuclear cells in skin ulcers, spleen, lymph nodes, lung, uterus, mammary gland (parenchyma and milk) and brain. By TEM, very large numbers of relatively small, coccoid bacteria were observed intra- and intercellularly in the genital ulcer. A Brucella isolate was obtained from brain and lung. The isolates showed catalase ,oxidase and urease activity, did grow in the absence of CO2 and agglutinated anti-A monospecific antiserum. The variable number tandem repeat (VNTR) profile of the strain was typical of *B. ceti*, in agreement with the biochemical typing. The present study suggests that the stranded animal suffered from bacteriaemia associated with *B. ceti* and is the first case described for the Belgian and northern French coastline. Many similarities appear between gross-lesions and microscopical findings between this case, and other cases of cetacean brucellosis described elsewhere in Europe. The presence of *Brucella* sp. antigens in mammary ducts and in skin ulcers may indicate ways of bacterial transmission between individuals. It raises the question of a risk of zoonosis when a cetacean is handled on the beach or in rehabilitation center.

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34. Activités agricoles familiales dans la ville de Lubumbashi (Katanga, RD Congo).

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Dans le but de quantifier la pratique agricole (culture et élevage) en milieu urbain, de déterminer les motivations et contraintes ainsi que les aspirations des agriculteurs, une enquête, financée par le Cecodel et menée par l'équipe du projet interuniversitaire ciblé (PIC) *Cabrilu*, a été réalisée en décembre 2005. 552 chefs de ménage résidant dans la ville de Lubumbashi (Katanga, RD Congo) ont été interrogés. Le questionnaire comprenait les points essentiels suivants : la localisation du ménage et l'identification de l'individu interrogé, les indicateurs du standard de vie, les activités professionnelles, les sources de revenus, la pratique de l'agriculture en milieu urbain, les principales contraintes rencontrées, la disponibilité des prestataires de services, les motivations et bénéfices retirés. Il apparait que 98 % des enquêtés pratiquent l'agriculture : 70 % mènent les deux activités, à savoir la culture végétale et l'élevage; 14 % sont seulement éleveurs et 14 % cultivateurs. Bien qu'étant, pour la majorité d'entre eux (75 % des enquêtés), une activité complémentaire, elle s'avère, pour plus de 70 % des enquêtés, indispensable à la survie du ménage. La volaille est la principale espèce élevée, suivie par la chèvre, tandis que la culture du maïs et l'horticulture prennent une part importante dans les activités de ces ménages. Toutes les classes sociales pratiquent l'agriculture. L'autoconsommation est la principale raison de cette activité, suivie par l'opportunité de revenus complémentaires. Les principales difficultés rencontrées par les familles sont la difficulté d'accès aux intrants (engrais, semences, aliments pour bétail) et les problèmes de maladies.

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35. Analyse de la croissance pré-sevrage des chevreaux de la race locale (Lubumbashi, RD Congo).

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Après la volaille, l'espèce caprine est la deuxième, en importance, à être élevée à Lubumbashi (RD Congo). Une étude a été menée afin de caractériser la croissance des chevreaux de race locale, de la naissance au sevrage. Elle a porté sur le suivi de 116 chevreaux. 83 mises-bas ont été enregistrées dont 62,65% portées simples, 34,94% portées doubles et 2,41% portées triples. La prolificité moyenne est de 1,42 chevreau par mise-bas. Le taux de sevrage est de 85,40%. Le poids moyen à la naissance et au sevrage (90 jours en moyenne) est respectivement de 1,77 ± 0,46 kg (n=116), et 5,65 ± 1,68 kg (n=99). Le gain quotidien moyen (gqm) de la naissance au 10^{e} jour, du 10^{e} au 30^{e} jour, du 31^{e} au 90^{e} jour est respectivement de 90,80 ± 49,45 g/jour, 53,10 ± 41,40 g/jour et 30,70 ± 25,20 g/jour. Le sexe, la taille de la portée, la saison de naissance, les interactions taille de portée X sexe et saison de naissance X taille de portée influencent significativement le poids pré-sevrage ($p<0,05$). Le taux moyen de mortalité pré-sevrage est de 14,65% (8,62% entre 0 et 30 jours; 6,66% entre 31 et 90 jours). Il est significativement ($p<0,05$) plus élevé pour les naissances multiples (23,40%) que pour les naissances simples (3,90%). Ces performances de croissance pré-sevrage sont moyennes au regard de ce qui est observé pour d'autres races et dans d'autres conditions d'élevage. Néanmoins, elles pourraient être améliorées notamment par la modification des conditions d'exploitation et par la sélection.

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36. Transforming growth factor beta 1 and its activating pathways in canine idiopathic pulmonary fibrosis.

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Idiopathic pulmonary fibrosis (IPF) is a progressive interstitial fibrotic disease, described in humans and in dogs. Etiology and pathogenesis of IPF are poorly known, even if a genetic basis is suspected in dogs because of the predisposition of the West Highland white terrier (WHWT). Serum transforming growth factor beta 1 (TGFB1) concentration is elevated in both healthy WHWTs and WHWTs with IPF, as compared to healthy dogs of various breeds. The aim of the present study was to quantify TGFB1 expression, as well as expression of proteins involved in TGFB1 activation, by quantitative RT-PCR, in lung tissue from dogs with IPF versus control dogs. Total RNA was extracted from lung tissues from 14 dogs with IPF (12 WHWTs, 1 Scottish terrier, 1 Lhassa Apso) and 11 control dogs (various breeds). IPF was confirmed by histopathology on all samples. Expression of TGFB1, 2 integrins (ITGB6 and ITGB8) and THBS1 was measured by qRT-PCR. Statistically significant differences between the groups were assessed using a Student t-test or a Mann-Whitney Rank sum test with significance defined as a $p < 0.05$. Expression of TGFB1 and ITGB6 was not statistically different between the two groups. Expression of ITGB8 was significantly lower ($p<0.001$) while THBS1 expression was significantly higher ($p=0.016$) in the IPF group relative to controls. This study highlights different activating pathways of TGFB1 in IPF lungs compared to control lungs with a shift toward an increased activation via THBS1 in canine IPF.

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37. CCL2 as a serum biomarker of idiopathic pulmonary fibrosis in dogs.

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Idiopathic pulmonary fibrosis (IPF) is an interstitial fibrotic pulmonary disease. The diagnosis is challenging and ultimately relies on lung histopathology. Identification of biomarkers specific for the disease would be very helpful. CCL2 (MCP-1) is a chemotactic cytokine for monocytes and a known biomarker of human IPF. In dogs with IPF, increased CCL2 expression has been described in lung tissue. The aim of the present study was to compare serum CCL2 concentration in dogs with IPF versus healthy dogs and dogs with other chronic pulmonary diseases. Thirteen dogs with IPF (ten WHWTs, two Scottish Terriers, one Yorkshire) mean age 12 years, range 8-15), nine dogs with eosinophilic bronchopneumopathy (EBP) (various breeds, 5 years, 1-12), ten dogs with chronic bronchitis (CB) (various breeds, 9 years, 1-13) and ten healthy WHWTs (9 years, 3-14) entered the study. CCL2 concentration in serum was determined by ELISA. Results in the different groups were then compared using non-parametric test (Mann-Whitney rank sum test). Serum CCL2 concentration was elevated in dogs with IPF (median; interquartile range = 528.8 pg/mL; 444.7-692.0) compared to healthy WHWTs (344.0; 254.5-415.5), ($p<0.001$). Serum CCL2 value in IPF dogs was higher than in EBP dogs (281.6; 163.9-416.5) ($p=0.009$) and than in CB dogs (277.7; 137.3-364.7) ($p=0.003$). The present study shows that (1) serum CCL2 values are significantly elevated in IPF dogs compared to healthy WHWTs; (2) serum CCL2 values are significantly elevated in IPF dogs compared to EBP and CB, suggesting that serum CCL2 could be a useful diagnostic biomarker of canine IPF.

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38. Doppler echocardiographic follow-up of three horses with congestive heart failure and treated with quinapril, digoxin and diuretics.

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Angiotensin converting enzyme inhibitors are recommended therapy in human and canine patients with heart failure (HF), but little is known concerning their efficiency to treat horses with HF. Enalapril has been shown to be poorly absorbed in horses and quinapril has been shown to decrease the severity of the insufficiency and to increase the stroke volume and the cardiac output in horses with mitral regurgitation (MR) without signs of HF. The objective of this cases report was to evaluate the effect of quinapril associated with routine treatment in 3 horses with severe MR and severe clinical and echocardiographic signs of HF. All horses were treated with quinapril 0,2mg/kg SID, digoxin 0,011mg/kg BID, and furosemide 1mg/kg BID. Standard echocardiographic and Doppler measurements were performed before treatment, and 1 and 4 weeks after starting treatment. Results were compared using paired T-test. All horses showed a transient clinical improvement after 1 week of treatment, but clinical signs deteriorated within the following weeks in 2 horses which were euthanized for ethical reasons. None of the measured echocardiographic and Doppler parameters showed individual improvement or were significantly different after 1 or 4 weeks of treatment as compared with values at admission, except for the pre-ejection to ejection time ratio of the aortic flow which decreased in each horse and was significantly lower after treatment. Despite the very limited number of studied cases, those results suggest that quinapril in association with digoxin and furosemide at the used dosage could be inefficient to control HF in horses.

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39. Description of a Body Condition Scoring Chart adapted to Belgian Blue Cattle.

