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FARAH

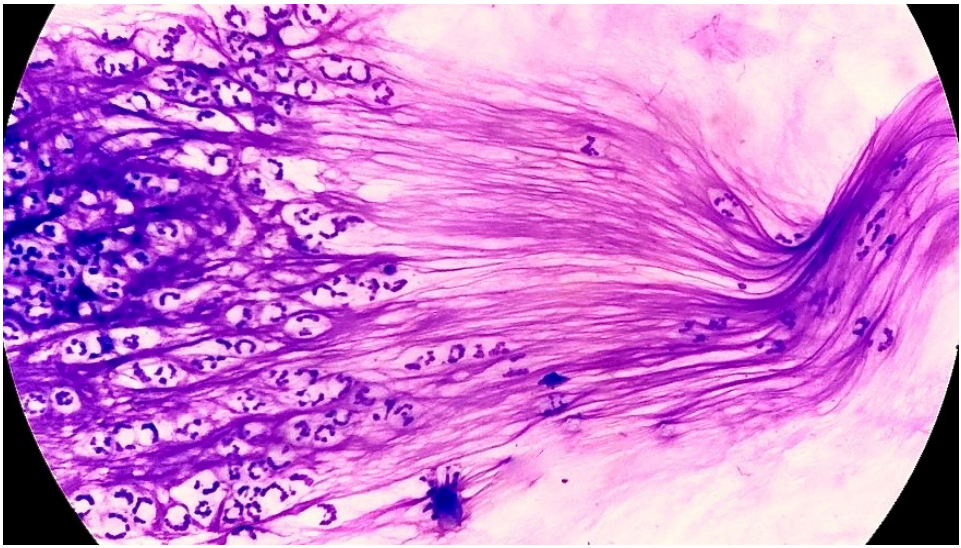
Proceedings of the 4th FARAH-Day

Faculty of Veterinary Medicine
(University of Liège - Belgium)

October 13, 2017

One Health

L'Animal et l'Homme, une même santé



Proceedings of the 4th FARAH-Day

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October 13, 2017

Edited by C. Bayrou, J.-F. Cabaraux, C. Delguste, C. Douny, C. Gatez, T. Jauniaux, L. Ludwig, J. Ponthier, A. Sartelet, D. Thiry, D.-M. Votion
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S. Vermeer, Clinical Dpt of Companion Animals and Equids, FARAH

Purulent mucus aspirated from dog lungs

Welcome to the 4th FARAH Day

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

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

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

Laurent Gillet, President of the FARAH

Frédéric Farnir, Vice-president

Dominique Votion, Secretary

Bienvenue à la 4ème journée du FARAH

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

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

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

Laurent Gilet, Président du FARAH

Frédéric Farnir, Vice-président

Dominique Votion, Secrétaire

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Program

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

09:00 - Opening and Welcome Speech

Dr Catherine DELGUSTE, *President of the Scientist Staff*

09:15 Invited speaker:

Winner of the "Etienne Baise award"

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

Chairmen: ***Thomas MARICHAL and Bernard TAMINIAU***

09:45 Conserved fever pathways across vertebrates: a herpesvirus delays fish behavioural fever through expression of a decoy Tnf-alpha receptor
Alain VANDERPLASSCHEN, Immunology-Vaccinology, FARAH

10:00 Identification of an essential virulence gene of cyprinid herpesvirus 3
Maxime BOUTIER, Immunology-Vaccinology, FARAH

10:15 Comparison of faecal microbiota of horses suffering from atypical myopathy and healthy co-grazers
Simona CERRI, Equine clinic, FARAH

10:30 Infection of European eel by Anguillid herpesvirus 1: from basic research to conservation programs
Natacha DELREZ, Immunology-Vaccinology, FARAH

10:45 - Coffee break and poster session 1 (Room P, building B45)

11:15 - Oral session 2: (lecture hall C, building B45)

Chairmen: ***Véronique DELCENSERIE and Stefan DELEUZE***

11:15 Rab guanine nucleotide exchange factor 1 (Rabgef1) restricts intestinal inflammation by limiting pro-inflammatory signals in Intestinal Epithelial Cells (IECs)
Sophie EL ABBAS, Cellular and molecular Immunology, GIGA

11:30 Participatory analysis of the swine value chain in the department of Ouémé and Plateau (Benin): characterization and innovations practices
Benoît GOVOEYI, Sustainable Animal Production, FARAH

11:45 ***Short talks***

Retrospective study on the influence of nephrosplenic space closure in recurrent colic
José-Manuel ARÉVALO-RODRIGUEZ, Equine clinic, FARAH

Mucosal immunity following vaccination with a CyHV-3 attenuated recombinant vaccine
Maxime BOUTIER, Immunology-Vaccinology, FARAH

The EEL4EVER project: Establishment of quarantine stations and evaluation of the effect of a recovery period before reintroduction of glass eels into the wild
Natacha DELREZ, Immunology-Vaccinology, FARAH

Aryl hydrocarbon receptor transactivation assays to study health impacts of exposure to mixtures of endocrine disrupting chemicals
Thi Que DOAN, Food Science, FARAH

Surgical co-transfers of genetically engineered pig embryos produced in vitro by ICSI-SMGT
Fabien ECTORS, Morphology and Pathology, FARAH

Muscle-derived stem cells injected in proximity to the recurrent nerve in horses – a potential treatment for laryngeal neuropathy
Alexia FOUREZ, Surgery and anaesthesiology of domestic animals, FARAH

Peroperative mesh obliteration of epiploic foramen to prevent recurrent entrapment of small intestine
Sigrid GRULKE, Equine clinic, FARAH

Crassicauda boopis in a fin whale (Balaenoptera physalus) ship-struck in the eastern North Atlantic Ocean
Laetitia LEMPEREUR, Infectious and parasitic diseases, FARAH

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

14:00 Invited speaker

J. CEUSTERS, JP. LEJEUNE AND D. SERTEYN

Cell therapy and veterinary regenerative medicine

14:30 - Oral session 3: (lecture hall C, building B45)

Chairmen: ***Fabrice BUREAU and Nicolas KORSAK***

14:30 In vitro evaluation of the competing effect of Carnobacterium maltaromaticum isolated from vacuum-packed beef with long shelf-life against three major food pathogens

Pedro Henrique IMAZAKI, Laboratory of food technology, FARAH

14:45 Evaluation of the effects of point mutation accumulation on the replicative fitness of a recombinant murine norovirus

Louisa LUDWIG, Veterinary virology, FARAH

15:00 A gammaherpesvirus protects against allergic asthma through the replacement of resident alveolar macrophages by regulatory monocytes

Bénédicte MACHIELS, Immunology-Vaccinology, FARAH

15:15 Latency-deficient AlHV-1 protects against malignant catarrhal fever in calves

Françoise MYSTER, Immunology-Vaccinology, FARAH

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

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

Chairmen: ***Géraldine BOLEN and Dominique CASSART***

16:00 Urodynamic and morphometric characteristics of the lower urogenital tract in growing male Beagle littermates

Mathilde PORATO, Clinical sciences, FARAH

16:15 Helminth-induced IL-4 expands virtual memory CD8+ T cells for early control of gammaherpesvirus infection

Marion ROLOT, Immunology-Vaccinology, FARAH

16:30 ***Short talks***

Comparison between swabbing devices in order to analyse the microbial flora found on surfaces by classical microbiology and 16S rDNA amplicon sequencing

Simone KRINGS, Food Science, FARAH

Staphylococcal chromosome cassette mec typing of methicillin resistant staphylococci from different samples collected in European, African and North-American countries

Cyrille NGASSAM TCHAMBA, Infectious and parasitic diseases, FARAH

Phenotypic, genetic and biochemical identification of mammary pathogens in milk samples from cows with mastitis in Wallonia (Belgium)

Cyrille NGASSAM TCHAMBA, Infectious and parasitic diseases, FARAH

Ear canal microbiota - a comparison between the healthy and atopic dog without any sign of active otitis

Jérôme NGO, Veterinary Dermatology, FARAH

CT-assisted retrograde urethrography in male dogs

Mathilde PORATO, Clinical sciences, FARAH

Deletion of macrophage-specific IL-4 receptor in schistosomiasis does not preclude Ly6Chigh monocytes as the main source of liver macrophages but impairs alternative activation and control of inflammation

Marion ROLOT, Immunology-Vaccinology, FARAH

Congenital Articular Rigidity outbreak due to ruminal dysfunction in a Belgian blue cattle herd

Arnaud SARTELET, Clinical Department of Production Animals, FARAH

17:15 - Awards

Dr Dominique VOTION, Board member of FARAH

17:30 - Closing session

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

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

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SHORT COMMUNICATIONS

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Veterinary public health

SUSTAINABLE LIVESTOCK PRODUCTION

COMPARATIVE VETERINARY MEDICINE

Oral presentations

Oral presentations

Conserved fever pathways across vertebrates: a herpesvirus delays fish behavioural fever through expression of a decoy Tnf-alpha receptor

Rakus K.[1,2*],Ronsmans M[1*],Forlenza M[3],Boutier M[1],Piazzon MC[3],Jazowiecka-Rakus J[1,4],Gatherer D[5],Athanasiadis A[6],Farnir F[7],Davison JA[8],Boudinot P[9],Michiels T[10],Wiegertjes GF[3],Vanderplasschen A[1]

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When infected by pathogens,endotherms and ectotherms can both increase their body temperature to limit the infection.Ectotherms do so by moving to warmer places,hence the term "behavioral fever".We studied the expression of behavioral fever by common carp infected by cyprinid herpesvirus 3 (CyHV-3) using multi-chamber tanks encompassing a 24-32°C gradient.We showed that carp maintained at 24°C died from the infection,whereas those housed in multichamber tanks all survived as a consequence of their transient migration to the warmest compartment.As the expression of behavioral fever occurred only at an advanced stage of the disease,we hypothesized that the virus might delay this phenomenon in order to promote its replication.This hypothesis was proved correct,and the delay mechanism was found to rely on the expression of a soluble viral decoy receptor for Tnfα encoded by CyHV-3 ORF12.This conclusion relied on three complementary observations:(i)a CyHV 3 ORF12 deleted recombinant induced an early onset of behavioral fever in comparison to wild-type CyHV-3;(ii)ORF12 expression product binds and neutralizes carp Tnfα;(iii)injection of anti-Tnfα neutralizing antibodies suppressed behavioral fever, and decreased fish survival in response to infection.This study provides a unique example of how viruses have evolved to alter host behavior to increase fitness.It demonstrates that behavioral fever in ectotherms and fever in endotherms are evolutionarily and functionally related through common cytokine mediators that originated more than 400 million years ago.Finally, this study stresses the importance of the environment in the host-pathogen-environment triad.

Identification of an essential virulence gene of cyprinid herpesvirus 3

Boutier M.[1], Gao Y.[1], Vancsok C.[1], Suarez M.N.[2], Davison A.[2], Vanderplasschen A.[1]

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The genus *Cyprinivirus* consists of a growing list of phylogenetically related viruses, some of which cause severe economic losses to the aquaculture industry. The archetypal member, cyprinid herpesvirus 3 (CyHV-3) causes mass mortalities worldwide in koi and common carp. A CyHV-3 mutant was described previously that is attenuated *in vivo* by a deletion affecting two genes (ORF56 and ORF57). The relative contributions of ORF56 and ORF57 to the safety and efficacy profile of this vaccine candidate have now been assessed by analysing viruses individually deleted for ORF56 or ORF57. Inoculation of these viruses into carp demonstrated that the absence of ORF56 did not affect virulence, whereas the absence of ORF57 led to an attenuation comparable to, though slightly less than, that of the doubly deleted virus. To demonstrate further the role of ORF57 as a key virulence factor, a mutant retaining the ORF57 region but unable to express the ORF57 protein was produced by inserting multiple in-frame stop codons into the coding region. Analysis of this virus *in vivo* revealed a safety and efficacy profile comparable to that of the doubly deleted virus. These findings show that ORF57 encodes an essential CyHV-3 virulence factor. They also indicate that ORF57 orthologues in other cypriniviruses may offer promising targets for the rational design of attenuated recombinant vaccines.

Comparison of faecal microbiota of horses suffering from atypical myopathy and healthy co-grazers

Cerri S.[1], Taminiau B.[2], Votion D.[3], Amory H.[1], Daube G.[2], Cesarini C.[1]
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The objective of this study is to characterize faecal microbiota of horses with atypical myopathy (AM) compared with healthy co-grazers (HcG). Fresh faecal samples were obtained from 6 horses (1 stallion, 3 geldings and 2 females; with a mean age of 11.8±10 years) with confirmed AM and from 6 HcG (4 geldings and 2 females; with a mean age of 13.6±8 years) during autumn-2016 and spring-2017 AM outbreaks in Belgium. Bacterial taxonomy profiling obtained by 16S amplicon sequencing of faeces was used to identify differentially distributed bacterial taxa between AM and HcG. Results were statistically compared using Welch's t-test with STAMP software. A total of 90,407 sequences were analysed and clustered to 8,066 operational taxonomic units. Bacterial populations were distributed between 17 phylas, although 20% of sequences could not be attributed to an existing phylum. Horses with AM harboured a significantly higher relative abundance of *Ruminococcaceae* family with a significantly lower *Lachnospiraceae* when compared to HcG. AM is caused by hypoglycin A intoxication, but only a part of horses pasturing in the same toxic environment develops the pathology, suggesting that there may be protective factors at the horse level. The results of this study show significant differences in faecal microbiota between AM cases and HcG, which could suggest that microbiota could play a role in the development or prevention of clinical disease. Results demonstrate that microbiota of AM affected horses is significantly different compared to HcG, and this difference could influence the development of AM. However, the role of microbiota, deserves further investigation.

Infection of European eel by Anguillid herpesvirus 1: from basic research to conservation programs

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The European eel (Anguilla anguilla) is a catadromous fish with a complex and fascinating life cycle. Over the last few decades, the number of eels reaching Europe has declined by 99%. The European eel is nowadays classified as a critically endangered species. Among the multiple factors contributing to this decline, viral infection caused by Anguillid herpesvirus 1 (AngHV-1) is thought to play a key role. In addition to its ecological impact, this virus is also causing important economic loses in the eel aquaculture sector. In the present project, we aim to study the infection of the European eel by AngHV-1 as a homologous host-virus model to address fundamental questions related to this member of the *Alloherpesviridae* family. This project is structured around three integrated work packages/objectives. In the first work package, we will use an In vivo Imaging system and a recombinant virus expressing firefly luciferase to address various aspects of pathogenesis: (i) the portal of viral entry into the host, (ii) viral spreading within the infected host, (iii) transmission of the virus between subjects and (iv) the effect of water temperature and the developmental stage of the host on the outcome of the infection. The second work package will consist of a multidisciplinary approach aiming to study the effect of the infection on the behavior of the host. Finally, in the third work package, we will investigate the role of ORF35 as a key virulence factor and the potential of ORF35 deleted recombinants as attenuated vaccine candidates compatible with mass vaccination and conservation programs.

Oral presentations

Rab guanine nucleotide exchange factor 1 (Rabgef1) restricts intestinal inflammation by limiting pro-inflammatory signals in Intestinal Epithelial Cells (IECs)

El Abbas S.[1], Beguin C.[1], Schyns J.[1], Pirottin D.[1], Tsai M.[2], Tam SY.[2], Galli SJ.[2], Bureau F.[1,3] and Marichal T.[1,3]

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Rab guanine nucleotide exchange factor (GEF-)1 (Rabgef1), a multifunctional protein whose *in vivo* functions remained unknown until recently, is highly expressed in mouse and human epithelial cells.

The aim of this study is to investigate the role of Rabgef1 in intestinal epithelial cells (IECs) and intestinal homeostasis in mice. We performed conditional deletion of Rabgef1 gene using the cre-lox system to obtain mice lacking Rabgef1 specifically in IECs (Rabgef1$^{IEC\text{-}KO}$), under the wild-type (WT) or the colitis-prone *Interleukin-10* (*Il-10*)-deficient background. In addition, we used the CRISPR-Cas9 technology to obtain a murine IEC line deficient in Rabgef1. Rabgef1$^{IEC\text{-}KO}$ mice under the WT background did not develop spontaneous intestinal abnormalities but exhibited an altered intestinal microbial composition associated with minor changes in IEC pro-inflammatory gene expression profile. Moreover, Rabgef1$^{IEC\text{-}KO}$ mice exhibited an increased susceptibility to inflammation in a dextran sodium sulfate (DSS)-induced model of colitis under the WT background, as well as in a constitutive model of colitis under the *Il-10*-deficient background. *In vitro*, we showed that mouse IECs lacking Rabgef1 significantly overexpressed several pro-inflammatory cytokines and chemokines as compared to control cells.

Taken together, these results support that Rabgef1 acts as a regulator of intestinal homeostasis and inflammation, and that dysregulated Rabgef1 expression could contribute to intestinal barrier dysfunction in inflammatory conditions of the gut.

