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Proceedings of the 7th FARAH-Day

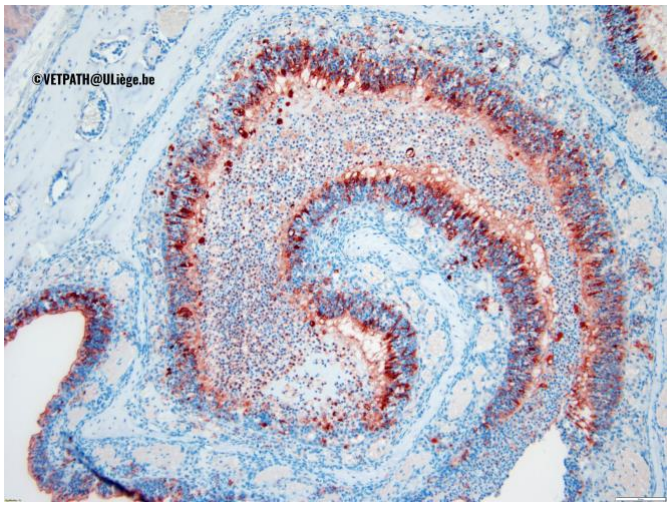
Faculty of Veterinary Medicine

(University of Liège - Belgium)

November 20, 2020

One Health

L'Animal et l'Homme, une même santé



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November 20, 2020

Edited by C. Bayrou, C. Cesarini Latorre, C. Delguste, C. Douny, C. Gatez, L. Gille, T. Jauniaux, V. Jergeay, L. Martinelle, J. Ponthier, A. Sartelet, D. Thiry, D.-M. Votion

Presses de la Faculté de Médecine vétérinaire de l'Université de Liège
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COVER PICTURE CREDITS:

"During the first hours after infection, the SARS-CoV-2 virus intensely replicates in the nasal turbinates, here in the hamster. Anti-nucleoprotein immunohistochemistry (red) counterstained with Mayer's hematoxylin (x10)."

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Welcome to the 7th 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 60 abstracts dedicated to fundamental, clinical and or applied researches.

Véronique Delcenserie, **Frédéric Farnir** & **Dominique Votion**.

Bienvenue à la 7ème 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 soixantaine de travaux ayant trait à la recherche fondamentale, clinique et/ou appliquée.

Véronique Delcenserie, **Frédéric Farnir** & **Dominique Votion**.

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Program

09:00 - Opening and Welcome Speech

09:05 - Etienne Baise Award

09:30 - Invited speaker

Prof. Thomas Marichal

"Lung myeloid cells: crucial regulators of homeostasis and immune-mediated diseases"

<u>10:00 - Oral session 1</u>

Chair: Bénédicte Machiels & Frédéric Farnir

10:00 ***Salem Djebala*** *- Infectious agents identified by real-time PCR, serology and bacteriology in blood and peritoneal exudate samples of cows affected by parietal fibrinous peritonitis after caesarean section*

10:10 ***Alphonse Dossou*** *- Estimation of Lead exposure of consumers of Wagashi Gassire (Peulh cheese) from southern Benin*

10:20 ***Céline Maquet*** *- Ly6Chi monocytes are key immunoregulators of gammaherpesvirus infection*

10:30 – Break

<u>10:50 - Oral session 2</u>

Chair: Marc Balligand & Jacques Mainil

10:50 Aline Fastrès - Identification of pro-fibrotic macrophages populations by single-cell transcriptomic analysis in West Highland white terriers affected with canine idiopathic pulmonary fibrosis.

11:00 Salem Djebala - Evaluation of the adequate timing to administrate an intramuscular prophylactic penicillin G (procaine benzylpenicillin suspension) in Belgian blue cow before the caesarean section realisation

11:10 Thibault Frippiat - In vitro assessment of the bactericidal effect of nebulized silver nanoparticles on Streptococcus equi subsp. zooepidemicus and Actinobacillus equuli subsp. equuli

11:20 Barbara Ghislain - Effectiveness of mowing or herbicidal spraying in the removal of Acer pseudoplatanus seedlings in an infected pasture to reduce risks of atypical myopathy for equids in spring

11:30 Lucie Gillard - Study of the importance of tegument proteins, and in particular of pORF63, in the biology of gammaherpesvirus infection.

11:40 Linde Gille - Retrospective analysis of intestinal obstruction in 83 calves: characteristics, survival rate and prognostic factors.

11:50 Meijiao Gong - Investigation of the role A10 gene in latently-infected CD8 T lymphocytes during bovine malignant catarrhal fever

12:00 – Lunch and poster session

<u>14:00 - Oral session 3</u>

Chair: Nicolas Korsak Koulagenko & Bernard Taminiau

14:00 ***Amira Preure*** *- A history of helminth exposure protects against pneumovirus infection in an IL-4Rα-independent manner*

14:10 ***Codjo Eustache Hounkpe*** *- Inventory of the production conditions of foods of animal origin sold in primary schools in the Mono department in Benin*

14:20 ***Maïlis Humbel*** *- Welfare evaluation of group-housed horses: preliminary results for active stables and tracks livery systems*

14:30 ***Valérie Jergeay*** *- Impact of diet and obesity on fecal microbiota in client-owned dogs: preliminary results*

14:40 ***Pauline Loos*** *- A gammaherpesvirus infection blocks the functionality of type 2 innate lymphoid cells in the context of HDM-induced airway allergy*

14:50 ***Louisa Ludwig-Begall*** *- The use of dry heat and methylene blue photochemical treatment to decontaminate face masks and filtering facepiece respirators inoculated with a SARS-CoV-2 surrogate virus*

15:00 ***Justine Eppe*** *- Treatment protocols and management of retained fetal membranes in cattle by rural practitioners in Belgium*

15:10 ***Thibault Frippiat*** *- Evaluation of the accuracy of a commercial heart rate monitoring system to detect RR-waves interval in Warmblood horses*

15:20 ***Constance Wielick*** *- The use of germicidal ultraviolet light, vaporised hydrogen peroxide and dry heat to decontaminate face masks and filtering respirators contaminated with a SARS-CoV-2 surrogate virus*

15:30 - Break

15:40 - Oral session 4

Chair: Véronique Delcenserie & Antoine Clinquart

15: 50 ***Irene Tosi*** *- Assessment of plasma acylcarnitine profiles in Alaskan sled dogs during submaximal multiday exercise.*

16:00 ***Bin Yang*** *- Investigating IL-4-dependent transcriptomic changes in CD8 T cells after exposure to helminth parasites at the single cell level*

16:10 – Invited speakers

Prof. Daniel Desmecht, Prof. Mutien-Marie Garigliany and Prof. Etienne Thiry

"About medical and veterinary interlocking worlds in COVID-19"

16:40 – COVID-19 initiatives at the Veterinary Faculty

Prof. Laurent Gillet

16: 55 - Awards and closing session

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Oral presentations

Infectious agents identified by real-time PCR, serology and bacteriology in blood and peritoneal exudate samples of cows affected by parietal fibrinous peritonitis after caesarean section

Djebala S.[1], Evrard J.[2], Gregoire F.[2], Thiry D.[3], Bayrou C.[1], Moula N.[4], Sartelet A.[1], Bossaert P.[1]

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The aim of the current study was to identify the pathogens that are potentially involved in parietal fibrinous peritonitis (PFP). PFP is a relatively common complication of cattle celiotomy, characterized by an accumulation of exudate in a fibrinous capsule within the abdominal or pelvic cavity; its aetiology is poorly understood. We have studied 72 cases of PFP, confirmed by a standard diagnostic protocol. Blood were collected to evaluate the presence of antibodies for *Mycoplasma bovis* (*M. bovis*), *Coxiella burnetii* (*C. burnetii*) and *Bovine Herpesvirus 4* (*BoHV4*) by enzyme-linked immunosorbent assays. Peritoneal exudate samples were obtained from the PFP cavity to perform bacteriological culture, and to identify the DNA of *M. bovis*, *C. burnetii* and *BoHV4* using real time polymerase chain reaction (qPCR). Bacteriological culture was positive in most of peritoneal samples (59/72); *Trueperella pyogenes* (*T. pyogenes*) (51/72) and *Escherichia coli* (*E. coli*) (20/72) were the most frequently identified. For *BoHV4*, the majority of cows showed a positive serology and qPCR result (56/72 and 49/72, respectively), in contrast to *M. bovis* (17/72 and 6/72, respectively) and *C. burnetii* (15/72 and 6/72, respectively), who were less frequently detected ($p<0.0001$). Our study proves that PFP can no longer be qualified as a sterile inflammation, since most PFP samples yielded a positive bacteriology and qPCR. Moreover, we herein describe the first identification of *BoHV4* and *C. burnetii* in cows affected by PFP. The exact role of these germs in the pathogenesis of PFP is not yet elucidated and requires further studies.

Estimation of Lead exposure of consumers of Wagashi Gassire (Peulh cheese) from southern Benin

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Most Beninese markets, especially those in the vicinity, are spaces where food products, often unpackaged, are exposed to the ambient air. In such environments, exhaust fumes and dust are sources of lead (Pb) contamination of foodstuffs. The main objective of this work is to predict the level of Pb exposure of Beninese adults through the consumption of Wagashi Gassire (WG) sold in markets in southern Benin. For this purpose, a sample of 15 WG including 9 red Wagashi Gassire (RWG) and 6 white Wagashi Gassire (WWG) were collected from three markets in Abomey-Calavi. Pb was determined by ICP-MS. The deterministic model was used to predict the level of exposure for an average consumption of WG. Results revealed the presence of Pb in all samples with median values of 0.048 mg/kg and 0.133 mg/kg for RWG and WWG respectively. For an adult of 60 kg body weight, the exposure is 0.16 µg/kg.bw/day and 0.44 µg/kg.bw/day for a daily consumption of 200 g RWG and WWG respectively. Thus, the margins of exposure are 9.4 (RWG) and 3.4 (WWG) for cardiovascular effects and 3.9 (RWG) and 1.4 (WWG) for nephrotoxic effects of Pb in adults. These data indicate an absence of risk. But they must be extended to all dietary sources of Pb so that measures can be taken to reduce its presence in the food chain.

Ly6C^{hi} monocytes are key immunoregulators of gammaherpesvirus infection

Maquet C.[1], Loos P.[1], Javaux J.[1], Mack M.[2], Gillet L.[1*] and Machiels B.[1*]

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Gammaherpesviruses (γHVs) represent highly prevalent human viruses as the best studied γHVs, Epstein-Barr virus and the Kaposi's Sarcoma-associated Herpesvirus, infect respectively some 90% and up to 40% of human populations. In most of the cases, these infections remain asymptomatic or associated with only mild-symptoms. Persistent viruses, such as γHVs, induce the development of immunomodulatory mechanisms avoiding the onset of a strong immune response by the host with potential deleterious effects. In some instances, persistent herpesvirus infections might even provide some benefits to the host. The understanding of mechanisms allowing setting a fine balance between the proinflammatory and immunomodulatory pathways induced by symbiotic viruses is of major importance while mechanisms underlying deleterious inflammation induced by highly pathogenic respiratory viruses are not well known yet. Indeed, a pulmonary infection with the Murid gammaherpesvirus 4 (MuHV-4), a γHV infecting mice, induces the recruitment of Ly6C^{hi} monocytes (MOs) from the bone marrow to the site of primary infection. In that context, we are investigating the role of Ly6C^{hi} MOs in the immune response against MuHV-4. By using complementary mouse models deficient for Ly6C^{hi} MOs, we highlighted the importance of Ly6C^{hi} MOs recruitment in the MuHV-4 lifecycle. Following pulmonary infection, the absence of Ly6C^{hi} MOs was associated with a stronger disease, higher levels of pro-inflammatory cytokines in blood and bronchoalveolar lavage and a modification of CD4 T cells population. We have shown that these recruited Ly6C^{hi} monocytes produce high levels of IL-10 suggesting regulatory properties and dampen inflammatory immune response. These results highlight that Ly6C^{hi} monocytes are key orchestrators of immune response following γHV infection.

Identification of pro-fibrotic macrophages populations by single-cell transcriptomic analysis in West Highland white terriers affected with canine idiopathic pulmonary fibrosis.

Fastrès A.[1], Pirottin D.[2], Fievez L.[2], Tutunaru A.C.[1], Bolen G.[1], Merveille A.C.[1], Marichal T.[2], Desmet J.C.[2], Bureau F.[2] and Clercx C.[1]
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Canine idiopathic pulmonary fibrosis (CIPF) is a not well understand disease which affects old West Highland white terriers (WHWTs) and mimics idiopathic pulmonary fibrosis (IPF) in man. Recent studies in IPF using the single-cell RNA sequencing (scRNA-seq) technique revealed the presence of profibrotic macrophages populations in the lung. Here we used the scRNA-seq to characterize disease-related heterogeneity within cell subsets of macrophages/monocytes (Ma/Mo) in the BALF of 5 WHWTs affected with CIPF in comparison with 3 healthy WHWTs. Five subsets of Ma/Mo were identified. Among them, a monocytes subset present in larger proportion in CIPF WHWTs showed a gene expression profile enriched for pulmonary fibrosis processes (PFPs) (normalized enrichment score (NES) = 1.85, q-value = 0.002). Eight genes associated with PFPs were significantly overexpressed in this subset including CCL2, SPP1, FN1, CCL3, TIMP1, IL1RN, CXCL8 and CCL4. A monocytes-derived macrophages subset enriched for PFPs (NES = 1.87, q-value = 0.007) was also identified with differentially expressed genes between CIPF and healthy WHWTs. Expression in CIPF dogs in this subset was enriched for PFPs (NES = 2.01, q-value = 0.008) with significant overexpression of 4 genes associated with PFPs including FN1, SPP1, CXCL8 and PLAU. ScRNA-seq analysis of BALF specimens from healthy and CIPF WHWTs identified pro-fibrotic Ma/Mo populations enriched in pro-fibrotic genes suggesting the implication of these subpopulations in CIPF processes. Overexpressed molecules were also identified that could be used as biomarkers and/or therapeutic targets in the future.