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Introduction: Body condition scoring (BCS) is increasingly used in cattle to get a general overview of their management. It estimates body fat reserves whose changes are worth-while indicators for main pathologies of dairy and beef cattle. Several BCS charts have been published for beef cattle but their use in Belgian Blue (BB) breed proved to be irrelevant due to the peculiarities of this extremely hyper-muscled breed. Consequently, it was necessary to define an easy to use chart, specific for BB. **Material and methods:** A 5-points scale (1 to 5 including ½ unit values) BCS chart was perfected, based on palpation and visual observation of body frame, muscle and fat cover of ribs, loin, hip and tail head. 275 BB cattle, 194 females and 81 males, aged 7 days to 9.75 years, were scored and results related to weight. Furthermore, scores obtained by different assessors were compared on 212 additional animals. **Results:** Mean BCS of the 487 animals was 3.26 ± 0.40, close to the standard of other BCS charts using the same scale. BCS changes correlated well with weight changes ($p < 0.1$), although BCS 3.5 and 4 in males >3y could not be discriminated regarding weight due to a too small strength. Only 13% of the scores obtained by several assessors showed a difference never exceeding 0.5 unit. **Conclusion:** This BCS chart for BB proves to be more reliable and easy to use than previous charts fitted for other breeds of beef cattle.

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

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

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41. Semen quality of stress negative Piétrain and Duroc boars in the tropics: the case of Vietnam.

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This study was carried out in Dong Hiep pig farm, North of Vietnam in order to evaluate the semen quality of stress negative Piétrain and Duroc boars. A total of 722 ejaculates from 13 homozygous (CC), 7 heterozygous (CT) stress negative Piétrain and 10 Duroc CC boars was collected between 2008 and 2012. The sperm quality was assessed on each ejaculate using ejaculate volume (VOL), spermatozoa motility (MO), sperm concentration (CO) and total number of spermatozoids (NT). Genetic type of boars, season, year and (season x year) as well as (genetic type x year) interactions were included in the model as fixed factors. The results show that the semen quality was influenced by all studied effects ($p<0.05$) except VOL for season ($p=0.45$) and season x year ($p=0.55$), and CO for genetic type ($p=0.93$). VOL and NT (291.74ml and 103.37×10^9spz) of Piétrain CC were higher than those (241.40ml and 84.58×10^9spz) of Piétrain CT and (228.05ml and 77.15×10^9spz) of Duroc ($p<0.001$) although the values of the 3 genetic groups are in the range of normal semen. MO, CO and NT tend to be higher in cold than in hot season ($p<0.001$). These results suggest that semen from Piétrain and Duroc boars could be used in tropical climatic conditions (particularly Piétrain CC) and that the semen quality could be improved through reduction of heat stress.

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42. Effect of body weight loss on pulmonary function assessed by 6-minute walk test and arterial blood gases in obese dogs.

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This study aimed to investigate the effect of body weight loss (BWL) on pulmonary function assessed by 6-Minute Walk Test (6MWT) and arterial blood gas values. Six experimental Beagles and 6 privately-owned dogs, all obese but otherwise healthy, were enrolled in a diet-induced BWL program. Physical examination, BW and Body Condition Score assessment, arterial blood gas analysis and 6MWT were performed when dogs were obese, and repeated with animals overweight and lean. Dogs were walked for 6 minutes. Heart rate (HR) and oxygen saturation (SpO2) were measured by pulse oximetry before the test, after three minutes of walk, and at 0, 1, 2, 3 and 5 minutes post-test. All dogs concluded the BWL program. BWL caused a significant increase in the walked distance ($P\leq0,05$) and a decrease in pre-test respiratory rate (RR) ($P\leq0,05$). Resting arterial blood gases were not influenced by BWL and neither did the pre-test HR and SpO2 values. Obese dogs showed the highest HR mid-test values ($P\leq0,001$) and the lowest SpO2 values recorded at 0 and 1 minute post-test. HR values registered at 1, 2, 3 and 5 minutes post-test were all lower in overweight and lean dogs. Obesity negatively affects the blood oxygenation level during and shortly after physical exercise in dogs, with subsequent HR increase. BWL induces a decrease in resting RR and it improves pulmonary function during exercise, even before achieving the targeted ideal BW. The 6MWT, but not pre-test arterial blood gas values, is an efficient tool to demonstrate the efficacy of BWL.

43. BTV-1/BTV-15 experimental challenge of calves previously infected with BTV-8.

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In order to study BTV-1 superinfection on BTV-8 infected animals, 8 Holstein calves, including 4 animals vaccinated against BTV-8, were infected with BTV-1, 5 months after an initial BTV-8 infection. Two calves were kept as controls. An incidental contamination of the inoculum with BTV-15 was discovered. The inoculum contained about 7 times more BTV-15 segment 2 RNA than BTV-1 did. Clinical signs were quantified. Viral RNA was detected by serotype specific RTqPCR. Calves were slaughtered 21 days post infection (dpi) and necropsied. Infected animals showed clinical conditions compatible with bluetongue disease. Three out of four non vaccinated calves had a more severe overall clinical outcome when infected with BTV-1/BTV-15 than with BTV-8 alone. BTV-1 and BTV-15 RNA have been detected in the blood as soon as 7 and 5 dpi, respectively, until the end of the experiment regarding BTV-15. The maximal copy number of BTV-15 segment 2 was about 80 fold higher than BTV-1. The necropsy revealed petechial haemorrhages in several organs. BTV-8 RNA could still be detected in organs of two calves; BTV-1 RNA only in the spleen of one calf and BTV-15 RNA in 16 organs belonging to 7 different calves. BTV-1 and BTV-15 are considered as classical serotypes, typically leading to subclinical or very mild disease in cattle. However, in this study the contemporarily infection with BTV-1 and BTV-15 was clearly pathogenic. Inoculation with different serotypes, as it can occur in the field, may lead to interference between virus strains and variations in the responses of animals.

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44. Phylogenetic relationships and evolutionary perspectives between time divergent and contemporaneous bovine noroviruses.

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Noroviruses are recognized as worldwide major agents of acute nonbacterial gastroenteritis in humans and genetically related viruses were also detected in stool samples from cattle. Five genogroups are currently described in the genus Norovirus and, within genogroups, sequences are further distributed into genotypes following genetic homology and phylogenetic relationships. In this study, stool samples from Belgian cattle (gathered during the years 2002-2003) were screened by RT-PCR. Norovirus sequences were detected and phylogenetically related to genogroups III genotype 2 noroviruses. The complete sequence of the bovine strain B309/2003/BE was also determined by primer-walking and bioinformatically compared to the original Newbury2/UK (bovine norovirus genogroup III genotype 2 reference strain) and to the Dumfries/UK (another bovine norovirus genotype 2 strain), detected in 1976 and 1994 respectively, giving some interesting features about genetic evolution of bovine noroviruses within their entire genome along a thirty year period. The results of this study also genetically evidenced that bovine noroviruses constitute a different lineage in the genus Norovirus along this time span, giving some interesting data for their risk analysis on Public Health.

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45. Influence of dietary selenium enrichment on performance and chemical composition of meat in Belgian Blue bulls.

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Selenium (Se) is a major structural component of various selenoproteins which contain a selenocysteine and a Se atom in their active site (except selenoprotein-P). They play an important role in many functions, such as antioxidant defense and the formation of thyroid hormones. Some of the metabolites, as methylselenol, play also a role in cancer prevention. The recommended intake of Se varies from 60 µg/day for women, to 70 µg/day for men. Soil deficiencies contribute to subsequent deficiencies in plants, animals and humans.Thus, it is necessary to find effective ways to improve the Se availability in food (meat in this work) by acting on the soil-plant-animal axis. So, three feeding trials were carried on with young fattening bulls offered a diet which was supplemented or not in Se. The Se supplementation was either on an organic form as Se enriched barley, spelt or linseed meal (3 groups of animals) or as selenite, a mineral form of Se (1 group of animals). The supplementation in Se did not affect the animal performance, the slaughter characteristics and the meat quality whatever the form of the added Se. The Se content in meat was significantly increased with the organic Se supplementation (480 vs 209 µg/kg DM). By contrast, there were no significant effects of the mineral supplementation (315 vs 290 µg/kg DM). There were however increases in the Se status as indicated by the Se improvement in plasma in both groups supplemented with Se even if the increase was smaller with the mineral supplementation.

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46. Resident CD11b$^+$Ly6C$^-$ lung dendritic cells are responsible for allergic airway sensitization to house dust mite in mice.

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Conventional dendritic cells (DCs) are traditionally assumed to be the prime inducers of airway allergy. Yet, several DC subsets exist in the lung and it remains unclear whether specific subsets are preferentially involved in the initiation of allergic responses. In order to test which lung DCs are able to induce allergic airway sensitization, we developed a novel model of airway allergy to house dust mite antigens (HDM) induced by the transfer of lung DCs isolated from mice exposed to intranasal HDM directly to naïve recipient animals. We further sorted individual lung DC subsets, and assessed for their respective pro-allergic potential. Among lung DCs, transfer of CD11c$^+$CD11b$^+$ DCs, but not of CD11c$^+$CD11b$^-$CD103$^+$ DCs, was sufficient to prime airway allergy. The CD11c$^+$CD11b$^+$ DC subpopulation was composed of CD11c$^+$CD11b$^+$Ly6C$^+$ 'inflammatory' DCs, whose numbers increase in the lungs following HDM exposure, and of CD11c$^+$CD11b$^+$Ly6C$^-$ DCs, whose numbers remain stable in the same conditions. Surprisingly, only CD11c$^+$CD11b$^+$Ly6C$^-$ DCs, and not CD11c$^+$CD11b$^+$Ly6C$^+$ DCs, were able to convey antigen to the lymph nodes and induce adaptive T cell responses and subsequent airway allergy. Our results thus support that lung resident CD11c$^+$CD11b$^+$Ly6C$^-$ DCs mediate allergic airway sensitization to the common aeroallergen HDM in mice.

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47. Ressources génétiques animales en Kabylie (Algérie).

