Book of abstracts

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Parallel session 1: From *in vitro* models to Immune Insights

Suzan Glänzer

Analysis of the impact of human milk extracellular vesicle isolation methods on their oxidative stress reducing potential

Abstract not available for publication

Imene Jridi

Canine liver organoids as a translational platform for modeling α-Amanitin hepatotoxicity and therapeutic discovery

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Background
α-Amanitin, the main toxin of *Amanita phalloides*, is a leading cause of fatal mushroom poisoning in companion animals, characterized by acute liver failure and high mortality rates. Hepatotoxicity results from toxin uptake into hepatocytes via organic anion transporting polypeptides (OATPs), followed by inhibition of RNA polymerase II and subsequent cell death. Despite its clinical relevance, experimental models to study α-Amanitin toxicity remain limited, hindering mechanistic understanding and therapeutic development. To address this gap, canine intrahepatic cholangiocyte organoids (ICOs) provide a clinically relevant and translational in vitro platform for mechanistic toxicology and early-stage drug evaluation.
Aim
To validate canine intrahepatic cholangiocyte organoids (ICOs) as a translational in vitro model for α-Amanitin-induced hepatotoxicity.
Methods
The effects of α-Amanitin on cell viability will be assessed using cell viability and lactate dehydrogenase (LDH) assays. OATP1b4 expression will be evaluated by quantitative PCR and immunohistochemistry in ICOs derived from three donors (Labrador retriever, Beagle, and Beagleton). Organoids will be cultured under both expansion and differentiation conditions and compared with the tissue of origin.
Results
Preliminary data show a dose-dependent decrease in organoid viability following α-Amanitin exposure. OATP1b4 expression is detectable in ICOs, supporting their suitability for modeling toxin uptake. These findings suggest that ICOs recapitulate key features of α-Amanitin-induced hepatotoxicity.

Conclusion
ICOs represent a promising translational platform for modeling α-Amanitin toxicity and enabling therapeutic screening. This approach supports the development of affordable and clinically applicable treatment strategies for companion animals.

Ruben Verstappen

Title: Establishing primary avian cell models from naturally deceased wild birds for avian virology research.

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The limited availability of avian-derived cell systems remains a major limitation for biologically relevant research on bird-associated viruses. In vitro studies of pathogens such as avian influenza viruses and flaviviruses are frequently conducted in mammalian cell lines, which fail to replicate the species-specific cellular environment of their natural avian hosts. This difference can limit physiological relevance and translational value.
Here we present an innovative strategy in which established cell isolation techniques are applied to naturally deceased wild birds in order to obtain primary cells for in vitro modeling. Instead of relying on experimentally infected animals or heterologous cell systems, we isolated and cultured primary cells directly from wild bird carcasses submitted to the Dutch Wildlife Health Center (DWHC). Cells of interest include bone marrow derived mononuclear cells (BDMCs), mononuclear cells from spleen, hepatocytes, and structural cell types such as fibroblasts. The primary objective is to evaluate post-mortem viability and in vitro culture potential of specific cell types, thereby determining whether carcasses of naturally-deceased wild birds can serve as a sustainable and ethically favorable source of primary cells.
Our first results appear to reveal a restricted post-mortem time interval during which PBMCs can be successfully isolated from bone marrow and cartilage and kept in culture successfully thereafter. Cells that survive harvesting, enzymatic pretreatment steps and seeding, show morphological characteristics that resemble those published for isolated chicken PBMCs and fibroblasts. This proof-of-concept indicates that naturally deceased wild birds represent a potentially promising resource for generating species-specific primary cell models.

Daniel Sáenz Fernández

25-Color flow cytometry panel for immunophenotyping T cell subsets in rheumatoid arthritis patients treated with an autologous tolerogenic dendritic cells therapy

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Rheumatoid arthritis (RA) is an autoimmune disease associated with chronic joint inflammation, pain, and dysfunction. Immune imbalance in RA reflects aberrant activation and regulation of immune cells, including dendritic cells (DCs) and auto-antigen-specific T cells. Its current treatment approach relies on immune suppression and disease-modifying anti-rheumatic drugs. However, these have a variety of side effects. Thus, the development of novel therapies that can restore the immune balance in RA is needed.

To this end, we developed a novel autologous tolerogenic dendritic cell (tolDC) therapy to induce antigen-specific tolerance and tested this therapy in a phase 1 clinical trial in 9 patients. Patients received two injections of tolDCs (week 0 and 4) and had a follow-up at weeks 8, 12, and 24 after the first injection. Blood samples were obtained before the first injection, with the injections, and in each of the follow-ups.

Here, we optimized a spectral flow cytometry panel to identify and characterize T cell subsets. The aim is studying the changes in the immune balance of pro- and anti-inflammatory subsets involved in RA pathogenesis in the patients receiving tolDCs. Dimensional reduction and cluster analysis showed on average a significant reduction in total pro-inflammatory Th17 cells (14.37% reduction) and a significant increase in anti-inflammatory Tr1 cells (19.60% increase) after the treatment. We also observe a significant reduction of RA-associated Th17.1 cells at 20 weeks after treatment (67.28% reduction). Furthermore, we observe a differential effect in anti-citrullinated protein antibodies (ACPA)+ and ACPA- patients, where ACPA+ patients undergo a significant reduction of pro-inflammatory Th1 cells at 20 weeks after treatment (22.37% reduction).

These results demonstrate changes upon autologous antigen-specific tolDC therapy in the T cell balance in RA patients that can be related to an improvement in the disease state, with a difference in the effects in ACPA+ and ACPA- patients.

Parallel session 2: From Drug Discovery to Safe Application

Jordi Martens

Evaluation of Clinically Relevant Lipid Nanoparticles for mRNA delivery in Osteoarthritic Joint Models

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Introduction: Osteoarthritis is a complex multifactorial joint disease characterized by chronic pain, cartilage degeneration, and low-grade synovial inflammation. Current treatments mostly provide symptomatic relief and may cause systemic side effects. Local administration of nucleic acid therapeutics using lipid nanoparticle (LNP) platforms may improve therapeutic efficacy, while reducing off target effects. In this study, we assessed the transfection capacity of clinically relevant LNP formulations, using enhanced green fluorescent protein (eGFP) mRNA and Cy5.5 fluorescent label, in models of increasing complexity.

Methods: Three LNP formulations containing different ionizable lipids: ALC-0315 (Pfizer/BioNTech), SM-102 (Moderna) and Dlin-MC3-DMA (Alnylam) were compared for mRNA delivery efficiency. Transfection was evaluated in canine articular chondrocytes (ACs) and synovial fibroblasts (SF) in monolayer culture, and in bovine articular cartilage and synovial tissue explants. To mimic an osteoarthritic microenvironment, cells and explants were pre-stimulated with pro-inflammatory cytokines before exposure to eGFP-mRNA-loaded LNPs.

Cartilage degeneration and inflammation were assessed by glycosaminoglycans (GAGs) and nitric oxide (NO) release, respectively. Transfection (eGFP expression) and LNP internalization (Cy5.5 fluorescence) were quantified 24 hours post-transfection by flow cytometry (FCM).

Results: Pro-inflammatory stimulation induced an OA-like phenotype, shown by increased GAG release from cartilage explants, and elevated NO production in both cartilage and synovium explants. In monolayer culture, all LNP formulations internalized and transfected ACs and SFs, with varying efficiency per formulation and cell type (1-15%). In explants, synovial tissue showed measurable internalization and transfection (approximately 15%), whereas cartilage explants showed no detectable eGFP and limited internalization.