Oral presentations

Evaluation of the adequate timing to administrate an intramuscular prophylactic penicillin G (procaine benzylpenicillin suspension) in Belgian blue cow before the caesarean section realisation

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Antibiotics (AB) are widely used to limit infectious complications after caesarean section (CS), the most common surgery in Belgian bovine veterinary practice. Most vets administer AB, mainly benzylpenicillin procaine suspension (BPG), during or after CS. However, to maximise their efficiency, AB should be administered preoperatively, and result in plasma concentrations above the minimal inhibitory concentration (MIC) throughout the surgery. We aimed to determine the time after intramuscular (IM) injection of BPG necessary to reach sufficient plasma concentrations, in order to rationalize prophylactic AB use in CS. Twelve non-pregnant Belgian blue cows received an IM injection of BPG (21000 IU/kg). Blood samples were collected in heparinized tubes from a jugular catheter at -15, 15, 30, 45, 60, 120, 240 and 480 minutes relative to injection, centrifuged and stored at -80°C. Plasma BPG concentrations were measured by high performance liquid chromatography.
Although MIC depends on the AB molecule and the germ, plasma BPG levels of 500 ng/mL are generally considered to inhibit the majority of bacteria encountered during CS. At 15 minutes after injection, plasma BPG levels were 668 ± 255 ng/ml (374 to 898 ng/ml), exceeded 500 ng/mL in 8/12 cows, and were close to 500 ng/mL in the other 4/12 cows. Plasma BPG concentrations reached a maximum of 1495±629 ng/ml (919 to 2882 ng/ml) at 60 to 240 minutes after injection, and remained elevated until 480 minutes after injection (1002 ± 323 ng/ml; 667 - 1642 ng/ml).
In conclusion, IM injection of BPG 15 minutes before CS is sufficient to reach satisfactory BPG concentrations from the beginning to the end of surgery.

In vitro assessment of the bactericidal effect of nebulized silver nanoparticles on *Streptococcus equi* subsp. *zooepidemicus* and *Actinobacillus equuli* subsp. *equuli*

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Antimicrobial resistance is increasing in both human and veterinary medicine. Bacteria can be part of the etiology of respiratory disorders in horses. Antimicrobial actions of silver have been largely described and are currently used in veterinary therapeutic applications such as wound dressings. The aim of this study was to assess the *in vitro* bactericidal effects of nebulized silver nanoparticles (AgNP) on 2 common equine respiratory bacteria, *Streptococcus equi* subsp. *zooepidemicus* (*S. zooepidemicus*) *and Actinobacillus equuli* subsp. *equuli* (*A. equuli*). Minimum inhibitory concentration of AgNP was first determined. Consequently, bacterial growth inhibition was tested after instillation or nebulization of low and high concentrations of AgNP. The bacterial growth was poorly inhibited at low concentration and completely inhibited at high concentrations of instilled AgNP. The bacterial growth was completely inhibited after nebulization of low concentrations of AgNP for *A. equuli* and high concentrations of AgNP for *S. zooepidemicus*. Therefore, nebulization of AgNP could represent a new therapeutic way against bacterial respiratory disorders in horses. Further investigations are required to assess the *in vivo* potential of nebulized AgNP and its possible toxicity.

Effectiveness of mowing or herbicidal spraying in the removal of *Acer pseudoplatanus* seedlings in an infected pasture to reduce risks of atypical myopathy for equids in spring

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Equine atypical myopathy (AM) is a seasonal intoxication of equids kept in pasture. The toxins are present in autumn and spring in respectively seeds and seedlings of some *Acer* tree species, of which *Acer pseudoplatanus*. Due to their ingestion, the mortality is high: 74%. As no specific treatment is known so far, prevention is the best solution. To decrease the risks of AM in spring, 2 mowing dates and 3 products for herbicidal spraying were tested to remove the seedlings of *A. pseudoplatanus* from an infected pasture. The number of seedlings were counted and the toxicity of remaining seedlings, the flora, grass height and final harvest were measured to compare treatments in terms of effectiveness to reduce the risks of AM but also to compare their advantages and disadvantages in terms of pasture management. A strong natural disappearance of seedlings was observed in the control. Yet, all treatments were successful to significantly decrease *A. pseudoplatanus* seedlings from the infected pasture. Remaining seedlings were toxic in all treatments. The delay before putting equids in the pasture was longer for herbicidal spraying than for mowing. The flora was mainly affected by herbicidal spraying, with a significant cover decrease in plants other than grass. Productivity of the pasture was significantly decreased by the latest date of mowing, whereas it was not affected by first date of mowing or herbicidal spraying. We recommend mowing to reduce risks of AM in spring for the following reasons: low environmental impact, early treatment allows a quicker use of the pasture, later treatments are still effective, ease of implementation and no need to hold a phytolicense (Belgium).

Study of the importance of tegument proteins, and in particular of pORF63, in the biology of gammaherpesvirus infection.

Gillard L.[1], Maquet C.[1], Myster F.[1], Lemmens I.[2], Tavernier J.[2], Machiels B.[1] and Gillet L.[1]

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Gammaherpesviruses (γHVs) are widespread viruses that cause lifelong infections in many mammalian species and represent a significant cause of diseases. Thus, in humans, Kaposi's sarcoma-associated herpesvirus (KSHV) is associated with several cancers and is therefore a major subject of research. Among the different constituents of the γHVs virions, tegument proteins play major roles in virus entry, morphogenesis and egress but also in early evasion of innate immune recognition. In order to identify new roles of KSHV tegument proteins, we performed a screening of the protein interaction between KSHV tegument proteins and a pool of 18,000 human proteins using a mammalian protein-protein interaction trap approach (MAPPIT). This analysis identified 347 potential interactions between KSHV tegument proteins and cellular partners. Based on the generated data, we specifically studied, via the murid herpesvirus 4 (MuHV-4) model, the functional importance of the interaction between the protein product of ORF63 and the heat shock protein 90, a molecular chaperone involved in the replication of many viruses and which inhibition induces positive therapeutic effects on KSHV-induced tumors. These results could allow a better understanding of KSHV lifecycle and promote the development of new therapeutic strategies against this virus.

Oral presentations

Retrospective analysis of intestinal obstruction in 83 calves: characteristics, survival rate and prognostic factors.

Gille L.[1], H. Casalta[1], C. Bayrou[1], S. Djebala[1], J. Eppe[1], A. Sartelet[1]

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Intestinal obstruction due to intestinal volvulus or intussusception is a feared disease in cattle medicine. Exploratory laparotomy is the only valid treatment option but has a low cost-benefit ratio due to the poor prognosis. The aims of this retrospective study were to evaluate short- and long-term survival rate of calves affected by a small intestinal volvulus (SIV), intestinal intussusception (II) or ceco(colic) volvulus(CV) and to determine prognostic parameters aiding decision-making. In total, 83 cases referred between 2013 and 2020 were analyzed. Thirty-five female calves and 48 male calves were included, aged between 3-340 days. In total, 24 II, 26 SIV and 33 CV were included. Twenty-nine (35%) were discharged. Of these, 24 (82.8%) survived at least one month after surgery, and 19 (65.5 %) survived more than 6 months. An age effect on diagnosis was present: II (75 %, n=18) were significantly more frequent in the preweaning group ($p<0.05$), CV were more present in the older age group (69.7%, n=23). Hyper L-lactatemia (>2.2 mmol/l) (OR= 3.98, CI: [1.25-13.4] $p<0.05$) and peritoneal fluid visible on ultrasonography (OR=7.15, CI:[2.1-26.67]$p<0.05$) were significantly linked with death during hospitalization, whereas hyperglycemia (>75 mg/dl) was as a protective factor (OR=0.29, CI:[0.093-0.82]$p< 0.05$). Hyperkalemia (K >5) resulted in death during hospitalization in 14 out of 16 cases (87.5 %). Only hyper L-lactatemia remained predictive for death when looking at long-term survival. ***Conclusion:*** The long-term survival of intestinal volvulus or intussusception was 22.8%. Hyperlactatemia and presence of peritoneal fluid on ultrasound can be used as prognostic factors.

Investigation of the role A10 gene in latently-infected CD8 T lymphocytes during bovine malignant catarrhal fever

Gong M.[1], Myster F.[1], Javaux J.[1], Van Campe W.[2], Roels S.[2], Mostin L.[2], Kerkhofs P.[2], Vanderplasschen A.[1], Dewals B.G.[1]

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Alcelaphine herpesvirus 1 (AlHV-1) is a gammaherpesvirus (γHV) asymptomatically carried by wildebeests (Connochaetes sp.). Upon transmission to various ruminant species including cattle, AlHV-1 is responsible malignant catarrhal fever (MCF), a deadly disease caused by latently infected CD8 T cells and resulting in a peripheral T cell lymphoma (PTCL)-like disease through unknown mechanisms. RNA-seq analysis was performed on CD8+ T cells purified from mock-infected or MCF-developing calves. Gene-set enrichment analysis highlighted enrichment of genes involved in TCR signaling pathway, interferon responses, cell division and chromatin remodeling, providing new insights on how AlHV-1 infection affects the phenotype of latently-infected cells. Interestingly, analysis of the viral genome coverage of RNA expression identified the expression of a few viral genes in CD8+ T cells. Among them, the predicted ORF A10 was highly expressed. A10 putatively encodes a 50 kDa type II membrane protein displaying a cytoplasmic tail containing ITAM and SH3 domains. A10 is reminiscent of oncogenic proteins expressed by other γHVs (KSHV K15, EBV LMP2A and SaHV-2 Tip). Thus, A10 might be involved in reprogramming infected T cells, resulting in their uncontrolled activation. To study the role of A10 in MCF, two recombinant viruses impaired for A10 expression were produced using the AlHV-1 BAC clone. Both constructs were fully sequenced by MiSeq sequencing to verify their genomic integrity and viruses were recovered by transfection into bovine MacT-Cre cells to further characterize their growth kinetics and plaque size over time in vitro. In the future, we will investigate the role of A10 in vivo.

A history of helminth exposure protects against pneumovirus infection in an IL-4Rα-independent manner

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Infections with helminth parasites modulate the immune response during viral coinfection. Here we have investigated the impact of helminth infections on the immune control of lethal viral pneumonia. Balb/c mice were treated with *Schistosoma mansoni* eggs or infected with *Nippostrongylus brasiliensis* to induce type 2 inflammation in the lung. After helminth exposure, mice were infected intranasally with the Pneumonia virus of mice (PVM). PVM belongs the *Pneumoviridae* family which also comprises human and bovine respiratory syncytial virus (RSV), and are responsible for severe respiratory distresses. Based on daily clinical scores and weight changes, we observed that helminth-exposed mice retained stable health conditions and weight, in contrast to PVM infected mice where the infection was deleterious and associated with increased monocytes and neutrophils in the lung. We further observed reduced viral titers in the lung at 6 days after PVM infection in helminth exposed mice, suggesting a control of the viral infection. To monitor virus infection overtime, a recombinant virus expressing a luciferase gene upon infection was produced (PVM-luc). Daily measurements of bioluminescence signals upon viral infection confirmed the reduced infection levels in the lung of coinfected mice. *Il4ra*$^{-/-}$ mice were also protected when PVM infection occurred 6 or 35 days after *N. brasiliensis* infection, demonstrating that the acquired protection was independent of IL-4Rα signaling and derived type 2 immunity. Further investigations need to determine whether the observed protective phenotype is due to a direct viral control and/or to the regulation of PVM-associated detrimental inflammation.

Inventory of the production conditions of foods of animal origin sold in primary schools in the Mono department in Benin.

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Foods of animal origin are true sources of protein for human nutrition. They are also subject to microbial contamination. In order to evaluate the microbiological quality of food of animal origin sold to children in primary schools in the department of Mono in the south of the Republic of Benin, a survey was carried out among food sellers or producers, by taking into account the fact that children are at risk. The objective of the survey was to make an inventory of the food of animal origin sold to children, the sources of supply and the hygienic conditions in which they are produced. To this end, interviews were conducted with 137 primary schools using a questionnaire created on the online collection platform called Epicollect5. A smartphone was used for this purpose. The study shows that the majority of the actors involved in the production and sale of food to school students are women (100%). The female vendors are unschooled (39.39%), enrolled in primary school (57.58%) or secondary school (3.03%). As for the animal food sold, we noted three types including fish (100%), eggs (65.23%) and sausage (45.52%). The saleswomen stated that they buy the food at the market (100%). Overall, food producers do not follow good hygiene and manufacturing practices in the kitchens. For this reason, training should be organized for them to raise their awareness of good hygiene and manufacturing practices in the kitchens in order to guarantee safe food for school children.