Participatory analysis of the swine value chain in the department of Ouémé and Plateau (Benin): characterization and innovations practices

Govoeyi B.[1,2], Moula N. [1], Kiki P.S.[1,2], Dotche I.O.[1,2], Youssao Abdou Karim I.[2], Antoine-Moussiaux N.[1]

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Rural poverty in sub-Saharan Africa remains a huge challenge despite the numerous agricultural development policies implemented. As pork demand is growing in Benin, it is necessary to assess the state of the value chain (VC) to envision its development. A participatory approach is here proposed to join this characterization to a stimulation of innovation dynamics. Four stages are included: i) Identification of actors and direct links along the VC, ii) Characterization of innovation practices, iii) Identification of bottlenecks and opportunities using innovation system framework, and iv) Measurement of agreement among VC actors about constraints and value-added sharing using proportional piling tool. Based on innovation practices, typologies or 'innovation profiles' were defined separately for three categories of actors: pig breeders (n=133), pork butchers (n=45) and input suppliers (n=25). Three innovation profiles were retained for breeders, two for butchers and two for input suppliers. For each category, the profiles qualified as "innovators" accounted for 51.1%, 84.4%, and 48.0%, respectively in breeders, butchers and input suppliers. The lack of professionalization was expressed as the major constraint by actors. The Kendall's coefficient of concordance (W) indicated that pork butchers gained the most part of the value-added, followed by input suppliers. It is here advocated that the present participatory method, while characterizing the value-chain in a rapid way, further sets the basis for the dialog between actors and the stimulation of innovation along the VC through an exchange platform.

In vitro evaluation of the competing effect of *Carnobacterium maltaromaticum* isolated from vacuum-packed beef with long shelf-life against three major food pathogens

Imazaki P.H.[1], Danielski G.M.[1,2], Daube G.[3], Ernlund Freitas de Macedo R.[2], Clinquart A.[1]
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Foodborne disease outbreaks are one of the leading causes of infections, hospitalisations and deaths provoked by pathogenic bacteria. Biopreservation is a hurdle which consists of the use of controlled microorganisms or its metabolites to preserve food and extend its shelf-life. Carnobacteria have been studied for their bioprotective properties since they can inhibit pathogenic and spoilage microorganisms. In this way, this study aimed to evaluate in vitro the bioprotective potential of *Carnobacterium maltaromaticum* (CM) isolated from vacuum-packed (VP) beef against *Escherichia coli* O157:H7, *Listeria monocytogenes* and *Salmonella Typhimurium*. Three CM strains isolated from VP beef with long shelf-life at a temperature near the freezing point were selected. The antimicrobial effect of CM against the pathogenic bacteria cited above was evaluated in co-cultures with or without EDTA at different temperatures. The antimicrobial effect of the cell-free supernatant of CM was also evaluated. The results indicate that the selected strains have an antilisterial activity, which is optimised at low temperatures. Moreover, when the strains were combined with EDTA, it was observed a slight inhibition of gram-negative bacteria (GNB). The inhibition with the EDTA treatment was possibly due to the capacity of this compound to destabilise the outer membrane of GNB, allowing CM to act against these bacteria. Finally, the evaluation of the antimicrobial activity of cell-free supernatant of CM did not highlight any inhibition effect against the tested pathogens. Thus, the antimicrobial activity of these CM strains is not related to bacteriocin production under the studied conditions.

Evaluation of the effects of point mutation accumulation on the replicative fitness of a recombinant murine norovirus

Ludwig L.F.[1,§], de Oliveira-Filho E.F.[1,2,§], Lu J.[3], Hosmillo M.[3], Mathijs E.[1,4], Toffoli B.[1], Di Felice E.[1,5], Goodfellow I.[3], Thiry E.[1], Mauroy A.[1,6] (*§ The two authors contributed equally to this work)*
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Noroviruses are recognised as a major cause of viral gastroenteritis in humans. Molecular mechanisms driving norovirus evolution are the accumulation of point mutations and recombination. Recombination can create great changes in a viral genome, potentially eliciting a replicative fitness cost (bottlenecking and faulty intragenomic cross talk), which must be compensated via the adaptive capacity of a recombinant virus. In this study, the capability of replicative fitness adaptation and genetic characteristics of a previously *in vitro*-generated recombinant murine norovirus (of WU20 and CW1) were evaluated at the start and end of ten *in vitro* passages. Our data provide evidence of viral adaptation in a known environment after a recombination event initially induced a fitness cost of an infectious recombinant. Replicative fitness regain of the recombinant was demonstrated by growth kinetics differences and increase of mean viral lysis plaque size after ten passages. Point mutations at consensus and sub-consensus population level (NS1/2,NS4,VP2) were characterised via NGS and putatively associated to the fitness changes. To investigate the effect of observed genomic changes in the context of a parental virus backbone, non-synonymous mutations were introduced via overlap mutagenic PCR into a plasmid containing wild type CW1 cDNA under control of a truncated T7 polymerase promoter. A DNA-based reverse genetics system allowed recovery of infectious virus at similar titres for all constructs, indicating that no mutation was so deleterious as to impair virus rescue. Passaging and phenotypic characterisation will allow us to pinpoint effects of mutations separately and combined.

Oral presentations

A gammaherpesvirus protects against allergic asthma through the replacement of resident alveolar macrophages by regulatory monocytes

Machiels B.[1,7], Dourcy M.[1,7], Xiao X.[1], Javaux J.[1], Mesnil C.[2], Sabatel C.[2], Desmecht D.[3], Lallemand F.[4], Martinive P.[4], Hammad H.[5], Guilliams M.[5], Dewals B.[1], Vanderplasschen A.[1], Lambrecht B.N.[5,6], Bureau F.[2], Gillet L.[1]*

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The hygiene hypothesis postulates that the increase in allergic diseases like asthma and hay fever is linked to reduced exposure to childhood infections. Here, we investigated how a gammaherpesvirus infection affects the subsequent development of allergic asthma. We showed that Murid herpesvirus 4 (MuHV-4) inhibits the development of House Dust Mite (HDM)-induced experimental asthma by modulating lung innate immune cells. Specifically, MuHV-4 infection caused the replacement of resident alveolar macrophages (AMs) by monocytes that were imprinted with regulatory functions in the bone marrow. These monocyte-derived AMs blocked the ability of dendritic cells to trigger a HDM-specific Th2 response. More generally, our results reveal that replacement of embryonic AMs by regulatory monocytes is a major mechanism underlying the long term training of lung immunity after infections.

Latency-deficient AlHV-1 protects against malignant catarrhal fever in calves

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Alcelaphine herpesvirus 1 (AlHV-1) persists in wildebeest asymptomatically but induces malignant catarrhal fever (MCF), a fatal lymphoproliferative disease upon transmission to several ruminants, including cattle. No vaccine against AlHV-1 is available until now while the consequences of this virus are important for the Massaï people. Experimentally, rabbit can be used to study the disease. One of the gene of AlHV-1, the ORF73, is known to be essential for the genome maintenance of gammaherpesvirus during latency. Using the rabbit model, the essential role of ORF73 for the development of MCF has been demonstrated. Moreover, a viral strain deleted for the ORF73 is able to induce the protection against a wild-type (WT) challenge in rabbits. Here, the potential vaccine candidate was tested in calves. An intranasal injection was performed in two groups of four calves with the ORF73-deleted virus or mock-infected supernatant. Next, the two groups were challenged by a WT strain. While three calves developed MCF after the WT challenge in the mock-infected group, no calf developed any clinical sign in the group primarily infected with the ORF73-deleted virus. These results suggest that the ORF73-deleted virus could be used as an attenuated vaccine in the field. *In vitro*, this strain (like the WT strain) growths very slowly and is strongly cell-associated. Further investigations are in progress to optimize the production yields of a cell-free attenuated ORF73-deleted virus that conserves its vaccine potential.

Urodynamic and morphometric characteristics of the lower urogenital tract in growing male Beagle littermates

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In dogs, castration is suggested to be detrimental to male continence. We studied urodynamic and morphometric characteristics of the urogenital tract in 6 growing male Beagle dogs to establish baseline values. They underwent EMG-coupled urodynamic tests and CT-assisted urethrography monthly from 4 to 12 months of age and at 15, 18, 21 and 24 months of age. Canine prostate specific esterase (CPSE) was measured in serum and prostatic wash and luteinizing hormone (LH) was measured in plasma. On UPPs, 26.35% of pressure peaks occurred along the penile urethra, 34.75% at the penis bone, 18.50% in the pelvic urethra where. Distribution of the peaks along the urethra varied significantly with age. Among 12 urethral pressure profiles, 17 EMG signals were synchronized with an urethral pressure peak, of which 12 occurred along penile urethra. Among 78 cystometry recordings, only 9 EMG signals were synchronized with bladder pressure peaks just before micturition. Integrated pressure (functional area) increased between 6 and 8 months of age. Urethral length increased between 6 and 7 months of age. CPSE concentration in prostatic wash varied with age. LH was better detected in plasma after 12 months of age. Bladder capacity in adults stabilized around 10 ml/kg. Results of this study indicate that in male Beagle dogs, urethral resistance and bladder capacity improved until 8 and 11 months of age, respectively. Not all urethral pressure peaks are attributable to striated muscle activity. Other structures may play a role in the maintenance of continence. Further investigation is needed to assess whether early castration might influence incontinence later in life.

Helminth-induced IL-4 expands virtual memory CD8$^+$ T cells for early control of gammaherpesvirus infection

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The first stages of host colonisation with pathogens often determine the efficacy of their control by priming effective adaptive immune responses. Immune imprinting during chronic infections by parasitic helminths can alter the control of bystander viral infections such as gammaherpesviruses. Here, we looked at how exposure to helminths affects the immune control of acute murid herpesvirus 4 (MuHV-4) infection. Viral replication was monitored by *in vivo* imaging and we observed that prior exposure to *Schistosoma mansoni* (Sm) eggs or *Nippostrongylus brasiliensis* resulted in a significantly enhanced control of acute pulmonary infection in Balb/c mice. Sm eggs did not significantly modify migration efficiency of lung dendritic cells to the draining lymph node but resulted in improved antigen-specific CD8$^+$ T cell lung responses after viral infection. Furthermore, early control of viral infection was directly dependent on the presence of CD8$^+$ cells on interleukin 4 (IL-4) signaling. IL-4 has been described as a cytokine able to drive the expansion of virtual memory CD8$^+$ T cells (T_{VM}). In our model, exposure to helminths induced the expansion of CD44^{high}CD62L^{high}CXCR3^{high}CD49d^{low} T_{VM} cells in the spleen. VM expansion in response to helminth exposure was dependent upon direct IL-4 signaling on CD8$^+$ T cells but did not result from the proliferation of naïve VMs. Thus, our data indicate that helminth-elicited IL-4 can promote effective anti-viral CD8$^+$ T cell responses through an enrichment of the VM compartment to position these cells at the front line of the anti-viral response.

Short communications

Short communications

Retrospective study on the influence of nephrosplenic space closure in recurrent colic

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Introduction—Nephrosplenic entrapment of the large colon (NSE) is a common cause of colic in horses. Although medical or surgical treatments are available, this type of displacement may have an important recurrence rate. Clinical data about the impact of nephrosplenic space closure on recurrent colic are sparse in the literature and is investigated in this study.

Material and Methods—Medical records of horses diagnosed with NSE in the Equine Clinic of the University of Liège were retrieved between 2004 and 2014. Horse signalment, the type of treatment (medical versus surgical) and the eventual realization of a preventive surgical closure of the nephrosplenic space were collected. Follow-up information was obtained by a telephone interview of the owners. Statistical analyses were performed using a Fisher's exact test with significance set at $P < 0.05$.

Results— Fifty-five horses were included in the study (49 % of survey responses). Mean follow-up was 36 months (6 to 112). No horses operated for nephrosplenic space closure has been reported to recur NSE. On those horses, there was a significant decrease in the total incidence of colic after the surgery compared to non-operated horses.

Discussion/Conclusion—Laparoscopic obliteration of the nephrosplenic space seems effective in preventing recurrence of NSE, and significantly lowers the overall incidence of colic. However, other types of displacement may occur.

Mucosal immunity following vaccination with a CyHV-3 attenuated recombinant vaccine

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Cyprinid herpesvirus 3 (CyHV-3) is the causative agent of a lethal disease in common and koi carp. In a previous study, we showed that the double deletion of ORF56 and ORF57 (Δ56-57) is associated with a safe and efficacious profile in vivo. In addition, In vivo imaging (IVIS) of vaccinated fish challenged with a wild type strain expressing luciferase (WT Luc) suggested that vaccination induces an immune protection at the portal of entry (fish skin). Here, we initiated the study of the mechanisms underlying this immune protection. First, the role of the skin mucus was investigated. Vaccinated fish were challenged with the WT Luc with or without a prior mucus removal treatment. This mucus treatment enhanced the WT Luc infection in mock-vaccinated fish but not in vaccinated fish. Clarified mucus extract (CME) was collected from both groups. The viral neutralizing activity of the CME was comparable in vitro suggesting a low adaptive immune response in the mucus. Interestingly, vaccinated fish develop a systemic adaptive immune response as shown by serum neutralization. To identify the relative contribution of systemic versus mucosal adaptive immune response, a fin explant model was developed. Fin explants from vaccinated fish exhibited potent immune protection against an ex vivo challenge. This ex vivo immune protection correlates with the in vivo protection. This second part of this study highlights that the Δ56-57 vaccine induces a mucosal adaptive immune response capable to block viral replication of a WT challenge in vivo and ex vivo. Interestingly, this model represents a remarkable opportunity to study fish mucosal immunity.

The EEL4EVER project: Establishment of quarantine stations and evaluation of the effect of a recovery period before reintroduction of glass eels into the wild

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Wild European eel populations have severely decreased reaching less than 1% of the estimated population in the 1980s. Since 2007 and the establishment of the "the eel management plan" by the European commission, restocking inland waters with glass eels is currently applied in Belgium. This plan does not take into account the roles of infectious diseases in wild European eel decline. Currently, glass eels used for restocking are released directly after their capture without systematic analysis of their health status. The aim of this study was to develop quarantine stations in order (i) to evaluate the benefit of a recovery period before reintroduction into the wild and (ii) to investigate the health and microbiological status of the batches released. With this goal in mind, four different conditions were tested (18°C or 24°C; with or without feeding). The impact of these conditions was investigated on the health of the fish, their microbiological quality and their survival rate after an experimental stress mimicking the reintroduction in the environment. The effect of 2 and 4 weeks of quarantine was tested. The survival rate was high in all conditions and comparable to a direct release of the fish without any quarantine time. Notably, the higher survival rates were associated with the two weeks recovery period. In conclusion, these quarantine conditions could be integrated into health surveillance strategy. These quarantine stations represent also valuable tools to adapt the management of the release in the environment according climatic conditions and human resources.

Aryl hydrocarbon receptor transactivation assays to study health impacts of exposure to mixtures of endocrine disrupting chemicals

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While most of the experimental studies investigated endocrine disruptors (EDs) as single compounds, there is a need for studying their mixture effect, which is more relevant to human exposure routes and impacts. This study aims to reveal the health impacts of exposure to mixture EDs at the level of the aryl hydrocarbon receptor (AhR) function, a key receptor to cope with xenobiotic compounds including EDs. In this study, DR-CALUX cell-based assays, wich are quick, relevant and effective tools using luciferase as a reporter gene, have been used to screen AhR transactivation activities i.e. agonistic and antagonistic of 29 different chlorinated, brominated and perfluorinated compounds listed as persistent organic pollutants (POPs) under the Stockholm Convention on Persistent Organic Pollutants (SCPOP). Three different reporter gene transformed cell lines (rat hepatoma H4IIE, human mammary gland carcinoma T47-D and human hepatoma Hep G2 cells) have been selected for this study. To evaluate the mixture effect, transactivation activities of individual EDs will, first, be identified. Then, each compound will be assigned for a response index. A mixture of the compounds with high indexes (>5% of the maximum response of the reference compounds is considered as positive) will be constructed for identifying their activity contribution for the total effects. Initially, there are variations among compounds and cell lines. HepG2 cell lines gave no responses with all 29 compounds, while H4IIE and T47-D cell lines shown some significant AhR agonistic activities with PBDE99 (EC50 of PBDE99=148.5 ±70.3 nM and 1866 ±1756 nM, respectively), while PCB138 activated H4IIE only; γ-HCH did the same with T47-D only. Interestingly, trans-nonachlor and alpha-chlordane inhibited the activities of endogenous AhR ligands with negative responses in H4IIE, but they are positive T47-D AhR ligands, activating AhR transactivation in T47-D.