Discussion and conclusion: These findings indicate that LNP performance is strongly influenced by tissue complexity and highlights the extracellular matrix as a critical barrier for LNP-mediated mRNA delivery. Cartilage's dense network appears considerably less permissive to LNP-mediated mRNA delivery than the looser synovial membrane. These tissue-specific differences highlight the need for tissue-specific targeting strategies for osteoarthritis therapy.

Naomi Benne

A liposomal inverse vaccine protects mice from arthritis and experimental autoimmune encephalomyelitis

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Chronic autoinflammatory diseases such as rheumatoid arthritis and multiple sclerosis lack curative therapies, and current immunosuppressive treatments often cause systemic off-target effects. To address this unmet need, we previously developed an "inverse vaccine" based on anionic phosphatidylglycerol liposomes carrying disease-specific peptide antigens conjugated to dexamethasone. Here, we assess the therapeutic efficacy and immunomodulatory mechanisms of this platform in two murine models of autoimmunity. In the proteoglycan-induced arthritis (PGIA) model, a single administration of liposomal dexamethasone-human proteoglycan peptide (Dex-K4-hPG) markedly slowed arthritis progression compared with an equivalent dose of free Dex-K4-hPG. Long-term follow-up showed that two injections provided superior protection over a single dose, whereas a third injection yielded no additional benefit. Flow cytometry of paw draining lymph nodes revealed reduced RORγt^{+}CD4^{+} T cells indicating a shift toward less pro-inflammatory T-cell responses. Histological staining of the knees of mice also revealed less damage in mice receiving 2 or 3 injections of liposomes. To evaluate the versatility of this platform, we generated liposomes encapsulating a Dex-myelin oligodendrocyte glycoprotein peptide conjugate (Dex-K4-MOG). High encapsulation efficiency (~60%) was again

achieved. In an experimental autoimmune encephalomyelitis mouse model, two injections of Dex-K4-MOG liposomes (one before disease induction and one after) strongly suppressed disease development compared with controls. Together, these findings demonstrate that antigen-specific inverse vaccination using anionic liposomes offers a modular and broadly applicable strategy for inducing long-term protection in multiple autoimmune disease models. The platform elicits regulatory immune signatures and suppresses pathogenic Th17 responses, supporting its potential as a tolerogenic therapeutic approach.

Stefanie Horst

Temporal Trends And Exposure Characteristics Of Paracetamol-Related Intoxication Reports In Dogs In The Netherlands (2020–2025)

Background: In recent years, paracetamol use in veterinary medicine in the Netherlands, particularly in dogs, has increased. This raises concern that accidental exposure, medication errors, and toxicity may also be increasing. National toxicovigilance data offer an opportunity to examine temporal trends and characterize exposure patterns and severity.

Hypothesis: We hypothesized no association between increasing clinical use of paracetamol in dogs and the frequency or severity of reported intoxications.

Methods: A retrospective study was conducted using reports of paracetamol exposure in dogs recorded by the Dutch Poisons Information Center (DPIC) between 2020 and 2025. Cases were classified by likelihood of exposure as unlikely, possible, or likely to distinguish suspected from probable exposures. Reported doses were standardized to mg/kg body weight and categorized as therapeutic (0-33 mg/kg), supratherapeutic (34-99 mg/kg), or potentially toxic (≥100 mg/kg). Temporal trends were assessed by calculating the proportion of paracetamol-related reports relative to all substance-related consultations.

Results: Between 2020 and 2024, 45.640 substance-related intoxication reports in dogs were recorded, including 503 cases of suspected paracetamol exposure. The proportion of paracetamol-related reports remained stable over time, ranging from 1.3% to 2.0% annually (β=0.1, 95% CI [-2.07;2.27],p=0.89).

The proportion of cases classified as highly likely exposure increased significantly over time (95% CI [0.03;0.19], p=0.02). Overall dosage distributions remained stable (p=0.45). While the absolute number of cases involving potentially toxic dosages (≥100 mg/kg) did not change over time (β=0.7, 95% CI [-7.07;5.61],p=0.74), their proportion among dog cases increased (β=0.007, 95% CI [0.0009; 0.012],p=0.03).

Conclusion: Despite increasing clinical use of paracetamol, toxicovigilance data do not indicate an increasing burden of paracetamol-related reports. However, the observed shift toward higher exposure and a relative increase in potentially toxic dosages underscore the importance of monitoring and risk mitigation.

André Joubert

Mechanistic evaluation of the monensin–tiamulin pharmacokinetic interaction in broiler chickens using a physiologically based kinetic model

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Monensin is a polyether ionophore coccidiostat incorporated into broiler feed at 100–125 mg/kg throughout the production cycle. Its concurrent administration with tiamulin, a pleuromutilin antibiotic used to treat Mycoplasma infections in poultry, is associated with severe toxicity. The pharmacokinetic basis involves competitive inhibition of CYP3A-mediated monensin metabolism by tiamulin, characterised in vitro but not yet evaluated at the whole-animal level. The aim of this study was to incorporate tiamulin-mediated CYP3A inhibition into an R-based broiler PBK model and quantify the resulting impact on monensin tissue concentrations during a realistic production scenario.

A physiologically based kinetic (PBK) model for monensin in broiler chickens, originally developed in Continuous Simulation Language by Henri et al. (2016), was translated into R and validated against the original model output. The R implementation closely replicated the CSL model across all tissues ($R^2 > 0.96$ for plasma, liver, and leg muscle).Tiamulin-mediated metabolic inhibition was incorporated into the model using a four-parameter Hill function fitted to in vitro inhibition data from Nebbia et al. (1999). A fixed hepatic tiamulin concentration of 200 µM was used to represent peak residue conditions, based on published EMA/CVMP residue data. A physiological cap on biliary clearance was introduced to prevent artefactual compensation under enzyme inhibition.

Under concurrent monensin feeding (125 mg/kg feed, 35-day production cycle) and tiamulin co-administration, predicted monensin concentrations were elevated by approximately 10% across plasma, liver, fat, and leg muscle during the treatment period. Given monensin's narrow safety margin of approximately 1.5-fold in poultry (EFSA, 2018), this pharmacokinetic interaction has meaningful implications for animal safety and warrants consideration when tiamulin treatment is required during monensin-medicated production.

This work demonstrates how mechanistic PBK modelling can translate in vitro enzyme inhibition data into whole-animal exposure predictions, supporting practical food safety risk assessment in veterinary medicine.

Parallel session 3: From Viral Emergence to Intervention

Janine T.A.T. Stienstra

Picornavirus-induced extracellular vesicles: Stimulators or evaders of the antiviral immune response?

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Introduction: Viruses can subvert the intrinsic properties of extracellular vesicles (EVs), lipid bilayer spheres secreted by cells to communicate with each other. Virus-induced EVs are known to affect viral spreading, but little is known on how they affect the function of immune cells. Better understanding of how they activate or inhibit immune pathways could shed light on the role of EVs in (chronic) viral infections. We investigated how EVs from picornavirus-infected cells affect human immune cell responses.

Methods: Cell lines were infected with two picornavirus species. Virus-induced EVs were isolated and incubated with human white blood cells (PBMCs). Their immune responses were assessed on cell, protein and mRNA level.