Oral presentations

Welfare evaluation of group-housed horses: preliminary results for active stables and tracks livery systems

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Individual horse stabling is often associated with social, forage and movement restrictions with concerning consequences on horse health and welfare and human safety. Group housing solutions in designed outdoor areas, such as active stables and tracks livery, are currently getting attention. The welfare of 376 horses was compared in 4 types of housing (individual stable, pasture, active stable and tracks livery) between 2016 and 2019, using a modified version of the AWIN Horse protocol with 26 indicators and non-parametric Fischer and Kruskal-Wallis tests ($p<0.05$). Multiple indicators confirmed the restrictive nature of individual housing, with lack of social interaction, forage and daily free movement. Also, horse mental health and human-horse relationships seemed compromised. Pastured horse welfare was overall good with some concerns about human-horse relationship, hoof care and sheltering. Active stables and tracks livery had intermediate results; they provided a satisfying level of comfort, overall good health and adequate coverage of social, movement and foraging needs. They seemed to promote better human-horse relationship. However, some concerns were raised: horses tend to be overweighed and had a higher number of skin lesions seeming correlated with interactions frequency. Also, stereotypies were observed in active stables, mostly directed to the automatic feeding systems and more vigilance behaviours were observed in both active stables and tracks livery. Those behaviours could be associated with impaired welfare. Further research is needed to confirm these results in larger cohorts and to determine potential causes for the concerns raised in this study.

Impact of diet and obesity on fecal microbiota in client-owned dogs: preliminary results

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Introduction. In human, gut microbiota composition plays a role on the development of obesity and its comorbidities. This study investigated the impact of obesity and a specific diet on dogs' fecal microbiota composition. **Animals, material and methods.** Fifteen lean (LD) (BCS = 5/9) and 28 obese (OD) (BCS ≥ 7/9) adult Labradors and Golden Retrievers were recruited. All dogs received for one month the same diet whose composition was (%DM): proteins 35, nitrogen-free extract 33, lipids 11, ME 300kcal/100g. MER was estimated according to dog diet history. Fresh fecal samples were collected at enrollment (V1) and 1 month later (V2), and frozen at -80°C (in a DNA stabilizer) until extraction and sequencing of microbial DNA. Results are expressed as mean (±SD) or median (Q1 – Q3). **Results and discussion**. The study was completed by 41 dogs.

Table 1. Body weight (BW) and BCS

	LD	OD
BW (kg)	31 ± 5	41 ± 7
BCS	5	8 ± 1

Table 2. Evolution of the core microbiota at phylum level (expressed in relative percentage – no statistical significance).

	OD at V1	Δ V2-V1 OD	LD at V1	Δ V2-V1 LD
Bacteroidetes	34 (25 - 59)	+	68 (45 - 75)	-
Fusobacteria	30 (7 - 48)	-	14 (7 - 22)	-
Firmicutes	20 (5 - 30)	+	11 (6 - 24)	+

These results (not significant) were in accordance with previous studie, and the impact of diet seemed (not significant) to be stronger in OD, whose microbiota has been suggested to be less stable. **Conclusion.** This study failed to show a statistically significant influence of obesity or diet on dog's microbiota.

A gammaherpesvirus infection blocks the functionality of type 2 innate lymphoid cells in the context of HDM-induced airway allergy

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The "Hygiene hypothesis" postulates that allergies could be prevented by some infections in early childhood. Gammaherpesviruses (γHVs) are among the most prevalent human viruses, they infect in early age and profoundly imprint the immune system of their hosts. Using Murid herpesvirus 4 (MuHV-4), a mouse model of human γHV infections, our laboratory recently showed that γHV infection inhibits the development of allergic asthma trough the replacement of resident alveolar macrophages by regulatory monocytes. However, the mechanisms by which airway allergy is controlled in that context are still ill-defined. Here, we showed that group 2 innate lymphoid cells (ILC2s) are massively affected by MuHV-4 infection. In particular, the number of pulmonary ILC2s was decreased for the long term and these cells displayed reduced capacity to respond to type 2 stimuli. These modifications appeared to be related to the microenvironment and especially to IFNγ. However, in contrast with reports from other viral infections, ILC2s from MuHV-4 infected mice did not exhibit any characteristic of plasticity towards an ILC1 phenotype but displayed decreased expression of the canonical Th2 transcription factor GATA-3. Finally, single cell transcriptome analysis revealed some specific modifications in ILC2s from MuHV-4 infected mice. Altogether, our results show that persistent γHV infections profoundly impact the functionality and composition of lung ILC2s in a potential dialogue with monocytes/macrophages.

The use of dry heat and methylene blue photochemical treatment to decontaminate face masks and filtering facepiece respirators inoculated with a SARS-CoV-2 surrogate virus

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In the context of the ongoing SARS-CoV-2 pandemic, the supply of personal protective equipment (PPE) remains under strain. To address this issue, re-use of face masks and filtering respirators has been recommended. While prior decontamination is paramount to safe re-use of these typically single use only items, access to costly and/or cumbersome decontamination equipment may be complicated in developing countries. With the aim of validating a reliable, inexpensive, and universally implementable method for mask and respirator decontamination, we recently joined forces with a multidisciplinary international team of researchers (DeMaND) to investigate the efficacy of both dry heat and photochemical treatment with the thiazine dye methylene blue. We assessed decontamination of infectious porcine respiratory coronavirus (PRCV), a BSL2 SARS-CoV-2 surrogate, in conjunction with six different mask or respirator types. PRCV-inoculated masks or respirators were either exposed to 75°C for 60 minutes (denaturation of viral proteins) or were sprayed comprehensively with a 10µM methylene blue solution and exposed to 12400 LUX for 30 minutes in a TERRA Research Centre LED Light Box (induction of viral RNA-protein crosslinkage and release of singlet-state oxygen). Viruses were recovered from sample materials and viral titres were measured in swine testicle cells. Dry heat and methylene blue treatment achieved reductions of PRCV titres by more than three orders of magnitude for all six, and four of six types of face covering, respectively. We show that both dry heat and methylene blue photochemical treatment present promising approaches for widely applicable PPE decontamination.

Oral presentations

Treatment protocols and management of retained fetal membranes in cattle by rural practitioners in Belgium

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Retained foetal membranes (RFM) is a common and economically important postpartum disorder in cows and a frequent cause of antibiotic (AB) use. To rationalize AB use, practitioners should consider clinical studies as well as national regulations. Scientific opinion leaders currently discourage intrauterine (IU) AB use and recommend systemic AB treatment of cows with RFM when clinical illness appears. Remarkably, ceftiofur is often mentioned in literature for systemic treatment, although this is in conflict with national AB regulations. We aimed to assess the therapeutic approaches of RFM by Belgian rural veterinarians in light of scientific literature and national guidelines. A digital survey was sent to Belgian cattle vets; 149 questionnaires (Wallonia: 78; Flanders: 71) were completed. Questions addressed socio-demographic data, case definition, therapeutic approach and treatment options for RFM.
Results show that vets commonly use AB for RFM treatment, both in cows with (dairy: 98%; beef: 99.3%) and without fever (dairy: 64.4%; beef: 78.5%). AB are often applied via the IU route, alone or in combination with a systemic treatment, in cows with or without fever. β-lactams are by far the most frequently used AB class for systemic treatment. In conclusion, there is room for improvement considering AB treatment habits of RFM. Particularly, the benefit of AB therapy in clinically healthy cows and of IU applied AB is doubtful. As for the molecule choice, practical routines are in line with national AB guidelines. The fact that beef cows with RFM are treated more commonly than dairy cows may be explained by the fear of complications in an incised uterus.

Evaluation of the accuracy of a commercial heart rate monitoring system to detect RR-waves interval in Warmblood horses

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Heart rate (HR) is used in exercising horses for the evaluation and monitoring of exercise fitness. HR is usually acquired in the field using heart rate monitor (HRM) or telemetric electrocardiogram (ECG). While HRM is commonly available and easy to use for horse owners and veterinarians, ECG is a more expensive equipment requiring experience. More recently, interest of heart rate variability (HRV) is increasing for both research and clinical aims. Parameters of HRV in horses are usually calculated from the interbeat intervals (IBI) obtained by ECG. The aim of this study was to determine the accuracy of a commercial HRM to detect R-waves and obtain accurate IBI in both resting and exercising horses. Simultaneous ECG and HRM recordings of 4 eventing horses, 5 dressage horses and 4 leisure ponies were performed under normal conditions. The length of the recordings was minimum 45 minutes, including at least 5 minutes of rest, walk, trot, canter and show intensity. IBI from the HRM was exported and aberrant data was deleted. IBI from the ECG was corrected manually using the ECG software. Agreement and concordance of IBI and HRV-parameters between both systems was first determined. Effects of variables was consequently examined. A total of 29761 IBI were recorded, and 29562 (99,33%) of the HRM recordings were within the 95% confidence interval (-4; 3 ms) of the reference ECG-recordings, with a median of -1 ms. Discipline and HR showed effect on the results. The commercial HRM used in this study detected accurately IBI and could be of interest as easy-to-use device for obtaining HRV-parameters in resting and exercising Warmblood horses and ponies.

The use of germicidal ultraviolet light, vaporised hydrogen peroxide and dry heat to decontaminate face masks and filtering respirators contaminated with a SARS-CoV-2 surrogate virus

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In the context of the ongoing severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) pandemic, the supply of personal protective equipment remains under severe strain. To address this issue, re-use of surgical face masks (SMs) and filtering facepiece respirators (FFRs) has been recommended; prior decontamination is paramount to their re-use. Here, we provide information on the effects of three decontamination procedures on porcine respiratory coronavirus (PRCV)-contaminated SMs and FFRs, presenting a stable model for infectious coronavirus decontamination of these typically single-use-only products. SM and FFR coupons and straps were inoculated with infectious PRCV and submitted to three decontamination treatments, UV irradiation, vaporised H_2O_2, and dry heat treatment. Viruses were recovered from sample materials and viral titres were measured in swine testicle cells. The effect of multiple rounds of decontamination was also evaluated. UV irradiation, vaporised H_2O_2 and dry heat reduced infectious PRCV by more than three orders of magnitude on mask and respirator coupons and rendered it undetectable in almost all decontamination assays. This is the first description of stable disinfection of SMs and FFRs contaminated with an infectious SARS-CoV-2 surrogate using UV irradiation, vaporised H_2O_2 and dry heat treatment. The three methods permit demonstration of a loss of infectivity by more than three orders of magnitude of an infectious coronavirus in line with the FDA policy regarding face masks and respirators. PRCV presents advantages of uncomplicated manipulation and utilisation in BSL2 facilities, thus being easily adaptable to other respirator and mask types.

Assessment of plasma acylcarnitine profiles in Alaskan sled dogs during submaximal multiday exercise

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Alaskan sled dogs are elite endurance athletes, covering up to 200 km/day on multiday races without showing overt signs of fatigue. Their muscle fibers composition (mainly slow-twitch), their diet (fat-based) and their activity (submaximal, prolonged exercise) would point out fat as predominant energy fuel. However, after an initial depletion of glycogen and triglycerides, with continuation of exercise, sled dogs switch their reliance from intra- to extra-muscular sources. Specifically, they show an increased stimulus for hepatic glucose output, likely sustained by gluconeogenesis and glycogenolysis, aiming both at fueling exercise and replenishing the depleted muscle glycogen. Carnitine and acylcarnitines (AC) play an essential role as metabolic regulators in energy metabolism; they serve as biomarkers in both physiological and pathological conditions. We assessed the effect of multiday exercise on plasma short (SC), medium (MC) and long (LC) chain AC in conditioned sled dogs. Our results show a chain-specific modification of AC profiles: LCAC maintained a steady increase, some SCAC increased during the last phase of the race and acetylcarnitine (C2) initially increased to decrease constantly afterwards. We speculated that SCAC kinetics could reflect an increased protein catabolism, C2 pattern could reflect its hepatic uptake for energy-generating purposes to sustain gluconeogenesis. Concerning LCAC, they could be exported by muscle to avoid their accumulation or distributed by liver as energy substrates. These findings spread a new light on sled dogs metabolism that appears carbohydrates- rather than fat-dependent and liver-centric during prolonged submaximal exercise.

Oral presentations

Investigating IL-4-dependent transcriptomic changes in CD8 T cells after exposure to helminth parasites at the single cell level

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Helminth infection can modulate the immune response to concurrent infections. Recent work from the laboratory has demonstrated that IL-4 during helminth infection could expand virtual memory CD8 T cells (T_{VM}), leading to a subsequently raised antigen (Ag)-specific CD8 T cell activation that enhanced control of viral infection. T_{VM} are foreign Ag-inexperienced with a "memory-like" phenotype displaying an enhanced response to Ag compared to naïve T cells. Here, we investigated how IL-4 can specifically affect gene expression in CD8 T cells after exposure to helminths at the single cell level. After IL-4-complex treatment or *Heligmosomoides polygyrus* infection, single-cell RNA-seq was performed on $CD8^+$ T cells enriched from the spleens of WT or CD8α-specific IL-4Rα knockout Balb/c mice ($E8^{iCre}Il4ra^{lox/lox}$) (n= 5). Individual cell hashing was performed using Totalseq anti-CD45/MHC-I antibodies before performing 10X Genomics encapsulation and high-throughput sequencing. Six datasets were obtained (WT-naïve, WT-IL-4c, WT-Hp, KO-naïve, KO-IL-4c and KO-Hp). Following exclusion of doublets and contaminating non-CD8 T cells, a total of 33,553 cells were obtained allowing detection of ±30-50k reads and 2,000 genes per cell. SingleR analysis was performed to identify naïve, memory and effector T cells. Then, memory T cells were further separated with Seurat in 15 clusters, among which one cluster was significantly affected by treatment and genotype, with signature gene regulation of T_{VM} like *Eomes, Ctla2, Ccr2, Ccr5, Il2rb*, *Gzma* and *Itga4*. Future investigation will help us understand which pathways are involved in IL-4-restricted activation of T_{VM} in the periphery.