Short communications

Surgical co-transfers of genetically engineered pig embryos produced *in vitro* by ICSI-SMGT

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We have previously generated genetically engineered (GE) mice by pronuclear injection of a BAC expressing the complete Bos taurus antiviral Mx system (boMx1 & 2) under the control of its natural regulatory elements (Garigliany et al., 2009). These mice displayed exceptional anti-viral activities against lethal infections with influenza A viruses. As swine are susceptible to produce new potentially life-threatening influenza variants, we now aim to generate boMx1&2-expressing pigs by the sperm mediated gene transfer technique, a method derived from intracytoplasmic sperm injection.

In 2016 & 2017, we generated 72 vitrified GE 6-day-old pig blastocysts after a complete *in vitro* procedure and in defined media (IVP-DM). Based on a previous screening, we expect that about 64% of these blastocysts have incorporated at least a fragment of the BAC, of which about 2/3 have incorporated its complete sequence (M'Boumba et al, FARAH-day 2016). These embryos were transferred in 2 recipient sows in August 2017. Because we were afraid of a lower ability of our GE/vitrified IVP-DM embryos to express and secrete the molecular signals required for maintaining gestation, we co-transferred 36 GE/vitrified embryos with 10 *in vivo* produced and vitrified blastocysts in each recipient. Surgically-speaking, the transfers were successful and the recovery of the surrogate mothers as well. Echographic evaluation of the recipients to check for ongoing gestation is scheduled in September.

Muscle-derived stem cells injected in proximity to the recurrent nerve in horses – a potential treatment for laryngeal neuropathy

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Recurrent laryngeal neuropathy is a common pathology of upper airways in horses.Affected horses show various degrees of atrophy in muscles innervated by the recurrent nerve.This atrophy comes from lesions of demyelination but also remyelination not related to clinical remission.Several studies in mammals have shown favourable applications of stem cells in neuropathies.The aim of the study is to evaluate the feasibility and safety of the injection of muscle-derived stem cells close to the recurrent nerve in healthy horses.Five Standardbred horses were used for this protocol.Muscle-derived stem cells were obtained from 4 horses through a muscular microbiopsy (Ceusters,2017).Horses were sedated with 10µg/kg detomidine and left recurrent nerve was approached with an insulated nerve block needle (Pajunk,Germany) under ultrasound control.Nerve stimulation (TOF watch SX, Alsevia,France) was applied at 2mA and progressively reduced.When motor response was lost at 0.5mA, 10*106 autologous muscle-derived stem cells were injected.An injection of 1ml of 2% lidocaine was performed in the first horse. Horses had their laryngeal function scored by blinded examiners to the treatment before and at day 1,7 and 28 after stem cell injection.The correct positioning of the needle was confirmed by the injection of lidocaine which resulted in temporary paralysis of the left arytenoid cartilage.Approaching the recurrent nerve with nerve stimulator is possible and well tolerated by the horses.None showed modification of their laryngeal function after the injection of stem cells.Further studies are needed to evaluate the effect of peri-neural injection of autologous muscle-derived stem cells in horses affected by laryngeal neuropathy.

Peroperative mesh obliteration of epiploic foramen to prevent recurrent entrapment of small intestine

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Entrapment of small intestine into the epiploic foramen (EEF) represents an important cause of strangulating small intestinal obstruction (SSI). Both the short and long-term prognosis seem to be less favourable than for other types of SSI. The aim of the study is to report a technique of mesh closure of the epiploic foramen (EF) during emergency coeliotomy and to report short- and long-term outcome of horses operated for EEF with mesh closure. A polypropylene net was rolled and fixed by one stitch to obtain a cone coated with a piece of resected omentum and inserted into the EF from the medial to lateral side at the end of the laparotomy. 12 horses with a mean age of 10 years suffering from EEF (one horse for the 3rd time within 6 months) underwent surgery for correction of entrapment and mesh closure. Post-operative complications (mainly paralytic ileus) occurred in 5 of the 12 horses. One horse was euthanized due to recurrence of severe colic (volvulus). Necropsy confirmed the mesh remaining in the EF. Long-term follow-up from 6 months up to 5 years was available in 11 horses and the outcome was favourable in 11 horses. One horse had an exploratory coelioscopy 5 months after surgery (due to another cause of recurrent colic, recurrent large colon displacement in the reno-splenic space) and fibrous closure of the EF was confirmed. One horse was euthanized 3.5 years after mesh placement due to unrelated reasons and the fibrous integration of the mesh in the EF was shown at necropsy. Mesh closure of EF during emergency coeliotomy avoids additional surgery and prevents recurrence of EEF in horses.

Crassicauda boopis in a fin whale (Balaenoptera physalus) ship-struck in the eastern North Atlantic Ocean

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On 9 November 2015, a juvenile male fin whale of 11.60m length was observed on the bulb of a merchant vessel in the Channel Terneuzen – Ghent (The Netherlands - Belgium). A severe parasitosis was present in the right heart ventricle and caudal caval vein. Parasites were identified as *Crassicauda boopis* based on macroscopic and microscopic observations. The sequence of the 18S rRNA gene obtained from the parasite samples was 100% similar to the sequence of the 18S rRNA gene from *C. magna* available on GenBank. While adults of *C. boopis* and *C. magna* are morphologically distinct and found at different locations in the body, the molecular analysis of the 18S rRNA gene seems insufficient for reliable species identification. Although numerous *C. boopis* were found, the cause of death was identified as due to the collision with the ship, as suggested by the presence of a large hematoma, and the absence of evidence of renal failure. The young age of this whale and the absence of severe chronic reaction may suggest that the infestation was not yet at an advanced chronic stage.

Short communications

Comparison between swabbing devices in order to analyse the microbial flora found on surfaces by classical microbiology and 16S rDNA amplicon sequencing

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The work in community kitchens involves several steps bearing the risk of transmitting pathogenic microorganisms from static or dynamic surfaces to the food. This study aimed at comparing the recovery efficiency of different sampling devices in terms of culture methods and culture-independent 16S rDNA amplicon sequencing for characterising the microbial flora. In order to mimic surfaces found in community kitchens, sterile stainless steel and polypropylene surfaces were spiked with a two-fold raw chicken meat dilution and several concentrations of *Escherichia coli*, *Salmonella Enteritidis* and *Bacillus cereus,* respectively. The ideal swab for kitchen analyses should recover the highest number of viable bacteria with the highest population diversity. Classical culture method showed that recovery was possible with all swabs, but that Sponge-Sticks had the highest recovery rates. The 16S rDNA amplicon sequencing revealed a high relative abundance of *Bacillus* genus in cotton pad, gauze pad and Sponge-Stick samples and low proportions of *Salmonella* genus in all samples. Analysis of *Escherichia* genus results was problematic for molecular analyses as it appeared as a source of DNA contamination of the reagents used during library creation. Furthermore, it was possible to decipher bias populations in the sponge samples (from neutralising buffer) and in controls (due to low amounts of DNA), allowing the exclusion of those in the following analyses, if needed. In the end, these results attest the similarity of population diversity in cotton pad, gauze pad and Sponge-Stick samples, leaving the final choice to the operator.

Staphylococcal chromosome cassette *mec* typing of methicillin resistant staphylococci from different samples collected in European, African and North-American countries

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Antimicrobial treatment against staphylococci has led to the selection of Methicillin Resistant Staphylococci (MRS) which represent a potential hazard in public health via the inter-*Staphylococcus* transferability of the mobile "Staphylococcal Chromosome Cassette" (SCC) carrying the *mec* genes encoding the resistance. The aim of this study is the identification of the MRS and the typing of their SCC*mec* in a collection of isolates coming from European, African and North-American countries. A total of 1168 staphylococci were isolated between 2005 and 2014 from different samples. After growing on "Chrom MRSA ID®" agar and testing to hybridization *mecA* and *mecC* genes, positive isolates have been submitted to PCR targeting the same two genes and the SCC*mec.* For the first two tests, a total of 128 isolates were positive, including 38 *S. aureus* for the both tests, 29 *S. aureus* for hybridization *mecA* only and 33 *S. aureus* growing on "Chrom MRSA ID®" agar only. One non-*aureus* was positive for the both tests, 16 non-*aureus* were hybridization *mecA*+ only and 11 non *aureus* were MRSA ID+ only. Moreover, 84 isolates were PCR *mecA*+ including 67 *S. aureus* and 17 non *aureus*. No isolate was tested positive for *mecC* gene. The *SCCmec* typing have shown a total of 57 positives isolates subdivided into 8, 3, 32, 11 and 3 isolates belonging respectively to the types II, III, IV, V and VII. The others 27 PCR *mecA* positive isolates were "not-typeable". The 57 isolates from Belgium, Japan and Niger can be considered as MRS(A) mediated by the gene *mecA* carried by SCC type II, III, IV, V and VII. The 27 "not-typeable" isolates will be submitted to the sequencing for more precision on their SCC.

Phenotypic, genetic and biochemical identification of mammary pathogens in milk samples from cows with mastitis in Wallonia (Belgium)

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Mastitis can be divided into contagious and environmental according to the bacteria responsible for the pathology. For the diagnostic of mastitis, many routine laboratories still use classical bacteriology with biochemical identification of mammary pathogens (API® sugar test or MALDI-TOF®) while others resort to PCR assays, like PathoProof® that can be applied on milk samples.

The aim of this study is to compare classical bacteriology (API® sugar test and MALDI-TOF®) and the PathoProof® Complete-16 kit PCR assay, for the identification of mastitis-associated bacterial species. Between January and March 2014, 182 milk samples were collected from 11 farms in Wallonia and cultured on Columbia blood, Chapman, Edwards and Mac Conkey agar. After 24h of incubation at 37 °C, colonies were pre-identified and submitted to identification by API® sugar test and MALDI-TOF®. A commercial real-time PCR assay (PathoProof®) was also used to identify and semi-quantify the DNA of mammo-pathogenic bacteria directly in the milk. Out of 233 isolates grew on Columbia blood agar, 130 also grew on Chapman's, 68 on Edward's, and 17 on Mac Conkey's agar. The comparison between Pathoproof® and Api test show that 143 samples were tested positive at both assays with a total or partial bacterial identification matching observed in 55 samples and concerning 7 bacterial agents. Now, 125 isolates that grew on chapman agar have been identified by the API test and MALDI-TOF, with 88 matching for the both tests and concerning 12 bacterial species. The rest of the isolates are being tested currently by Maldi-tof in order to compare the results with those of the Pathoproof® and the Api® sugar test.

Ear canal microbiota - a comparison between the healthy and atopic dog without any sign of active otitis

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Background:The canine skin microbiota has been recently explored using new microbiological techniques, showing a difference of bacterial population composition between healthy and atopic dogs (non-affected skin). Little is reported concerning the ear canal microbiota.

Hypothesis/Objectives: The study evaluates the ear microbiota in healthy dogs and atopic dogs without any sign of active otitis.

Animals:Nine healthy dogs, owned by veterinary students and eleven atopic dogs from our referral practice without active otitis are prospectively enrolled.

Methods:Ear swabs from the two groups are used for global bacterial DNA extraction. V1-V3 hypervariable 16S rDNA amplicon libraries are prepared for each sample and sequenced with MiSeq Illumina sequence(V3 kit).Taxonomical assignation and clustering are performed with Mothur and SILVA database.

Results:An auricular dysbiosis is observed in the atopic dogs, with increased abundance of *Staphylococcus spp.*(p=0.02) and a decreased percentage of *Escherichia spp*. (p = 0.04). The bacterial diversity and richness, expressing the number of different species identified, are not statistically different in the two groups. On the contrary, the evenness using the Simpson Evenness Index is significantly higher in healthy dogs(p=0.04), showing a more evenly distributed total of bacteria in the number of genera identified.Conclusions and clinical importance:The bacterial genera present in the two groups are statistically distinct. In the atopic group, *Staphylococcus spp.* is over represented. The observed results in the ear canal are similar to those reported on the skin.

Short communications

CT-Assisted retrograde urethrography in male dogs

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Urethral lesions can be difficult to localise due to surrounding structures superimposition with conventional retrograde urethrography technique. We aimed to describe a CT-assisted retrograde urethrography technique in male dogs. CT were performed monthly in 6 intact male Beagle dogs, from 3 to 12 months of age and at 15, 18, 21 and 24 months of age. Each dog was anaesthetised and positioned in left lateral recumbency. An 8-Fr Foley catheter previously filled with sterile 0.9% saline solution was introduced into the urethra and stabilised by inflating the balloon just caudally to the external urinary meatus with 1.5 mL of air. It was connected to a power injector with a coil tube and filled with iodinated contrast medium (approximately 17.5 mg of I/ml). The power injector was set at a flow rate of 0.3 mL/sec and a pressure of 50 psi. The injected volume was the smallest volume between 5 mL/kg and the bladder threshold volume (determined by previous urodynamic tests). Acquisitions were performed during injection once the volume had reached 50% and 90% of the injected volume. Injected volume was 5 mL/kg in adult dogs. Urinalysis was systematically performed and no dog was diagnosed with cystitis nor haematuria. No dog had signs of incontinence/dysuria at the end of the protocol and blood tests were normal. The urethra was easily visualised on its entire length on each acquisition. Total time of the test was maximum 15 minutes. This standardised technique displays a stable urethrogram by a gentle and safe expansion of the urethral lumen in a controlled way regarding bladder filling. This could be clinically relevant for neoplastic/traumatic/congenital lesions diagnosis.

Deletion of Macrophage-specific IL-4 Receptor in Schistosomiasis does not preclude Ly6C^{high} Monocytes as the Main Source of Liver Macrophages but impairs Alternative Activation and Control of Inflammation

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During *Schistosoma mansoni* infection in mice, IL-4 receptor-dependent alternatively activated macrophages (aaMφ) response mediate host protection. Here, we used *LysM^{cre}Il4ra−/^{lox}* mice to determine whether the absence of Mφ-specific IL-4 receptor signalling modifies the dynamics of Mφ responses in liver granulomas after *S. mansoni* infection. Liver inflammatory response was investigated after low-dose *S. mansoni* infection. We observed significantly increased total leukocyte numbers in the liver by week 8 in *LysM^{cre}Il4ra−/^{lox}* mice while mice survived the infection similarly to littermate controls. Over the course of infection, we observed that CD11b^{low}MerTK^{+}CD64^{+} resident Küpffer cells (KCs) were severely reducing in term of numbers independently of IL-4Rα signalling. While KCs lowered, Ly6C^{hi} monocytes were recruited in the liver from 6 weeks pi, proliferate strongly at week 8 and acquire CD64 expression. The number of Ly6C^{hi} recruited monocytes were significantly higher in *LysM^{cre}Il4ra−/^{lox}* mice than littermate controls. From 8 weeks pi, CD11b^{low} KCs seemed to be replaced by CD11b^{high}MerTK^{+}CD64^{+}F4/80^{+} Mφ, probably derived from recruited Ly6C^{hi} monocytes. At week 8 pi, expression of Ym1 and Relm-alpha was significantly reduced in hepatic CD11b^{high} Mφ of *LysM^{cre}Il4ra−/^{lox}* mice compared to littermate controls. These results suggest that recruited monocytes differentiate into Mφ at the cost of resident KCs independently of IL-4Rα and validate the use of *LysM^{cre}Il4ra−/^{lox}* mice to study IL-4Rα-dependent activation of monocyte-derived granuloma Mφ during schistosomiasis.

Congenital Articular Rigidity outbreak due to ruminal dysfunction in a Belgian blue cattle herd

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Congenital articular rigidity (CAR) is due to a limitation of the movement of the joint before birth. This affects the posture and flexion of the limbs in the newborn calves. Different etiologies are described but none of these forms would correspond to the entity encountered in Belgian blue cattle breed (BBCB). Se and I deficiencies are implying in calves vitality affecting *in utero* mobility. Nowadays,CAR seems to be principally due principally to fetomaternal disproportion and trace elements deficiencies. We herein report the outbreak of CAR due to ruminal dysfunction in a BBCB herd. Data analysis, recorded between Aug. and Dec. 2016, revealed that 17 out of 120 born calves suffered from CAR, two from generalized arthrogryposis, one from cardiomyopathy and about 30% from weak calf syndrome. Based on farm inspection and laboratory investigations, we highlighted a trace elements deficiency despite the sufficient complementation. A ruminal dysfunction and impaction could be due to the very poor quality of the forages, the distribution of the diet promoting a sorting behavior and the insufficient water access. The ruminal impaction could explain the lack of abdominal space in the late pregnancy and increase the *in utero* calf hypomobility. Moreover, the ruminal dysfunction decreases macro and micronutrients absorption despite the sufficient intake and could explain the protein-energy malnutrition identified by the increase of the NEFA and the decrease of urea and blood glucose in late pregnancy. Moreover, the poor quality of the maize silage with a lot of indigestible fibers and by-starch encourage intestinal glucose absorption and then fattening of the cows. This case report enhance the importance of the monitoring of ingested and metabolized food in beef herd health status to compare it with calculated ration. Ruminal impaction associated with fattening decreases significantly the space for the calf leading to CAR. Ruminal dysfunction could however make micronutrients insufficient.