Results: After stimulation with virus-induced EVs, PBMCs secrete the strong antiviral cytokines IFN-α and IP-10/CXCL10. Using blocking strategies we discovered that IFN-α is triggered by virus particles inside EVs while IP-10 is elicited by other EV-cargo. Among the immune cells, monocytes take up most of the EVs and consequently differentiate into antiviral FcγRIII^{+} subsets. Naked viruses, but not EV-enclosed viruses, can be opsonized with neutralizing antibodies and trigger FcyR^{+} immune cells to secrete pro-inflammatory cytokines needed for viral clearance.

Conclusion: Picornavirus-induced EVs induce a powerful set of antiviral cytokines during the initial stages of infections. However, in the presence of neutralizing antibodies, the immune response to EV-enclosed virus is severely hampered, possibly leading to chronic inflammation. The obtained knowledge will therefore lead to a better understanding of the role of virus-induced EVs during the course of picornavirus infections.

Tabitha Hoornweg

Testing the safety and efficacy of an EEHV1A subunit vaccine in Asian elephants

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Abstract

Elephant endotheliotropic herpesviruses (EEHV) are a group of seven elephant-specific herpesviruses. While adult elephants are latently infected with at least one EEHV (sub)species, often without showing clinical signs, EEHV infections in young elephants may lead to an acute, fatal hemorrhagic disease (EEHV-HD), which is a major cause of young elephant deaths worldwide.

Over the last years we have shown that antibodies play a crucial role in protection against EEHV-HD. Elephants less than one year of age have comparable high EEHV-specific antibody levels as their dams and do not develop EEHV-HD upon infection. As soon as these maternal antibodies start to wane, risk of EEHV-HD significantly increases. Antibody levels against the viral protein gH/gL correlate particularly well with protection against EEHV-HD. Only young elephants with very low antibodies to gH/gL of a specific EEHV (sub)species develop disease when infected with that particular EEHV species.

The EEHV gH/gL heterodimer naturally forms a complex with a third protein gO, called gH/gL/gO, which functions in host cell recognition. Since antibodies that can prevent host cell recognition are likely to be protective, we developed an adjuvanted subunit vaccine using EEHV1A gH/gL/gO as the antigen. Initially, vaccine safety was tested in 6 (sub)adult elephants living in Dutch zoos using a prime/boost regimen. The vaccine only caused very mild side effects and induced a clear rise of EEHV1A gH/gL(/gO)-specific antibody levels in the vaccinated elephants.

We next proceeded to vaccinate young elephants with low antibody levels to EEHV1A gH/gL, i.e. the group still at risk of developing EEHV1A-HD, as part of a vaccine efficacy study. All young elephants vaccinated to date (n=9) developed a clear EEHV1A-specific antibody response. To assess whether vaccination protects against EEHV1A-HD, antibody levels and development of EEHV1A-HD will be monitored in the participating elephants over the coming years.

Keywords: Asian elephant, EEHV, EEHV-HD, subunit vaccine, gH/gL/gO

Esmée Janssen

Effectiveness of portable air cleaners on airborne microbial markers in primary school classrooms: results from a cluster-randomized controlled trial

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High occupancy and prolonged classroom attendance can impair classroom air quality and consequently increase respiratory health risk. Laboratory and modelling studies suggested that portable air cleaners (PACs) may reduce microbial bioaerosol concentrations, though field-based evidence on their effectiveness remains limited.

Starting the winter of 2023/2024 and continued in winter 2024/2025, 180 classrooms across 29 Dutch primary schools were clustered and randomized to obtain high-efficiency particulate air (HEPA) or Plasma/Ionization PACs (clean air delivery rate ~400 m^3/h), or no PACs. During a three-week baseline and three three-week intervention periods per school, airborne dust was sampled using electrostatic dust fall collectors and analyzed for human-associated bacteria, a general bacterial marker, and seasonal viral markers using (RT)-qPCR. Impact of intervention with PACs on microbial aerosol concentrations is determined by mixed regression analysis, taking into account interdependency of data.

Baseline microbial aerosol levels were similar within classroom clusters, corroborating appropriate clustering. Relative to control classrooms, HEPA-filter PACs in classrooms were associated with 9% lower total bacterial load (geometric mean ratio [GMR] = 0.91, 95% CI 0.75–1.11), and Plasma/Ionization PACs with 5% lower load (GMR = 0.95, 95% CI 0.78–1.17), neither statistically significant. Restricting analyses to timepoints with consistent operational PAC usage slightly increased effect estimates to 12% (HEPA GMR=0.88 (95%CI 0.72-1.09)) and 9% (Plasma/Ionization GMR=0.91 (95%CI 0.74-1.12)). Species-specific bacterial markers were often near or below the limit of quantification, and viral markers were rarely detected. Species-specific probability of detection was not significantly associated with either PAC type, and effect directions showed no consistent pattern across markers.

Results suggest PACs did not significantly affect airborne bacterial or viral concentrations under dynamic bioaerosol behavior in primary school classrooms. These findings underscore the importance of evaluating PACs in real-world settings to determine feasibility of air cleaning interventions.

Harsh Gupta

Known enemy or new threat? A tussle between persistence and introduction of PRRSV strains on pig farms.

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Porcine reproductive and respiratory syndrome virus (PRRSV), a rapidly evolving RNA virus, poses significant challenges to the global swine industry, causing substantial economic losses and persisting despite ongoing control efforts. The viral dynamics on farms involve a complex interaction between circulating endemic strains and newly introduced variants, which may have a considerable genetic difference from older strains, alongside the influence of modified live vaccines. However, the frequency of new strain introductions and the role of cross-immunity and vaccine-induced immunity in determining whether introduced strains persist and take over by becoming dominant or fade out remains poorly understood. As the Netherlands aims to eradicate PRRSV, improved insight into these dynamics is essential.

To quantify the rate of PRRSV introduction on farms and identify predictors of strain persistence and takeover, Bayesian phylodynamic methods were applied to 519 partial nucleotide sequences collected from 75 anonymized pig farms in the Netherlands between 2018 and 2025. Sixteen farms with four or more sequences were included in the analysis of persistence and introduction, while the other sequences served as background for the analysis.

Strains persisted on farms for a median of 0.98 years (SD 2.23), while on seven of the 16 farms, a strain persisted for ≥4 years. The median rate of introduction was 0.32 (0.714 IQR) strains per farm per year, though not all introduced strains persisted on farms (take-over). In total, seven strains on seven farms took-over and persisted, for a median of 1.17 years. Ongoing analyses aim to identify predictors of strain takeover, such as antigenic distance between strains, and vaccination strategies. Farms with persistent strains may benefit from improved internal biosecurity, while those with frequent introductions need to prioritize strengthening external biosecurity. Overall, our findings highlight the importance of phylogenetically informed methods in understanding PRRSV dynamics and supporting farmers in eradication efforts.

Poster session abstracts

Peter Reinink

When blocking fails: revealing the limits of eDNA amplification blocker specificity.

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Environmental DNA (eDNA) studies rely on sampling DNA traces from air, water, or soil to identify species presence in environmental communities. However, the DNA signal from highly abundant species or common contaminants can mask those of rarer taxa, hindering detection. Blocking these dominant signals during polymerase chain reaction (PCR) amplification has therefore become a key strategy in eDNA and ancient DNA research. Previous work demonstrated that blocking primers containing a C3 spacer element can effectively suppress amplification of one target species in mixed DNA samples (Vestheim et al., 2008). Primers based on this principle are now frequently applied in mitochondrial (MT)-12S and MT-16S rRNA gene barcoding (next generation sequencing) approaches to reduce “off-target” sequencing by for instance human or livestock DNA (Calvignac-Spencer et al., 2013; Lynggaard et al., 2022). Despite widespread use, the blocking specificity of these primers has not yet been fully evaluated.