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La Kabylie est une région côtière montagneuse du nord-est algérien. Ce poster présente les principales espèces et races élevées dans cette région. Les ovins sont représentés par la race Tazegzawt, répertoriée récemment. Elle est reconnaissable à ses tâches noires à reflets bleuâtres, son nom kabyle signifiant bleu. Son poids peut dépasser 30 kg à 6 mois. La population locale de chèvres n'a pas fait l'objet de descriptions. Toutefois, la chèvre locale est adaptée aux massifs montagneux. Elle est de petite taille avec un poids vif d'environ 25 kg. Elle présente de longs poils et différentes couleurs de robe. La race bovine locale est issue de l'adaptation de la race brune de l'Atlas à un climat tempéré. Elle possède un petit gabarit (de 250 à 300 kg) et une robe grise ou allant du fauve brunâtre au rouge-brun. Par l'industrialisation de la production de volailles, les poules locales ont subi une érosion génétique sévère. Elles présentent ainsi une grande variabilité phénotypique et sont de taille moyenne (autour de 1,5kg). Le lapin local connaît une situation similaire et présente un poids de 2 à 3 kg. Il existe une tradition apicole importante en Kabylie. Les types d'abeilles exploitées ne sont toutefois pas encore caractérisés. Toutes les races citées ici sont menacées par le croisement ou l'abandon. Leur caractérisation génétique et zootechnique est nécessaire pour l'amélioration des systèmes de production familiaux, plus particulièrement en régions montagneuses, et la conservation de la biodiversité.

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48. Caractérisation des performances d'élevage et de production de deux variétés de la race Ardennaise.

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La poule Ardennaise est une race emblématique de la biodiversité avicole belge. Dans un contexte mondial favorable à la conservation des races locales d'animaux domestiques, cette étude est consacrée à la comparaison de deux variétés de la race Ardennaise (Noire dorée et Noire Argentée). La comparaison est réalisée par le biais de trois études: (1) caractérisation morpho-biométrique des deux variétés; (2) caractérisation de la croissance et de la qualité de la carcasse et de la viande; (3) suivi du taux de ponte pendant 52 semaines et l'étude de la qualité des œufs à 30, 45, 60 et 75 semaines d'âge. Des différences significatives ont été enregistrées au niveau des poids corporels, du grand diamètre du tarse, de la longueur du tarse et de la taille de la crête des deux variétés. La variété de la poule Ardennaise n'influence pas significativement les caractères quantitatifs et qualitatifs de production de viande (rendement, poids après abattage, pH, couleur de la viande). Elle influence cependant significativement le poids de l'œuf entier, le poids du blanc, le pourcentage du blanc, le pourcentage du jaune, le rapport jaune/blanc et le pH du blanc ($p<0,05$). Pour plusieurs caractères morphologiques, d'engraissement et de ponte, les deux variétés ne présentent aucune différence significative. Il serait intéressant de compléter cette étude par une analyse moléculaire permettant de préciser le degré de similitude génétique entre les deux variétés et éventuellement avec les autres variétés de la race.

49. Quality assessment of marketed eggs in Basse Kabylie (Algeria).

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Quality variations of retailed eggs are widely reported. This study aims at assessing the quality of eggs according to the marketing channel in the department of Bejaia (Algeria). In spring and summer 2012, a total of 3330 eggs were bought in 30 stores divided into 3 categories: 10 supermarkets (1146 eggs), 10 public markets (1048 eggs), and 10 shops (1136 eggs). The eggs weights differed significantly with the marketing channel with 58.9±0.14, 61.2±0.13 and 62.8±0.13 g for public markets, shops and supermarkets, respectively ($p<0.001$). Although the shell thickness was similar for all marketing channels, the proportion of damaged eggs was higher in public markets (9.0%), intermediate in shops (7.3%) and lower in supermarkets (5.7%; $p<0.05$). The yolk/albumen ratio was significantly higher for eggs from supermarkets (48.1%) compared to the other channels (around 47.4%; $p<0.05$). The freshness of the eggs, measured by the Haugh method, was lower in public markets (74.3 units), intermediate in shops (77.6 units) and higher in supermarkets (79.9 units; $p<0.05$). The price of eggs, expressed in Algerian Dinar (AD) per kg, was significantly lower in public markets (124 AD/kg) compared to the two other channels (around 131 AD/kg; $p<0.05$). One can conclude that egg quality in Basse Kabylie differs significantly between marketing channels with higher quality observed in supermarkets. The lower quality of eggs in public markets is associated with lower price. Eggs from shops present an intermediate quality. A one-year study would allow studying both the potential seasonal effect and compare intrinsic variability across marketing channels.

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50. *Alcelaphine herpesvirus 1* Semaphorin 7A-homologue is a 140 kDa secreted glycoprotein.

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Alcelaphine herpesvirus 1 (AlHV-1) is a γ-herpesvirus that persists asymptomatically in its natural host, the wildebeest. However, AlHV-1 transmission to a large number of susceptible ruminants, including cattle, results in the development of a lethal lymphoproliferative disease named malignant catarrhal fever (MCF). The A3 gene of AlHV-1 encodes a putative semaphorin-homologue protein termed AlHV-Sema. Although first identified as proteins involved in neuronal development, newly identified immune semaphorins have been shown to be crucial in various phases of the immune response. Semaphorins are secreted or membrane-associated glycoproteins characterized by a conserved amino-terminal Sema domain. Sequence and conformational predictions showed that AlHV-Sema is an homologue protein of the immune semaphorin 7A (Sema7A), a GPI-anchored protein involved in T cell-mediated inflammatory responses. Despite the high similarity between AlHV-Sema and Sema7A, the viral protein is predicted to be secreted and to signal *via* different receptors. It is therefore unclear whether AlHV-Sema shares similar functions with Sema7A. Here, we studied the expression of AlHV-Sema *in vitro*. First, we Fc-tagged the entire A3 coding sequence cloned in a pTorsten expression vector and demonstrated that AlHV-Sema is a 140 kDa secreted glycoprotein. Secondly, we used the BAC clone of AlHV-1 to introduce a Fc-tag sequence in frame with the A3 coding sequence and showed that AlHV-Sema is expressed and secreted during AlHV-1 infection *in vitro.* Further analyses will be developed to determine whether AlHV-Sema have immune regulating functions.

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51. Kinetics of quinalphos residues elimination in water and fish tissues from tank cultured silver barb (*Barbonymus gonionotus*).

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From surveys conducted in the framework of the DELTAQUASAFE project, it appeared that Quinalphos, an organophosphorus insecticide, is one of the most used pesticides in rice-cum-fish production systems, leading to the possible contamination of fish, and possible negative consequences on fish and consumer's health, because of the presence of residues. In order to estimate the quinalphos residues levels in cultured silver barb, we have conducted an experiment involving fish reared in 60 L glass tank, with 4 treatments: control, 0.086 ppm, 0.172 ppm and 0.43 ppm of quinalphos in water, and repeated 6 times. The results obtained from the analysis of the water sampled during the experiment show a quick decrease of quinalphos concentration in water, for the 0.086 and 0.172 ppm treatment. After 28 days, no residues were detected any more for the 0.086 ppm treatment, while low levels (2 to 6 ppb) were still measured in 0.172 and 0.43 ppm treatment. In fish tissue, however, the results showed that, after 1 day of application, the quinalphos residues levels were much higher than concentration of this compound applied into water. After 28 days of quinalphos application, the residues of the compound were still high, even in the lowest dose, with 31.3±38.7 ng/g of quinalphos in fish tissue.

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52. Management of vaginal stenosis by ballooning dilation under endoscopic guidance after vaginourethroplasty in a dog.

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This original case report describes a vaginourethroplasty performed via an episiotomy access and management of postoperative stenosis by endoscopic ballooning dilation. A 9 year old female Bouvier was presented for severe dysuria. On vaginal examination, a mass was palpated at the urinary meatus (UM). Biopsies revealed an undifferentiated urothelial cell carcinoma. Vaginourethrography showed an irregular urethral wall extending 4 cm cranial to UM. Diagnostic work-up showed no evidence of distant metastasis. A vaginourethroplasty was performed via an episiotomy. The urethra was dissected up to 1 cm cranial to the lesion. A urinary catheter was placed postoperatively. Two days after discharge, the urinary catheter was accidently removed. The dog presented a weak urinary stream and abundant urinary leakage after micturition. Vaginoscopy and vaginourethrography showed severe stenosis at the vestibulo-vaginal junction caudal to the urethral opening. An „urovagina" was suspected. Three ballooning sessions were performed. The balloon was placed by endoscopy and dilation was performed during 5 minutes. The size of the balloon was increased each time. Triamcinolone (0.5ml; 40 mg/ml) was injected at 3 locations of the stenosis. Owner rapidly reported improvement of urinary symptoms with a strong urinary stream and urinary leakage consisting of a few drops after micturition. Nine months postoperatively, the dog presented pollakiuria and stranguria. Local disease progression was diagnosed and the dog was euthanized. Vaginourethroplasty via an episiotomy access is a valuable technique to remove distal urethral masses in selected cases and ballooning dilation is a valuable option to manage postoperative vaginal stenosis.

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53. Urodynamic investigation by telemetry in Beagle dogs: validation and effects of oral administration of current urological drugs.

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The objectives were to evaluate the use of telemetry for urodynamic investigation in dogs and to determine its usefulness for toxicologic studies. Conventional diuresis cystometry was performed in six adult continent female Beagle dogs before surgical implantation of telemetric device. Continuous urodynamic telemetric recordings were performed during 8 days and 8 nights and during the night at days 0, 1, 8 and 15 after phenylpropanolamine, estriol, bethanechol, oxybutynin or duloxetine administration. During filling phases and micturitions, abdominal, intravesical and detrusor threshold pressures, and electrical activity of the smooth urethral muscle were recorded. Threshold bladder volume (Vth) and compliance were measured and presence of involuntary detrusor contractions (IDC) was recorded. Vth values recorded by telemetry were significantly lower than values obtained by diuresis cystometry. Better repeatability of telemetric measurements was observed during night than during day. During night, the frequency of IDC was lower and Vth values were higher than during day. After 15 days of phenylpropanolamine administration, a decreased detrusor threshold pressure associated with an increased Vth was observed, although significance was not reached. Opposite results were obtained after estriol administration. No significant changes in bladder function were observed after bethanechol or oxybutynin administrations. After duloxetine administration, a significant increased Vth was observed at days 1 and 15 compared to day 0. The electromyographic investigation was inconclusive. In dogs, the repeatability of nocturnal telemetric recordings could provide more interpretable results in urologic research. Further studies are needed to confirm the positive effect of duloxetine on bladder function in dogs.