Posters

Veterinary Public Health

1. Assessment of the efficiency of the phage PEV2 against *Pseudomonas aeruginosa* by titration in the *Galleria mellonella* model

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Pseudomonas aeruginosa is a ubiquitous gram-negative bacteria forming biofilms and living in soil and water. *P. aeruginosa* is the first bacterial species isolated from dog's ear canal that cause secondary infections during chronic otitis. The management and treatment of *P. aeruginosa* in dog otitis is particularly complicated given the high antimicrobial resistance. The aim of this work is to evaluate the efficacy of one bacteriophage (PEV2) against a clinical isolate of *P. aeruginosa* using an in vivo model of *Galleria mellonella* larvae. A preliminary experiment was performed to assess the optimal inoculation dose of *P. aeruginosa*. In a second experiment, the optimal multiplicity of infection (MOI) was assessed by injecting larvae with the optimal inoculation dose and 4 different MOI (50000, 5000, 500, 50). The MOI 5000 and 50000 were selected for the main experiment, which consisted in monitoring the evolution of the concentration of phages and bacteria contained into the larvae. For this experiment, 6 groups of 10 larvae were injected. All groups were separately mixed and the titration of *P. aeruginosa* and PEV2 was performed every 24h during 72h. An increase in phage titer was observed after 24h and 48h with both MOI (5000 and 50000). At the same time, there was a smaller increase in the titer of *P. aeruginosa* after 24h for MOI 50000 compared to 5000. The *P. aeruginosa* titers were lower with MOI 50000 and 5000 compared to the positive control. No phage was detected in the phage control group. These results show that PEV2 is active against *P. aeruginosa* in an in vivo model, *Galleria mellonella*, even if it did not result in an elimination of bacteria at the MOI tested.

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2. 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 of bovine origin, and a major bovine milk oligosaccharide (BMO), the 3'-sialyllactose (3'SL), modulated virulence gene 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 gene expression 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.

3. Infection of European eel by Anguillid herpesvirus 1: social interactions of yellow eels contribute to viral transmission through biting

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Over the last few decades, the number of European eel (*Anguilla anguilla*) reaching Europe has declined by 99%. Infection caused by Anguillid herpesvirus 1 (AngHV-1) is thought to play a key role in this decline. The present study aimed to investigate the pathogenesis of AngHV-1 in his natural host using *in vivo* bioluminescent imaging. With that goal in mind, we produced a recombinant strain (hereafter called LucGFP strain) encoding a luciferase-GFP reporter expression cassette. Using this recombinant, we first investigated the sensitivity of different developmental stages of European eel (glass eels, elvers and yellow eels) after inoculation by immersion in water containing AngHV-1. These experiments led to the following observations: (i) Glass ells are not sensitive to AngHV-1 infection through the natural route tested. (ii) Inoculation of elvers led to few positive subjects expressing bioluminescence on the gills and jaws with no spreading of the infection between subjects according to time. (iii) Inoculation of yellow eels led to infection of all subjects expressing bioluminescence signal on the gills, periodontal mucosa and skin foci mainly distributed on the tail and the head. These observations suggested that the gills and the periodontal mucosa represent portals of entry of the virus into naïve subjects and that biting could also contributes to transmission of the virus between subjects. These hypotheses were tested using two experimental models. First, yellow eels were infected by immersion in infectious water than kept in group or isolated. This experiment demonstrated that skin foci of bioluminescence were observed only on eels housed in group. Second, infected eels expressing bioluminescent signals on the periodontal mucosa were used to experimentally bite naïve subjects. Inversely, naïve eels were used to experimentally bite the skin of infected subjects. These experiments demonstrated efficient transmission of the infection in both ways: from infected mouth-to-naïve skin and from infected skin-to-naïve mouth. These experiments also demonstrated that transmission through biting leads to the induction of a systemic infection comparable to the one observed after inoculation by immersion in infectious water. Together the results of the present study demonstrated two modes of transmission of AngHV-1 relying on the role of water as an abiotic vector and on bites between subjects.

4. Treatment with a CXCR2 antagonist imprints mouse lungs to prevent key inflammatory features of house dust mite-induced allergic asthma

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The lung is a vital organ that supports breathing, must protect the host against inhaled pathogens and toxins, without mounting a disproportionate response against harmless allergens like house dust mite (HDM), underlying allergic asthma development. The lung is full of immune cells, including circulating neutrophils retained in the microvasculature and interacting with endothelial cells. Moreover, neutrophils are now thought to contribute to a wide range of physiological processes and have the ability to regulate immune responses. Yet much remains enigmatic about the functions and fate of neutrophils in healthy naive lungs. Here, we found that treatment with an antagonist of the receptor CXCR2 (α-CXCR2), a major player of neutrophil recruitment, 2 weeks before sensitization with HDM, protects mice against airway eosinophilia and lung inflammatory cell recruitment in HDM-challenged asthmatic mice. Intriguingly, mucus production by epithelial cells and the type 2 immune response profile was not affected by α-CXCR2 treatment in asthmatic mice. Twenty-for hours after HDM, the recruitment of monocytes and the numbers of monocytes-derived cells were drastically decreased in mice treated with α-CXCR2 two weeks before, while the levels of *Gmcsf* mRNA in epithelial cells were increased in α-CXCR2 mice as compared to controls, suggestive of defects in endothelial cells rather than in epithelial cells induced by α-CXCR2. We will now investigate the dependency of these results on neutrophil responses, as well as neutrophil-mediated imprinting of the lung microenvironment.

5. Detection of CTX-M-1, CTX-M-2 and CTX-M-9 extended-spectrum-β-lactamase-encoding genes with triplex PCR in bovine *Escherichia coli* isolates collected during 3 calving seasons

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Extended-spectrum-β-lactamase (ESBL) confer a resistance to some antibiotics classified as critical in human medicine, like 3rd/4th generation cephalosporins. It remains important to follow the evolution of ESBL resistance, especially in enterobacteria, since the use of critical antibiotics in livestock is regulated (2016). Therefore, the aim of the study was to identify the ESBL-encoding genes in *Escherichia (E.) coli* from young calves with an ESBL profile at the disk diffusion assay. During 3 calving seasons (A: 2017-2018; B: 2018-2019; C: 2019-2020), 158(A), 152(B) and 91(C) ESBL *E. coli* were collected at ARSIA from calves with enteritis or septicaemia. Based on previous study with microarrays, where only *bla*$_{CTX-M}$ genes, coding for cefotaximases, were detected, all 401 *E. coli* of the collection were tested with PCR for the different genes coding for the CTX-M-1, CTX-M-2 and CTX-M-9 groups. The results showed that the CTX-M-1 group is the most prevalent with 101(A), 84(B) and 61(C) positive isolates, followed by the CTX-M-2 and the CTX-M-9 groups (with respectively 26(A), 24(B), 14(C) and 23(A), 37(B), 14(C) positive isolates). Only 4(A) and 6(B) isolates were negative. Genes coding for CTX-M-1 and CTX-M-2 groups were simultaneously detected in 4(A), 1(B) and 1(C) isolates, like CTX-M-2 and CTX-M-9 in 1(C) isolate. To conclude, the *bla*$_{CTX-M}$ genes are the most prevalent ESBL-encoding genes in our collection and these coding for the CTX-M-1 group come in first, as described in the literature. The PCR-negative *E. coli* will be further tested for others ESBL-encoding genes, if any. Moreover, a large study with whole genome sequencing is already planned for next months.

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6. Genomic analysis of several new Anguillid-herpesvirus-1 isolates reveals the first insights into the evolution of this virus and suggests that known Cyprinivirus species have much lower core gene diversity compared to Herpesviridae species

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Anguillid herpesvirus-1 (AngHV-1) belongs to the *Cyprinivirus* genus within the *Alloherpesviridae* family and is the causative agent of haemorrhagic disease in European eels (*Anguilla anguilla*) and Japanese eels (*Anguilla japonica*). To date, the genomes sequence of only two AngHV-1 isolates have been published. In this study, we sequenced seven additional AngHV-1 isolates of different geographical origin, revealing low genomic variability and two main genetic lineages among isolates. Further analysis indicated five putative recombination events between these isolates, one of which suggested the existence of a third, yet unidentified lineage. The addional genomic data was also utilized in the comparison of core genes to other *Cyprinivirus* and *Herpesviridae* species. We observed significantly lower core gene diversity among *Cyprinivirus* species, which we speculate might be due to more recent divergence times or lower evolutionary rates of these species. With a view to generating tentative estimates for these, we explored whether models of *Cyprinivirus* evolution that involve the calibration (or dating) of internal nodes under assumption of co-speciation with hosts, could explain the observed patterns diversity while also being compatible with existing hypotheses regarding when these *Cyprinivirus* species emerged. Ultimately, we found estimates to be implausible, which may be principally due to lack of support for co-speciation assumptions. However, deriving purely relative estimates in the absence of such assumptions indicates that the AngHV-1 diverged earlier and has a lower evolutionary rate relative to that of other *Cyprinivirus* species, which also corresponds to differences we observed in selective pressure. Notably, these observations are also remarkably congruent with expected differences in epidemiology between AngHV-1 and other *Cyprinivirus* species, which itself may be reflective of the fact that AngHV-1 is an anguillid pathogen, and thus unique among *Cyprinivirus* species. Collectively, this represents the first insights into the evolution of AngHV-1 relative to that of other *Cyprinivirus* and *Herpesviridae* species.

7. Determination of pesticide residues in Tunisian wine grapes by LC-MS/MS and GC-MS/MS

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Pesticides are one of the most popular solutions to manage vine phytosanitary problems. A recent study carried out on Tunisian table grapes shown that the current use of these chemicals induced residues detection at levels above the European maximum residue level (MRL). This study aims to evaluate pesticides residue levels in Tunisian wine grapes. Fifty-three wine grapes samples were collected from two major Tunisian wine grapes producing regions (Grombalia and Bouargoub), then homogenized, extracted and analyzed by either liquid (LC) or gas (GC) chromatography (depending on the target molecule) coupled to tandem mass spectrometry (MS/MS). About 64% of the samples contain at least one active substance belonging majorly (65%) to the fungicides class. The main detected fungicides are boscalid, thiophanate-methyl, fenhexamid, carbendazim, iprovalicarb, and metalaxyl at concentration between 0.01 and 0.7 mg kg^{-1}. The insecticide imidacloprid was detected in 13 samples at levels below the MRL. Besides, 32% of the samples exceed the MRL for at least one active substance. This exceedance was noticed for 4 active substances: iprodione (MRL = 0.01 mg $kg^{-1)}$ (3 samples between 0.06 and 0.3 mg kg^{-1}), chlorpyriphos ethyl (MRL= 0.01 mg kg^{-1}) (10 between 0.01 and 0.15 mg kg^{-1}), fluazifop (a sample at 0.013 mg kg^{-1}; MRL = 0.01 mg kg^{-1}) and omethoate (a sample at 0.02 mg kg^{-1}; MRL = 0.01 mg kg^{-1}). These first results show a part of the pesticides applied to Tunisian wine grapes thanks to their presence in the fresh wine grapes at harvest. The number of pesticides detection as well as the number of MRL exceedance seem to be higher in the Tunisian table grapes than in Tunisian wine grapes.

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8. Efficacy assessment of *PEV2* phage on *Galleria mellonella* larvae survival after inoculation with a *Pseudomonas aeruginosa* dog otitis isolate

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Dog external otitis is a worrying problem in veterinary medicine due to the emergence of antibiotic resistance. Phage therapy is put forward as a promising alternative or addition to the current anti-microbials. In this context, the efficacy of *PEV2* phage against a *Pseudomonas aeruginosa* strain, isolated from a dog otitis, was assessed in an *in vivo Galleria mellonella* larvae survival model. To study the impact of *PEV2* on the survival of the infected larvae, the optimal *P. aeruginosa* inoculation dose, resulting in the death of 90% of the larvae within 4 days, was assessed in a preliminary experiment. A total of 210 larvae were inoculated with different bacterial dilutions and the optimal inoculation dose was found to be 4 CFU/4µl. In the main experiment, 270 infected larvae were injected either with different concentrations of *PEV2* or with marbofloxacin. Larvae survival was checked at 24, 48, 72 and 96h post inoculation. Survival curves were generated with R Commander software. No significant survival improvement was observed with *PEV2* therapy in the infected larvae groups. Indeed, the generated Kaplan-Meier curves showed that the rate of living larvae was significantly higher in the control groups compared to the infected-treated group. The only exception was the group with infected larvae treated with marbofloxacin, in which the survival rate was higher than for the other infected larvae groups. These results show an ineffectiveness of *PEV2* phage on the survival of *Galleria mellonella* larvae infected with *P. aeruginosa*. One hypothesis could be that the phage is able to kill the bacteria but the bacterial lysis release some toxic materials such as endotoxins.