In this study, we assess the specificity of commonly used MT-12S and MT-16S blocking primers through quantitative PCR (qPCR) using isolated DNA from a diverse panel of single host species. Our results reveal that achieving selective blocking of a target species in complex, multi-species samples may be challenging. Several widely used blockers display limited or even absent specificity, allowing unintended cross-reactivity / non-intended blocking during amplification. Based on these findings, we highlight key molecular features that may improve the design and effectiveness of species-specific blocking primers.

This work provides new insights into the limitations of current meta-barcoding blocking approaches and offers practical guidance for developing more precise tools to reduce unwanted amplification in eDNA studies.

Lisa Verhoeven

Interleukin-6 and Tumor Necrosis Factor Alpha Synergistically Drive Differentiation Plasticity in Hepatocellular Carcinoma by Inducing a Progenitor-Like Cell State

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Keywords: hepatocellular carcinoma, cell state plasticity, inflammation, drug tolerance.

The liver parenchyma consists of hepatocytes, cholangiocytes, and bipotential progenitor cells (BPCs). BPCs can differentiate into hepatocytes and cholangiocytes upon extensive liver injury, and are positive for the cell surface marker CD44. Liver cancer frequently arises in a background of chronic inflammation and injury, and enhanced CD44-expression in hepatocellular carcinoma (HCC) has been linked to therapy tolerance. Therefore, it is of particular relevance to investigate whether IL-6 and TNFα can reprogram HCC cells into a dedifferentiated, drug tolerant CD44+ cell state, and to dissect the underlying molecular mechanisms. Here, we show that IL-6 and TNFα synergistically induce a transient CD44+ cell state in Huh7 cells. Using targeted inhibitors, we demonstrate that this process is dependent on NF-κB signaling. Moreover, the induction of the CD44+ cell state is accompanied by increased progenitor marker expression, and decreased HNF4α expression, suggesting dedifferentiation towards a more progenitor-like state. The mechanisms through which CD44 mediates dedifferentiation of HCC cells remain to be investigated. Our findings contribute to a more thorough understanding of the mechanisms underlying cell state plasticity in HCC, and facilitate the design of more effective therapies aimed towards preventing the formation of a drug-tolerant, CD44+ HCC cell population.

Joaquin Montilla Rojo

Developing a hPSC-derived teratoma in vitro model for hPSC malignancy assessment: current possibilities and limitations

Human pluripotent stem cells (hPSCs) have a myriad of potential clinical applications due to their capacity to self-renew and differentiate towards any of the embryonic germ layers. However, hPSC-derived cell products have shown to have the risk of tumor formation when used *in vivo*. To circumvent this issue, the safety of their use needs to be addressed, which includes the evaluation of the intrinsic hPSC malignant potential *in vitro.* Due to our limited knowledge on the underlying mechanisms causing this malignant potential, this assessment has traditionally been performed focusing on the histological traits of malignancy by xenografting the cells into immunodeficient mice (teratoma assay), for later evaluating the tissues found in the resulting tumor. Although informative, this assay faces scrutiny due to the lack of standardization of the procedure, the long waiting times and high related costs as well as the limited predicted value and questionable ethics behind animal testing. Here, we aimed at developing a reliable, standardized and fully animal-free *in vitro* model able to recapitulate the teratoma assay for addressing the histologic features of hPSC malignancy in an efficient manner. We generated embryoid bodies (EBs) as basic models of unbiased cell differentiation from validated malignant and safe stem cells, and exposed them to xeno-free and xeno-derived culture conditions. These were then compared on the basis of their capacity to generate structures displaying tissues with the same histopathological traits as those observed upon *in vivo* xenografting. Immature, fully differentiated, and mixed tissues were obtained in EBs from malignant stem cells, safe hPSCs, and iPSCs with impaired differentiation, respectively, cultured in both xeno-derived and -free conditions, although with low efficiency. Our results suggest that it is possible to develop an *in vitro* system able to recapitulate (immature) teratoma formation using xeno-free differentiation conditions, although efficient tissue formation can be improved potentially by better mimicking the mouse injection site microenvironment through co-culture and hydrogel embedding conditions.

Bram Tuinte

Host cell type specificity determines the efficiency of picornavirus spreading via extracellular vesicles

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Introduction: Picornaviruses can be packaged in extracellular vesicles (EVs) released by infected host cells, which shields them from neutralizing antibodies. However, there is limited knowledge in how packaging in an EV influences the efficiency with which virions infect new cells. Picornaviruses are known to infect and cause disease in multiple tissues. Virions are therefore likely packaged in EVs from multiple different cell types. We here investigate how cell type specificity of EVs may shape the efficiency of EV-mediated spread of Coxsackievirus B3 (CVB3), a picornavirus that causes viral myocarditis.

Methods: We compared the infectivity of extracellular vesicle (EV)–enclosed versus naked CVB3 , across different combinations of EV-producing and recipient cell types (HeLa, Huh7, U87, and human iPSC-derived cardiomyocytes), using a luciferase-reporter virus to quantify infection. Mock- and CVB3-induced EV proteomes were analyzed by mass spectrometry.

Results: CVB3 infectivity of EV-enclosed virions was strongly dependent on EV-donor cells. While EV-enclosed CVB3 from HeLa, Huh7 and cardiomyocytes poorly infected recipient cells, U87-derived EVs could efficiently transmit infection to new cells. We used comparative mass-spectrometry analysis of U87 and HeLa EV-virus to identify candidate EV proteins that enhance or reduce virus infection. We identified common CVB3-specific induced proteins that could be linked to virus-induced EV biogenesis pathways. Interestingly, the pool of cell type-specific virus-induced proteins strongly differed between U87 and HeLa EVs, with different functional pathways being enriched in each of the EV types.

Conclusion: Overall, our data indicate that the efficiency with which EV-enclosed viruses spread depends on the type of cell that produced the EVs. We identified substantial differences in the proteome of EVs that facilitate or reduce infectivity, which may explain their different contribution to viral spread. Further investigation of conserved and cell-specific CVB3-induced proteins will be important to understand how EVs contribute to picornavirus infection and pathogenesis.

Camille Bonhomme

Development of a smart hydrogel with photo-editable mechanical and chemical properties for volumetric bioprinting

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Volumetric bioprinting (VBP) enables the fabrication of complex cell-laden architectures at high printing speeds in a layer-less fashion. Recently, this technology was refined to precisely pattern (bio)active molecules inside (bio)printed constructs post-fabrication. A key challenge in biofabrication lies in replicating the time-dependent biochemical and mechanical changes occurring in the extracellular matrix during tissue maturation or disease progression. Therefore, bioresins for VBP, able to replicate these changes over time, are needed. In this study, a dual-functionalized tyramine and norbornene gelatin (GelTN) is developed for spatiotemporally controlled patterning of thiolated compounds within the printed structures. The dual wavelength bioresin is photo-crosslinked via green light-mediated di-tyrosine bond formation, leaving the norbornenes available for post-printing modifications via blue light-mediated thiol-ene click chemistry, allowing precise control over the crosslinked hydrogel's properties.

VBP-printed 3D constructs were fabricated in less than 30 seconds. A 2-fold increase in stiffness was observed in gels grafted with DTT, and the ability to fine-tune the stiffening degree of the gel was further evaluated by varying the thiol-to-norbornene ratios. Furthermore, SH-PEG_{5000}-Cy3 was successfully photografted into complex 3D architectures, enabling the creation of constructs with intricate biochemical patterns. Finally, while hMSCs' morphology showed rounder cells in stiffened gels, their viability remained high after the grafting process (85.0±5.1%), confirming the hydrogel's platform biocompatibility.