Posters

Veterinary public health

Posters

1. Atypical polypoid leiomyosarcoma in an Ouessant ewe

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Smooth muscle tumors of the myometrium are well known tumors frequently described in ruminants. Leiomyosarcomas are less common than their benign counterparts but are not unusual in cows and especially in goat with a higher frequency of the tumor in older Saanen suggesting a inherited component. We herein present an atypical uterine polypoid leiomyosarcoma in an Ouessant ewe found at the end of a clinical investigation for chronic vaginal bleeding. The diagnosis was performed by histopathology and immunohistochemistry on samples of the tumor taken after ovariohysterectomy by midline celiotomy under volatile general anaesthesia. The macroscopic aspect was unusual with large exophytic masses with a deep black surface filling the uterine lumen. Interestingly, a similar description of such an atypical leiomyosarcoma has been made in a single Suffolk ewe with chronic vaginal bleeding (Vemireddi et al., 2007). These descriptions of atypical uterine leiomyosarcomas suggest that this entity might be more frequent in sheep. This tumor should be included in the differential diagnosis of a chronic vaginal bleeding and discharge in sheep.

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2. Assessment of Trace elements contamination in grilled and smoked chicken processed and consumed in Burkina Faso

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Heavy metals are toxic compounds produced naturally or from human activities. Environment pollution is the main pathway contamination of food by heavy metals, including meat. Among heavy metals, lead, cadmium, arsenic mercury are the most toxic and have been discussed by several studies. Among heavy metals, Cd and As are classified as carcinogenic for humans (Group 1) and Pb is classified as possible carcinogenic for humans by the International Agency for Research on Cancer. In 2010, the European Food Safety Authority reported human exposure to Pb is through diet and result in his bioaccumulation which could involve cardiovascular, renal, endocrine, gastrointestinal, immune and reproductive system (EFSA, 2010). This study aims to assess heavy metals contamination in smoked and grilled chicken consumed in Burkina Faso. Ten trace elements (lead, cobalt, chromium, cooper , cadmium, silver , nickel, iron, zinc and manganese) were assessed in 103 grilled and smoked chicken samples with flame atomic absorption spectrometry method using wet digestion with a mixture of nitric acid and chlorhydric acid.

The study revealed contamination of all samples by heavy metals. 88% of the samples showed level of Pb ranging between 0.03 and 0.77 mg/kg. Cd is found in 91% of samples with level ranging between 0.03 and 24.06 mg/kg. The highest level of Pb and Cd exceed 0.1 mg/kg and 0.05 mg/kg, the maximum limits set by regulation ((EC) No. 1881/ 2006)) respectively for Pb and Cd.

3. Metabolites from media supplemented with bovine milk oligosaccharides and fermented by Bifidobacterium mongoliense modulate virulence of pathogens

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Whey, a by-product of dairy-industry, contents complex oligosaccharides (BMO) similar to human milk oligosaccharides and are mainly represented in colostrum by 3'-sialyllactose (3'SL). Cell free spent media (CFSM) obtained from culture of *Bifidobacterium crudilactis*, a bifidobacteria of bovine origin, and 3'SL modulated virulence genes expression of intestinal pathogens. This study focused on the capacity of *B. mongoliense*, another bifidobacteria of bovine origin, to metabolise BMO, especially 3'SL, and to exert an antivirulent effect through cell-free spent media (CFSM). *B. mongoliense* FR/49/F/2 isolated from cow raw milk cheese, was grown on media supplemented with glucose (MG), whey rich in BMO (MWh) or 3'SL (M3'SL), as sole source of carbon. A media without carbohydrate (MwG) was used as a control. Next, the filtrated supernatants were tested against virulence gene expression of *Escherichia coli* 0157:H7 ATCC 43890 (*ler, fliC, luxS, stx1* and *qseA* genes) and *Salmonella Typhimurium* ATCC 14028 (*hil, ssrB2* and *sopD* genes) using RT-qPCR. *B. mongoliense* was able to grow in presence of whey (8.3 ± 1.0 log cfu/ml after 48 hours of growth at 37 °C) and 3'SL (6.7 ± 0.3 log cfu/ml after 48 hours of growth at 37 °C). Regarding *E. coli* O157:H7, CFSM obtained from MWh caused a down-regulation of all the tested genes: l*er, fliC, luxS, stx1* and *qseA* (-2.2, -5.3, -2.4, -2.5 and -4.8, respectively). Regarding *S. Typhimurium,* CFSM obtained from M3'SL down-regulated *hil* and *sopD* genes (-2.2 and -4.2, respectively). In conclusion, BMO or 3'SL combined with *B. mongoliense* could be an interesting synbiotic to maintain or restore the intestinal health of young children.

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4. Impact of 3'-sialyllactose and Bifidobacterium crudilactis on infant microbiota and Escherichia coli O157:H7 virulence modulation, using the SHIME® model

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In a previous work, cell free spent media (CFSM) obtained from culture of *Bifidobacterium crudilactis*, a bifidobacteria from bovine origin, and a major bovine milk oligosaccharide (BMO), the 3'-sialyllactose (3'SL), modulated virulence genes expression of *Escherichia coli* O157:H7. The aim of this study was to evaluate this effect directly on microbiota. The gastrointestinal model SHIME® was inoculated with feces from a young child and four treatments were successively administrated: 3'SL, *B. crudilactis*, 3'SL and *B. crudilactis* simultaneously and CFSM from 3'SL and *B. crudilactis* culture. Collected samples have been analysed for SCFA concentrations using HPLC, and microbiota composition using pyrosequencing. In addition, impact of SHIME® samples have been assessed on *E. coli* O157:H7 virulence genes expression. The results showed that SCFA levels were stable during the experiments with mainly production of acetate, propionate and butyrate. Metagenetic analysis showed a microbial diversity in transverse (TC) and descending colon (DC) close to feces, dominated by *Bacteroides, Prevotella* and *Fusobacterium*, while the ascending colon (AC) showed a microbial diversity dominated by *Veillonella*. Probiotic treatment with *B. crudilactis* seemed to increase proportions of bacteria beneficial to host health (*Prevotella, Lactobacillus, Lachnospiraceae, Prevotella, Bacteroides, Akkermansia*). Also, SHIME fractions tended to down-regulate virulence genes of *E. coli* O157:H7 (*ler, fliC, luxS, stx1* and *qseA*). Interesting effects have therefore been highlighted after this first run. However, those trends have to be validated with the further replicates on the SHIME® system.

5. A NMR-based metabolomics study of minced pork meat inoculated with *Brochothrix thermosphacta, Leuconostoc gelidum* and *Pseudomonas fragi*

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In order to control food waste, studies have highlighted the importance of monitoring the microbial diversity of food. The aim of the current study was to assess meat spoilage through the evolution of bacterial counts and changes in the metabolic profile of minced pork meat using Proton Nuclear Magnetic Resonance (1H-NMR). Microbiological assessment and metabolomics analysis were carried out in minced pork meat samples stored under food wrap (FW) and under modified atmosphere packaging (MAP, 70% O2 – 30% CO2) at 4, 8 and 12°C. All samples were irradiated and then inoculated separately with three dominant bacterial strains isolated from previous aging tests: *Brochothrix thermosphacta, Leuconostoc gelidum* and *Pseudomonas fragi*. For all conditions, non-inoculated samples are also analysed. The metabolomics signature of samples obtains by Orthogonal Partial Least Square Discriminant Analysis (OPLS-DA) revealed a clear discrimination between: (1) the packaging conditions, (2) the temperature storage, (3) the inoculated and non-inoculated samples, and (4) the type of strain inoculated. The results evidenced a noticeable increase in acetate and glycerol for *B. thermosphacta*; betaine and lactate for *L. gelidum*; threonine and glycine for *P. fragi*. Exploration of the correlation of these metabolites with microbial counts (Spoilage Value at 7.0 log CFU.g-1) suggested their use as possible spoilage indicators in future analysis. These results support the use of NMR-based metabolomics as a valuable tool to provide information on pork meat spoilage and to follow intrinsically the evolution of the metabolomics pattern linked to a specific strain in a complex bacterial ecosystem.

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6. Clinical and haematological alterations in foot and mouth disease virus naturally-infected domestic water buffaloes in Vietnam

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Foot and mouth disease (FMD) has been one of the most important epizootic disease in several cloven-hoofed animals including buffaloes, causing severe economic losses in Vietnam. This study aimed at observing clinical indicators, physiological parameters, hematological and blood chemistry values of 10 buffaloes naturally infected with FMD virus (FMDV). The study was undertaken from January 2015 to February 2016 in Hanoi, Vietnam. Results show that buffaloes infected with FMDV displayed significantly higher fever, increased respiratory rate and increased heart frequency compared to healthy animals. Moreover, FMDV-infected animals always showed high fever and vesicular stomatitis, often showed edging nail congestive inflammation and, sometimes only, blisters on the nipple. Hematological indicators showed that FMDV-infected buffaloes were dehydrated (increased total protein). Besides, infected animals also underwent anemia as mean corpuscular hemoglobin and mean corpuscular hemoglobin concentrations were plummeted. Total white blood cell count plummeted too, especially the number and proportion of neutrophils. Meanwhile the number and proportion of lymphocytes (lymphocytes) sharply increased compared to healthy animals. Infected buffaloes also displayed signs of liver, heart and kidney damages as judged by concentrations of liver enzymes and creatinine increased quite clearly.

7. A case of splenic myelolipoma in a dog

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An older neutered female German Shepherd dog was referred to our Department for necropsic evaluation after euthanasia for chronic renal insufficiency. Apart from chronic renal lesions and gastrointestinal ulcerations, the macroscopic examination showed multiple off-white multifocal splenic nodules. Histologic examination revealed multiple, well-demarcated, unencapsulated masses of hematopoietic elements admixed with mature adipose tissue, consistent with a diagnosis of myelolipoma. This benign tumor is rarely reported in dogs in the literature and is more often observed in the form of a single encapsulated nodule. The other possible sites where these tumors can be seen are adrenal glands and organs retaining hematopoietic activity. Although several hypotheses have been proposed, the exact origin of myelolipoma still remain unknown.

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8. A Gammaherpesvirus Infection Protects from Pneumovirus-Induced Immunopathologies

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

9. Epidemiological study on the impact of in hive chemical contaminations on honey bee mortality

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Europe and particularly Belgium are strongly impacted by honey bee mortality. Colony success depends partly on the management of the ectoparasitic bee mite, *Varroa destructor,* which historically has been achieved using acaricides that have an impact on honey bee health. Despite honey bees being managed as a domestic pollinator, the impacts of the use of veterinarian pesticides has often been overlooked as a possible factor influencing bee mortality. In an attempt to better understand the in hive pesticide burden, a pesticide screening was realised in foundation wax and a hazard quotient was calculated. The pesticide burden in wax was correlated to bee mortality using a logistic regression model. A face-to-face questionnaire was designed and implemented in a representative randomized (n=200), stratified sample of beekeepers in Belgium. The variables obtained where integrated in a logistic regression model (univariate and multivariate) and correlated to the declared mortality rates in order to identify risk and protective factors. A Classification and Regression Tree Analysis was used to confirm the first analysis. The contamination level found in foundation wax raise our concern. Nevertheless, no correlation was found between pesticide burden in wax and bee mortality. The benefit of pesticides in controlling *Varroa* infestations should be considered as regard to their toxic effects on bees. Proper diagnosis of *Varroa* infestation rates should be generalised before using acaricides with parsimony. More efforts are needed in research to characterise the total pesticide burden, which bees are confronted to in wax, beebread, pollen, nectar, water, honey and propolis.

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10. Epidemiological study on the impact of beekeeping management practices on honey bee mortality in Belgium

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Europe and particularly Belgium are strongly impacted by honey bee mortality. Colony success depends strongly on beekeeping management practices. Despite honey bees being managed as a domestic pollinator, the impacts of beekeeping management practices has often been overlooked. In an attempt to understand the impact of bee management practices on bee mortality in Belgium, we correlated the variables obtained from a face-to-face questionnaire, to winter mortality rates. A face-to-face questionnaire was designed and implemented in a representative randomized (n=200), stratified sample of beekeepers in Belgium. The variables obtained where integrated in a logistic regression model (univariate and multivariate) and correlated to the declared mortality rates in order to identify risk and protective factors. A Classification and Regression Tree Analysis confirmed the regression analysis results. We present the first evidence of a relationship between beekeeping management practices and bee mortality. The results show that the main factors protecting honey bee colonies are the resilience of the beekeepers, the hive type, the equipment hygiene, wintering in proper conditions that includes the use of a partition, the colony strength, winter monitoring and last but not least, an appropriate integrated pest management. Proper diagnosis of *Varroa* infestation should be generalised before treatments. More efforts are needed in beekeeper training to promote good beekeeping practices and achieve early identification of clinical signs of disease.

11. Genomic and biologic comparison of Cyprinid herpesvirus 3 strains

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Since its emergence in the late 1990s, CyHV-3 is causing negative ecological impacts and severe economic losses to the common and koi carp industries worldwide. To date, the genome of only 4 strains of CyHV-3 are available in the Genbank. No comparison of the biological properties of these 4 strains such as viral replication *in vitro* or virulence *in vivo* have been reported. Here, we sequenced 7 new strains of CyHV-3 representative of the genetic diversity reported in the viral species. Moreover, we compared their ability to growth in cell culture (growth assays and plaque size assays) and their virulence in vivo. The data were used to revisit the phylogenetic relationship existing between CyHV-3 strains and to establish correlation existing between genetic and biological traits.

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12. Characterization of a murine model of replicative adenovirus based oral vaccination

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Oral vaccination offers many immunological and practical advantages. Nevertheless, oral immunization may be hampered by oral tolerance mechanisms. A solution to this problem lies in the use of viral vectors. Since the 1970s, alive, orally administered adenovirus serotype 4 and 7 vaccines are effectively used to protect U.S. military personnel from severe respiratory diseases caused by these viruses. Replication-competent adenoviruses appear therefore as promising vectors for the development of oral vaccines. Until now, as human adenoviruses replicate efficiently only in a highly restricted host range, researches on this topic have suffered from the lack of reliable animal models.

In this study, we used mouse adenovirus type 1 (MAV-1) to develop a small animal model for oral replicative adenovirus vaccines. We firstly characterized adenovirus oral infection in mice. No clinical signs were observed following MAV-1 oral administration. Nevertheless, viral DNA was detected by qPCR in various organs, showing that the virus efficiently infects by this route. Furthermore, this infection generated a specific and neutralizing humoral response. We then evaluated the protection induced by MAV-1 oral infection against a respiratory homologous challenge. Our observations showed that oral immunization prevents the weight loss due to an intranasal infection. Moreover, histological and qPCR analysis showed a protection against lung inflammation and viral replication. Altogether, these results show that MAV-1 offers a reliable model for oral vaccination based on replicative adenoviruses. This model provides a valuable tool to assess the potential of adenoviruses as oral vaccine platforms.

13. Prevalence of genes encoding classical and extended spectrum β lactamases (BLA, AmpC and ESBL) and carbapenemases (CPE) amongst pathogenic and non-pathogenic *Escherichia coli* from calves in Wallonia

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β lactamase (BLA) enzymes can be summarily classified into four main groups of inactivation spectrums: classical BLA (BLA_C) inactivating original narrow and large spectrum β lactams, extended cephalosporinases (BLA_{AmpC}) including different cephalosporins, cephamycins and β lactamase inhibitors, extended spectrum BLA (BLA_{ESBL}) including 3rd/4th generation cephalosporins but not βlactamase inhibitors, and carbapenemases (BLA_{CPE}) also including carbapenems. Further classification in families and variants is based on the molecular structure, the precise inactivation spectrum and the encoding gene sequence. The BLA_{ESBL} and BLA_{CPE} enzymes represent the highest public health hazard since 3rd/4th generation cephalosporins and carbapenems are amongst the critical antibiotics in human hospital medicine. The recent decrease of resistance to β lactams in animal bacteria is therefore a positive observation, but the actual evolution, decline or emergence, of the different families of *bla* genes (bla_C, bla_{AmpC}, bla_{ESBL} and bla_{CPE}) is unknown. This research project will identify, based on their β lactam resistance profiles by the disk diffusion assay, the different *bla* gene families present in bovine pathogenic *Escherichia coli* isolated over two calving seasons from cases of enteritis, septicaemia and mastitis, using micro-arrays and PCR. In parallel, the bla_{AmpC} and bla_{ESBL} genes present in non-pathogenic *E. coli* isolated from cases of enteritis will be identified after growth on selective agar plates. All pathogenic and non-pathogenic *E. coli* harbouring similar *bla* genes will be compared for their clonal relationship by virulotyping, phylogenetic grouping, MLST, PFGE and/or WGS.