9. Two SNP alleles encoded by Cyprinid herpesvirus 3 ORF131 are subject to antagonistic selection in cell culture and in natural environment with one variant contributing to the negative selection of the other

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Cyprinid herpesvirus 3 (CyHV-3) is a member of the *Alloherpesviridae* family and the causative agent of a lethal disease in common and koi carp. When comparing the replication of seven CyHV-3 strains in cell culture, we observed that only three of them expressed syncytia. Interestingly, these three strains do not share a monophyletic origin. Syncytium formation by herpesviruses is mediated by expression of virion transmembrane proteins (VTP) on the surface of infected cells. Analysis of VTP sequences among CyHV-3 strains revealed that the three strains forming syncytia shared mutations in both ORF27 and ORF131. Mutations in ORF27 varied between the three strains but were systematically associated with the disruption of the ORF. In contrast, the three strains expressing syncytia encoded the same SNP causing one amino acid substitution in pORF131 at position 183 with Alanine (A) and Threonine (T) correlating with syncytium and non-syncytium phenotypes, respectively. Through the production of CyHV-3 recombinant strains, we demonstrated that ORF131 SNP A determines the syncytium phenotype. The recombinants produced were also used to compare the viral fitness associated with the two ORF131 SNPs both *in vitro* and *in vivo*. These experiments led to the following observations: (i) Compared to ORF131 SNP T, SNP A was associated with a higher CyHV-3 fitness in cell culture (higher production titer and larger plaque size). (ii) Co-infection experiments in cell culture demonstrated that the SNP A genotype rapidly out competes SNP T over several passages. (iii) The higher fitness of SNP A over T in co-infection experiments *in vitro* was demonstrated to partially rely on an increased ability of SNP A to prevent superinfection of infected cells. (iv) *In vivo* experiments revealed that SNP T was associated with a higher virulence (higher morbidity, higher mortality and higher viral charge) than SNP A. (v) Interestingly, SNP T was shown to inhibit superinfection more rapidly than SNP A *in vivo*. Together, the results of this study demonstrated that CyHV-3 ORF131 SNP A and T are subject to antagonistic selection *in vitro* and *in vivo* with the fittest variant contributing to the negative selection of the other.

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10. The use of germicidal ultraviolet light, vaporised hydrogen peroxide and dry heat to decontaminate face masks and filtering respirators contaminated with an infectious norovirus

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In the context of the ongoing severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) pandemic, reuse of surgical masks and filtering facepiece respirators has been recommended. Their reuse necessitates procedures to inactivate contaminating human respiratory and oral pathogens. We previously demonstrated decontamination of masks and respirators contaminated with an infectious SARS-CoV-2 surrogate via ultraviolet germicidal irradiation, vaporised hydrogen peroxide, and use of dry heat. In the present investigation, coupons and straps from masks and respirators were inoculated with infectious murine norovirus (MuNoV) and submitted to the same three decontamination treatments. The effect of two to four rounds of decontamination was also evaluated. We show that these same methods efficiently inactivate MuNoV, a more resistant, non-enveloped oral virus. All three methods permit demonstration of a loss of viral infectivity by more than three orders of magnitude in line with the FDA policy regarding face masks and respirators. Inactivation of a norovirus, the most resistant of the respiratory and oral human viruses, can predict the inactivation of any less resistant viral mask or respirator contaminant.

Posters

11. Role of neuro-modulated neutrophils in lung repair

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Tissue repair and remodelling of injured lung represent crucial steps to restore respiratory function. Such processes are tightly regulated and highly dependent on cytokines and growth factors secreted by immune cells. Abnormalities in such immune pathways result in defects in the resolution of wound-healing responses and the accumulation of fibrotic damage that chronically affects lungs' compliance and oxygen permeability. Respiratory viral infections such as SARS-Cov-2 or influenza infections are known to activate fibrogenic pathways, although the mechanisms involved remain unclear. While the nervous system has been found to modulate immune response and wound healing, its relationship with lung repair is not completely understood. Here, we show that influenza virus infection leads to long-lasting increase in nerve fibers and in the activity of nociceptors, i.e. the nerves involved in pain signaling. Moreover, single cell RNA sequencing and flow cytometry analyses highlighted a cluster of neutrophils exclusively present in the lungs of mice infected with influenza virus 15 days before. These neutrophils exhibit a unique phenotype characterised by high expression of MHC-II and the co-stimulatory molecules CD80 and CD86, as well as of proteins involved in tissue repair and angiogenesis (TGFbeta1, VEGFR-1), suggesting that neutrophils participate in lung repair. Finally, in influenza-infected mice, resiniferatoxin ablation of nociceptor resulted in a significant loss of this particular phenotype and in impaired remodelling responses. These data support that neuro-immune crosstalk may play an important role in lung repair and remodelling by regulating neutrophil functions.

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12. Isolation of Lactococcus garvieae from a Dog with Chronic Respiratory Disorders: Genetic Comparison with Human, Animal and Environmental Isolates

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Described in 1986, *Lactococcus* (*L.) garvieae* is a zoonotic fish pathogen that can cause bacteraemia and endocarditis in humans and has been isolated from healthy or diseased domestic animals.
The aims of this study were to (i) identify to *L. garvieae* one isolate from a dog with chronic respiratory disorders; and (ii) compare this canine isolate with fish, human and other animal *L. garvieae* isolates after Whole Genome Sequencing. Bacchus, a 16 year-old male dog suffered from chronic upper and lower respiratory problems. All treatments failed and the dog was finally euthanized. The nasal discharge was sampled and an abundant and mixed growth was obtained on Columbia and Edwards' agars with a majority of greyish alpha-haemolytic 1-mm wide colonies representing 80% of the culture. These colonies were identified to *L. garvieae* by MALDI-TOF and WGS. A phylogenetic tree builded on the 34 *L. garvieae* genomes available in GenBank and based on MLST genes showed that all isolates are distributed within 4 gene clusters (GC) and that Bacchus isolate belongs to the GC1. The Bacchus isolate was more closely related to a camel isolate from Australia and to a fish form India. No capsule encoding genes was detected, raising the question of the role of the capsule in the virulence. Several putative virulence genes were detected and no gene related to the different levels of resistance to beta-lactams or to clindamycin could be identified by RAST annotation. This study reports the presence of a *L. garvieae* in dog and allows, for the first time, to study the full genome of a dog isolate. Further studies are needed to conclude on the zoonotic potential of this isolate.

13. The N-terminal region of Cyprinid herpesvirus 3 ORF104 inhibits the formation of stress granules induced by various stress stimuli

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Eukaryotic cells express specific mechanisms that block protein synthesis when facing various stress stimuli including viral infections. Stress granules (SG) are cytoplasmic inclusions into which stalled translation initiation complexes are dynamically recruited in cells subjected to stresses. Different types of stress (e.g. oxidative stress induced experimentally by arsenite, heat or viral infection) induce SG formation through the eIF2α-dependent pathway (via activation of five different eIF2α kinases: GCN2, PKR, PKZ, PERK and HRI) or through the eIF2α-independent pathways involving numerous other factors.
Cyprinid herpesvirus 3 (CyHV-3) is a member of the *Alloherpesviridae* family infecting common and koi carp. Recently, we observed that CyHV-3 inhibits the formation of SGs induced by arsenite treatment in infected cells. Using BAC cloning recombination technologies, we identified CyHV-3 ORF104 as one of the two proteins responsible for the inhibition. ORF104 protein consists on a N-terminal (Nter) intrinsically disordered region and a C-terminal kinase domain. The N-terminal region contains a NLS and a NES suggesting that ORF104 protein is able of nuclear-cytoplasmic shuttling. To unravel the mechanism of action of ORF104, we performed ectopic expression of ORF104 or recombinant forms of ORF104 in Hela cells. These experiments led to the following observations: (i) Cytoplasmic but not nuclear expression of ORF104 inhibits arsenite induced SGs. (ii) Cytoplasmic expression of ORF104 Nter domain is sufficient to inhibit arsenite induced SGs. (iii) Expression of ORF104-Nter domain fused to two 2 NES (ORF104-2NES-Nter) further increases the inhibition of arsenite induced SGs. (iv) Expression of ORF104-Nter domain in which the NLS has been deleted is no longer able to inhibit the formation of arsenite induced SGs. (v) Hela cells expressing ORF104-2NES-Nter were submitted to various stress stimuli such as heat shock, dithiothreitol (DTT) and poly I:C treatment, which active HRI, GCN2 and PKR, respectively. Interestingly, ORF104-2NES-Nter inhibited SG formation induced by all these various stimuli. Together, the results suggest that CyHV-3 ORF114 Nter domain inhibits SG formation by a mechanism acting on the common pathway downstream of the activation of the eIF2α kinases: GCN2, PKR, and HRI or even further downstream on the common pathway between the eIF2α-dependent and the eIF2α-independent pathways.

14. Photoconvertible "green to red" fluorescent proteins: a powerful tool to track the intracellular mobility of Cyprinid herpesvirus 3 ORF112 protein in living cells

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Dendra2 and mEos3.2 are photoconvertible "green to red" monomeric fluorescent proteins derived from corals. Neosynthesized proteins express green fluorescence when excited at 488 nm. Interestingly, their exposure to 405 nm light induces irreversible "green to red" fluorescence conversion. Converted molecules emits red fluorescence in response to 568 nm excitation. Here, we investigated the use of Dendra2 and mEos3.2 as tools to track the intracellular mobility of the ORF112 protein encoded by Cyprinid herpesvirus 3 (CyHV-3). ORF112 is essential for replication of CyHV-3 in cell culture. Subcellular localization of ORF112 in infected cells evolves according to the time post-infection from a nucleolic distribution, to multifocal nucleoplasmic foci and finally cytoplasmic aggregates. First, we investigated the possibility to express photoconvertible fusion proteins consisting of Dendra2 or mEOs3.2 fused to the N-term of CyHV-3 ORF112 (hereafter called, Dendra-ORF112 and mEOs-ORF112, respectively). Ectopic expression demonstrated that both constructions led to photoconvertible proteins exhibiting a subcellular localization similar to wild type ORF112. Next, we aimed to produce recombinant strains of CyHV-3 expressing Dendra-ORF112 or mEOs-ORF112 instead of wild type ORF112. We reached this goal using BAC cloning technology and recombination in eukaryotic cells. For both constructions, we were able to produce a recombinant strain expressing the fusion protein thereby demonstrating that the fluorescent tags did not affect the essential functions of ORF112. Subcellular localization of Dendra-ORF112 and mEOs-ORF112 expressed in infected cells was similar to wild type ORF112. Together, the results described above strongly support the use of the CyHV-3 recombinants produced to track the subcellular mobility of ORF112 in living cells.

Sustainable livestock production

15. Effect of dried mealworms (*Tenebrio molitor*) larvae and olive leaves (*Olea Europeae L.*) on growth performance, carcass yield and some blood parameters of Japanese quail

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The aim of this study was to investigate the effect of *Tenebrio molitor* (TM) and/or olive leaves (OL) supplementation to the diet of quail on their growth performance, carcass yield and some blood parameters. One hundred and forty-four 1-day-old Japanese quails (body weight: 29.9±0.46 g, mean ± SE) were divided into four groups of 36 chicks, with three replications. The chicks in Group 1 were fed with a standard commercial diet (Diet1); Group 2 received the Diet1 diluted with 3% replacement of dried *Tenebrio molitor* (TM) larvae (Diet2); Group 3 received the Diet1 with 3% of olive leaves (Diet3); and Group 4 received the Diet1 with 3% of TM and 2% t of OL (Diet4). Feed and water were provided *ad libitum*.
The results showed that TM and OL inclusion promoted growth performance of quails at 5 weeks of age (205.0 vs. <192g; P=0.001). Feed conversion ratio (FCR) of the group 3 was significantly (P <0.01) reduced compared to the others groups (2.78 vs. >3). The overall mortality was not significantly different (at around 6.25%; P>0.05) for any of the dietary regimens. Carcass [71.5-74.3%], gizzard [2.5-2.7%], heart [1.1-1.2%] and giblets [6.8-6.9%] yields were not significantly (P>0.05) influenced by the diet. No significant effect of the diet was observed for blood constituents (TP: 3.0-3.2 g/dL; Alb: 1.3-1.4 g/dL; Glob: 1.6-1.7 g/dL; Alb/Glob: 0.76-0.80%; Creatinine: 0.25-0.28mg/dL; Urea: 6.53-6.81g/dL) and lipid profile (TC: 190.7-197.0mg/dL; TG: 214.6-220.2mg/dL; HDL: 54.3-56.1mg/dL; LDL: 91.6-99.1mg/dL; VLDL: 42.4-45.1mg/dL).
In conclusion, the results demonstrated that the supplementation with TM (3%) and OL (2%) of quail diet improve body weight at 5 weeks old, reduce FCR and did not negatively influence carcass yield and blood parameters of Japanese quail.

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16. Sperm morphology and motility parameters of wild Barbary macaque, *Macaca sylvanus*, in the National Park of Gouraya in Algeria, preliminary results.