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54. Toe-heel and medio-lateral hoof balance in sound ponies at the walk and trot on a hard versus a deformable surface.

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Equine pressure plate analysis has been limited to measurements on a hard surface, whereas equine athletes routinely perform on a soft, deformable surface. To explore the feasibility of analysis on arena footing, 5 sound unshod ponies (281 ± 33 kg) were walked and trotted over a 2m-pressure plate (RSscan International) under 2 different conditions: firstly, the plate was only covered with a 5-mm rubber mat, whereas in the second session, an additional 50-mm layer of sand and synthetic fibres was placed over the measuring area. Five measurements of each forelimb were recorded at the walk and trot, and hoof prints were divided in a toe and heel region and in a medial and lateral zone. Toe-heel and medio-lateral hoof balance of the vertical ground reaction force were calculated throughout stance (126 Hz). Hoof balance on the hard and soft surface were compared at impact, mid-stance and at the end of the stance phase using a Kruskall-Wallis test with Bonferroni correction. Hoof balance curves on the hard and soft track presented similar shapes, however, at impact, there was more even load distribution between the toe and heel region on the soft surface. The effect on medio-lateral balance was less pronounced: at impact, there was more even load distribution between lateral and medial on the soft surface at walk, but not at trot. The pressure plate permitted dynamic evaluation of hoof balance on a deformable surface at the walk and trot, thereby opening perspectives for evidence-based evaluation of farriery on arena footing.

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55. Identification of MicroRNAs encoded by *Alcelaphine herpesvirus 1* in a Lymphoblastoid Cell Line using Small RNA Deep Sequencing.

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Oncogenic γ-herpesviruses encode multiple microRNAs (miRNAs), some of which are expressed during latency and target cellular genes involved in lymphoproliferation or apoptosis. *Alcelaphine herpesvirus 1* (AlHV-1) is a γ-herpesvirus that persists asymptomatically in its natural host, the wildebeest. However, AlHV-1 transmission to a large number of susceptible ruminants, including cattle, results in the development of a lethal lymphoproliferative disease named malignant catarrhal fever (MCF). Our recent results suggested that MCF lesions are induced by the uncontrolled proliferation of latently infected CD8+ T cells. However, the pathogenic mechanisms involved remain unknown. Prediction analyses identified multiple pre-miRNAs in non-coding regions of the AlHV-1 genome. We therefore performed a combined approach of small RNA cloning and next-generation RNA deep sequencing to identify the expression of 34 putative miRNAs in a bovine lymphoblastoid cell line originating from a calf developing MCF. The AlHV-1 miRNAs were clustered in two regions and mainly encoded by the reverse-strand of the L-DNA. Four miRNAs were found at the 5'-end of the genome and a second cluster comprising 28 miRNAs originated from a region containing no predicted protein coding regions. Two additional small RNAs originating from the forward strand of the pr-DNA were also detected. These preliminary results indicate that AlHV-1 encodes the largest number of miRNAs among the gammaherpesvirus subfamily. Additional analyses should determine (i) the putative cellular targets of these miRNAs, (ii) whether these miRNAs are also expressed in the lymph nodes of MCF-developing animals and (iii) whether AlHV-1 miRNAs have a role in MCF.

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56. Impact of common calf diseases on mortality and carcass traits in white veal calves.

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Little is known on the effect of common calf diseases on mortality and carcass traits in the white veal industry, a highly integrated production system, currently criticized for intensive metaphylactic use of antimicrobials. The objective of the present study was to determine the impact of bovine respiratory disease (BRD), diarrhea, arthritis and otitis on the economically important parameters of mortality, hot carcass weight (HCW), carcass quality, fatness degree, meat color and carcass value. A total of 3519 calves on 10 veal herds were followed. The incidence risk of BRD, diarrhea, arthritis and otitis was 4.8%, 5.3%, 1.5% and 1.6%, respectively, and 5.7% of the calves died. BRD resulted in a 8.2 kg reduction in HCW, a lower carcass value, a lower fatness degree and an increased mortality risk. With an increasing number of BRD treatments, these losses increased dramatically. Additionally, calves treated multiple times for BRD, were more likely to have low carcass quality and undesirable red meat color at slaughter. Arthritis increased the mortality risk, but only reduced HCW when associated with BRD. Otitis did not affect any of the studied parameters. Diarrhea increased the mortality risk, reduced HCW by 8.8 kg on average and decreased carcass quality. Present results indicate a significant negative impact of BRD, diarrhea and arthritis on economically important parameters in white veal calves, even under the contemporary high levels of antimicrobial coverage.

57. Superovulation in the mare with commercially available pFSH.

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To date, equine superovulation is still unsatisfactory. This study aims at assessing commercially available pFSH (Stimufol®, Merial, Belgium) for superovulation in mares. The study was conducted during (3 cycles/5 mares) and out (2 cycles/4 mares) of the breeding season. The first untreated cycle served as control group. Mares were short-cycled with 125 μg of cloprostenol 7 days post-ovulation and received 6.25 mg of pFSH IM twice daily for 2 days. Ovulation was induced when a follicle reached 30mm with either hCG or busereline. Ovaries were scanned daily until induction and then twice daily until all follicles >25mm had ovulated or disappeared. Kruskal-Wallis Test was used and significance was established at $p<0.05$. Ovulation rates for controls were 1.6 and 1.2 during and out of the breeding season respectively and were not statistically different, allowing results to be pooled. Ovulation rates for the treated cycles were 2.9 and 1.5 during and out of the breeding season respectively. The treated cycles' ovulation rate was higher than controls' ($p= 0.025$). Ovulation rate of treated cycles during the breeding season (2.9) was also higher than that of untreated cycles during the same period ($p<0.01$) but not out of it. pFSH seems to increase ovulation rates if administered during, but not out, of the breeding season. Further studies with larger numbers, should identify if this is due to a needs being adapted or if this just reflects individual variation, as Stimufol® might prove inexpensive and easy to superovulate mares.

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58. Relationship between horses' temperament and stress during a jumping competition.

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During equestrian competitions, stress may affect riders and horses, and compromise health, welfare and/or performance. Previously, we quantified stress level during a show-jumping event by using salivary cortisol concentration (SCC) on horses and riders, and demonstrated relationships between stress and performance. During this study, horses' temperament (n=13) was assessed, using behavioural tests and a horse personality questionnaire. We found significant Spearman's correlations ($p<0.05$) between some temperament traits, and horses' SCC during competition. Saliva was sampled at rest (4 samples per horse) during a rest morning for individual horse basal SCC. During competition, 5 samples were taken on each horse: before warm-up (at 9:00AM), just before entering the competition ring, and 20, 40 and 60 minutes after. The mean SCC was (0.89±0.20nM) at rest and (1,26±0,52nM) during competition. The mean SCC discharge (SCCcompetition/SCCrest) was (146±70%) and was actually correlated to behaviours associated with "curiosity": time spent near the novel-object ($r_s=0.61$), time spent manipulating novel-object ($r_s=0.57$), time spent with head oriented to the ground during open-field test ($r_s=0.55$). Furthermore, the SCC peak is correlated with the "curiosity" assessed by riders ($r_s=0.57$). The SCC seems thus to be related to a curiosity temperament trait, rather than to an "anxiety", as first expected. As the horses' SCC was linked with higher performance, horses with higher curiosity related behaviours and temperament traits performed better. However, this conclusion needs to be put in perspective. Riders' stress was linked with poor performance, contrary to horses. Future studies are thus needed to assess relationship between temperament, stress, performance and welfare of riders and horses.

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59. Gaseous emissions from fattening pigs offered an ad libitum high-fibre diet and kept on fully slatted floor: preliminary results.

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According to the literature, the diet composition of livestock can influence polluting gas emissions from agriculture. The aim of this preliminary study was to measure gaseous emissions from fattening pigs offered an ad libitum high-fibre diet (HFD) and kept on fully slatted floor. A batch of 24 fattening pigs was divided into two homogeneous groups randomly allocated to a treatment: conventional cereals-based diet or sugar beet pulp-based diet (HFD). With HFD, a significant decrease of animal performance was observed (837 vs. 962 g for the average daily gain). With pigs offered HFD, gaseous emissions per pig were significantly lower for NH_3 (-30%, 6.64 vs. 9.47 g/d; $P<0.05$) and significantly greater for CH_4 (+40%, 6.46 vs. 4.60 g/d; $P<0.05$). The emissions of N_2O (0.34 g/d), CO_2equivalent (0.27 kg/d), CO_2 (1.68 kg/d) were not significantly influenced by the diet. Due to a more important microbial activity with HFD, the lower NH_3-emissions could be attributed to the shift of a part of excreted nitrogen from urine (as urea) to faeces (as protein form), and to a lower slurry pH explained by the increase of volatile fatty acid content. The higher CH_4-emissions could be explained by a greater production in the digestive tract and in the slurry due to fibre fermentations. In conclusion, HFD allowed decreasing NH_3- and increasing CH_4-emissions. However, in terms of climate change, this increase was offset by the decrease of indirect N_2O-emissions due to NH_3-emission decrease, as indicated by the similar CO_2equivalent-emissions in the two groups.

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60. Impact of the floor type on emissions of ammonia and greenhouse gases from pig houses.