Utilizing the developed two-wavelength hydrogel, we demonstrated the ability to perform both VBP and volumetric photografting using bio-orthogonal thiol-ene chemistry, distinct photoinitiating systems, and light sources. The ability to selectively stiffen or photo-pattern gels allows the possibility of controlling the gel's mechanical and (bio)chemical properties over time. This spatiotemporal control allows for studying specific cells' behavior as shown by embedding hMSCs. The combination of the dual-wavelength bioresin and photo-grafting technology shows promise for the development of complex platforms mimicking the dynamic nature of native tissues and organs.

Marie Veltman

Cartilage oligomeric matrix protein for enhanced cartilage integration: Investigations using a novel tissue on-a-chip model

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Lateral integration of cartilage implants is crucial for durable tissue restoration and does not occur spontaneously. Using partial enzymatic digestion, improved integration can be observed. Still it remains unclear how cartilage can be interconnected on a microstructural level. Therefore, we aimed to develop a cartilage tissue on-a-chip model in which we could reliably study and optimize conditions modulating the formation of a structured collagen network. As the formation of the structurally functional collagen network relies on the presence of various ECM components, we also aimed to investigate the effect of supplementing our model with cartilage oligomeric matrix protein (COMP). COMP binds various matrix proteins enhances collagen fibrillogenesis. Recombinant Strep-tagged COMP was obtained through HEK293-EBNA cell culture and affinity-purification. The COMP concentration and purity was estimated by Coomassie blue staining of SDS-PAGE gels. To study integration, an interface was created in equine femoral cartilage biopsies, by cutting with a scalpel across the explant. Hereafter, explants were placed into the chip. To evaluate the effects on tissue integration, explants were exposed to a combination of partial hyaluronidase digestion and COMP supplementation. Explants were cultured in the chip in chondro-permissive media. COMP was successfully produced, as confirmed by Western blot. A polydimethylsiloxane (PDMS) chip was developed, functioning as a holder for cylindrical shaped cartilage explants (d = 5mm). The chip is an open system, allowing easy sample loading and medium change, and has a customizable height, for dealing with tissue variety. Cartilage explants could effectively be cultured in the chip, in which repeatable histological integration was observed after 14 days of culture. We developed a new cartilage tissue on-a-chip model for studying lateral cartilage integration, which was compatible with enzymatic treatment and protein supplementation. Further investigations will focus on electron microscopy imaging and the microstructural consequences of COMP treatment on the integration.

Jose Alberto Aguilar Briseno

Characterization of mosquito-derived extracellular vesicles during Usutu virus infection

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Orthoflaviviruses are enveloped, arthropod-borne viruses that cause significant morbidity and mortality worldwide. Usutu virus (USUV), a member of this group, infects birds and mammals, and represents a direct health concern in Europe. Infections of USUV and other flaviviruses transmitted by mosquitos are facilitated by saliva components injected in the skin upon mosquito bites. It has been hypothesized that small lipid bilayer-enclosed vesicles (Extracellular Vesicles (EVs)) in saliva from infected mosquitos modify cells of mammalian hosts and facilitate virus replication and spreading. However, research in this area is strongly hampered by technical challenges to separate host EVs from virions, which strongly overlap in size and biophysical/biochemical properties. Here, we designed a strategy for separating mosquito EVs and viruses, using USUV infection of mosquito Aag2 cells as a model. Particle subsets were separated using high-resolution density gradient centrifugation, and single density fractions were analyzed for viral RNA, viral proteins, and mosquito proteins. Nanoparticle flow cytometry analysis identified two EV populations that could be separated from virions based on differences in density. One of the EV subsets was enriched for syntenin-1, a key molecule involved in EV biogenesis and secretion. Interestingly, the other EV subset was enriched in USUV non-structural proteins. To test whether mosquito derived EVs could influence human immune cell function, we investigated EV interactions with peripheral blood mononuclear cells (PBMCs). We found that monocytes were the main cell type mediating the uptake of mosquito-derived EVs. Altogether, we developed a strategy to purify EVs produced by USUV-infected mosquito cells. This strategy could pave the way to further study their roles in infection during mosquito bites.

Charlotte Brice

Development of an iPSC-derived foetal liver-like niche in a chemically defined hydrogel for the assessment of HSC homing and expansion

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Most *in vitro* haematopoietic models focus on recreating the adult bone marrow niche, however, the foetal liver serves as a critical reservoir for definitive haematopoietic stem cell (HSC) expansion prior to bone marrow colonisation. A recently established protocol demonstrated the development of an induced pluripotent stem cell (iPSC)-derived foetal hepatic-haematopoietic niche, relying on Matrigel for 3D maturation/self-organisation. Replacing Matrigel with a fully defined and printable hydrogel would enable greater control over niche architecture and would allow investigation of how tissue organisation influences HSC behaviour. Therefore, the aim is to investigate the use of HybriGel, a gelatine-derived covalent-supramolecular biomaterial shown to support organoid proliferation and cell migration.

After initiation of the differentiation protocol, cells were embedded in HybriGel/Matrigel. Resulting morphologies and spatial structure were evaluated using brightfield and immunofluorescence (IF) imaging, and emerging cell populations were quantified by fluorescence-activated cell sorting (FACS). Additionally, through IF imaging and FACS, the model was evaluated for its capacity to support homing/expansion of donor haematopoietic stem and progenitor cells (HSPCs), both when using HybriGel- or Matrigel-derived niches.

Brightfield imaging revealed the progressive development of hepatocyte, hepatobiliary, and cholangiocyte organoids. Evaluation of HybriGel variants with different stiffness and viscoelastic properties revealed that softer matrices were associated with increased cell death and fragment dissociation, indicating that matrix mechanics influence niche stability during differentiation. FACS analysis demonstrated no significant differences in major cell populations between Matrigel and HybriGel. Donor-derived HSPCs seeded together with both the niches demonstrated homing and exhibited early expansion, with an increase in the proportion of committed progenitor cells.

These findings support HybriGel as a tunable, fully defined platform for foetal liver niche engineering, enabling more reproducible and mechanistic studies of human HSPC-niche interactions, with next steps focusing on leveraging its printability to fabricate perfusable architectures for dynamic culture and expansion of HSPCs.

Hristo Ivanov

MASH-in-a-dish. 13C-tracing of itaconate metabolism in steatotic hepatocyte organoids

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Abstract:

Metabolic dysfunction–associated steatohepatitis (MASH) is a severe medical condition which affects up to 5% of the world population. It is characterised by elevated liver steatosis, chronic inflammation, oxidative stress and fibrosis. In this study, we explore the role of itaconate, an anti-inflammatory and antimicrobial immunometabolite, produced by Kupffer cells (liver residential macrophages), on the progression and severity of MASH. Healthy hepatocytes metabolize itaconate to citramalate, which is then broken down by the enzyme CLYBL (citramalyl-CoA Lyase) into pyruvate and acetyl-CoA. We hypothesize that this process is impaired under steatosis, which could be another factor in their progression towards MASH.

To model itaconate signalling in MASH, we used primary murine hepatocyte-derived organoids (HepOrgs) as a model. HepOrgs were treated with free fatty acids (palmitate and oleate) and free cholesterol to induce steatosis. To understand itaconate metabolism, we performed liquid chromatography-mass spectrometry (LC-MS)-based fluxomics and metabolomics with 1 mM U-13C5 itaconate in steatotic and control HepOrgs.