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Barbary macaque is an endangered species of primate with declining wild populations. Understanding the reproduction of this species are important information in conservation approaches. The current knowledge on the reproduction of Barbary macaque is limited to behavioral and endocrine reports. The present work aims to analyze, for the first time, sperm parameter in wild Barbary macaques including motility values and morphometry values of individual spermatozoon. Semen was collected by a non-invasive method after a copulation event at Gouraya National Park (Bejaia, Algeria). The seminal coagulum ejaculated externally of three wild adult individuals was collected from the ground in an Eppendorf using forceps and stored at environmental temperature during transport to laboratory. Then, the samples were kept at 37°C for 1 hour to allow liquefaction. The seminal coagulum was washed with 600 µL of sodium chloride solution at 0.9%. Sperm motility parameters were evaluated using the computer sperm analyzer system. Photomicrographs were taken at a magnification of 1000× using a microscope equipped with a camera to analyze morphometry parameters. Linear dimensions were measured using ImageJ 1.47 software. The results of the mobility analysis were as follows: VCL = 26.92 µm/s, VSL= 8.28 µm/s, VAP= 14.12 µm/s, ALH= 1.66 µm, BCF= 2.03 Hz. Morphometric analysis (in µm) showed the following results: head area= 117.11, head length= 5.8, head width= 3.3, midpiece length=12.1 and tail length=47.3. This study provides the first preliminary data on the sperm of Barbary macaque monkeys. More studies are needed to better understand male fertility in this species, particularly in relation to anthropogenic impacts.

Posters

17. Daily grazing area of goats browsing a North Moroccan forest rangeland

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In the Southern Mediterranean areas, especially in Northern Morocco, goats are exclusively or partially conducted in extensive farming systems in which most of their forage resources provide from woody pastures. Effective management of goats grazing on these heterogeneous forest rangelands requires a clear understanding of the spatial distribution patterns of animals. The current research was conducted in forested rangelands of Chefchaouen, Northern Morocco, which was grazed mainly by goats. The daily pattern of grazing areas of goats was characterized for three days during each contrasting grazing season (spring and summer). In each season, eight goats were randomly selected and fitted with GPS collars. The daily grazing area was calculated using Kernel analysis. During spring, goats tended to select lower elevation feeding stations and traveled shorter distances, whereas they tended to select higher elevation feeding station sites and traveled longer distances during summer. The daily grazing area explored by goats during summer was on average 8 ha larger than that recorded during spring. Seasonal variation in the daily grazing area of goats was inversely related to the forage availability. The interaction of these findings with several parameters of the feeding behavior of goats will allow us, in the future, to understand the grazing behavior of goats better.

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18. Nutritional composition, *in vitro* digestibility, and metabolizable energy of shrubs grazed by goats in a North Moroccan forest rangeland

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Woody species contribute mainly to the diet of grazing goats browsing in forested rangelands of Northern Morocco. Chemical composition, *in vitro* digestibility, and metabolizable energy (ME) of shrubs, the most selected by goats, were studied during three seasons (spring, summer, and autumn). Three independent samples of each consumed parts of shrubs were analyzed. The nutritive quality of shrubs content fluctuated among seasons and species. The dry matter (DM) ranged between 227 and 691 g DM /kg plant, and the highest values were observed in the dry season while the Organic Matter content recorded more than 848 g/kg DM. The Crude Protein level varied from 53 g/kg DM for *E. arborea* in summer to 196 g/kg DM for *C. villosa* in autumn. The highest Neutral Detergent Fiber (NDF) and Acid Detergent Fiber (ADF) contents were recorded in *C. villosa* during the three seasons and the highest Acid Detergent Lignin (ADL) content in *E. arborea* during the dry season. Although, the lowest concentrations of ADF and ADL were noted in *R. ulmifolius* during autumn, while the lowest NDF content was in *C. crispus* during the three seasons. The *C. villosa* in autumn was the less digestible species with an *in Vitro* Organic Matter Digestibility of 359 g/kg and *L. stoechas* in spring was the more digestible with 717 g/kg. *E. arborea* had the lowest ME with 4 MJ/kg DM in the dry season. However, the highest energy level was observed in *L. stoechas* in the green season with 10 MJ/kg DM. It is concluded that the shrubs *A. unedo, C. villosa, C. crispus, E. arborea, L. stoechas,* and *R. ulmifolius* have a good potential in the feeding of goats.

19. A non-common case of parietal fibrinous peritonitis in Belgian blue heifer without a history of laparotomy

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A 19 months old Belgian Blue heifer was referred to the Veterinary Clinic of Liege University. The heifer was 2 months pregnant by artificial insemination performed by the farmer, and presented hyperthermia, anorexia and weight loss. Rectal palpation revealed a large, depressible abdominal mass. Diagnosis of parietal fibrinous peritonitis (PFP) was made by ultrasound, revealing a liquid and fibrin filled cavity attached to the abdominal wall. Blood analysis indicated an inflammatory status. Analysis of a PFP fluid sample confirmed bacterial contamination. Treatment, apart from fluids, antibiotics and non-steroidal anti-inflammatory drugs, consisted of surgical drainage and repeated flushing of the cavity. Complete resorption of the cavity was observed after 5 weeks. The heifer remained pregnant but died 7 months later, after elective caesarean section, due to generalized peritonitis. This is the first report of PFP in an animal without a history of laparotomy. The PFP may have been caused by an insemination induced trauma.

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20. Effect of alternative feed resources on rumen liquor microbiota of goat kids: olive cake and cactus cladodes

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In Northern Morocco, forest rangelands are the major feed resources for goat's livestock. These rangelands are characterized by annual and seasonal variation, causing low herds' productivity. Thus, it is necessary to diversify goat livestock feeding by alternatives feed resources incorporation. Olive cake (OC) and cactus cladodes (CC) are two unconventional resources widely available in the Mediterranean area and Northern Morocco that could take place in ruminants' diet. However, their effects on ruminal microbiota were rarely investigated. This work aims to evaluate the effect of OC and CC on rumen's bacterial community of goat's kids. Forty-four animals aging three months were divided into four groups receiving diets containing either 35% OC, or 30% CC, or 15%OC and 15%CC instead of a conventional concentrate diet. These animals were slaughtered after three months of the experiment, and the rumen liquor was collected. According to the results, alpha diversity (richness, evenness, and reciprocal Simpson indexes) was not affected by the inclusion of OC and CC. These results were confirmed by the lack of diet effect on beta-diversity (non-metric multidimensional scaling (nMDS) plot, analysis of molecular variance (AMOVA), homogeneity of molecular variance (HOMOVA), and permutational multivariate analysis of variance (PERMANOVA). The diet had a slight effect on the operation taxonomic unit by affecting three bacterial genera ($P < 0.05$). Thus, goat kids' rumen liquor seemed to be able to adapt to alternative feed resources incorporation and could be included in the diet of goat kids in times of drought or feed shortage.

Posters

21. Effect of olive cake and cactus cladodes on fatty acids of goat's products (meat and milk)

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In Northern Morocco, goat herd feeding is mainly based on forest rangelands. These rangelands are characterized by seasonal variability and severe degradation, causing low herd productivity. To resolve this problem, ruminants' feed diversification is recommended. The olive cake (OC) and cactus cladodes (CC) are two unconventional feeds available in the Mediterranean area. These resources are known for their secondary compounds that could affect the products' quality. Their effects on goat meat and milk fatty acids (FA) were rarely investigated. This work aims to evaluate these effects. In the first experiment, 33 goat's kids aging 3 months were divided into 3 groups receiving diets containing either 35%OC, or 30%CC, instead of a conventional concentrate. After 3 months, animals were slaughtered, and meat samples were collected. In the second experiment, 33 lactating goats were divided into 3 groups receiving diets with a conventional feed as a control, and test groups received respectively 20%OC, and 30%CC. Over 3 months, a manual milking was performed fortnightly to collect milk samples. In meat intramuscular fat, 35%OC increased C6:0, C8:0, C10:0, C18:3n-3, C20:2 and C22:2 contents ($P<0.05$), while 30%CC decreased C6:0, C8:0, C10:0 and C15:1 contents ($P<0.05$). However, FA groups, ratios, and indexes of meat fat were not affected by diet. In milk fat, 20%OC increased C18:1n-9, monounsaturated and n-9 FA, and decreased 9t-C18:1 ($P<0.05$). However, 30%CC increased C15:0, C18:1n-9 and C21:0, and decreased C4:0, 9t-C18:1, 6t-C18:2, C20:0 and poly-unsaturated FA ($P<0.05$). Thus, OC and CC could take place in goat diet without a negative effect on milk and meat quality.

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22. Complementary feed resources used in small ruminant farming systems in Benin (West Africa)

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A socio-economic survey was carried out in 640 small ruminant breeding units located in eight communes, four in North and four in South of Benin. This survey aimed to inventory the feed resources used by breeders in order to quantify and qualify the feeding practices and the sustainability of these farms. The SPSS23.0 software was used to realise a categorical principal component analysis of the collected data for the selection of the relevant variables, which were submitted to a Two-step cluster to obtain the breeding group. Differences between the breeding groups in the use of different feed resources were verified by the Kruskal Wallis test. As results, five different clusters of breeders were highlighted: goat breeding (18.6%), goat and sheep breeding (11.3%), sheep breeding (6.6%), goat and sheep breeding associated with agriculture (20.5%), goat breeding associated with agriculture (43.5%). Agro-industrial by-products and crop residues provided the additional feed resources used to complement the fodder diet. The most important supplements were maize bran, cassava peelings, sorghum dregs, peanut and cowpea tops (dried or no) with significant differences ($p \leq 0.05$) in the use of these feed supplements according to the different breeder clusters and seasons. While there is evidence that breeders well understand the benefits of using these feed supplements, these have little knowledge of how to provide appropriate quantity and how to manage these resources rationally.

23. Effects of the incorporation of olive in feed on the zootechnical performance and biochemical parameters, of the broiler turkeys

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The objective of this study was to evaluate the effects of olive leaves OL (*Olea europaea*) supplementation in broiler turkeys diet, on their zootechnical performance; biochemical and hematological parameters; and welfare/oxidative status. A total of three hundred sixty 3-weeks-old male and female turkeys were reared under the same environmental conditions from week 3 to week 15. Turkeys were randomly divided into three groups, i.e. OL0 control group receiving a basic diet containing 0 % OL; OL3 and OL6 groups, receiving basic diet supplemented with 3% and 6 % of OL, respectively.
The results obtained showed that diets supplemented with 3% of OL have significantly improved body weight ($p<0.0001$) comparing to OL0 and OL6 groups. On the other hand, the feed conversion ratio for the OL0 control group was significantly lower ($p<0.05$) compared to the OL3 and the OL6 groups ($2.59<2.73$). The average cholesterol level was significantly ($p<0.05$) lower in OL3 and 0L6 comparing to OL0 (1.30 g/l; 1.29 g/l vs. 1.39 g/l, respectively). The level of albumin was significantly ($p<0.05$) different between OL6 (12.90 g / l) and OL0 group (13.67 g / l). The overall mortality was not significantly affected by a diet ($P>0.05$). Spleen, heart and abdominal fat weights were significantly heavier in the control group compared to OL 3 and OL6. No significant difference ($p> 0.05$) was recorded between the 3 experimental groups for carcass yield, gizzard and liver weights.
In conclusion, the supplementation of 3% OL into the basic broiler turkey improved their body weight and did not negatively influence carcass yield and blood biochemical parameters

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24. Production of cow's milk and its transformation into wagashi gassire in the commune of nikki (Benin)

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Traditional *wagashi gassiré* cow's milk cheese is one of the most remarkable products of cattle breeding. It is consumed and sold in all regions of Benin. This research therefore relates to the production of milk and its transformation into *wagashi gassiré*. This research aims to identify the different perceptions related to the breeding and production of *wagashi Gassiré.* She also describes the production methods of *wagashi gassiré*. Mixed in nature, quantitative and qualitative methods were used. Documentary research, questionnaire, interview and observation allowed data to be collected from 140 key players. JOFFE Helene's theory of perception was used to better identify the different perceptions. From the analysis of the data, it emerges that breeding is a cultural practice and the main source of wealth; but it is a risky profession and very threatened because of the repetitive conflicts between herders and farmers. *wagashi gassiré* is an identity product whose production methods include variants. The breeding and production of *wagashi gassirè* are activities with multiple functions in rural and urban communities.

Posters

25. Detection of *Ascaris suum* in slaugthered pigs from Walloon indoor, outdoor or organic pig farms using coprology, serology and liver scoring

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From October to December 2019, the presence of *Ascaris suum* was detected in 55 Walloon indoor (32), organic (18) or outdoor (5) pig herds. Faeces and sera from maximum 10 fattening pigs per farm were collected and liver scoring was performed. Coprological examinations using McMaster test allowed to estimate the number of eggs per gram of faeces (EPG) individually (n=450) and for maximum 5 pooled samples (n=81). Serological analysis (n=518) using Elisa test SERASCA® were performed. A total of 1583 livers were scored using 4 scores: 0 (no milkspots), 1 (<10 milkspots), 2 (>10 milkspots) and 3 (most of the liver surface covered with milkspots). A phone survey was addressed to farmers concerned by the study.
In 48% of the tested pig farms *Ascaris* eggs were identified in faeces: 12 indoor pig farms out of 30 tested (40%), 11 organic pig farms out of 17 (65%) and 2 outdoor pig farms out of 5. Serological analysis has shown that 8 herds/10 have been exposed to the parasite whatever the herds type. Indoor, 25% herds had a low or absent contamination level, 25% had a moderate level of infection and 50% herds presented a high infection level. In organic herds, herds proportion were respectively 17, 28 and 56%. In outdoor pig farms, the 5 tested herds were highly exposed to Ascaris suum. According to liver scoring 96% groups of pigs tested had liver lesions but in 40% tested groups only score 1 was registrated. The present results and the response to the survey will allow more specific anti-*Ascaris suum* strategies.