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14. Preliminary assessment of the risk linked to polycyclic aromatic hydrocarbons ingestion through smoked and dried smoked fish consumption in Benin

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Polycyclic Aromatic Hydrocarbons (PAHs) are chemical contaminants produced by incomplete combustion of organic matter during thermal treatment of food including smoking of fish. Among PAHs, Benzo(a)pyrene (BaP), carcinogenic to humans (Group 1, IARC) received a particular attention due to its toxic effects including carcinogenic tumours formation (EFSA,2008). In Benin, smoking of fish is performed using traditional process, which leads to PAHs production and fish contamination, resulting in potential health problems for operators as well as consumers. This study aims to assess consumers risk due to PAHs intake through smoked fish (SF) and dried smoked fish (DSF) consumption. Fifteen PAHs were analyzed in nineteen samples of SF and DSF, using HPLC coupled with Fluorescence detector. A deterministic approach was used to calculate the margin of exposure (MOE) (which is the ratio between the benchmark dose lower confidence limit and the estimated daily intake) based on two scenarios. Scenario n°1 was using the average PAHs levels found in SF and DSF, while scenario n°2 was using only maximum PAHs levels. In each scenario, MOEs (BaP and sum of 4 PAHs (BaP, benz(a)anthracene, benzo(b)fluoranthene and chrysene)) were found to be below 10 000, which means a concern for consumer's health. The lowest MOE-BaP and MOE-4PAHs recorded were 35 and 25, respectively, showing a very high risk, as the MOE is to be above 10 000 to consider that there is no risk, in case of carcinogenic compounds.

15. Contamination by polycyclic aromatic hydrocarbons of grilled pork processed with traditional grilling methods using *Acacia auriculiformis* wood

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Polycyclic aromatic hydrocarbons (PAH) are toxic compounds produced in pork during grilling processing. Among PAH, Benzo(a)pyrene (BaP) is recognized as carcinogenic for humans. BaP, chrysene, benzo[a]anthracene and benzo[b]fluoranthene are 4 PAHs relevant according to both their occurrence in food and their toxicity (EFSA, 2008). In this study, we investigated PAH content in grilled pork from two traditional grilling methods (direct and indirect) previously identified using *Acacia auriculiformis* wood as fuel. Fifteen PAHs, selected as priority PAHs in food by European Union in 2002, were analysed on freeze-dried samples through extraction with accelerated solvent extraction, purification on solid phase extraction columns cartridges and injection on a High Performance Liquid Chromatography coupled with Fluorescence detector. Analysis of variance of Kruskal-Wallis using Statistica 7.1 was used to compare PAH concentration and significance difference was accepted at $p < 0.05$. The results revealed a contamination of raw and grilled pork with BaP and the sum of PAH4 which exceed maximum limits set by Commission Regulation ((EC) No. 1881/ 2006). BaP and sum of PAH4 recorded in grilled pork ranged respectively between 2.4 and 53.6 µg/Kg for BaP and 53.8 and 300.6 µg/Kg for sum of PAH4. No significant difference ($P>0.05$) was observed in BaP and sum of PAH4 in direct and indirect grilled pork samples showing that the grill used for each method lead to same PAH amount. This study shows evidence of public health issue and necessity of improvement of the grilling process.

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16. Mass stranding of 10 long-finned pilot whales (*Globicephala melas*) on the beach of Calais (France)

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A mass stranding of 10 long-finned pilot whales occurred on November 2nd 2015 on the beach of Calais (France). They were found ashore alive at low tide but 7 of them died (3 adult females and 4 males) within few hours, while the 3 smallest individuals survived and could be released at sea 3 h after their discovery. Necropsies were started 24h after the stranding. All specimens were in good nutritional status with empty gastro-intestinal tract, except one where few otoliths and 1 cephalopod beak were found. Significant observations were subcutis congestion and hemorrhages, presence of parasite cysts (presumably *Monorigma sp*.) in the blubber and the peritoneum, lung congestion and edema with moderate hemorrhagic froth in airways. One female had skin ulcers infested by *Isocyamus delphinii* and disseminated chronic ulcers in the stomach. Although pilot whales are one of the most frequent species involved in mass stranding, this is the first reported case in northern France. The pilot whales occurring in all deep waters areas of the north-eastern Atlantic except the eastern English Channel and the southernmost part of the North Sea. Only 7 other single strandings of pilot whale have been recorded on this area since 1970. The topography of the northern France coastline is complex with shallow waters, numerous sandbanks and treacherous differences in tides. This area is totally unfamiliar for deep divers like pilot whales that have probably be grounded by the falling tide, trapped between sandbanks. Finally, this mass stranding should be considered with unusual strandings that occurred in the North Sea during the 2015-2016 winter such as sperm whales and narwhal.

17. Study of the interplay between a gammaherpesvirus infection and innate lymphoid cells in the context of type 2 immunity

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Environmental conditions are a key factor in shaping the host immune system. In particular, the early bacterial microbiome and the virome are determinant for immune development and influence health in child and adulthood. Persistent gammaherpesvirus infections profoundly imprint the immune system of their hosts. We recently observed that infection by Murid herpesvirus 4 (MuHV-4) inhibits the development of House Dust Mites (HDM)-induced allergic asthma in mice through the alveolar recruitment of regulatory monocytes. Group 2 innate lymphoid cells (ILCs) play a major role in the initiation, the maintenance and the memory of type 2 immune responses. Their activation can be triggered by allergens as well as viruses such as influenza, rhinovirus and respiratory syncytial virus. Their plasticity may exacerbate anti-viral immunity, which may have adverse consequences in respiratory diseases. Here, we investigated the different subset of ILCs in mock and MuHV-4 infected mice upon HDM treatment. Interestingly, we observed a reduction of ILC2s and an augmentation of ILC1s in the MuHV-4 infected groups, suggesting a possible plasticity between ILC2 and ILC1. Our results support the hypothesis that modification in numbers, proportions or functions of ILC2 following some virus infection could impede initiation, maintenance or recall of Th2 cells during asthma.

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18. Identification of plasmid-located *mcr*-like genes in collections of colistin-resistant bovine and porcine *Escherichia coli*

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In 2015, the *mcr-1* plasmid-located gene that confers resistance to polymyxins, including colistin, was identified in one porcine *Escherichia coli* in China (Liu YY et al., 2016). During the following months the *mcr-1* gene was identified by PCR in avian, bovine, human and porcine *E. coli* isolates in different countries. Moreover in 2016 an *mcr-2* gene was identified in another porcine *E. coli* in Belgium (Xavier BB et al., 2016). The purpose of this work was to identify mcr genes in a collection of 410 animal and human *E. coli* growing on agar plates containing 1 µg/ml of colistin by DNA colony hybridization, PCR and NGS. A total of 31 isolates tested positive by the colony hybridization assay: 8 bovine (9%) and 25 porcine (23%) *E. coli* isolated in 2009 and 2010. Of them 7 bovine and 7 porcine isolates tested positive with the *mcr-1* PCR whereas 16 porcine isolates tested positive with the *mcr-2* PCR. But one bovine isolate and two porcine isolates tested negative with both PCR. NGS of 18 *E. coli* confirmed the presence of a plasmid-located *mcr-1* gene in the 2 bovine and 7 porcine *mcr-1* PCR-positive *E. coli* and the presence of a plasmid-located *mcr-2* gene in the 7 porcine *mcr-2* PCR-positive *E. coli* that were sequenced. Conversely no *mcr*-like gene was identified in the bovine probe-positive PCR-negative isolate and the NGS results of the 2 porcine probe-positive PCR-negative isolates are soon expected. So far the results mean (i)that the *mcr-1* gene was already present in cattle and pig farms and the *mcr-2* in pig farms in Belgium years before their description; and (ii)that other *mcr*-like genes may exist.

19. Detection and Quantification of Biogenic amines in Cambodian Smoked Freshwater Fish

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Freshwater fish, highly perishable food, has been traditionally processed and smoked to give special smoke flavor and crispiness to fish flesh and also preserve it for longer shelf-life. The quality of fish can be downgraded due to improper storage conditions and manufacturing practices following the fishing and prior to smoking production process. Biogenic amines could be produced consequently in fish and fish products by bacteria through decarboxylation of free amino acids. These could affect the quality of finished smoke fish products. Particularly, histamine and tyramine have been considered as the most toxic among the biogenic amines causing allergic reactions including difficulty in breathing, itching, rash, vomiting, fever and hypertension to consumers. Therefore, a preliminary study was carried out to assess the quality of smoked freshwater fish in terms of contaminated biogenic amines in Phnom Penh local markets. Out of nine markets, Oreussey market was a main target for diversification of smoked fish as it possessed the highest number of stores (24) commercializing these products as retailers and wholesalers. Five types of smoked fish including Trey Andoeng (*Clarias spp.*), Trey Kaes (*Micronema spp.*), Trey Riel (*Cirrhinus spp.*), Trey Slek Reussey (*Paralaubuca typus*) and Trey Changvachnot (*Osteochilus spp.*) were commonly available in the market and collected for further investigation. The samples were subjected to analysis by Ultra High Performance Liquid Chromatography coupled to fluorescence detection. The results obtained were compared with the maximum acceptable concentrations allowed by the European legislation.

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20. Ticks and tick-borne diseases associated to cattle transhumance in West Africa

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Livestock production is one of the most important pillar of the West African countries economy. It contributes up to 44 % to agricultural gross domestic product. In Burkina Faso and Benin, it is practiced by more than 80 % of people and 95% of farmers, respectively. However, in these countries, the cattle production sector is facing important constraints such as the negative effects of ticks and harboured protozoan parasites, namely *Babesia spp*., *Theileria spp.* and *Ehrlichia ruminantium*. The purpose of this study is to assess the impact of the transhumance on the spread of ticks, especially *Rhipicephalus (Boophilus) microplus*, and associated diseases transmitted to cattle between Burkina Faso and Benin.

Therefore, in 2016-2017, ticks and blood were collected on 490 cattle in three border provinces of Burkina Faso which represent the transhumance departure zone to Benin. Additionally, 456 cattle were sampled in four border departments of Benin. Thus, in the study areas tick species and associated blood parasites presence were evaluated in the departure and arrival zone of transhumance. Furthermore, localities names, GPS coordinates and climate data of each sampling site have been registered during surveys.

Ticks were morphologically identified and subsequently submitted to DNA extraction for parasitic screening. Blood smears and DNA samples were also analyzed for haemoparasites research.

The results aim to contribute to cattle health improvement for an increment of the breeding production and productivity, and therefore will participate to the improvement of cattle-breeders live conditions in West Africa.

21. Development of a propidium-monoazide-based method to correlate positive RT-qPCR signals to infectivity of murine norovirus particles

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Human noroviruses, genus *Norovirus*, family *Caliciviridae*, are a major viral cause for gastroenteritis outbreaks. Molluscs, which filter contaminated water and accumulate noroviruses in their hepatopancreas, are a typical vector for human infection. The in vitro culture of human noroviruses is not viable for routine analysis. Thus, the murine norovirus is used as surrogate. RT-qPCR, the established molecular method for detection of human noroviruses in food, does not allow the distinction of infectious and non-infectious viruses. Our study addresses this issue by combining the use of an intercalating agent, propidium monoazide (PMA)-pretreatment and RT-qPCR on murine noroviruses. PMA has been shown to enter only viruses with compromised capsids, subsequently binding to nucleic acids, and inhibiting PCR amplification. Viruses with intact capsids nevertheless remain impermeable and detectable via RT-qPCR. A protocol for the inactivation of norovirus samples via different methods and the subsequent set-up of a PMA-qPCR has been achieved. Initial PMA-RT-qPCR results obtained for the murine norovirus model in a simple matrix (PBS), will be further adapted for detection in a complex matrix (mussel hepatopancreas), from which intact viruses must be extracted before analysis. The current ISO-norm for extraction incorporates a capsid-damaging treatment with proteinase K and impairs the recovery of infectious viruses. An optimisation of a newer approach implementing glycine-elution and PEG-concentration is currently ongoing for better infectious virus recovery. The final objective is to detect and quantify only infectious norovirus in naturally contaminated mussels by PMA-RT-qPCR.

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22. Biosecurity practices in cattle farming: level of implementation, constraints and weaknesses

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A recent shift from curative towards preventive medicine occurred leading to an increased importance of biosecurity to better prevent and control infectious diseases. Existing studies report a low implementation level of biosecurity measures (BSM) by cattle farmers. Barriers such as cost, usefulness, importance, workload and knowledge were investigated but the decision-making processes of the farmers is yet to be clarified. The objectives of this study were to investigate for the different BSM: their level of implementation, the correlation between its perceived importance and its effective implementation and the main reasons leading to its non-implementation. A stratified randomised survey was conducted in 100 farms in Belgium. A descriptive analysis of the data was performed. The differences between dairy and beef herds in terms of BSM implementation levels and the correlation between importance that farmers give to a BSM and its level of implementation were assessed in Stata14® by means of chi square tests and the Spearman's correlation test respectively. There is a generally low level of implementation, especially for BSM preventing indirect transmissions. Some measures show a significant difference of implementation level between beef and dairy herds and a positive correlation exists between the farmer's perception of a BSM importance and its effective implementation. Efficiency, feasibility and usefulness are the most frequent constraints mentioned. The level of influence of other factors in the decision making process should be further investigated and evidence based studies would be useful to convince the farmers of the BSM efficiency and relevance.

23. Rural veterinarian's perception and practices in terms of biosecurity across three European countries

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The implementation of biosecurity measures in the animal health context aims at limiting the risk of introduction and spread of diseases. Veterinarians play a major role as key informants for cattle holders, key players of disease control and eradication programs, as well as key risk factor for diseases dissemination. Many biosecurity studies highlighted professional visitors such as representing a high risk factor in terms of disease introduction in animal facilities but very few studies focused on the implementation level of biosecurity measures by veterinarians. An on-line survey was implemented in 3 EU countries (Belgium, France, Spain) in order to assess the biosecurity practices of rural veterinarians. A descriptive analysis of data and a scoring system were applied to assess the implementation level of measures. The influence of different factors on the implementation level of biosecurity measures was investigated through a negative binomial regression model. The study identified different strengths, weaknesses, possible constraints and solutions in terms of veterinary perspectives. Veterinarians are considered as key informants by the farmers and could play a more active role in terms of guidance and improvement of biosecurity at farm level. Based on the survey outcomes, 2 factors seem to influence significantly the implementation level of measures:the country of practice and the veterinarian's perception level of biosecurity. The biosecurity stages with the lowest application level,therefore representing the biggest threats, were bio-exclusion (preventing disease introduction) and bio-containment (controlling within herd transmission).

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24. Assessment of the polycyclic aromatic hydrocarbons contamination of *kilichi*, a dried grilled meat produced in Niger

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Polycyclic aromatic hydrocarbons (PAHs) are toxic compounds produced by incomplete combustion of organic matter during thermal meat processing including beef grilling. Among the 4 PAHs currently found in food (Benzo[a]Anthracene (BaA), Chrysene (CHR), Benzo[b]fluoranthene (BbF) and Benzo[a] pyrene (BaP)), BaP is the most toxic due to its carcinogenic effect in human (EFSA, 2008). Thirty samples of *kilichi* (24 beef *kilichi* and 6 camel *kilichi*) were collected in five areas of Niger according to the coating process identified from previous study (16 samples were coated *kilichi* and 14 were uncoated *kilichi*). Fifteen priority PAHs of EU were assessed using High Performance Liquid Chromatograph coupled to fluorescence detector (HPLC-FLD). The average level for the sum of PAHs was 31 µg/kg. Benzo[a]anthracene, Benzo[c]fluorene and chrysene were particularly found in high concentration in the both types of *kilichi.* However, their concentration is higher in the coated *kilichi* (1.5 to 2 times) than in uncoated *kilichi*. The results also showed that 43 % of *kilichi* samples were non-compliant to the European permitted level of BaP (2 µg/Kg) while for PAH4, 53 % of samples were above 12 µg/kg, which is the maximum limit set by European Commission Regulation (EC No. 1881/ 2006).The highest levels of BaP and PAH4 recorded in the coated *kilichi* were respectively 7.1 and 60.7 µg/kg, showing possible health concern for the consumer. Further studies of improvement of the grilling process are needed to reduce PAH formation.

25. Implementation of the *Galleria mellonella* larvae model to evaluate phage therapy against *Klebsiella pneumoniae*

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Klebsiella pneumoniae (*K. pneumoniae*) is an encapsulated Gram negative bacillus belonging to the family *Enterobacteriaceae. K. pneumoniae* strains display a large number of capsular serotypes. It is an opportunistic pathogen causing severe therapeutic challenges due to the continuous emergence of multi-drug resistant strains. The aim of this work is to develop an infection model of *Galleria mellonella* larvae with *K. pneumoniae* strains of interest to evaluate phage therapy strategies.