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This study aims to compare slatted floor (SFS) and bedded floor systems (BFS) regarding emissions of ammonia (NH_3) and greenhouse gases (GHG), i.e. nitrous oxide (N_2O), methane (CH_4), and cumulate GHG, expressed in CO_2-equivalents (Eq-CO_2) for weaned piglets, fattening pigs and gestating sows. Two batches of 80 piglets, five batches of 32 fattening pigs and three batches of 10 sows were housed successively. Each batch was divided into two homogeneous groups kept in two separate rooms fitted with either SFS or BFS. With SFS, the space allowance was 0.31, 0.75 and 2.50 m²/animal for piglets, fattening pigs and sows, respectively. With BFS, the space allowance was 0.54, 1.21 and 2.50 m²/animal, respectively, and the average amount of straw was 190, 400 and 915 g/animal per day, respectively. The average body weight was 15.4, 67.4 and 210.9 kg, respectively. Emissions were measured by infra-red photoacoustic detection. For piglets, daily emissions associated to SFS and BFS were 0.38 and 0.74 g NH_3/animal ($P<0.01$, s.e.m.=0.06), undetected and 0.03 g N_2O/animal ($P>0.05$, s.e.m.=0.03), 0.91 and 0.75 g CH_4/animal ($P>0.05$, s.e.m.=0.11), 22.0 and 32.9 g Eq-CO_2/animal ($P>0.05$, s.e.m.=8.7), respectively. For fattening pigs, daily emissions were 6.15 and 12.99 g NH_3/animal ($P<0.001$, s.e.m.=0.73), 0.54 and 1.11 g N_2O/animal ($P=0.068$, s.e.m.=0.21), 16.15 and 15.91 g CH_4/animal ($P>0.05$, s.e.m.=0.75), 563 and 727 g Eq-CO_2/animal ($P=0.090$, s.e.m.=66), respectively. For sows, emissions were 12.77 and 9.05 g NH_3 ($P<0.05$, s.e.m.=1.04), 0.47 and 2.27 g N_2O ($P<0.01$, s.e.m.=0.32), 10.15 and 9.20 g CH_4 ($P>0.05$, s.e.m.=0.49), 393 and 907 g Eq-CO_2/animal ($P<0.01$, s.e.m.=94), respectively.

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61. Prevalence of naturally occurring cartilage defects in the ovine stifle (knee).

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Purpose: to determine the prevalence, anatomical location, nature and severity of cartilage defects in the stifle within a population of ewes enrolled for research. **Methods**: 100 hind limbs from 50 crossed Texel ewes aged 7 months to 11 years. Articular surfaces of the distal femur, proximal tibia and patella were examined by gross observation. Investigators scored the abnormalities of the cartilage using OARSI recommendations in 26 anatomic areas. For each anatomical location where lesions had been identified, osteochondral slabs were obtained for histopathology. Microscopy was performed to characterize structure of cartilage and bone, and confirm the classification of lesions performed by gross observation. The most severe lesion observed at gross anatomy in each of the 26 anatomic regions was used to score the articular surface of that region. Grading of cartilage defects within each knee was obtained by the addition of the scores of all regions. **Results**: Five anatomical locations where defects were frequently identified: central third of the medial femoral condyle, axial aspect of the central third of the medial tibial condyle, axial aspect of the central third of the lateral tibial condyle, middle third of the articular surface of the patella and the distal third of the medial femoral condyle. Statistical trend was observed for weight on the scores of cartilage defect within each knee. Scores increased significantly with age in this population. There was no effect of side. **Conclusion**: Score 2 defects can be significantly prevalent in stifles in a population of sheep enrolled for research.

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62. A flow-cytometric study on the effect of myeloperoxidase on stallion spermatozoa.

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Myeloperoxidase (MPO) is a pro-oxidant enzyme that has been associated with keratinized cells and with decreased post-thaw motility in stallion semen. The aim of the study was to determine effects of addition of MPO on motility, mitochondrial potential, apoptosis induction, membrane and acrosome integrity in equine semen. Three stallions were used and semen was collected four times. Extended semen was processed for density gradient centrifugation. Purified pellet was re-extended to $100x10^6$spermatozoa/ml: one sample was used for control and active human MPO was added in the other two samples to 5 or 50ng/ml concentrations. After 2 hours, motility was analysed with Computer Assisted Semen Analysis and cytometric analyzes were perfomed to assess mitochondrial potential, apoptosis, membrane, and acrosome integrity. Statistical differences ($p<0.05$) were determined using Kruskall-Wallis test. No effect of the stallions was observed on parameters assayed in this study. Unlike total motility, progressive motility was decreased in both MPO concentrations ($p<0.001$). MPO addition had no effect on membrane and acrosome integrity. No differences were detected for percentages of spermatozoa having polarised or depolarised mitochondria. Apoptosis was not increased by treatments. Our results agree with previously published effects of in vitro ROS production systems with xanthine oxidase, showing an effect on motility but no influence on mitochondria and membrane or acrosome integrity. However, membrane lipoperoxidation was increased by ROS in previous studies, and it could be linked to impaired motility also observed in our protocol. Further studies with increasing concentrations of added MPO should be conducted to correlate motility with lipoperoxidation.

63. Changes in mitochondrial respiration of cultured equine myoblasts induced by serum of atypical myopathy affected horses: a preliminary study.

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Atypical myopathy is a frequently fatal seasonal pasture myopathy that is now recognised in about 20 countries. Up to now, the aetiology of this emerging condition remains unknown. Atypical myopathy exhibits biochemical changes consistent with a multiple acyl-CoA dehydrogenase deficiency (MADD). These biochemical changes in MADD result, among others, from defects in several mitochondrial dehydrogenases that are involved in the β oxidation pathway. Although MADD in humans is considered a genetic disorder, the cause of this lipid storage myopathy in horses might result from a toxin that mimics this dysfunction of fatty acid oxidation. The pathophysiology of atypical myopathy is not yet well established but from epidemiological studies, it is undoubted that the cause is linked to the environment. We hypothesized that, at onset of disease, the unknown toxin is still circulating in blood and thus sera collected from atypical myopathy affected horses should contain the compound that alters the mitochondrial function. We monitor mitochondrial respiration in cultured skeletal myoblasts with high-resolution respirometry with or without addition of serum of atypical myopathy affected horses (n = 2). In this *preliminary* study, we observed a dose-dependent inhibition of the mitochondrial respiration (up to the full inhibition of the respiration) which was not induced by serum of healthy controls (n = 2). **Perspectives**: Elucidation of the pathophysiological mechanism associated to atypical myopathy in grazing horses and *in vitro* testing of potential therapeutic compounds.

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64. DNA vaccination as a powerful method for production of customized antibodies.

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A DNA vaccine is a bacterial plasmid engineered to express an encoded protein following *in vivo* administration and subsequent transfection of cells. The classical protocols for generating antibodies are using proteins/peptides as antigens. By contrast to protein/peptides synthesis and purification, DNA plasmids are easy to produce in high quantities at a high level of purity; they are stable and rather inexpensive to produce. We sought to optimise this technology for production of customized antibodies using human CD134L (CD134LH) as carrier. A plasmid encoding for this nonself type II transmembrane carrier linked to the antigen of interest was used. The CD134LH has the advantage of forming trimmers at the cell surface and this oligomeric presentation of the antigen has been shown to induce a strong antibody response even against self-antigens. ORF134 from Cyprinid herpesvirus 3 (CyHV-3) and ORF73 from Alcelaphine herpesvirus 1 (AlHV-1) were cloned in the DNA plasmid. The expression of the antigens on the cell surface was controlled by staining with available antibodies directed against the carrier. Subsequently, the plasmids were used for immunising mice using a protocol that consist to inject the plasmid IM followed by electroporation. Immunised mice antisera were tested for specific antibody raised against the cloned antigen by flow cytometry staining of cells infected with wild type (WT) virus or ORF134/ORF73 deleted (Δ) virus. These tests demonstrated the potential of this approach to produce customized antibodies.

65. Vitamin D status, calcium and protein metabolism in dogs fed Bone and Raw Food (BARF) or commercial pet food.

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This study aimed to assess the nutritional composition of nine BARF diets and to investigate the dogs' vitamin D status, Ca and protein metabolism, compared to those of a control group of eleven experimental Beagles fed commercial petfood (NO BARF).
The nutritional composition of the BARF diets was estimated to evaluate whether the individual intake of protein, Ca, P and vitamin D was in accordance to the NRC (2006) recommended allowances (RA). Dogs were submitted to a physical examination and a blood sample was taken for the measurement of serum Ca, P, PTH, 25-vitD, the marker of bone resorption CTX, total proteins, urea and creatinine. One BARF diet resulted deficient in Ca whereas 7 were slightly deficient in vitamin D. In BARF dogs the protein supply exceeded the RA, moreover they consumed diets containing a higher protein level than NO BARF. No differences in serum 25-vitD, Ca and PTH were detected between groups. BARF dogs showed higher P and higher CTX serum concentrations. No differences in TP level were detected between groups. Urea and creatinine resulted significantly higher in BARF dogs. We conclude that the slight vitamin D deficiencies observed in BARF diets did not eventually lead to blood hypovitaminosis D. The higher serum urea and creatinine values observed in BARF dogs may be indicative of the higher protein consumption and of a more developed muscular mass, respectively. Further investigations are deemed to verify whether the lower bone turnover activity of NO BARF dogs was due to their poor exercise.

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66. Variation in TGF-beta1 blood levels according to the breed predisposition to the idiopathic pulmonary fibrosis in healthy dogs.