Steatotic organoids exhibited higher total intracellular levels of palmitate, alanine, arginine, tryptophan, L-carnitine, S-adenosyl-methionine, but lower levels of GSSG and ophthalmic acid. Healthy organoids showed significantly higher enrichment of 13C-labelling in metabolites involved in the tricarboxylic acid (TCA) cycle or response to oxidative stress (glutathione, GSSG, ophthalmic acid and some of their precursors), consistent with active itaconate degradation to pyruvate and acetyl-CoA by CLYBL and TCA cycle in the mitochondria. The data shows that healthy HepOrgs could be able to incorporate carbons from itaconate in synthesizing intracellular antioxidants while itaconate degradation is impaired in steatotic HepOrgs. These findings will be further validated with 13C5-itaconate fluxomics in HepOrgs deficient for CLYBL, the key enzyme for itaconate degradation.

Peter Scherpenisse

Microbiome analysis at the IRAS One Health Microbial-laboratory – an overview of the generic workflow

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Microbiome analysis determines the composition of micro-organism communities (bacteria, archaea, viruses and fungi) within an environment. At IRAS we mainly study the environmental microbiome directly (air, water and soil) or we study the compositional changes of the microbiota inside hosts niches (e.g. airway, gut, milk) due to some form of external exposure or changes in hygiene and farming conditions. Most microbiome analysis at IRAS-lab uses deep-sequencing of barcoding gene amplicons which identifies the differences in the DNA of high variability regions of a barcode gene (typically multiple variable 16S rRNA gene regions for bacteria/archaea, and ITS1 for fungi).

The typical workflow is to carefully collect samples (including field blanks) and extract (low-biomass) DNA. After qPCR for quantity, barcode genes are amplified, indexed and deep-sequenced (Illumina short-read) after which the sequences are quality controlled and assembled into pseudo-reads. These pseudo-reads are annotated using a variety of databases and taxon abundances are determined for semi-quantitative analysis. Currently, Illumina-based short-read sequencing is being replaced by long-read Oxford nanopore technology allowing deep-sequencing of full-length barcoding genes.

Here, as a use case we present the data analysis workflow applied for a goat milk quality around parturition study, where microbiota changes in feces and milk (individual and bulk milk) were determined and compositions were explored. Data analysis was done in R using the *DaDa2, phyloseq* and *vegan* up to *ANCOM-BC2* packages. A Generic pipeline that checks sequence quality, makes exploratory barplots at various taxonomic levels, remove contaminants, explores rarefaction, determines alpha diversity for the number of taxa and distribution within groups, beta-diversity analysis for the compositional differences between groups and finally differential abundance analysis was applied. As a result, we identified compositional changes after parturition as well as differences between farms, and show which specific fecal bacterial taxa were involved in these changes.

Elysia Yau

Mapping the metabolic toolkit of milk-derived extracellular vesicles through proteomic analysis

Abstract not available for publication

Maarten Delemarre

Bovine Organoids to improve Animal Health and Food Safety

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Background

The dietary composition and supplements given to cattle are constantly evolving, leading to an increasing number of compounds that cows are exposed to. This development creates a need for deeper understanding of the underlying pharmacological mechanisms, with implications for animal health and potential impacts on human health through the consumption of beef and milk. The current standard for studying the transfer of compounds from feed or medication to cows relies on animal experimentation, which is both costly and time-consuming. However, current *in vitro* and *in silico* models for cows are limited in scope and application.

Aim

The goal of this project is to develop a generic bovine kinetic modelling platform capable of predicting the transfer of compounds from veterinary medicines, supplements, and feed to bovine tissues. This physiologically based kinetic (PBK) model is intended to provide a new method to study the efficiency and safety of veterinary medicines and feed.

Methods

To generate data for the PBK model, three *in vitro* organoid models were established: bovine intestinal-, hepatic-, and mammary gland organoids. These organoid models are cultured on hollow fiber membranes to form polarized layers of epithelial cells, enabling transport and metabolism assays that determine absorption, metabolism, distribution, and excretion (ADME) parameters for relevant compounds. The acquired ADME parameters will serve as input for the bovine PBK model.

Outcomes

The development of a bovine PBK model, along with the use of in vitro organoid systems, is expected to offer a cost-effective and ethical alternative to traditional animal experiments. These models can simulate functions of bovine organs, providing more precise and ethical research. It will enable more thorough and detailed analysis of ADME parameters, leading to improved accuracy in predicting compound transfer and metabolism in cattle. These methods can increase understanding of (veterinary) pharmacology and will contribute to animal welfare and human health.

Noah Riem

CRISP2 oligomerisation and stability depends on zinc and cholesterol

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Mammalian spermatozoa are highly specialised cells responsible for the delivery of paternal genetic material to the oocyte[1]. Cysteine-rich secretory protein 2 (CRISP2) is a sperm-specific member of the CAP (CRISPs, antigen 5, pathogenesis-related 1) superfamily of proteins. CRISP2 is incorporated into the perinuclear theca during spermatogenesis, where it contributes to highly condensed protein assemblies in the sperm head.[1,2] During the subsequent maturation, spermatozoa are exposed to a distinct biochemical environment characterised by high zinc concentrations and a cholesterol-rich plasma membrane, both of which are associated with structural stabilisation.[3,4,5]

Upon entry into the female reproductive tract, this environment changes substantially. Zinc availability decreases, while membrane cholesterol is progressively depleted during capacitation, enabling membrane remodelling and fusion competence.[3,4]

Here, we demonstrate in vitro that CRISP2 oligomerises in a zinc-dependent manner, suggesting that zinc may stabilise CRISP2-containing assemblies formed in elongating spermatids. Based on this observation and previous reports of sterol binding by CAP family proteins[5,6], it appears that zinc and cholesterol co-ordinately regulate CRISP2 oligomerisation and stability: high zinc levels in the male genital tract promote oligomer formation and stabilisation, whereas decreasing zinc especially in the oviduct cause gradual oligomer destabilisation and decondensation. In parallel, cholesterol dynamics may modulate CRISP2 function through CAP domain-mediated interactions, potentially linking membrane remodelling to intracellular structural changes.

To investigate this mechanism, a *Δhem1 Saccharomyces cerevisiae* model has been used to express CRISP2–GFP. This model allows controlled manipulation of sterol availability and intracellular zinc levels whith continuous imaging of CRISP2 oligomerisation behaviour and oligomer stability at the molecular level.

Together, this work provides mechanistic insight into how changes in zinc and cholesterol across sperm maturation and capacitation may contribute to the dynamic regulation of CRISP2 assemblies, supporting the balance between structural stability and timely decondensation required for successful fertilisation.