Three selected bacteriophages have been isolated from wastewater plants of Paris against two *K. pneumoniae* strains of epidemiological interest, two against the ST258 (phages K?-ULIP47 and K?-UL-IP54) and one against ST23 (phage K1-ULIP33). The host range of these 3 bacteriophages shows a high specificity for the *K. capsular* type. In a 1st *in vivo* experiment, 110 larvae have been used to assess the optimal inoculation dose of *K. pneumoniae* to use for phage therapy experiments. A dose of 104 CFU/10µl results in a 70-90% killing of larvae in 4 days. In the 2nd and the 3rd experiments, 480 larvae were inoculated to assess both prophylactic and curative treatment of these bacteriophages. More than 80% of survival is observed in the larvae treated with the bacteriophages at a multiplicity of infection of 10 compared to the non-treated *K. pneumoniae* infected control in which over 90% of larvae died. The group of larvae inoculated with bacteriophages only showed comparable survival rate as the PBS control group. Both prophylactic and curative groups showed similar survival rates.These results show that *G. mellonella* could be used as a preliminary model to test phage therapy against *Klebsiella* infection.

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26. Identification of O80:H2 enteropathogenic and Shigatoxigenic *Escherichia coli* (EPEC and STEC) from intestinal contents of healthy cattle and diarrhoeic calves in Belgium

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Human Attaching-Effacing (AE) Shiga toxin (Stx)-producing *Escherichia coli* (AE-STEC) belong to dozens of somatic serogroups whose O26, O103, O111, O121, O145, O157, and O165 ones ("the gang of seven") are the most frequent and pathogenic. Some of them can also cause diarrhoea in young calves. Other AE-STEC serogroups can emerge in humans like the O80:H2 serotype recently in France. Enteropathogenic *E. coli* (EPEC) share the production of the AE lesion with AE-STEC and are associated with diarrhoea in humans and different animal species, including young calves too. The purpose of this study was to assess the prevalence of O80:H2 among bovine (AE-)STEC and EPEC by: (i) identifying the O80:H2 serotype with PCR targeting the genes coding for the O80 somatic (wzy_{O80}) and H2 flagellar ($fliC_{H2}$) antigens; and (ii) comparing the bovine positive isolates with human O80:H2 AE-STEC with PCR targeting the *eae*, *stx1* and *stx2* gene subtypes. Two collections were screened: (i) 153 AE-STEC, 104 EPEC and 10 STEC isolated from diarrhoeic calves at ARSIA from November 2008 to June 2015 and (ii) 118 AE-STEC, 435 EPEC and 191 STEC isolated from healthy cattle at two slaughterhouses in 2014. The serogroup O80 was detected in 42 calf EPEC, and not among calf (AE-)STEC, neither among (AE-)STEC or EPEC isolates from healthy cattle. These 42 calf EPEC harboured the $fliC_{H2}$ and the *eae*ξ genes, like the human O80:H2 AE-STEC. Further studies must be designed to: (i) identify the clonal relationship of bovine and human O80:H2 EPEC and AE-STEC; (ii) understand whether calf O80:H2 EPEC are AE-STEC derivatives or precursors; (iii) perform other surveys in healthy cattle at slaughterhouses and farms.

27. Proteomic and functional analyses of the virion transmembrane proteome of cyprinid herpesvirus 3

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Virion transmembrane proteins(VTPs) mediate key functions in the herpesvirus infectious cycle. Cyprinid herpesvirus 3(CyHV 3) is the archetype of fish alloherpesviruses. The present study was devoted to CyHV-3 VTPs. Using mass spectrometry approaches, we identified 16 VTPs of the CyHV 3 FL strain.Mutagenesis experiments demonstrated that eight of these proteins are essential for viral growth *in vitro* (ORF32, ORF59, ORF81, ORF83, ORF99, ORF106, ORF115, ORF131), and eight are non-essential (ORF25, ORF64, ORF65, ORF108, ORF132, ORF136, ORF148, ORF149). Among the non essential proteins, deletion of ORF25, ORF132, ORF136, ORF148 or ORF149 affects viral replication *in vitro*, and deletion of ORF25, ORF64, ORF108, ORF132 or ORF149 impacts plaque size. Lack of ORF148 or ORF25 causes attenuation *in vivo* to a minor or major extent, respectively. The safety and efficacy of a virus lacking ORF25 were compared to those of a previously described vaccine candidate deleted for ORF56 and ORF57(Δ56 57). Using quantitative PCR, we demonstrated that the ORF25 deleted virus infects fish through skin infection and then spreads to internal organs as reported previously for the wild type parental virus and the Δ56 57 virus. However, compared to the parental wild type virus, the replication of the ORF25 deleted virus was reduced in intensity and duration to levels similar to those observed for the Δ56 57 virus.Vaccination of fish with a virus lacking ORF25 was safe but had low efficacy at the doses tested. This characterization of the virion transmembrane proteome of CyHV-3 provides a firm basis for further research on alloherpesvirus VTPs.

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

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

29. A case of swinepox in a wild boar (*Sus scrofa*) in Belgium

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A male wild boar, less than one year old and shot because of abnormal coat, showed multiple dark brown to black skin lesions up to cherry size spread over the whole body, including perineum and the external genital tract. On basis of necropsy, the boar was heavily infested by respiratory and digestive helminths, *Metastrongylus spp.* and *Ascaris suum*, respectively, but no other inside lesion was seen. From papules and crusts, histopathological examination, bacterial investigation and multi-sequencing were performed.

Histologically, a severe exophytic dermatitis with crusts and bacterial foci was observed. For bacteriology, the skin swab revealed the involvement of *Staphylococcus aureus* as secondary infection. No parasite was seen based on cutaneous scrapings and trichograms. Finally, the skin-lesion involved agent was identified as swinepox virus by sequencing.

Swinepox virus (SWPV), which replicates only in swine, belongs to the *Suipoxvirus* genus of the *Poxviridae f*amily. It's the etiologic agent of a skin disease of pigs, characterized by generalized pustular lesions and associated with high rates of illness (>80%) but low death rate (<5%). In domestic pigs, it occurs mainly with poor management and housing conditions. *Swinepox* virus oubreaks are often reported in farmed pigs but, at this day, only one case of swine pox in a wild boar (*Sus scrofa*) has been described (Austria, 2004). In the wild, infestation with *Haematopinus suis* could promote the infection.

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30. Ticks and tick-borne diseases in transhumance cattle between Burkina Faso and Benin: Socio-epidemiological aspects

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Livestock production has an important role in the economies and people daily life within West African countries. In this area, the cross-border transhumance represents one of main animal production strategy. . It contributes to the production of more than 65% of beef, 40% of meat of small ruminants and 70% of milk. Vectors and Vector-borne diseases are the main constraint to the development of livestock in West Africa. Transhumance can favour vectors and vector-borne diseases spreading within and/or across countries. For instance, following its introduction in Ivory Coast and Benin around 2002-2004, the cattle tick *Rhipicephalus* (*Boophilus*) *microplus* invaded others border West African countries (i.e. Burkina Faso, Mali, Niger, Nigeria and Togo). Transhumance implication in such spreading is suspected as well as that for others tick species and related transmitted pathogens.

The present work aims to investigate transhumance role in ticks and tick-borne diseases spreading between Burkina Faso and Benin. Therefore, 90 herds have been sampled in the study area (46 in the Est region of Burkina Faso and 44 in the North of Benin) and 29 of Burkina Faso's herds have been followed during the last transhumance season to Benin.

Partial results show three transhumance routes between Burkina Faso and Benin and we found two species of ticks (*Rhipicephalus lunulatus* and *Boophilus annulatus*) in Burkina Faso's herds during they stay in Benin. These partial results also show that Est region of Burkina Faso is free of *Rhipicephalus* (*Boophilus*) *microplus*.

31. The major envelope glycoprotein of Murid Herpesvirus-4 promotes sexual transmission

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Gammaherpesviruses are important human and animal pathogens. Infection control has proved difficult because the key process of transmission is ill-understood. Murid herpesvirus-4 (MuHV-4), a gammaherpesvirus of mice, transmits sexually. We show that this depends on the major virion envelope glycoprotein, gp150. Gp150 is redundant for host entry and, *in vitro*, it regulates rather than promotes cell binding. We show that gp150-deficient MuHV-4 reaches and replicates normally in the female genital tract after nasal infection, but is poorly released from vaginal epithelial cells and fails to pass from the female to the male genital tract during sexual contact. Thus, we show that regulation of virion binding is a key component of spontaneous gammaherpesvirus transmission.

Sustainable livestock production

1. Contribution to the study of some mesoparasite in goats of Laghouat area (Algeria)

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The present study aims to investigate mesoparasites in goats of Laghouat region and studying the variation of parasite prevalence according the age (≤2 years or ≥3years), the sex (males or females) and the breed (local or exotic) of animals. The study was carried out over a period of 3 months (January, February and March 2017) on 61 faecal samples taken from 61 goats. Different methods were used: direct examination, flotation method, sedimentation method, modified Ziehl – Neelsen staining technic and Mac Master technic, for the identification and quantification of parasites eggs.

General parasite prevalence was 44.26%. Several types of mesoparasites were revealed: *Cryptosporidium spp*. (27.87%), *Giardia spp.* (18.03%), *Nematodirus spp*. (13.11%), *Eiemeria spp.* (13.11%), *Strongyloides spp.* (3.28%) and finally *Trichuris spp*., *Moniezia spp.* with the same proportion (1.64%). The average intensity was 587.50 ± 91.61 opg for *Nematodirus spp.*, 550.00 ± 133.63 opg for *Eimeria spp.*, 363.64 ± 63.60 opg for *Giardia spp.* and 375.00 ± 35.35 Opg for *Strongyloides spp*. The statistical analysis did not show difference between sex, age and breed on the total prevalence and frequencies of parasites observed (p>0.05).

Despite the moderate values of the prevalence and the intensity of parasites recorded, they should be taken seriously to avoid adverse effects on the health and profitability of goat herds.

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2. Abdominal steatonecrosis in a twelve-year old Highland bull

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Steatonecrosis, also called lipomatosis or abdominal fat necrosis (AFN), is frequent in cattle, but is often an accidental finding during transrectal palpation or slaughtering process. We herein describe an abdominal fat necrosis in a twelve-year old Highland bull. A 12-year old Highland bull was referred to the Clinic for Ruminants of the University of Liège for anorexia, scant feces since few days. At the arrival, the clinical examination revealed a high rectal temperature. Special abdominal examination highlighted an absence of digestive sound and ruminal contractions and a large firm mass was felt at the flank succussion. Rectal palpation revealed a large mass located around the left kidney and smaller masses around the intestinal tract. Transabdominal ultrasonography showed a heterogeneous hyperechoic mass with undefined shape and a hyperechoic omentum. The right-flank celiotomy confirmed the AFN with colic obstruction. Due to the generalized and severe lesions, the bull was euthanatized. The necropsy revealed the presence of hard necrotic fat masses surrounding and stenosing spiral and distal colon, a thickened hard great omentum including necrotic fat masses and the left kidney was completely included in fat necrotic mass. Histopathological examination showed typical lesions of fat necrosis. AFN is a common finding in domestic animals and humans. Excessive fattiness of abdominal tissue in the growing stage, disturbance in lipid metabolism, genetic predispositions and fescue toxicity have been associated with the occurrence of AFN. Isoprothiolane appears to be an effective treatment to AFN in cattle. To our knowledge it is the first report of AFN in highland cattle.

3. Goat grazing in northern Morocco: problem or solution?

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In northern Morocco, silvopastoral areas are characterized by pastoral activities, and the goats find some or all their feed rations. Overgrazing is listed as a main factor of silvopastoral degradation by local authority and several authors. Goat cannot only be regarded as a problem for silvopastoral ecosystems. A survey, with local population and local authority, were conducted in several rural communes of northern Morocco, to understand the actual factors of silvopastoral degradation. According to all interviewed persons: agriculture development, authority policy, cannabis cultivation, deforestation, drought, fire, overgrazing, population growth and soil erosion are the most cited drivers of silvopastoral degradation. Local population and local authority have the same perception that soil erosion, drought, fire and population growth are factors of silvopastoral degradation. Goat grazing can be considerate as a solution and not just a problem. In some forest areas, goat grazing contributes to a reduction of burned areas by elimination of inflammable shrubs. Herd mobility, as an ecological rationality, is considered as a response to unexpected variability in pasture production and/or animal nutritional needs. Despite all forms of degradation, silvopastoral areas in northern Morocco continue to play a support of all livestock activities, considered an inevitable source of goats feeding.

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4. Dong Tao chicken: characteristics of an indigenous breed with big legs in VietNam

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The objective of this study is to determine morphological characteristics and reproductive performances of Dong Tao chicken, an indigenous breed in Vietnam. It is also a foundation for a rehabilitation of Vietnamese local chicken breeds. Semi-structured interviews were conducted on 120 households raising purebred Dong Tao chicken, including 96 raising less than 50 hens, 17 raising 50 – 100 hens and 7 raising more than 100 hens. Morpho-biometric characterization was based on measurement of 240 adult individuals, including 40 males and 200 females over six months of age. The body parameters were recorded in accordance with the FAO recommendations (2012). Egg production performances were recorded on 10 chicken family flocks (one cock and five hens per family) on 10 households. Chickens were reared in traditional livestock farming system. Results showed that Dong Tao chickens have a large variety of phenotypes, as indicated by their five feather colors, two skin colors and three comb types. However, their reproductive performances were low (12.7 eggs/clutch and 5.7 clutches/year). Therefore, due to these modest performances and to the presence of more productive exotic breeds, the Dong Tao breed is currently endangered. Moreover, its reproductive performances should be improved before considering *in-situ* conservation.

5. Effect of the method and the period of oil extraction on *in vitro* digestibility of olive cake in northern of Morocco

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In Northern Morocco, goat population is the most dominant livestock. His feeding is based on forest rangelands and characterized by strong seasonal variability responsible of low productivity. Therefore, it is necessary to diversify and improve its diet. Olive cake is an oil extraction by-product that is widely available and can be a ruminant feed. Olive cake is produced by three methods and for limited period. This work aims to determine the effect of oil extraction method and period on digestibility of olive cake. Twenty-seven samples were collected from 3 mills by each extraction method (mechanical pressure, centrifugation 2 and 3 phases) during three periods (November, December and January). *In vitro* digestibility was performed using gas production method of (Menke et al., 1979) improved by Menke and Steingass (1988). Samples were incubated in glass syringes that contained rumen fluid and incubation solution that conserved in water bath in 39°C during 72 hours. Gas production was recorded after 2, 4, 8, 12, 24, 48 and 72 hours. Studied digestibility parameters were dry and organic matter digestibility, microbial biomass production, partitioning factor and volatile fatty acid. Extraction method affected digestion kinetics and the digestibility parameters except partition factor and volatile fatty acids. Olive cake obtained by 2 phases method had the highest digestibility. For extraction period, gas production decreased significantly per period. Period had no effect on digestibility parameters except for volatile fatty acids. Two phases olive cake is most suitable for feed but has a reduced shelf life, which requires the development of a suitable conservation mode.

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6. Evolution of pre and post-operative lactatemia as prognostic indicator of digestive surgeries in cattle

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Digestives disorders are particularly common in cattle. A previous study showed that the level of blood lactate (BL) measured before digestive surgeries is irrelevant as a prognostic value to estimate the survival of adults bovines. Here, we propose to determine whether variations of this parameter could be used as a prognosis predictor when measured before and after the surgery.

The aim of this study is to determine whether the levels of BL could be used as a postoperative prognosis value in digestive surgeries of adult bovines.

This retrospective study was performed on 52 bovines referred to the veterinary clinic (CVU). The level of BL was measured before (LAC1), 12h after (LAC2), and 48h after (LAC3) the surgery. Individuals were divided in two groups: positive outcome (IP) and negative outcome. Changes in the level of BL over time were investigated within each group and the differences in this parameter were compared between the two groups. Finally, ROC curves were drawn to estimate the prognostic values of punctual levels of BL and changes in the level of BL. The level of BL significantly decreases over time within each group and postoperative levels are significantly lower in the group with the IP ($p < 0,05$). The ROC curves show that four parameters based on the levels of BL are useful (i.e. AUC > 0,5) to predict the outcome: LAC2 (AUC: 0,707), LAC3 (AUC: 0,851), the decrease (%) between LAC1-LAC2 (AUC :0,692) and LAC1-LAC3 (AUC :0,814). A IP is predicted when the level of BL is < 1.73 mmol/l or when the decrease of BL is > 50% 12h after surgery or when the level of BL is < 1,94 mmol/l or when the decrease of BL is > 70,5% 48h after surgery.

The level of BL measured after the surgery and its changes over time allow reliable predictions of the outcome of digestive surgeries. The quality of the prediction is however higher 48h after the surgery.