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Idiopathic pulmonary fibrosis (IPF) is a progressive and chronic fibrotic lung disease with an unknown etiology, a poorly understood pathophysiology and a poor prognosis. This pathology has been described in humans but also in dogs where there is a clear breed predisposition. Indeed, canine IPF affects mainly terriers, particularly the West Highland White Terrier (WHWT). Preliminary results have shown higher blood level of TGF-β1 in WHWT suffering from IPF compared to asymptomatic WHWT. The aim of this study was to assess how serum levels of TGF-β1 vary according to breed predisposition to IPF in healthy dogs of various breeds, with variable susceptibility to IPF. Therefore, serum TGF-β1 levels were measured by an ELISA test in 94 healthy dogs from nine breeds. The overall breed effect was statistically evaluated using an ANOVA 1 and the different breeds were compared two by two using a multiple comparison test (Holm-Sidak). There was a significant breed effect ($P<0.001$) on serum TGF-β1 levels. In WHWT, TGF-β1 level was higher ($P<0.001$) compared to all other breeds except the Scottish Terrier. In the Scottish Terrier,TGF-β1 level was higher ($P<0.001$) compared to the non-predisposed breeds, as the Bichon and the Bobtail that presented TGF-β1 levels higher ($P<0.001$) compared to the Whippet, Shepherd Malinois and Labrador. The wide variation in serum TGF-β1 basal level amongst breeds might be in favor of our hypothesis, proposing high serum TGF-β1 level as one of the predisposing factors required for initiation and development of IPF in canine.

67. Sensitivity and permissivity of carp (*Cyprinus carpio*) to cyprinid herpesvirus 3 infection during development.

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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 permissivity of common carp (*Cyprinus carpio*) to the CyHV-3 infection from hatching to juveniles stages. Taking advantage of the recombinant strain we dispose, KHV 136 LUC which expresses the firefly luciferase (LUC), we have shown by bioluminescence imaging that larvae exhibit positive signals as soon as 24H post-infection at hatching and the number of positive larvae increases with time. Concerning mortalities, there are differences in delay and importance of virulent effect between the larval and the juvenile stage. So, we can conclude from this first study that, contrary to what previous publication suggested, carp larvae are susceptible to CyHV-3 infection directly after hatching and their sensitivity to virulence seem to increase with the developmental stage. To complete these results, we produced a recombinant expressing the beta-galactosidase which allows by diverse staining, microscopic visualization of infected cells as well as fluorescent-activated cell sorter analysis. This recombinant represents a tool to precise the results of the first study but also, brings the opportunity to investigate later other critical points of viral pathogeny.

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68. Development of Purkinje Cells in the Ovine Brain.

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Purkinje cells are involved in many vital functions within the body. Twenty ovine fetuses ranging from 2 to 5 months of gestation, two lambs in the first week after birth and three adult sheep were studied.Sections of the cerebellum were stained with haematoxylin and eosin, cresyl violet and KlÜver–Barrera. This study indicates that Purkinje cells began to appear after the 15th week of gestation. There were varying degrees of development of Purkinje cells in different zones of the cerebellum. Our findings in sheep fetuses suggest that the maturation of Purkinje cells starts in the caudal regions of the cerebellum and that the process begins in the vermis before it does in the cerebellar hemispheres. The alignment of Purkinje cells was found to be very regular in the caudal regions of the cerebellum. A partial absence of Purkinje cells in the rostral regions of the cerebellum was observed in both sheep fetuses and adult sheep. In the first post-natal week, some ectopic Purkinje cells were found in the white matter of the cerebellum.

69. Beneficial effects of probionts in marine fish larvae and their modes of action.

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The use of antibiotics in aquaculture has resulted in antibiotic resistance, involving environmental and human health risks. The use of beneficial bacteria or probionts might be an effective alternative in disease prevention and production enhancement. The use of probionts in aquaculture is popular as a numerous amount of scientific studies have reported improved survival and growth of fish larvae when supplied with probionts. However, there is a lack of knowledge on the modes of action of probionts and their interaction with the aquatic host. Understanding the mechanisms of action is of paramount importance to utilize the right probiotic strain to prevent specific diseases. As such aquaculture production will be improved and the side-effects associated with the use of antibiotics reduced. The objective of this study is twofold. First, we will select and optimize probiotic isolates. The potential probiotics will be cultured from healthy larvae and adult fish. The predictive screening tools used to assess probiotic potential will be: 1) the ability to adhere and colonize the fish intestine; 2) the ability to suppress the growth of several important marine fish pathogens and 3) a lack of invasiveness or toxicity. Secondly, we will unravel the modes of action of the probionts, selected in the first objective, with a focus on disease resistance in the host organisms, applying innovative techniques e.g. gnotobiotic larval model systems, immune priming and laser capture microdissection. Sea bass (*Dicentrarchus labrax*), the major marine fish cultured in southern Europe, will be employed as a model for marine fish.

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70. Morphological and morphometric analyses of the suspensory ligament in Standardbreds.

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Ultrasound techniques allow examination of some parts of the suspensory ligament (SL) but "anomalies" are regularly observed. Their significance is not known. Few studies have described the relationship between ultrasonographic appearance and the exact morphology in histological sections. The aim of this study is to develop good techniques for cutting, staining, and showing the variation in the tissue composition within the SL. The SLs from the right limbs of 11 horses were collected. Samples were taken from cross-sections at six levels of the SL and they were embedded in paraffin or in Tissue-Tek®. Most of the paraffin sections were shredded. By using the cryosection technique, some freezing artifacts (holes) appeared. Therefore, a technique of freezing with cryoprotection was carried out, which produced the best results. Hematoxylin-phloxine-saffron gives a good contrast of colors between the tissues observed allowing the use of an image analysis program. The percentage of each tissue within the SL for each section and for six levels of the ligament was calculated. Results were analyzed by SAS software. The muscle tissue (PMT) and adipose tissue (PAT) decreased significantly ($p < 0.0001$), whereas the connective tissue (PCT) increased significantly ($p < 0.0001$) with age and when descending from the proximal to the distal level of the SL. The PMT was significantly higher ($p < 0.0001$) in females than males, while the PCT was significantly higher ($p < 0.0001$) in males than females. The PAT was significantly higher ($p = 0.0278$) in hindlimbs than in forelimbs.

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71. Critères d'appréciation des reproducteurs en systèmes d'élevage traditionnel du zébu Azawak dans les zones d'Abalak, Filingué et Niamey (Niger).

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Au Niger, le zébu Azawak fait l'objet d'un programme de sélection au sein de la Station de Toukounous, créée en 1954. Si de substantiels progrès en termes de production individuelle ont été atteints, ce matériel génétique nécessite d'être diffusé à travers le pays. Cette étude caractérise les critères d'appréciation des géniteurs Azawak auprès de 120 éleveurs d'Azawak non-améliorés au sein de trois contextes d'élevage différents: pastoral (Abalak, n=38), agricole (Filingué, n=54) et périurbain (Niamey, n=28). Près de 65,5% des éleveurs interrogés apprécient l'Azawak pour sa production laitière, sa valeur socioculturelle, l'esthétique de la robe, la facilité d'engraissement et sa docilité pour la traction. Les motifs mentionnés par les 34,5% d'éleveurs affirmant ne pas apprécier le zébu Azawak amélioré sont son exigence en compléments alimentaires et en soins vétérinaires et son inadaptation à la transhumance. Les critères majeurs du choix du géniteur sont la longueur de la queue (60,0%), la grosseur du fourreau (58,2%), la performance laitière des descendants (55,0%), ainsi que leur bonne conformation (48,3%) et leur docilité (46,7%). La réforme des mâles par l'éleveur est dictée par le comportement d'errance (67,7%), l'indocilité (51,9%) et la mauvaise conformation des descendants (42,6%). Les déterminants de l'appréciation des reproducteurs par les éleveurs sont le fondement des objectifs et critères de sélection. L'inadéquation entre les deux résulte en une absence de diffusion du matériel amélioré. La compréhension de ces contraintes à la diffusion permettra leur prise en compte au niveau du programme de sélection ou des autres services d'élevage.

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72. *Culicoides* (Diptera : Ceratopogonidae) : important vectors of cattle diseases. Control trials in Belgium.

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Culicoides (biting midges) are known vectors of several diseases amongst livestock. Since 2006, European countries faced Bluetongue (BTV) and recently Schmallenberg (SBV). These diseases brought huge economic losses. In Belgium, in parallel with epidemiological control, control methods for culicoides populations had been tested. A study was conducted in two steps (Phase I and Phase II). Phase I evaluated the direct treatment of livestock with biocidals. Biting midges were caught around and on the animals and their numbers compared. Phase II tested physical control methods (insects traps) and biocidals as treatments for stables. Efficiency of different traps was compared with the OVI-trap (UV light trap) as reference. Efficiency of biocidals was evaluated with one OVI trap.

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73. Flatfish fishery : impact & challenges.

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Sole (*Solea solea*) and plaice (*Pleuronectes platessa)* are by far the most targeted marine organisms by the Belgian fishermen. Both demersal fish species are mainly caught using beam trawls with tickler chains. However, this fishing technique has several disadvantages including seabed disturbance, excessive discards and high fuel consumption. Alternative passive techniques such as fly shooting, gill nets or long lines encounter much less this problems, but they are hardly economically feasible or too dependent on the weather conditions to be fully effective. Adequate long-term solutions hence are indispensable to ensure a sustainable and profitable future for the flatfish fishery. The most promising alternative meeting both the fisherman's aspirations and the need for ecological progress is pulse fishing. This technique replaces the tickler chains by electrodes towing over the sea floor and inducing electrical pulses, which elicit an upward movement of the fish enabling its catch without spading the bottom. Pulse fishing, using high frequency and voltage pulses, is currently evaluated for catching sole. Hitherto, several plus points are discernible in comparison to the classical trawl fishery: marked decrease in seabed disturbance, reduction of bycatch and halved fuel consumption. Unfortunately, also negative effects such as dislocated spinal cords, hemorrhages and mortality were observed in certain exposed fish species, especially cod. These adverse effects need to be tackled in order to be able to define pulse fishing as an environmental friendly fishing technique. Further studies hence are needed to define and optimize pulse characteristics for stimulating flatfish that are not harmful for exposed marine organisms.

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74. *cis*-Acting Inhibition of MHC class I-restricted Epitope Presentation by *Alcelaphine herpesvirus 1* genome maintenance protein.