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Ni Made Wedayanti

Presence and Tissue Tropism of Avian Coronaviruses in Wild Birds in the Netherlands

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Avian coronaviruses (AvCoVs), encompassing Gammacoronaviruses and Deltacoronaviruses, have been detected in wild birds and domestic poultry. Gammacoronaviruses (GCoVs) are associated with infectious bronchitis in chickens and are detected mostly in wild waterfowl, while Deltacoronaviruses (DCoVs) are present more extensively in wild birds, poultry, and mammals. DCoVs spillover to humans and domestic pigs has been documented, resulting in acute enteric clinical signs. These findings underscore the importance of understanding the virus circulation and transmission in wild birds. Despite previous studies in Europe suggesting varying prevalence across different avian species, the tropism of the AvCoVs in wild birds remains poorly characterised. This study aims to investigate the presence and tropism of AvCoVs in tissues of 150 wild birds from the Passeriformes, Pelecaniformes, Anseriformes, and Charadriiformes orders that were found dead in the Netherlands between 2019 and 2024. Tissues collected during necropsy and stored in the biobank of the Dutch Wildlife Health Centre were tested by nested two-step Reverse Transcriptase (RT) PCR using PanCoV primers targeting the RdRp gene. Positive samples were confirmed by Sanger sequencing and assigned to their genera. Coronaviruses were detected in 10 of the 150 birds (6.7%). DCoV were detected in the intestines of three song thrushes (*Turdus philomelos*), and two Eurasian spoonbills (*Platalea leucorodia*), one of these spoonbills was also found positive in the lungs. Five birds from the Anseriformes group were found positive for Gammacoronaviruses in their intestines. In all samples from the Charadriiformes group tested negative. These findings highlight the circulation of AvCoVs in wild birds and underscore the need for further research into their pathogenic role and potential for interspecies transmission.

Keywords: coronaviruses, wild birds, RT-PCR, virology

Aileen Blom

Assessing neuronal plasticity in the mPFC after cannabis exposure using ImageJ

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Cannabis is the world's most widely used substance of abuse, and the use of cannabis is especially high among adolescents. During adolescence, the brain undergoes maturation, and this includes the medial prefrontal cortex (mPFC), a brain region that is crucial for executive functioning and cognitive control. Beside this, the endocannabinoid system plays an important role in the development of the PFC, which suggests that the adolescent brain may be particularly sensitive to the effects of cannabis. While previous research has shown that other substances of abuse can affect the dendritic organization of the pyramidal neurons in the mPFC, the impact of cannabis exposure on these neurons remains elusive. Therefore, this project aims to elucidate the effects of cannabis exposure on mPFC pyramidal neurons using a male adolescent rat model. Animals were exposed to either a low or high dose of cannabis vapour for four consecutive weeks, followed by extraction of the brains. Then, the Golgi-Cox method was used to stain the neurons, including the pyramidal neurons in the mPFC. These pyramidal mPFC neurons were subsequently analysed using ImageJ. The neurons were assessed on the following six characteristics: the convex hull surface and volume of apical and basal projections, the total length and volume of the apical and basal projections and the cell body, and the branch number and order for the apical and basal projections. Additionally, the branching complexity was determined through the so-called Sholl analysis. The results of this study will provide more insight into the impact of cannabis on the neuronal plasticity of the mPFC, which is relevant for understanding the impact of cannabis on the development of the brain and behaviour of adolescents.

Stefanie Horst

Global Practices And Perceptions Regarding Acetaminophen Use In Veterinary Medicine: Preliminary Findings From An International Survey

Background:

Acetaminophen is widely used in human medicine and is increasingly applied off-label in veterinary species, particularly when non-steroidal anti-inflammatory drugs are contraindicated. However, the extent, context, and perceived safety of its clinical use across animals remain poorly characterized. Better insight into current practice may help identify safety concerns, regulatory barriers, and missed opportunities for appropriate clinical use.

Hypothesis: We hypothesized that acetaminophen use in veterinary medicine varies across species, clinical settings, and geographic regions, and is influenced by perceived safety, regulatory availability, and clinician familiarity.

Methods: An anonymous, web-based international survey was developed to investigate the clinical use of acetaminophen, including indications, dosing practices, perceived efficacy, safety concerns, and regulatory access among veterinarians across all fields of practice. The 26-item survey included multiple-choice, Likert-scale, and open-ended questions addressing clinical indications, species treated, dosing protocols, treatment duration, frequency of use, adverse effects, regulatory status, and self-assessed pharmacological knowledge. The questionnaire was reviewed by board-certified veterinary anesthesiologists and clinical pharmacologists for clarity and clinical relevance. Data collection began in November 2025 and remains ongoing. As an international exploratory survey, the study aimed to obtain broad representation across species, practice settings, and geographic regions rather than to meet a predefined minimum sample size. Preliminary results are descriptive in nature; more detailed quantitative analysis will be performed once data collection is complete.

Results: At the time of analysis, approximately 180 responses from 18 countries had been received. Preliminary evaluation suggests variability in reported acetaminophen use and perceptions among respondents. Final analyses will further characterize patterns across species, practice settings, and regions.

Conclusion: This study will provide an international overview of current veterinary acetaminophen use and help identify knowledge gaps, species-specific concerns, and areas for future clinical guidance.

Fleur Somers

Exploring the interaction between lipid nanoparticles and decellularized notochordal cell-derived matrix for intervertebral disc regeneration

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Low back pain due to intervertebral disc (IVD) degeneration is the major cause of years lived with disability (1). Current treatments focus on pain relief, lifestyle changes, physiotherapy, or surgery as a last option (2). Therefore, a treatment that can functionally restore nucleus pulposus (NP) tissue is crucial. The gelatinous and proteoglycan-rich NP is found at the center of the disc, where NP extracellular matrix is secreted by nucleus pulposus cells (NPCs). However, matrix degradation and changes in composition while aging limit maintenance of healthy NP matrix (3). IVD degeneration is observed both in dogs and humans (4). Therefore, developing a therapy that can benefit both species is of great importance. The NC-CHOICE project focuses on developing an injectable cell-free therapy that delivers rejuvenating mRNA to NPCs to (re)stimulate healthy matrix production. To ensure successful delivery to NPCs, mRNA is encapsulated in lipid nanoparticles (LNPs). These are incubated with porcine decellularized notochordal cell-derived matrix (dNCM) (5) to mimic the disc environment. Biomolecules (as proteins and GAGs) in dNCM will adsorb to the LNP surface forming a biomolecular corona (BMC). The BMC affects the physiochemical properties of LNPs (size and surface charge) as well as LNP-cell interactions by means of cellular association and mRNA delivery (6). This study aims to elucidate tissue-specific interactions between LNPs and dNCM by studying BMC effect on mRNA translation. We will use LNPs that displayed the best eGFP translation in dog NPCs. LNPs were incubated with dNCM followed by isolation of LNP-corona complexes for BMC characterization. Single-protein coating will be performed to study individual protein effects on LNP transfection and to explore the possibility of using a synthetic corona in the future. Altogether, this approach will contribute to a long-term regenerative treatment for low back pain by optimizing NP matrix restoration.

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Estefanía Lozano-Andrés

Calibrated high-sensitivity flow cytometric analysis reveals differences in extracellular vesicle concentrations and fluorescence signals related to the limit of detection across instruments

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Introduction: High-sensitivity flow cytometry analysis of extracellular vesicles (EVs) is rapidly advancing. To generate reliable and comparable EV data across instruments and laboratories, robust calibration methodologies are essential. Here, we evaluated instrument calibration on two high-sensitivity flow cytometry systems using well-characterized reference materials and biological EV samples.

Methods: Violet side scatter was calibrated using NIST-traceable polystyrene beads (Thermo Fisher) and fluorescence signals were standardized with antibody capture beads (ACB, Cellarcus) using FCMpass software. First, we validated the calibration model using non-fluorescent silica nanoparticles (siNPs, 50–200 nm, nanoFCM) and EGFP-expressing recombinant EVs (EGFP-rEV, Exosome standards, Sigma) on an Enhanced Small Particle (ESP) Aurora (Cytek Biosciences) and a CytoFLEX Nano (Beckman Coulter). Second, we analyzed EVs derived from human MDA-MB-231 breast cancer cells intrinsically expressing CD63-mClover3 and using an anti-CD63 AlexaFluor647 antibody.