7. Physico-chemical and Microbiological quality of Goat milk commercialized in informal market in Laghouat, Algeria

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The goat milk is less or not industrialized in Algeria especially in Laghouat region where commercialization is made in unknown conditions (informal markets). Thereby, this work aimed to evaluate its physico-chemical and microbiological quality according to criterion recommended by Algerian standards except the Listeria monocytogens. A total of 52 samples of raw milk taken from different informal markets in Laghouat city, were analyzed during February and Mars 2017.

The results revealed that the dray matter depends on the fat and protein contents that this milk contains; the density is related to the not fatty solids; the freezing point was high and approaching 0°C; the fat content was high which could be related to the low milk production (concentration phenomenon). On the other hand, the protein content was low, probably in relation to an energy deficit of food ration.

The microbiological results showed the absence of pathogenic bacteria as Salmonella and Clostridium. However, for the others microbiological criterion and according to sampling plan, unsatisfactory quality was reported: fecal coliforms and Yeasts and molds (100%), total mesophilic aerobic flora (73%), faecal streptococci (50%) and Staphylococcus aureus (4%). Therefore, the quality of goat milk handing from the farm to market is probably linked to failure of hygiene practice. At the end of this work, we recommend breeders and sellers to respect the good practices of hygiene and milking.

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8. Characteristics of goat production systems and effect of Boer crossbreeding on body weights of goats in the five agro ecological zones of Burundi

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To provide an overview of local goat farming systems and to evaluate the effect of Boer crossbreeding in goat's body weight, 320 farmers were surveyed in the five agro ecological zones of Burundi and 929 adult goats were measured. Clustering analysis of the farming systems separated dry lowlands (high mobility of animals, large flocks and market driven objective) from humid highlands (low mobility of animals, small mixed flocks and low market characteristics). This may be due to the longer dry season in lowlands than in highlands which limits the production of crop forages and the relative availability of pastures areas. Moreover, in humid zones, the lack of pastures areas due to high human pressure and the need of manure for agriculture decrease the mobility of animals. A fixed effect model was used to evaluate the effect of Boer crossbreeding adjusted for age, sex, grazing system and agro-ecological zone. Least square mean weights of Boer crossbreds were higher by 4.74 kg than weights of indigenous goats which is lower than expected. Hypotheses may be that imported bucks were not pure breed Boer and that management conditions were insufficient for the animals to express their genetic superiorities. Moreover, goats were weightier in lowlands than in highlands. In conclusion, goat husbandry seems better developed in lowlands than in highlands and crossbreeding didn't enhance performances of local breed as expected. The advantages and disadvantages of a selection program within indigenous breed are discussed as an alternative to the crossbreeding program.

9. Effect of genetic European taurine ancestry on milk yield of Ankole-Holstein crossbred dairy cattle in mixed smallholders system of Burundi highlands

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Different breeding systems associated with specific bovine genetic resources coexisted in Burundi. To prepare the development of a national action plan for the improvement of bovine genetic resources in Burundi, we wished to perform a genetic characterization of Ankole and Ankole x European crossbred individuals and to assess the effect of European ancestry on milk productivity of cows kept under the mixed crops livestock system. To that end, we genotyped 37 Ankole and 138 crossbred individuals on 42,636 SNPs and combined these genotypes with those from 21 cattle breeds, representative of the bovine genetic diversity. We also measured milk yield not suckled and estimated the suckled milk. Given the results, we confirmed that the indicine x African taurine admixed origin of the Ankole in Burundi and showed that crossbred individuals present a high proportion of European ancestry (57% on average). As the proportion of European ancestry increased, milk yield increased by 0.03 ± 0.01 l/d, but to a lower extent than expected. We also observed that breeders were unable to evaluate correctly the European proportion for their livestock. Our results may provide useful information for objective dairy breeding in Burundi. As an example, an ex-situ conservation program for Ankole within the framework of value chains is proposed as an accompanying strategy to improve the sustainability of the crossbreeding program.

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10. Quality assessment of marketed eggs in Hanoi (Vietnam)

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Poultry farming is the first sector of livestock that has been industrialized. The poultry sector plays an important role in the GDP of Vietnam. It is the second largest livestock after the pig livestock. The production and consumption of eggs in Vietnam is estimated as 5.64 billion and 102.6 eggs per person per year respectively. The aim of this study was to assess the quality of eggs in relation to the chicken breed and different marketing channels in Hanoi, Vietnam. In total, 431 chicken eggs were purchased from four different marketing channels in Hanoi, including households (89 eggs), supermarkets (152 eggs), public markets (130 eggs) and small grocery stores (50 eggs). Out of the 431 above-mentioned eggs, 119 eggs came from the commercial chicken breeds and 312 eggs from traditional local breeds. The quality of eggs for a consumer is represented by its cleanliness, nutritional quality, freshness, and price. A series of measurements is carried out just after their purchase. According to the breed and the marketing channel, highly significant differences ($P < 0.05$) were found in the freshness of the eggs (Haugh units), egg prices, eggs weight, egg shell, the white and the yolk of the egg. Though the chicken breed and marketing channels do not significantly affect ($P > 0.05$) the freshness of the eggs, however, they have a significant effect ($P < 0.05$) on the size of the eggs marketed in Hanoi. The results of this study show an important diversity in marketing channels and marketed eggs in Hanoi.

11. Comparison of the effects of using *Opuntia ficus-indica* powder on growth performance and serum composition of the Broiler Chicken in Algeria.

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In Algeria, the ingredients used in the broiler feed are exclusively imported from abroad, which affects negatively the production cost of the chicken meat and its sale price in the market at national level. Because of the wide diversity in soil and climate, Algeria has a substantial number of plants which can be used in animal feed. This work is part of the valuation of the feed potential of the barbaric fig tree, widely present in the Algerian rural landscape, for the broiler chicken. The Opuntia ficus-indica is known for its edible fruits and for use of its fleshy leaves or "racquets" as fodder especially during periods of drought. It is also used to control the water and wind erosion as well as for the protection and improvement of soil fertility in arid and semi-arid regions. The aim of this study was to measure the effects of Opuntia ficus-indica powder on growth performance, serum composition and carcass yield of broiler chickens. The experiments were performed in a private poultry farm in the Chemini region (Wilaya de Bejaia). In this study, 120 Ross-308 day-old male chicks were monitored. They were divided in 3 groups (Group 1, 2 and 3) according to the specific diet (4 x 10 chicks / group). The group 1 was offered commercial feed. The group 2 and group 3 were offered the same commercial feed as group 1 but 5% and 10% of the commercial feed was replaced by the Opuntia ficus-indica powder respectively. The Opuntia ficus-indica powder did not show any negative effect ($p>0.05$) on the final body-weight, average daily gain and carcass yield. However, it decreased ($p<0.05$) the biochemical parameters -blood concentration- (plasma glucose, uremia, cholesterol and triglycerides). In conclusion, the Opuntia ficus-indica powder has the potential to be used in poultry feed to reduce the cost of broiler feed in Algeria.

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12. Effects of pig diets containing rice distiller's by-product on growth performance, carcass characteristics and meat quality

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The aim of this study was to evaluate the effects of fattening pig diets containing rice distiller's by-product (RDP) on growth performance, carcass characteristics and meat quality. A total of 24 castrated male crossbred pigs male Duroc ×female (Landrace × Yorkshire) were used for the experiment. Pigs were divided randomly by weight and litter into 3 diets, with 4 replications of 2 pigs in each pen. Pigs were fed one of 3 diets including DAR0 (control), DAR15 (15% RDP in dry matter) and DAR30 (30% RDP in dry matter) during 8 weeks. The results showed that the diets with RDP tended to affect average daily gain ($P=0.09$) and feed conversion ratio ($P=0.08$), while feed cost was decreased ($P<0.001$). Carcass traits was not significantly different between the diets, however back fat thickness was increased by RDP levels ($P=0.03$). Drip loss of *Longissimus dorsi* muscle at 24 and 48 hours was increased ($P<0.05$), whereas there were not effect on pH value and meat color at 45 min, 24 and 48 hours *postmortem*. Muscle lipid content was increased ($P<0.01$), whereas protein content was not influenced by RDP in diets. This suggests that using diet with 30% RDP for fattening pigs can improve lipid content of *Longissimus dorsi* muscle and reduce feed costs while not affecting growth performance and meat quality.

13. Effect of parity on metabolic profile of Algerian local rabbit doe

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Feeding plays an crucial role in success of a rabbit breeding. Good nutrition status is essential to mutch an intensive breeding and production rabbit does.

The aim of this work was to study the blood biochemical parameters of thirty-three (33) local rabbit does raised in Algerian breeding conditions during the first two lactations. The females were followed at the Technical Institute of Livestock (ITElv) of Algiers. The reproductive rate applied was the semi-intensive one (mating at 10-day *post partum*). Pregnancy diagnosis was performed by abdominal palpation at 10 days *post coitum*. A total of 260 blood samples were taken from marginal ear vein at 01, 04, 12 and 19 days *post partum* during the first two parities. The 260 samples were evaluated for glucose, triglycerides, cholesterol, total protein and urea at the ENSV biochemistry laboratory.

The results showed that parity had not effect on the blood's triglyceride and cholesterol levels. However, the blood glucose levels were significantly higher in rabbits at the first lactation ($p < 0.05$). The blood levels of total proteins and urea were significantly higher during the second lactation ($p < 0.05$). In addition to the effect of parity, the day of sampling had a significant ($p < 0.05$) effect on glucose, cholesterol and total protein levels during both lactations, with no effect on urea and triglycerides levels.

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14. Inventory of Beekeeping in the Algerian north (Tizi-ouzou and Bejaia)

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The objective of this study is to make an inventory of the honey production in the counties of Bejaia and Tizi-Ouzou in northeastern Algeria. To achieve this, a survey was carried out on 31 beekeepers (14 in Bejaia and 17 in Tizi-Ouzou). The survey shows a social, cultural and religious role for 61.29% of the beekeepers. The beekeeping is primarily held by men (93.55%). It is the basic source of income for 64.52% of the respondents. The average age of the beekeepers is 42.90years (Min-Max: 28-67 years, Median: 40.5 years). The main products of the hive are honey (100%), swarms (16.13%), royal jelly (9.68%) and propolis (9.68%). The average honey production per hive is 7.70kg (Min-Max: 0.4-15kg hives, Median: 10kg). The average number of hives per beekeeper is 42.20 hives (Min-Max: 3-300 hives, Median: 17.50 hives). The average selling price of one kg of honey is 4000.00 DA / kg (Min-Max: 2000-5000DA; Median: 4000DA). The phenotype of the bee reported by 74.19% of the surveys is of small size with a long body and dark pigmentation corresponding to the breed "*Apis mellifera intermissa*". Two apiculturists described another phenotype corresponding to the "*Apis mellifera major*". The factors behind the motivations for beekeeping are consumption of honey (100%), income generation (90.32%), hobby (58.06%) and conservation of biodiversity (22.58%).The multiple constraints associated with several diseases, notably Varroase (mentioned by 80.65% of beekeepers), cause difficulties for the breeders. Thus they cannot profit maximum from beekeeping. Other constraints which were reported are; forest fires (35.48%), wasps (32.26%), absence of beekeeping professionals or technicians (29.03%), harsh and cold winters with snow (19.35%), high density of hives in the region (16.13%) and uncontrolled spreading of pesticides and crop protection products at farms (12.90%).The economic situation of the Algerian beekeepers can be optimized by improving the production potential of the local bees.

Comparative veterinary medicine

Posters

1. Canine muscle derived mesenchymal stem cells: minimally invasive process, characterization and future clinical use

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Mesenchymal stem cells are commonly produced from bone marrow or adipose tissue samples. Unfortunately the sampling of bone marrow and adipose tissue is invasive and does not always result in a sufficient number of cells.

Recently, our team proposed an alternative method for the sampling, isolation and culture of skeletal muscle-derived mesenchymal stem cells (mdMSC). This method is easily applied in practice and transposable to various species.

The purpose of this study was to obtain and characterize the canine muscle derived stem cells from a muscle microbiopsy.

Eight Beagle dogs were used to test the innocuity of an injection of stem cells into the intervertebral disk. The process to obtain the mdMSC included explant of the sample, Percoll density gradient for isolation and subsequent culture of the cells.

After 4 weeks, we obtained 3 million of mdMSC in 6 of the 8 samples ready to be reinjected or to be frozen. The cells were characterized by their capabilities to differentiate into chondroblasts, adipocytes and osteoblasts. Differentiation in other cell types (myoblasts, neurons,…) are still in progress for dogs but are already proved in other species.

This novel easy-to-use technique will facilitate the use of autologous cell therapy in dogs. To date, several studies have reported interesting results in several pathologies such as osteoarthritis, corneal ulcers, renal and cardiac dysfunction and urinary incontinence.

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2. Canine gangrenous mastitis : series of three patients

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This report describes and compares the clinical presentations of three post-parturient bitches diagnosed with gangrenous mastitis, their treatments and their cost-effectiveness. Mastitis is an inflammation of the mammary gland found mainly in lactating females. While more common in ruminants, gangrenous mastitits is rare in the bitch. They are typically presented with anorexia, hyperthermia, swollen and necrotized mammary tissue. One Sheltie (S), one NewFoundland (NF) and one Boxer (B) were presented at the Veterinary Clinic of University of Liège. The S was in decompensated septic shock 36 hours after ovariohysterectomy on 4 puppies dead during labour. Three crackling mammary glands with patchy blue discoloration were involved. The NF, 14 days post-partum, had stopped milking her pups 3 days earlier because of painfull mammary glands. At admission she was alert with hyperthermia. One left gland was purplish, swollen and indurated but necrosis only appeared 2 days later. The B, 1 month post-partum, 4 days before presentation seemed weak and reluctant to move. At admission, it was prostrated with one open necrotized mammary gland. The S was stabilized then treated with negative wound pressure therapy after progressive debridement and wet to dry bandages. 1 surgery for debridement before closure of the wound sufficed for the NF. The B underwent 2 surgical debridements before closure with a drain left in place 4 more days. The treatment choice depends on the number of mammary glands involved, the patient's shock status and the evolution of necrosis. Optimal treatments' choice is based on these criteria and the cost-effectiveness of repeated surgeries and anaesthesia.

3. Possible effect of environment on lung microbiota in the healthy West Highland white terrier.

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Over the past few years, the number of publications about lung microbiota has considerably increased due to the advancement of new molecular technologies. In dogs, only 2 studies in healthy experimental beagles have been published. Interestingly, we recently reported major differences in the lung microbiota composition and diversity between experimental healthy beagles and healthy client-owned West Highland white terriers (WHWT) suggesting an impact of the breed or of the living environment on lung microbiota composition. Accordingly, to further assess the effect of living environment, the aim of the present study was to compare lung microbiota data obtained in healthy WHWT (n=5) living in Finland with those previously obtained in healthy WHWT (n=5) living in Belgium. For this purpose, bronchoalveolar lavage was retrospectively selected. Metagenetic analysis were performed after DNA extraction and sequencing on a MiSeq Illumina sequencer. Taxonomical assignation and microbiota community analysis were done with MOTHUR V1.35 with an OTU clustering distance of 0.03. Data analyses demonstrated that the same 4 major phyla *Proteobacteria, Firmicutes, Actinobacteria* and *Bacteroidetes* predominated in both groups of dogs. Significant but subtle differences were observed between groups. Results of the present study do not demonstrate a clear impact of the living environment on lung microbiota composition. In conclusion, analysis of larger cohorts of healthy dogs with various living conditions appears necessary.

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4. Antibiotics residues determination in meat samples from Pichincha – Ecuador using an optimized two – plate screening method

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The accomplishment of controls to identify antibiotic residues in animal food products to guarantee food safety is a topic widely studied; however, these analytical controls have not been established by authorities in Ecuador. At present, there is a national monitoring plan focused in the determination of the level contamination produced by antibiotic residues in the main animal food products consumed in the country. To contribute to this goal, a screening microbiological method of the main antibiotics used in animal production, called New two – plate test (NTPT) is being optimized. This method uses two plates that contains two medias at different pH, inoculated with *B. Subtilis* as control strain. To increase the method screening capacity, the first factor modified was the quantity of media used in each plate, in this case it was used 5 mL of media instead of the 8mL recommended by the method. With this variation, there were tested several concentrations of antibiotics standard solutions, and the results showed that 50 µL of standard with the same concentration presented a bigger bacterial growth inhibition in the plates that contain 5 mL of media. The same tendency was observed as in Penicillin as Oxytetracyclines standard solution tests. In the case of sulfonamides, besides to vary the media quantity, it was necessary to modify the media composition, since in this case the media contains trimethoprim (TMP) solution. The different tests showed that using 26 ng of TMP/mL of media instead of 30 ng/mL did not produce false positives. Finally, the use of a water:methanol mix to prepare the sulfonamides standard solutions seems to give better results than to use just methanol.