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γ-Herpesviruses persist as latent episomes in actively dividing lymphocytes. Their consequent need to express a viral genome maintenance protein (GMP) during latency presents a potential immune target. However, the GMPs from several γ-herpesviruses have evolved related strategies to limit their own MHC class I epitope presentation to cytotoxic T lymphocytes (CTLs). *Alcelaphine herpesvirus 1* (AlHV-1) is a γ-herpesvirus that persists asymptomatically in its natural host, the wildebeest. However, AlHV-1 transmission to a large number of susceptible ruminants, including cattle, results in the development of a lethal lymphoproliferative disease named malignant catarrhal fever (MCF). We recently demonstrated that the AlHV-1 GMP-homologue encoded by ORF73 is highly expressed during MCF and that the impairment of its expression renders AlHV-1 unable to induce MCF. With its 1300 aa, AlHV-1 ORF73 is the largest γ-herpesvirus GMP described to date and contains a large acidic internal repeat region that could be involved in the *cis*-acting CTL evasion mechanism. Here, we sought to determine the CTL evasion properties of AlHV-1 ORF73. We first performed bioinformatic analyses to characterize the protein domains. Then, we used an *in vitro* assay to demonstrate that ORF73 severely limits the presentation at the cell surface of an MHC class I-restricted epitope linked to ORF73 in *cis*. These results suggest that AlHV-1 has developed mechanisms to evade cytotoxic anti-viral response during latency. The exact mechanisms explaining the presentation defect remain to be deciphered as well as the role of the *cis*-acting CTL evasion mechanism of ORF73 in the pathogenesis of MCF.

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75. Selection criteria as used by owners-breeders of racehorses in Algeria.

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Due to positive externalities and the need to work on a large scale, animal breeding is usually carried out by breeders associations or states. In Algeria, racehorse breeding is in the hands of unorganized breeders-owners. Through a survey conducted with 461 owners-breeders between 2009 and 2011, this study investigates their perceptions, objectives and practices regarding selective breeding. Racehorse breeding is a full-time professional activity for a third of interviewees. The holdings are small-sized with 77% owning one or two mares. The regular practice of insemination could categorize breeders according to their professionalization (38.4% professional vs. 61.6% occasional breeders). Seniority in the sector was also used to classify breeders, considering as "young" the breeders under 10 years experience (38.8%) and as "senior" those above 10 years (61.2%). More than professionalization, seniority shows a significant impact on practices and objectives. Thus, seniority influences breed choice (young breeders tend to specialize while senior own both Arabian and Thoroughbreds; $p<0.001$), age at first foaling (sooner among senior breeders; $p<0.01$), information sources considered for selecting stallions (senior use more diversified sources; $p<0.01$), the importance granted to the price of insemination (greater for the young breeders; $p<0.001$), the importance granted to the ranking compared to earnings (the ranking being more important to young breeders, $p<0.001$), and the priority given to breeding (young breeders give rather priority to a buy-race-resell activity; $p<0.001$). Finally, racehorse breeding in Algeria is poorly professionalized, the only financial goal being cost coverage. Despite inappropriate practices, an interest for selection is noticed.

76. Evaluation of the non-genetic factors of the racing performances of Arabian horses in Algeria.

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Selective breeding of Arabian horses in Algeria is not yet well developped. No accurate estimation of the breeding values of racing horses is carried out untill now. The estimation is based on one or several measurable traits, linked to race performance. To allow for an accurate estimation, it is important to determine the part of the phenotypic variability that is due to non-genetic factors (environmental). This first study estimates phenotypic correlations between the recorded traits in order to deduce to what extent the different measures bring different information on animal performance. Then it estimates the influence on performance of non-genetic factors such as sex, age, year of performance and the interactions between these various factors. From 1995 to 2007, the data related to flat racing was collected. The General Linear Model was used to identify and quantify the non-genetic factors affecting racing performances. Three traits were used: two earnings traits (the logarithm of annual virtual earnings: LAEV and the logarithm of average annual virtual earnings per start: LAEV/S), and one ranking trait (the ranking transformed and normalised: PERF). Significant high positive correlations was observed between the three traits ($p<0.001$), which thus give account of similar informative aptitudes. The effects of sex, age, year as well as the interactions between age and sex and between age and year turned out to be significant for the three traits ($p<0.05$). These results indicate the need to adjust the earnings and ranks traits in order to use them as criteria of selection of racing Arabian horses.

77. Typology of cattle farms and animal genetic resources in periurban Bamako, Mali.

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In response to Livestock Revolution, dairy production in periurban areas of developing countries is changing rapidly. To characterize this development around Bamako (Mali), this study establishes a typology of production systems with a special focus on animal genetic resources. The survey included 53 dairy cattle farms from 6 periurban sites. It was conducted in 2012 through two visits, in dry and crop harvest seasons. The average farm numbered around 31 cattle and owned 8 ha crop land. Estimated through gross margin on milk sale per year, the profitability of the farms proved highly diverse, with a maximum around 4 million FCFA and four farms getting negative margins. The multivariate analysis helped disentangle the diversity of situations, pointing to a continuum of intensification levels. Feeding strategy was crucial, accounting for about 85% of total expenses. The use of artificial insemination and tight veterinary follow-up were other crucial parameters. Thirty genetic profiles were identified, from pure local breeds to different levels of crossbreeding. Purebred animals raised were Fulani zebu (45.8%), Maure zebu (9.2%), Holstein (3.0%), Azawak zebu (1.3%), Mere zebu (0.5%) and Kuri taurine (0,1%). Holstein-crossbred represented 30.5% of the total number of animals (19.0% Fulani-Holstein, 11.2% Maure-Holstein and 0.3% Kuri-Holstein). Montbeliarde, Normande and Limousine crossbreds were also found (6.6%, 0.7% and 0.3% of total, respectively). The high diversity of situations shows the rapid change at play. Although strongly anchored on local breeds, the periurban dairy systems included a diversity of exotic cattle, showing an uncoordinated quest of breeders for innovation.

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78. Clinical assessment of alfaxalone in rabbits. Preliminary results.

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The aim of this study was to describe induction of general anaesthesia in rabbits using alfaxolone without oxygen supplementation. Seven healthy adult female New Zealand White rabbits were first premedicated with fentanyl (0.0125 mg/kg) and droperidol (0.625 mg/kg) administered intramuscular in the lumbar region 20 minutes prior to induction. Alfaxolone was administered intravenously in the lateral auricular vein at a dose 3 mg/kg injected over 60 seconds using a syringe driver. After blind intubation, rabbits were breathing spontaneously room air. Respiratory and cardiac parameters were assessed by measuring the duration of post-induction apnoea, respiratory rate, oxygen saturation of haemoglobin in the peripheral blood (SpO_2), pulse rate and end-tidal CO_2. The degree of anaesthesia was monitored by evaluating ear and paw pinch reflex, as well as ocular signs such as nystagmus, exophthalmia and the loss of palpebral and corneal reflexes. Quality of induction was good without any excitation. Intubation was quick and easy in all rabbits. Ocular signs recorded after induction had a high rate of variation among individuals as was the time to reoccurrence of the swallowing reflex. Two rabbits showed apnoea and all rabbits showed varying degrees of hypoxemia, with lowest SpO2 values recorded at 42%. In conclusion, alfaxolone should only be used as an induction agent in rabbits when oxygen supplementation and the possibility of intermittent positive pressure ventilation can be assured. The induction dose should be adapted to the response of the individual, as there was a considerable variation in anaesthetic depth among the rabbits.

The Institutional Animal Care and Use Ethics Committee of the University of Liège approved the protocol used in this study. The "Guide for the Care and Use of Laboratory Animals", prepared by the Institute of Laboratory Animal Resources, National Research Council and published by the National Academy Press, was followed carefully throughout the experiments.

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79. Lesion development in a new intestinal loop model indicates the involvement of a shared *Clostridium perfringens* virulence factor in hemorrhagic enteritis in calves.

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Clostridium perfringens associated enterotoxaemia is a highly fatal disease in fast growing suckler and veal calves. In the present study an intestinal loop model was developed to study the pathogenesis of the disease. A collection of *Clostridium perfringens* isolates was tested for their ability to induce the typical hemorrhagic enteritis lesions in the loops. Intestinal loops were injected with logarithmic *Clostridium perfringens* cultures with, or without, a milk protein based commercial milk replacer for calves. Both isolates from bovine enterotoxaemia cases and from calves without pathological changes in the gastrointestinal tract were used, as well as different isolates from non-bovine origin. All tested isolates were capable of inducing hemorrhagic enteritis-like lesions provided they were injected in combination with milk replacer, while all control loops remained negative. In addition, time-course experiments were conducted using an isolate from an outbreak of bovine enterotoxaemia. Histological examination showed that lesion development was initiated by loss of epithelial sheets, followed by congestion of the capillaries, starting within 30 minutes after inoculation. Hemorrhages and mucosal necrosis developed in a later phase. These lesions are similar to those observed in natural cases of bovine enterotoxaemia. It can thus be concluded that hemorrhagic enteritis lesions can be induced by *Clostridium perfringens* isolates from diverse origin in an intestinal loop model. This suggests that the lesions are caused by the action of a virulence factor(s) that is present in many *Clostridium perfringens* isolates, underscoring the importance of environmental factors in the development of the disease in the field.

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80. Renewed interest in porcine heart and pulmonary vein anatomy in an experimental model for atrial fibrillation treatment.