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26. Effect of seeding rate on seed yield and its components of sulla flexuosa (Hedysarum flexuosum) in the Northwest of Morocco

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Sulla flexuosa (Hedysarum Flexuosum L.) is a marginal plant used traditionally by farmers in the Northwest region of Morocco. These farmers can multiply this plant themselves in order to become self-sufficient in seeds. This work aimed to study the effect of different sowing rates on the seed yield and its components. The experiment was carried out in an experimental plot of the INRA regional centre of Tangier. Plants were sown at four seeding rates (15, 20, 25 or 30 kg/ha). A randomized complete block design with three replicates was adopted. The number of pods per infructescence and articles per pod, and the pod length were statistically similar for all seeding rates. However, the number of infructescences per plant, the number of pods per plant, the weight of thousand seeds, the weight of hundred articles, and the weight of fifty pods were statistically similar with the two lower seeding rates (15 and 20 kg/ha), compared to the results obtained with the two higher seeding rates (25 and 30 kg) that presented lower values. The decreasing in pod numbers per plant could be due to competition between plants in high seeding rates. The seed weight per plant was heavier with a sowing rate of 20 kg/ha and lighter for the other densities. Use of low seeding rates gave good seed yields which are economically interesting, since the weight of sulla thousand seeds is low; dehulling of sulla seeds being time-consuming. This study showed that yield parameters were negatively influenced by the increase of the seeding rate. According to our experiment, a seeding rate of 20 kg/ha is the best one for obtaining higher seed yields.

27. Ecological characterization of the natural habitat of sulla flexuosa (Hedysarum flexuosum) in the Northwest of Morocco

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Sulla flexuosa (Hedysarum Flexuosum L.) is a marginal plant used traditionally by farmers in the Northwest region of Morocco. Describe its natural habitat could help to understand the relationship with its morphological and agronomic characteristics and to facilitate its selection for cultivation. This work aimed to describe soil and environmental characteristics of Sulla natural habitats. A survey and collection mission were realized in Mai and July 2018. Sulla was encountered in sites where soil pH varied between 7.03 and 8.89 (i.e. rather alkaline); soil humidity between 1.55 and 7.30%; organic matter between 0.76 % and 3.46%); carbon between 0.28 and 2.18%, nitrogen between 0.02 and 0.26%; total limestone (CaCO3) between 0.54 and 21.01%; phosphorus between 7.4 and 13,6 ppm; and electrical conductivity between 0.06 and 0.11 mmho/cm. C/N ratio varied between 4.49 and 67.77; the mean value being higher than 10 this value indicates a healthy soil where microbial life was active. Concerning the climatic parameters, annual rainfall was above 600 mm for all sites and altitude varied between 26 and 358 m. Based on all these parameters, a dendrogram was established, showing similarities between sulla collection sites. This study highlighted the variability of soil parameters indicating sulla adaptability to grow on different soil types and environments, which could facilitate its cultivation in other Moroccan areas.

Comparative veterinary medicine

28. Equine Atypical Myopathy: Prevention is still the key

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Equine atypical myopathy (AM) is an environmental intoxication due to the ingestion of hypoglycin A found in seeds and seedlings of *Acer Pseudoplatanus* in Europe. Risk and protective factors were defined previously. Although several studies about this pathology are carried out, no specific treatment is known yet. The mortality rate remains high and, consequently, prevention is still the key to preserve horses from this severe intoxication. A systematic literature review was performed and epidemiological data was collected via standardized questionnaires. A total of 127 documents and 2371 cases were included in this study. Analysis of the data indicates that the risk can be decreased through different measures. First, contact with toxic plant material should be avoid (removal of seeds, prohibition of grazing in contaminated areas, parcels creation, no manure and/or harrowing). Secondly, by using or creating low risk pastures which are lush pastures without watercourses and/or freestanding water and without *Acer Pseudoplatanus* in the vicinity. Thirdly, it is advised to stable horses during risky periods or when the weather is inclement and/or to limit the grazing time (i.e. less than six hours a day). Fourthly, by revising feeding practice: it is advised to provide supplementary feeds (with riboflavin), a salt block, water from distribution network or stored in a tank, free-toxin hay or silage in a net in order to avoid contact with seeds or seedlings. These preventive measures should be followed for a period of 3 months starting in October to prevent "autumnal cases" and in March for "spring cases".

29. Does obesity influence blood pressure parameters in client-owned dogs? Preliminary results

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Introduction. Obesity in dogs is a growing nutritional disease with several presumed adverse health effects such as systemic hypertension. The aim of this study was to compare blood pressure (BP) in healthy lean (LD) and obese (OD) adult dogs. **Materials and methods.** Fifteen lean (BCS 5/9) and 28 obese (BCS ≥ 7/9) client-owned Labradors and Golden Retrievers were recruited and declared healthy. BP was measured by oscillometry (ACVIM guidelines). Data were analyzed with Kruskal-Wallis and Mann Withney *U* tests. Results are expressed as mean (±SD) or median (Q1–Q3). **Results.** Dogs were: 23 females (17 neut.) and 20 males (14 neut.).

Table 1. Body weight and BCS in LD and OD

	LD	OD
Body weight (kg)	30.2 ± 4.5	40.3 ± 7.0
BCS (/9)	5	7.8 ± 0.6

No significant difference was found for age (p=0.13) and activity (p=0.09, data not shown)

Table 2. BP parameters (mmHg) in LD and OD

	Lean	Obese	p-value
Heart rate (bpm)	98 (86-110)	112 (105-135)	0.017
SYS	142 (126-182)	161 (141-172)	0.49
DIA	70 (65-120)	89 (72-103)	0.17
MAP	96 (89-138)	115 (96-127)	0.29

Discussion. No correlation was found between obesity and blood pressure, like in previous studies, where it has been more related to age, concurrent diseases, exercise, size, breed, and temperament of the dog. **Conclusion.** This study failed to show differences in blood pressure parameters between LD and OD of similar breeds.

30. Tailored programs make weight loss achievable in naturally obese client-owned dogs: preliminary results

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Introduction. Canine obesity is a growing problem inducing concurrent diseases and a shortened lifespan. Experimental weight loss (WL) programs show high success rates, but do not represent the daily practice. This is why this study attempted to fit to field conditions. **Materials and methods.** Twenty-eight naturally obese (BCS≥7/9) adult Labradors and Golden Retrievers were recruited. The composition of the diet was (%Dry Matter): proteins 35, nitrogen-free extract 33, lipids 11, metabolizable energy 300kcal/100g. After a transition phase of 1 month, daily metabolizable energy requirement (MER) were calculated 132xideal body weight ($BW^{0,75}$) with correction factors depending on BCS. Dogs were weighted twice a month and assessed at the clinic every 3 months, with adjustments to the diet when necessary. Data are expressed as mean (±SD), or median (Q1–Q3). **Results.** Twenty-four dogs completed the study.

Table 1. Results of the WL program

Initial BW	39±6kg
Final BW	34±5kg
Initial BCS	8±1
Final BCS	6±1
WL	12±4%
Duration WL	27±10weeks
WL rate	0.45 (0.35 – 0.61)% per week
Energy Allocation for WL	71±9kcal/kg ideal $BW^{0,75}$

Discussion. This rate is slower than in experimental studies (environmental factors easier to control), but corresponds to previous clinical results. **Conclusion.** This study showed that WL programs are achievable in clinical practice, with a constant monitoring and tailoring of the diet to individuals.

31. Impact of obesity on fecal short chain fatty acid concentration in client-owned dogs: preliminary results

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Introduction. Obesity in pets is a growing nutritional disease. Enhanced capacity to produce short chain fatty acids (SCFA) has been related to obesity. The aim of this study was to characterize fecal SCFA concentration in lean (LD) and obese (OD) dogs. **Materials and methods.** Fifteen LD (BCS=5/9) and 28 OD (BCS≥7/9) healthy adult Labradors and Golden Retrievers were recruited. Fecal samples were obtained from overnight fasting dogs fed their usual diets. Samples were frozen at -80°C until analysis of SCFA concentration (gas chromatography coupled to mass spectrometry). A *t* test and a Mann–Whitney U test were performed, and results expressed as mean (±SD) or median (Q1–Q3). **Results**. Dogs were: 23 females (17 neut.) and 20 males (14 neut.).

Table 1. Body weight (BW) and BCS in LD and OD

	LD	OD
BW (kg)	30.2 ± 4.5	40.3 ± 7.0
BCS (/9)	5	7.8 ± 0.6

Table 2. Feces' analysis

	OD	LD	p-value
DM	68 (±4)	71 (±5)	0.017
pH	6.6 (±0.4)	6.5 (±0.5)	0.4
Acetate	94 (±31)	86 (±36)	0.52
Propionate	78 (±25)	71 (±27)	0.40
isoButyrate (iC4)	2.9 (2.3-4.3)	1.8 (1.0-3.0)	0.032
Butyrate (C4)	23 (±9)	14 (±6)	0.003
isoValerate (iC5)	4 (2.9-5.7)	2.5 (1.2-4.2)	0.027
Total	205 (±65)	178 (±69)	0.25

Discussion. The accumulation of C4 and abundance of major C4-producing bacterial groups have already been reported to increase with the intake of fiber and obesity.
Conclusion. This study suggests that fecal concentrations of C4, iC4 and isC5 can be higher in OD.

32. Peripheral blood mononuclear cells do not reflect skeletal muscle mitochondrial respiration in horses

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In human medicine, skeletal muscle mitochondria and their link to a variety of conditions have been investigated extensively. In horses, using respirometry, mitochondrial dysfunction has been reported in horses affected by equine atypical myopathy and polysaccharide storage myopathy.
It was recently suggested that peripheral blood mononuclear cells (PBMCs) may reflect mitochondrial bioenergetics in muscle. Blood sampling and subsequent PBMC analysis is a less invasive alternative to muscle biopsies. Therefore, respirometric measures were recorded for both types of samples in order to compare mitochondrial respiration in equine PBMC and skeletal muscle fibers. Skeletal muscle microbiopsies and whole blood were sampled in six horses of different breeds. Microbiopsies were taken at 5 cm depth in the long head of the *triceps brachii*. Muscle fibers were processed and analyzed following a standardized procedure. Whole blood was collected in 9 ml EDTA tubes and PBMCs subsequently isolated with a density-separation medium (Lymphoprep™), washed and analyzed within 4 hours of blood draw. Respiratory capacities were assessed using a specific substrate-uncoupler-inhibitor titration protocol for analysis of oxidative phosphorylation and electron transfer capacity. The flux control ratios (*FCRs*) and substrate control ratios (*SCR*) were calculated for both PBMCs and muscle fibers. Neither *FCR* nor *SCR* showed a correlation between the two samples (Spearman's correlation $p>0.05$). Even though PBMC may potentially be an indicator of overall metabolic health, our results do not support the theory that circulating PBMC cells can replace muscle biopsies for studies of skeletal muscle mitochondrial function in horses.

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33. Geo-spatial representation of the distribution of *Salmonella* Dublin in Wallonia.

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Introduction: *Salmonella enterica* subspecies *enterica* Dublin (SD) is a serotype adapted to cattle which can cause a very high mortality rate, especially among calves, and heavy economic losses for producers (Harvey et al., 2017). This bacterium has not been studied in Belgium, or only to a very limited extent. However, among the farms in Wallonia, cattle farms are the most numerous. The main objective is therefore to draw up an initial inventory of the situation of SD in Wallonia, its distribution by commune in relation to the number of cattle farms present per commune. Method: Using of ARSIA data that reported bovine samples tested positive to SD from ARSIA laboratory analysis between 2011 and 2019 as well as the cattle farms present in 2015 on the Walloon territory. QGIS software is used for geo-spatial representation. Results: Almost all of Wallonia is reached. It is mainly the Ardennes-Fagne-Famenne-Condroz region. Significant relation between communes affected by SD and number of cattle farms present per commune was found. The areas with the highest density of cattle farms are generally those most affected by SD.

Posters

34. Evaluation of the sensitivity of a new diagnosis method of *Salmonella* Dublin in cattle: lymph node bacteriological culture.

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Introduction: A sensitivity of 70% is achieved in bacteriological culture on faecal matter for the detection of *Salmonella enterica* subspecies *enterica* Dublin (SD) in calves (Nielsen, 2013). This sensitivity drops to approximately 20% in adulthood (Nielsen, 2013). The detection of healthy carrier animals is essential in controlling the spread of SD. A new method consisting of the bacteriological culture on lymph nodes is considered. The aim of this preliminary study is to determine whether this new method would be more sensitive or at least as sensitive as bacteriological culture on faeces (the current method) for the detection of SD in calves. Method: The autopsy of 13 calves aged 10 days to 4 months was made by ARSIA. A systematic sampling of the pre-scapular (left and right) and pre-crural (left and right) lymph node was performed on each calf in order to perform a bacteriological culture for the detection of SD. A bacteriological culture for the detection of SD in the faeces contained in calves' intestines was also systematically performed. An aerobic bacteriological culture was systematically carried out on certain organs in order to serve as a Gold Standard for the study. Results: The gold standard reported 10 positives calves to SD. Bacteriological cultures on lymph nodes returned positive to SD for at least 2 lymph nodes/4 for the all 10 positives. The sensitivity is therefore 100%in lymph nodes whereas in faeces the sensitivity is 70%. However, no significant difference was detected between the two tests ($p = 0.08$). The new method is therefore at least as sensitive as the old one for calves.