5. Development of tools and assays to facilitate the study of CyHV-3 ORF12 and its interaction with the carp immune system

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Cyprinid herpesvirus 3 (CyHV-3) is the causative agent of a lethal, highly contagious disease in common and koi carp (*Cyprinus carpio*). The genome of CyHV-3 is a 295-kb double-stranded DNA molecule, encoding for 156 potential protein-coding open reading frames (ORFs). Herpesviruses are well known to encode for multiple proteins that are involved in immune evasion mechanisms. In the CyHV-3 genome, such candidate genes include the ORF12, and ORF134, which encode a TNF receptor homolog and an IL-10 homolog, respectively. To investigate the function of ORF12, and understand its role with the fish immune system, tools and assays have been developed. Firstly, a pCMV-ORF12-HA plasmid has been constructed and successfully validated by Western Blot using anti-ORF12 and anti-HA antibodies. Secondly, a real-time PCR assay has been developed and performed by analysing cDNA samples generated from an *in vitro* infection model. For this, CCB cells were infected with CyHV-3 deletion (12Del) mutant or a revertant virus (12Rev) (where the ORF12 was restored). Cells were dissolved in TRI-Reagent for further analysis at 3, 6, 12, 24 and 48h post infection. RNA was extracted and cDNA synthesised, in order to perform real-time PCR analysis and to investigate the expression of several key immune genes. Preliminary results showed an interesting pattern, where the expression of certain immune genes (e.g. interferon gamma) was affected by both mutants, whereas the expression of other immune markers (e.g. tumor necrosis factor alpha) was only affected by one mutant but not the other. Overall, this on-going work provides an insight into the involvement of ORF12 as a TNF receptor homolog.

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6. Predictive value of pre and postoperative urethral pressure profiles for long-term continence in dogs with ectopic ureters

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Introduction : Long-term continence rate can be unsatisfactory after surgical correction of ectopic ureters (EU) in dogs. Urodynamic investigation has been proposed to assess urethral function. Our objective was to evaluate the usefulness of pre- and postoperative urethral pressure profiles (UPP) in predicting long-term continence in dogs with EU.

Materials and Methods : Medical records of dogs that underwent surgical correction of EU and UPP between 2012 and 2016 were reviewed.

Urodynamic parameters included Maximal Urethral Closure Pressure (MUCP), Anatomical Profile Length (APL), Functional Profile Length (FPL) and Integrated Pressure (IP = MUCPxFPL).

Results : Fourteen females were included in the study. Depending on the type of EU, neoureterostomy with dissection of the intramural portion (17 intramural EU) or ureteroneocystostomy (3 extramural EU) were performed. Pelvic bladder was diagnosed in 5 dogs and colposuspension was performed during the same surgery.

A continence score (1= incontinent, 2= continent with sporadic episodes of incontinence, 3= continent) was assessed at the preoperative and postoperative UPP (mean 1.7 months postoperatively). The continence score, APL, FPL and IP values were significantly increased at the postoperative time. Recurrence of urinary incontinence was observed in 8 dogs after a mean follow-up time of 4 months and none of the urodynamic parameters was predictive for the recurrence.

Discussion/Conclusions : In this study, pre or postoperative UPP was not a predictive procedure for recurrence of urinary incontinence after surgical correction of EU.

7. Diagnosis of clitoral adenocarcinoma by computed tomography in a dog presented with hypercalcemia

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An 11-year-old female dog was presented with a history of dysorexia, polyuria/polydipsia, weakness and weight loss. At physical examination, a mass was palpated in the left inguinal region. Rectal palpation was unremarkable. Blood work revealed increased total and ionized calcium. Abdominal ultrasonography showed left medial iliac and left superficial inguinal lymph nodes enlargement. Lymph nodes fine-needle aspirations (FNA) showed a cellular population similar to apocrine gland anal sac adenocarcinoma (AGASA), and were consistent with adenocarcinoma metastasis. Full body computed tomography revealed a small soft-tissue mass at the level of the clitoris, and enlarged left medial iliac and bilateral superficial inguinal lymph nodes. FNA of the clitoral mass showed similar cytological findings as the lymph nodes. A tentative diagnosis of primary clitoral adenocarcinoma with regional lymph node metastasis was made. En-bloc excision of the clitoris with diode laser and excision of affected lymph nodes were performed. Histopathology confirmed the diagnosis of primary clitoral adenocarcinoma with lymph node metastasis. The owners declined adjuvant chemotherapy. One month postoperatively, polydipsia/polyuria reoccurred. The left medial iliac lymph node was enlarged and was cytologically identified as a metastasis. Six months after surgery, the dog presented with dysorexia and weakness and was suspected of recurrence of hypercalcemia. The owners declined further investigation and the dog was euthanized. Clitoral adenocarcinoma shares cytological, histological and clinical similarities with AGASA and should be part of the differential diagnosis of hypercalcemia in female dogs.

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8. Management of extensive burns following an elective procedure in a dog

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Severe thermal burn injuries can be induced by patient's warming devices during or after surgery. In some cases, burns may involve large areas of skin and primary wound closure may not be possible. Second intention healing or reconstruction techniques may be necessary to close the wound. We report the management of an iatrogenic burn by heating pad occurring during an ovariectomy in a 6-month-old female Maltese. The dog suffered from a full-thickness burn involving about 27% of its total body surface area extending from the dorsal surface of the neck to the base of the tail, up to the mid-thorax, abdomen and the origin of the four limbs. Local care with sterile NaCl 0.9% irrigation and silver sulfadiazine wound dressings was initially performed together with analgesic, nutritive and antimicrobial supports. After a week, closure of the wound was planned by use of a skin stretching technique. Surgical debridement of the wound was realized and Velcro straps were sutured on both sides of the wound to relieve skin load on either side of the skin defect. One week later, once healthy granulation tissue was obtained, horizontal mattress sutures were applied to further close the wound. One month after admission, complete closure of the wound was attempted using single interrupted pattern sutures reinforced with vertical mattress sutures. A triangle of skin located at the level of the base of the tail was impossible to close due to excessive tension. Punch grafts harvested from the dorsal surface of the neck were placed within the wound bed to accelerate epithelialization. The dog was discharged two months after admission with complete wound healing.

9. Release of Neutrophils Extracellular Traps as a main trigger for asthma onset

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The etiology of asthma is still unknown. Yet, some environmental factors like viral infections and exposition to low doses of lipopolysaccharide (LPS) increase the risk of disease inception. These two risk factors induce a recruitment of neutrophils into the lung. Neutrophils can form neutrophils extracellular traps (NETs) which could initiate pathological conditions. Here, we investigated the role of NETs as potential asthma inducers in specific pro-Th2 environmental risk factors like respiratory viral infections and low LPS doses exposures. We developed two mouse models, a virus-induced asthma model and a model of asthma promoted by low LPS doses. In these models, only previously infected mice or mice exposed to low LPS doses displayed the characteristics of asthma after sensitization and challenge to house dust mite. Then, we tested the correlation between respiratory viral infection or low LPS exposure and NETosis. Viral infection and exposition to low but not to high LPS doses recruited neutrophils expressing CXCR4 in the lung of exposed mice. These $CXCR4^{high}$ neutrophils released NETs. The role of NETs in asthma onset was then demonstrated using NETosis inhibitors in our models as infected or low LPS doses exposed mice exhibited strong decrease of all asthma features when treated with NETs inhibitors compared to non-treated mice. Finally, to address how NETs could promote allergic sensitization, we analysed the distinct subpopulations of lung dendritic cells (DCs) in our models. We observed, during the NETosis phase, a recruitment of monocytic derived DCs (moDCs). In conclusion, we have demonstrated an unexpected role for NETs in asthma onset by recruiting lung moDCs.

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10. Loss of transfer RNA U34 modifying enzymes impairs hematopoietic stem and progenitor cell differentiation and function

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Hematopoietic stem and progenitor cells (HSPCs) require fine-tuned protein translation initiation rates for their normal maintenance and function. Conserved modifications of the wobble uridine base (U34) in transfer RNAs catalyzed by the Elongator complex are required for optimal speed and fidelity of protein elongation, but their biological importance in mammalian stem and progenitor cells remains unexplored. We therefore studied the impact of loss of activity of Elongator on HSPC differentiation and function. Hematopoietic-cell-specific loss of Elongator in conditional knockout mice resulted in shortened lifespan associated with hematopoietic failure. Loss of Elongator caused mild apoptosis and accelerated differentiation of bone marrow multipotent and oligopotent progenitors, resulting in blood and bone marrow pancytopenia. In contrast, Elongator-deficient hematopoietic stem cells (HSCs) were not negatively impacted in the steady-state, although they showed diminished engraftment potential in irradiated hosts. Mechanistically, loss of Elongator did not result in detectable alterations in global protein synthesis rates in any HSPC subset. Rather, Elongator-deficient HSPCs displayed enhanced activity of the stress integrator and apoptosis and cell cycle regulator p53. Loss of p53 rescued bone marrow function in Elongator-deficient mice. Therefore, this study supports the notion that Elongator activity is required in distinct HSPC subsets to avoid aberrant p53 activation, which otherwise results in discrete loss of function phenotypes in HSCs and downstream progenitors.

11. Clinical outcome after tracheal resection and anastomosis for correction of a tracheal stenosis in a sport pony.

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Objectives: To report our experience using tracheal resection and anastomosis to treat a severe trauma-induced tracheal stenosis involving 2 tracheal rings causing a loud respiratory noise at rest in a pony. Methods: After exposure of the trachea and placement of stay sutures, the 2 tracheal rings involved in the stricture were removed. On both remaining segments of the trachea, the mucosa was turned back over the open end and sutured to the adventitia. The head was then flexed and tracheal ends were apposed using stainless steel wires placed without mucosal penetration. The anastomosis was completed by a simple continuous suture of absorbable material on the adventitia. A closed suction drain was applied in the soft tissue before routine closure of the subcutaneous and cutaneous layers. A martingale was applied before the recovery from anaesthesia. The pony was discharged 1 month after surgery. Control endoscopies were performed during hospitalisation and at 7, 10, 17, 21 and 30 weeks and 1 year after surgery. Results: A moderate cicatrix recurred. The excessive tissue was injected with triamcinolone and then with 4% formaldehyde during the first endoscopies and remained stable thereafter. Some stainless steel broke and caused the formation of a self-resolving seroma caudally to the trachea. A moderate respiratory noise was still audible at low-intensity work, which was resumed 4 months postoperatively. However, work was stopped before achieving the preoperative level because the pony developed a lameness. Conclusions: Although a moderate tracheal cicatrix recurred after surgery, tracheal resection and anastomosis clearly improved the quality of life of this pony.

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12. Case report: Acquired permanent blindness secondary to an orbital abscess resulting from a dog bite

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Purpose: To describe permanent blindness secondary to an orbital abscess. Methods: A four-year old Staffordshire Bull-Terrier was presented for dysorexia and apathy with an acute periocular swelling four days after having fought with a dog. General physical examination revealed hyperthermia, left mandibular lymph node adenomegaly, halitosis, painful buccal opening, exophthalmos and two punctate skin wounds ventrally to the left eye. Menace response, direct pupillary reflex (PLR) and dazzle reflex were absent from the left eye. Slit lamp examination and indirect ophthalmoscopy of this eye revealed a hyperaemic, swollen and prolapsed nictitating membrane, chemosis, ulcerative keratitis, mydriasis and an indented posterior eyewall. The right eye was unremarkable except the absence of an indirect pupillary reflex. Results: On ultrasound and CT-scan a large orbital and periorbital abscess was suspected and confirmed by cytology. Oral and ventral trans-conjunctival orbital drainage was performed, as well as a temporary partial tarsorrhaphy. Medical treatment consisted of amoxicillin-clavulanic acid, metronidazole, carprofen, opioids, topical gentamycin and artificial tears. The orbital abscess and the corneal ulcer healed uneventfully but left blindness and PLR anomalies persisted. Three weeks and three months later, ERG's were performed on both eyes and were within normal limits. The control CT-scan at three weeks postoperatively was consistent with a left optic nerve enlargement. At three months postoperatively, ophthalmoscopy showed signs of disk atrophy. Conclusions: An orbital abscess secondary to a facial dog bite can result in permanent blindness due to optic nerve compression, inflammation and/or atrophy.

13. Ultrasound-guided bladder biopsy: technique description in 13 dogs.

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Fine-needle aspiration under ultrasonographic (US) guidance is controversial in case of transitional cell carcinoma as seeding of neoplastic cells along the needle track has been reported in dogs. Biopsies can be performed under cystoscopic guidance using rigid or flexible endoscope. Cystoscopes are however not always available and introducing biopsy forceps with a cystoscope in small patients can be challenging.

The aim of this study was to report an US-guided bladder biopsy technique in dogs with bladder masses. Records from dogs that underwent bladder US-guided biopsy were reviewed. Biopsies were performed with reusable flexible forceps graspers, using a 10Fr urinary catheter as a guide, or by introducing directly the forceps into the urethra for small dogs.

Thirteen dogs were included in the study (2 males and 11 females). Mean body weight was 18.1kg, ranging from 4.9kg to 34.5kg. The procedure was easily performed in 12 dogs. No signs of bladder hematoma, uroabdomen or urethral rupture was observed. All biopsies samples allowed histopathological diagnosis and all biopsies were diagnostic. Masses location into the bladder was varied. The ureter was involved in 54%. In one male dog, the forceps grasper was too rigid and too large to be introduced fully and blocked at the level of the ischiatic curvature. This dog was a jack-russel cross-breed dog and the smallest dog of this sample (4,9kg vs 14,5kg for the other male dog).

The present study is the first report of an US-guided bladder biopsy technique in male and female dogs with or without urinary catheter guidance for forceps introduction. This methods is to be considered in dogs presented with a bladder mass. However, due to their anatomy, small male patients may not be eligible for this technique.

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14. Clinical outcome after surgical correction of cleft palate by laryngeal tie-forward in 2 horses.

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Cleft palate is a rare congenital defect in horses. In most cases, very young foals present with severe clinical signs of dysphagia. Surgical intervention consists in palatoplasty considered as a salvage procedure. Only a few cases of cleft palate in adult horses are described. Laryngeal tie-forward, a surgical technique used to treat dorsal displacement of the soft palate, has been described only in one case with cleft palate with poor outcome. Our study describes laryngeal tie-forward as a treatment option for the cleft palate in two adult horses not suitable for palatoplasty. Clinical records show two 8 y.o. jumping horses presented for respiratory noise, exercise intolerance, cough and alimentary nasal discharge. Endoscopy showed a moderate soft palate defect and a laryngeal tie-forward was performed. At 4 months and 3 years after surgery a control endoscopy was done. In both horses postoperative endoscopy showed a visible reduction of the gap between soft palate and the larynx so that the epiglottis was covering the soft palate defect. One horse was examined 4 months after surgery, the other 3 years after surgery and a residual respiratory noise during exercise or some cough were still present but lighter than prior to intervention, their performances had remarkably improved. In both horses, initial symptoms significantly improved after surgery and owners were satisfied. Reduction of the soft palate defect was still effective, even several months after surgery. Our study shows that some horses can grow up to adult age with moderate defects of the soft palate with clinical signs of variable severity and laryngeal tie-forward seems an interesting treatment option.

15. Investigation of the nasal microbiota in healthy dolichicephalic dogs and dogs with sinonasal aspergillosis (SNA)

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Sinonasal aspergillosis (SNA) is characterised by a destructive rhino-sinusitis in the absence of fungal deeper tissue invasion. In humans a role of the bacterial microbiota in the regulation of host immune responsiveness to fungi has been hypothesised. The objective of our study was to characterise the microbiota present in nasal cavities of dogs with SNA compared with healthy dogs. Nine dolichocephalic dogs diagnosed with SNA and 10 healthy age-and breed-matched dogs were included. DNA was extracted from a sterile swab introduced in the nasal cavity under general anaesthesia. Metagenetic analysis was performed on V1-V3 hypervariable region of 16S rDNA after total bacterial DNA extraction from nasal specimens and sequencing on a MiSeq Illumina sequencer. Taxonomical assignation and microbiota community analysis were done with MOTHUR V1.35 with an OTU clustering distance of 0.03. Differences of population abundance between groups were assessed using multiple t tests with Holm-Sidak multi-test correction. Sequencing revealed that *Proteobacteria* and *Firmicutes* were the two most predominant phyla (80%) in both groups. The remaining 20% were composed of *Bacteroidetes* and *Fusobacteria* in diseased dogs, and of *Actinobacteria* almost exclusively in healthy dogs. At family level, a higher abundance of *Lactobacillaceae* were found in SNA dogs, while *Moraxellaceae* predominated in controls. Results of the present study demonstrated the presence of nasal microbiota alteration in dogs affected with SNA in association with an increased bacterial richness. Whether such changes are a cause or a consequence of the disease warrants further investigation.

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