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Atrial fibrillation is the most common cardiac arrhythmia diagnosed in man, resulting in substantial morbidity and mortality. Atrial fibrillation is frequently initiated by triggering foci in myocardial sleeves extending into the pulmonary vein antra. Currently, the efficacy and risks of catheter ablation as an ultimate treatment option for the most obstinate forms of atrial fibrillation are actively debated. The search for more performance and safer ablation techniques is hampered by a lack of in-depth data on the fine anatomical architecture of the pulmonary veins in any experimental animal model considered. In this context, 50 pig hearts were by dissection anatomically examined to determine the pulmonary vein topography and variability. In all examined cases, the pulmonary veins derived from the right cranial and intermediate lung lobes drained into the right atrium through a common septal ostium. More variation was found in the branching pattern and the cardiac entrance of the pulmonary veins draining the other segments of the lungs. To analyse the stretch tolerance of the pulmonary vein orifices, as a prerequisite for using expandable intraluminal ablation devices, biomechanical stretch tests were carried out. The pulmonary veins could be stretched up till 170% of their original diameter without apparent histological damage. From 180% onwards however, myocardial damage could be detected microscopically. These results provide fundamental data essential for the development and in vitro, in silico and in vivo testing of a new surgical technique in the treatment of atrial fibrillation.

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81. Could Belgian horses be screened for West Nile fever by the use of the clinical examination?

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Introduction: West Nile virus (WNV) is an emerging virus causing neurological signs in humans and horses (West Nile fever – WNF). WNV has not yet been encountered in Belgium, but is a genuine threat, considering the presence of migratory birds and suitable vectors. In order to identify possible future cases early and mobilize rapid responses, syndromic surveillance is considered useful. However, it is difficult to recognize WNF clinically. **Aims:** Identify clinical variables that could be indicators for WNF in Belgian horses. **Cases**: French horses with confirmed WNF (N=33). Control group I (N=65): Belgian horses with neurological problems, consisting of cases with a definitive diagnosis excluding WNF (control group II; N=34), and cases without a definitive diagnosis but highly suspected free from WNF. **Statistical analysis**: Clinical findings were compared with a Fisher's exact test with Bonferroni correction between groups and classification and regression tree (CART) analyses was performed with WNF as dependant variable. **Results**: Besides ataxia (73%), the neurological signs in WNF cases were variable. Also hyperthermia (39%) was not consistently encountered. WNF cases suffered significantly less from recumbency, hypermetria and cranial nerves deficits. In addition, the CART analyses identified the month of disease occurrence, absence of recumbency and absence of hypermetria as useful predictors with high sensitivity. **Discussion and conclusions**: Although the clinical picture of WNF was highly variable, this study suggests that clinical information can be used as a screening tool for syndromic surveillance for WNF in Belgian horses. Nevertheless, laboratory confirmation of clinically suspected cases remains necessary.

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82. Genome-wide RNAi screening identifies cellular genes involved in Murid Herpesvirus 4 cycle.

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Gammaherpesviruses are archetypes of persistent viruses that have been identified in a range of animals from mice to man. They are host-range specific and establish lifelong latency of immunocompetent hosts. Most of the gammaherpesvirinae members are associated with neoplastic diseases. For example, the best studied gammaherpesviruses are Human herpesvirus 4 and 8 that are respectively associated with Burkitt's lymphoma and Kaposi's sarcoma. By opposition to its human counterparts, Murid herpesvirus-4 (MuHV-4) is able to replicate to high titers in cultured cells and is therefore an excellent candidate for studying gammaherpesvirus cycle. RNA interference (RNAi) is a natural process that cells use to turn down, or silence, the activity of specific genes by inhibition of mRNAs. The selective and robust effect of RNAi on gene expression makes it a valuable research tool in cell culture because synthetic dsRNA introduced into cells can induce suppression of specific genes. RNAi may also be used for large-scale screens that systematically shut down each gene in the cell. Here, we describe the use of high troughput screening by reverse transfection of cells of 17 820 small interfering RNAs (siRNA), which, combined to fluorimetry explored 5940 human genes (Ambion silencer druggable genome). The data were analysed for each siRNA (3/gene) and the results were obtained by bioinformatics. 67 genes appeared to be potentially involved in MuHV-4 cycle and were specifically retested. Among these genes, proteins encoded by NAT8 and MFSD5 recently emerged as membrane trafficking proteins and could therefore be involved in cellular trafficking of viral particles.

83. Molecular detection of HAV by a new one step real time RT-PCR.

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Hepatitis A virus (HAV) is a RNA virus with a single-stranded positive sense genome. Belgium and European countries in general, are countries with a low prevalence and the majority of adults can be infected. Viral loads in food samples are lower than in clinical samples and their detection requires refined molecular detection methods. A one step real-time RT-PCR to detect HAV, with new primers (HAV F2 and HAV R2) and probe (HAV P2) was performed directly on HAV diluted suspensions and on food samples (dates) and was compared with a ready-to-use commercial kit. Before the one step real time RT-PCR, a preliminary step combining concentration of viral particles with polyethyleneglycol and centrifugation was used on food samples. Real time RT-PCR one step with HAV F2/R2/P2 is more efficient but less sensitive than the commercial kit. It could be used to confirm a positive sample or to detect HAV in an unknown sample. With cell cultured HAV, the limit of detection (LOD) is 10^2 $TCID_{50}$/ml and in food samples, LOD is between 10^4 and 10^5 $TCID_{50}$/ml. Several hypotheses could explain these results: the loss of viral particles during the extraction process, the low efficiency of RNA extraction and interference of food on molecular detection. Molecular detection of virus in food samples remains a challenge and the protocol of extraction should be improved and adapted at each food category to increase the sensitivity of detection in food matrices characterized by a low viral contamination.

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ADDENDUM

84. Genotypic and phenotypic characterisation of Methicillin-Resistant *Staphylococcus intermedius* and *pseudintermedius* (MRSI and MRSP) isolated from dogs and cats in Japan

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Staphylococcus (S.) intermedius and *pseudintermedius* are two species responsible for skin infections in dogs and cats. Both species can rarely cause infections in humans, usually following infected animal contacts. Recently, Methicillin-Resistant *S. intermedius* (MRSI) and *S. pseudintermedius* (MRSP) have emerged as significant nosocomial pathogens in companion animals. Their rising incidence makes them an alarming problem since there is the limited therapeutic option for animals, or even for human. The aim of this study is to investigate MRSI and MRSP presence in *S. intermedius* and *S. pseudintermedius* and to compare their genotypes and phenotypes characteristics.
Two hundreds *S. intermedius* and *pseudintermedius* isolated in Japan from cats and dogs were tested for methicillin resistance. Positive isolates were characterised for virulence genes, biofilm formation and antibiotics resistance. Besides, the strains were typed by SCC*mec*-typing, *spa*-typing, PFGE and MLST.
Out of the 200 tested strains, 27 were methicillin-resistant. PFGE results showed that most positive strains are not closely related. However they are shared between 4 main groups according to MLST and SCC*mec*-typing. Most strains showed strong biofilm formation. Finally, MRSI and MRSP were resistant to nearly 80% of the antibiotics. Virulotyping and *spa*-typing are currently on-going.
In conclusion, results obtained showed that MRSI and MRSP are present in the studied animals and don't seem to form a homogeneous group. Phenotypic features as strong biofilm formation and high antibiotics resistance probably help bacteria to infect and persist in animals and veterinary hospital. Moreover, such strains could represent a risk for pets owners and veterinarians.

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85. Immature equine oocyte vitrification

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Vitrification is a cryopreservative method based on an infinite increase of viscosity of water obtained after a short time exposure to high concentration level of cryoprotectants associated with a very fast cooling in liquid nitrogen.
This method was chosen as a standard for the equine immature oocyte cryoconservation. Actually its large size, its low membrane permeability and its high lipid content interfere with water and cryoprotectant inflows and with the survival of the cell. That is why the success oocyte cryopreservation is poor and needs to be improved.
The first stage of this study was conducted in order to test the effect of cumulus cells removal on meiotic competence (ability to reach the metaphase 2) and oocyte cryopreservability. As previously shown, the present study confirmed a positive effect of the cumulus cells on *in-vitro* maturation and a protective effect during vitrification.
The second stage of the study compared two vitrification protocols based on dimethyle sulfoxyde, ethylene glycol and sucrose. Base solution was for the first one a usual saline solution (Dulbecco's phosphate buffered saline), and for the second one a solution commercialized for slow freezing of equine sperm containing glycerol and egg yolk plasma. Maturation rate obtained with the commercialized solution was higher than with the usual one and similar to the non-vitrified control group.
The final stage of the study addressed the oocyte volume variations during both protocols of vitrification. Oocyte volume decreased significantly during the two protocols, but the reduction was significantly more important with the usual solution.

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86. Effect of chronic confinement stress on sperm quality in Eurasian perch.

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Stress plays a key role in the ability of fish to perform reproduction. Given the huge variability in fish reproductive strategies, large variability in the effects of stress on reproductive efficiency could be expected. Eurasian perch (*Perca fluviatilis*) is mainly represented in Europe and reared under intensive rearing conditions (e.i. high confinement), which are known to impair with many physiological function. In perch, stress induces an increase of cortisol, but little is known regarding the effect of stress on male reproductive capacity. The aim of the present study was to examine the impact of chronic confinement stress on sperm quality of perch during the final maturational period.

Perch breeders were reared in 3 confinement conditions (0.70 m^3, 0.5 m^3 and 2 m^3) in duplicate (except for 0.70 L) at the same density (12 ind/m^3) from September 2011 at a 50:50 sex ratio. The 5th and 26th March, 3 males from each tank were randomly handled, anesthetized and sperm collected by stripping. 100 µL were dissolved in extender, sperm concentration assessed by counting spermatozoa with a Burker's cell and sperm motility determine by CASA system. Velocity parameters studied were % progressive (Prog) and motile (MOT) spermatozoa and VAP, VCL and VSL. Sperm concentration decreased with confinement from 70.5±17.2 x10^9 to 61.5±9.9 x x10^9. VAP (from 32.6 to 40.2 µm/sec), VSL (from 23.5 to 30.0 µm/sec), VCL (from 81.6 to 89.5 µm/sec), MOT (from 56.0 to 85.0 %) and PROG (from 21.5 to 28.7) were not significantly influenced by the confinement level.

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