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35. *Trichophyton equinum* as a new agent of guttural pouch mycosis in a horse

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Guttural pouch mycosis is a rare disease characterized by fungal plaques that develop within the guttural pouches of horses. *Aspergillus spp.* are by far the most common fungi isolated from guttural pouches affected by mycotic infection.
An 8-year-old anglo-european gelding was referred to the Equine Clinic of Liege's University to investigate bilateral mucopurulent nasal discharge. Extensive bilateral guttural pouch mycosis was diagnosed after endoscopic and cytological examination of guttural pouch material. *Trichophyton equinum* was isolated from a diphteritic plaque sample using Sabouraud's dextrose agar. The horse was treated with repeated guttural pouch debridement and topical enilconazole (1% solution into 0,9% sodium chloride infused on the plaque). To minimize hemorrhagic risk, an arterial embolization was successfully performed confirmed by an angiogram. Some weeks later, the horse was presented again to the equine hospital for severe epistaxis and central nervous system deficits, and it was euthanized because of poor prognosis. Necropsy revealed progression of the infection to the point of lysing the septum and both ostia of the pouches. Origin of the bleeding was not identified but could have been caused by neovascularization or aberrant vascularisation. From the authors' knowledge, this is the first documented clinical case in which *Trichophyton equinum* was isolated as the causative agent of guttural pouch mycosis.

36. Uroabdomen secondary to chronic urethral obstruction secondary to intravesical gossypiboma

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Gossypiboma (retained surgical sponges) are common intraperitoneal foreign body but no case of intravesical gossypiboma has been described in veterinary medicine. They can stay clinically silent for years and their diagnosis is challenging. A 9 years old neutered female Labrador is presented in emergency for lethargy, anorexia, pollakiuria, and hematuria for 5 days. The dog had history of cystitis and underwent ovariohysterectomy 3 years ago because of a pyometra. A uroabdomen is diagnosed after abdominal POCUS, analysis of the abdominal free fluid and urine. Urinanalysis revealed pyuria, hematuria and presence of bacterias but no bacteria were found in the peritoneal effusion. Uroabdomen were succesfully medically treated. Abdominal ultrasonography and vagino-uretrography revealed a small cranial vesical tear, two parietal (cranial pole and vesical neck) masses in the bladder with diminution of urethra's diameter and regional adenomegaly. FNA of lymph nodes and US–guided through urinary catheter vesical biopsies were consistent with reactionnal lymph node, and vesical ulcers with severe firbrinopurulent inflammation and presence of non organical material (suture thread is suspected). An explorative coeliotomy and cystotomy were planned but during the preparation, transurethral protrusion of retained surgical gauze is seen and removed through cystoscopy. The dog went home with NSAID and antibiotics.
In this case there was no history of cystotomy, the origin is probably transmural migration of an intraperitioneal gossypiboma into the bladder. This is in our knowledge the first description of intravesical gossypiboma without history of cystotomy removed through cystoscopy.

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37. Hypoglycin A and methylenecyclopropylacetyl-carnitine transfer to milk: a potential exposition of unweaned foals

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Atypical myopathy (AM) is non-exercise-induced rhabdomyolysis syndrome that strikes grazing equids on a seasonal rhythm. It was discovered that AM occurs following ingestion of the toxin hypoglycin A (HGA) mainly present, in Europe, in seeds and seedlings of the sycamore maple (*Acer pseudoplatanus*). Both HGA and its active metabolite methylenecyclopropylacetyl-carnitine (MCPA-carnitine) have been detected in blood and/or urine of affected horses. Previously, it was published that HGA can even be detected in the serum of unaffected co-grazing horses while MCPA-carnitine was found at either very low levels (or below the detection limit) in the serum of these healthy co-grazers, confirming the existence of subclinical cases.

The objective of this study was to determine whether HGA and/or MCPA-carnitine are present in milk from grazing mares exposed to sycamore maple trees in pasture.
Four mare/foal couples were sampled; blood and milk. Both hypoglycin A and MCPA-carnitines were detectable in all but one of the milk samples.
To our knowledge, this is the first study to describe HGA and MCPA-carnitine transfer to the milk.
This unprecedented observation could partially explain cases of unweaned foals suffering from AM.
However a transplacental transfer of the toxin cannot be excluded.

HGA and MCPA-carnitine mare's milk contamination, besides being a contamination pathway for foals, could constitute a risk for food safety especially with the increasing mare and donkey's milk consumption in Western Europe. Further studies will be needed in order to evaluate a potential contamination of other species' raw milk or dairy products.

Posters

38. Imaging features of unusual expansile bone lesions as metastasis of a mammary tubulopapillary carcinoma in a dog

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A 6-year-old, spayed female, Basset Hound had been presented for 2-days history of subacute paraplegia. Mastectomy secondary to mammary carcinoma was reported 9 months prior to admission. Neurological assessment was consistent with a compressive myelopathy localized in the T3-L3 spinal cord segment.
A lateral view of the thorax was performed to exclude gross metastatic process. No pulmonary metastasis was visible but expansile osteolytic lesions were observed in the extremity of the spinous process of T2, T6 and T7 vertebrae.
The differential diagnoses for expansile osteolytic lesions included giant cell tumour, benign bone cyst, aneurysmal bone cyst, fibrous dysplasia and bone abscess. Because of the history, vertebral metastasis was added to the differential diagnosis.
CT scan of the spine was performed and confirmed expansile osteolytic lesions in thoracic spinous processes. A large, vascularized mass invaded the vertebral canal on T13 and compressed the spinal cord. Lytic areas were observed in vertebrae, femoral heads and wing of ilium.
Due to the poor prognosis the dog was euthanized.
Post-mortem MRI was made to document the case.
Post-mortem cores were obtained from the spinous process of T6 and T7, and were consistent with metastasis of mammary carcinoma.
Mammary neoplasia is the most common tumour in bitches. Bone metastasis are rare in case of mammary carcinoma and are usually located in the vertebral bodies due to the greater vascularisation. Expansile spinous process metastases had not been described in mammary carcinoma. When an expansile osteolytic lesion is observed, bone metastasis should be added to differential diagnosis, especially if mammary tumour is present.

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39. Feasibility of pulmonary micro-CT in dogs

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Micro-CT is an imaging modality that allows a better resolution than High Resolution Computed Tomography (HRCT). Micro-CT permits visualisation of micrometric structures while HRCT only gives an anatomic visualisation of them. Micro-CT images can be compared to histology. Micro-CT is used in human medicine to characterize the pulmonary lesions more accurately than with HRCT. Some studies in humans and mice compare HRCT, micro-CT and histology of healthy lungs and ill lungs. These studies give better knowledge and understanding of the underlying causes of HRCT changes.
Only one article used micro-CT to assess lung lesions in dogs. The aims of this study were to assess the feasibility of micro-CT in dogs and to compare images obtained by HRCT, micro-CT and histology/to present similarities and differences between HRCT, micro-CT and histology images.
Lungs of healthy beagle dogs were removed, inflated to a pressure of 20cmH2O, frozen in liquid nitrogen vapor and stored at -80°C. HRCT was performed on the inflated frozen lung. The frozen lungs were cut into 2 cm slices and cores were taken. Micro-CT and histological images were performed for each core and compared to the HRCT.
Micro-CT is a feasible technique in dogs. It provides information about terminal bronchi and alveola that are not visible with HRCT. It can help to make the bridge between HRCT, physiopathology and histopathology in dogs, and to characterize the type of the pulmonary lesion. Such an improved knowledge of the significance of HRCT lesions will allow to use serial HRCT in patients with lung diseases for non invasive assessment of the progression of the disease as well as of the benefit of targeted therapies.

40. Geolocation and temporal distribution of equine atypical myopathy cases in Belgium and France, from year 2006 to 2019.

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Atypical myopathy (AM) is a severe and often fatal muscle disorder arising from ingestion of toxins contained in sycamore maple (*Acer pseudoplatanus*) seeds and seedlings. Progresses in research to diagnose and treat AM after toxins ingestion are moving forward, but the acuteness of the disease with a fatality rate of about 75% reinforces the need for preventive measures.
The condition is a seasonal disorder. Ingestion of samaras in autumn and seedlings the following spring causes two subsequent clinical case series, with a majority of cases observed in autumn.
Our objective is to describe geographic and temporal distribution of cases in Belgium and France based on 14 years (2006-2019) of AM reporting by owners and veterinarians faced with a case via the AMAG (Atypical Myopathy Alert Group; http://www.myopathie-atypique.be) and/or RESPE (*Réseau d'Epidémio-Surveillance en Pathologie Équine*; https://respe.net) websites.
During this period, 1904 cases were declared in Belgium (n = 730; 38.3%) and France (n = 1174; 61.7%) from which 1373 (72.1%) were autumnal. While the annual number of declared cases varies a lot, the increasing number of declarations from 2006 to 2019 suggests that AM is emerging. From the recorded cases, 1004 (52.7%) have been geographically located, at the pasture level, corresponding to 365 cases (50.0%) in Belgium and 639 (54.4%) in France. A temporal information, based on the appearance of the first clinical signs was available for 1262 cases (66.3%).
Climate factors are essential for trees and plants cycle, sycamore maple is no exception. Based on geographic/temporal distribution of AM cases, next step will be to identify the relation between the number of cases declared, by year and region, and climate anomalies.

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41. Characterization of brain myeloid cells in the exacerbation of experimental multiple sclerosis by Murid Herpesvirus 4 latency

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Multiple sclerosis (MS) is a devastating chronic inflammatory disease of the central nervous system (CNS). While it has a high prevalence, its aetiology is still poorly understood. MS is a multifactorial disease caused by a combination of genetic, immunological and environmental factors. The best-documented and most strongly associated environmental risk factor for MS is Epstein-Barr virus (EBV). However, it is not known yet how EBV contributes to the development of MS. As EBV does not replicate in vitro and does not have any animal infection model, related animal viruses, such as Murid Herpesvirus 4 (MuHV-4), could help us to address the same question in a more accessible form. Here, we compared the development of experimental autoimmune encephalomyelitis (EAE), an animal model of MS, in mock and MuHV-4 infected mice. We showed that MuHV-4 infection prior to EAE induction leads to a significantly worse clinical outcome compared to mock infected mice. Moreover, a change in CNS-infiltrating CD11b+ cells was observed and higher expression of MHCII on CD11b+ cells after MuHV-4 infection compared to mock infected mice. In parallel, we also observed major alterations in circulating myeloid cells in MuHV-4 EAE mice compared to their mock infected counterparts. All of the observed results were dependent on latency of the virus, proved by using a latency deficient strain (ORF73). Based on this model and on this preliminary characterization, we want to understand how EBV increases overall MS risk. In the end, this project could warrant the development of EBV target therapies for MS and emphasize the involvement of myeloid cells in MS pathogenesis.

Posters

42. Could alterations in radiographic symmetry and sharpness be useful indicators of proximal subchondral bone lesions in the equine proximal phalanx? – A pilot study

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Introduction
Stress lesions of the proximal phalanx (P1) are common in sport horses and can lead to catastrophic breakdown injuries. Radiography remains the first line imaging technique to evaluate this structure.
Aims
To evaluate the intra- and inter-observer agreement in the evaluation of 1. the sharpness of the subchondral/trabecular bone interface of the proximal subchondral bone (SCB) plate in the equine P1, and 2. the lateromedial symmetry of the proximal SCB plate of P1.
Methods
Eleven isolated front limbs from 7 horses were included. A unique dorso15°proximal-palmarodistal oblique view was acquired with the limb placed in a physiologic position. SCB/trabecular bone interface and lateromedial symmetry of the proximal SCB plate of P1 were evaluated by 4 experienced readers according to a semi-quantitative grading scale. Intra- and inter-observer agreement was calculated.
Results
Intra- and inter- observer agreement was moderate to good for most of the parameters.
Discussion
The overall moderate to good intra- and inter-observer agreement is encouraging. The limited number of cases might explain the failure to highlight agreement for 2 measurements (sharpness of the medial SCB plate within the same observer, and lateralization of the thicker portion of P1 within different observers).

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43. Flexor surface of the distal sesamoid bone: do we see the same at ultrasound and magnetic resonance imaging?

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Introduction
The assessment of the flexor surface of the distal sesamoid bone is of clinical interest in early stages of navicular disease.
Aims
The aim of this study is to compare imaging findings on the flexor surface of the distal sesamoid bone at ultrasound (US) and magnetic resonance (MR) imaging.
Methods
Low field MR images of 13 isolated limbs were compared to their respective transcuneal US images. Isolated limbs were dissected after imaging to assess the macroscopic appearance of the flexor surface.
Results
Overall fibrocartilage thickness was better assessed on MR images and corresponded well to post-mortem in isolated limbs. Transcuneal US abnormalities on the flexor surface were identified when changes in shape of the palmar aspect of the distal sesamoid bone occurred due to the presence of an irregular synovial fossa or an erosion. Both imaging techniques demonstrated changes of the compact palmar bone.
Discussion
These results suggest an interest in combining MR imaging and transcuneal US for assessment of the flexor surface of the distal sesamoid bone as findings in the two modalities are complementary.

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