Results: After calibration, light-scatter detection showed that the CytoFLEX Nano detected smaller siNPs (68 nm) than the Aurora ESP (91 nm), consistent with estimated limits of detection (45 nm and 88 nm, respectively, assuming EV RI = 1.38 and using core-shell modeling). Despite these differences, both systems demonstrated strong agreement when analyzing EGFP-rEVs, with median detected sizes of 125 nm and 132 nm and consistent concentrations of ~5×10^{9} particles/mL across a 16-fold dilution series. Next, breast cancer–derived EVs revealed intrinsic mClover3 fluorescence, though with different brightness and modeled abundances between instruments (~3-fold), while parental EVs remained negative as expected. Antibody staining confirmed surface CD63-mClover3 expression and revealed a double-positive EV subpopulation.

Conclusion: Side-scatter and fluorescence calibration substantially improve comparability between high-sensitivity flow cytometers despite differences in optics, electronics, and fluidics. Although instrument-specific LODs varied, brightly fluorescent EVs (RI = 1.42, e.g., EGFP-rEV) produced concordant concentration measurements across platforms. For smaller and dimmer EVs, however, instrument performance diverged to a higher extend, emphasizing the need for rigorous calibration and definition of LOD to ensure robust and interpretable EV data.

Jasper van den Ende

Coxsackievirus B3-infected intestinal epithelial barriers release apical and basolateral extracellular vesicles with distinct contributions to pathogenesis

Abstract not available for publication

Alizée Martin

HOPE: Harnessing Ovarian tissue Preservation for a safe and viable Engraftment

Alizée Martin, Alessia Longoni, Riccardo Levato, Mabel Beitsma, Marta de Ruijter Villani

Pediatric cancer survival now exceeds 83%, shifting research focus toward the long-term gonadotoxic effects of chemotherapy[1]. Ovarian tissue (OT) cryopreservation is the sole fertility preservation strategy for prepubertal patients[2]. This method entails surgical oophorectomy, followed by isolation and sectioning of the ovarian cortex into strips for cryopreservation. Autologous re-transplantation of the cryopreserved OT strips, has led to over 200 natural births[2]. However, fertility restoration fails in 60% of the patients, largely due to delayed revascularization of the transplanted OT, leading to hypoxia[3]. Moreover, in some cancer types, such as leukemia, the 60% of cryopreserved OT contain malignant cells[4]. Since availability of human ovarian tissue is limited, this project aims at using a bovine slaughterhouse ovarian model to develop (1) a non-invasive malignant cells detection system and (2) a strategy to promote rapid revascularization of the OT strips post transplantation. We were able to identify via rtPCR extracellular biomarkers (fusion gene) indicative of leukemia in the cell free DNA extracted from the supernatant of the cancer cell line Kasumi-1. The next step will be to test sensitivity and specificity of this non-invasive detection assay in conditioned medium during OT strip isolation from ovaries microinjected with a known number of Kasumi-1 leukemia cells. To enhance graft survival through revascularization, two complementary strategies are studied. Ovarian graft size will be reduced into smaller and more easily revascularized functional follicular units (FFUs). FFUs will be encapsulated in a novel delivery system composed by jammed microspheres chemically functionalized with VEGF to improve graft survival by promoting patients' blood vessel recruitment. Preliminary analyses have shown that OT strips can be reduced in size without excessively impacting follicle yield, moreover, the novel delivery vehicle show increased amount of nascent capillary junctions when cultured in vitro with endothelial cells in comparison to a bulk gel.

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Elizaveta Zadirako

Identifying extracellular vesicle subset signatures in human milk and plasma by single particle high-sensitivity flow cytometry

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Introduction: Extracellular vesicles (EVs) are membrane-enclosed nanoparticles that transport biomolecular cargo reflecting the status of the cell of origin. Consequently, EVs are heterogenous in composition, size, and molecular cargo. EVs are present in all biofluids, including blood and milk, making them a promising diagnostic tool. However, EV analysis in minimally processed biofluids is complicated due to the presence of other colloidal structures with partially overlapping characteristics and tailored EV-enrichment strategies are needed. Recent developments in high-sensitivity flow cytometry allows for single particle level analysis, making it possible to identify specific EV subsets. We here explored different workflows for EV-enrichment, fluorescent staining and high-sensitivity flow cytometric analysis to define an optimized and validated workflow for robust EV analysis in biofluids.

Methods: EVs were isolated from human plasma and milk using size exclusion chromatography (SEC), density gradient ultracentrifugation (DGU), or a combination of both. Lipid staining was performed using MemGlow488 dye on enriched EV samples, and excess dye was removed using SEC, DGU or micro-SEC methods. Finally, samples were measured on CytoFLEX Nano and Cytek Aurora ESP platforms. FCMPASS software was used to convert arbitrary units to standard units to allow EV data comparison between instruments. NIST traceable polystyrene beads of known sizes (100-453 nm) were used to approximate the diameter of EV particles.

Results: Single EVs were detected upon isolation from plasma and milk using SEC, DGU or a combined approach on both platforms. By performing calibrated measurements, we were able to define the limit of detection (LOD) and the approximate size distribution of the EV samples measured on both platforms. We identified biofluid specific EV-signatures and found that the CytoFLEX Nano is the most sensitive in detecting small EVs (between 52-250 nm).

Conclusion: Calibrated measurements are essential when comparing different flow cytometric platforms and defining LODs. By optimizing the whole workflow from sample preparation, labeling and calibrated analysis we identified biofluid specific EV signatures based on fluorescence and light scattering signals.

Chantal van der Meer

Developing a 3D dog synovial membrane *in vitro* model to study osteoarthritis heterogeneity

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Osteoarthritis (OA) is a chronic joint disease that affects both humans and dogs. Complicated by its heterogeneous nature, therapeutic options for OA remain limited. Representative *in vitro* models could provide insight into complex OA mechanisms, such as inflammation of the synovial membrane (SM), which is a key factor in OA pathogenesis. While 2D synoviocyte models provide basic insights, a 3D culture system could offer a deeper understanding of processes affected during OA progression, such as synoviocyte organisation and synthesis of synovial fluid components, including lubricin. This study aims to develop and characterise synovial spheroids and evaluate their potential as a model for OA heterogeneity.

Synovial spheroids were generated by culturing fibroblast-like synoviocytes (FLS), obtained through enzymatic digestion of both healthy and OA-affected dog SM, in Matrigel. Different concentrations (0, 0.1, 1, or 10 ng/ml) of transforming growth factor-beta (TGF-β) were supplemented to stimulate lubricin secretion. The development and morphology of spheroids, harvested after 7 and 21 days of culture, were investigated using immunohistochemistry for the phenotypic marker CD90, as well as the functional markers lubricin and collagen type I.

The generated spheroids self-organised into a lining and sublining, indicating an intrinsic, stable cell-to-cell adhesion of FLS. Low concentrations of TGF-β stimulated spheroid development, but additionally caused fibrosis-like changes within the lining. Furthermore, higher concentrations of TGF-β shifted the expression of CD90, found in the sub-lining of the SM, from the lining to the sublining of the spheroid.

Future work will evaluate the effects of inflammatory stimuli (TNF-α, IL-1β and OA SM-conditioned medium) on spheroid development and morphology. Additionally, RNA analysis will be performed to assess changes in gene expression related to inflammation and synovial function.

Overall, these results demonstrate that synovial spheroids can be successfully generated from canine SM, representing a promising *in vitro* model for studying OA-associated synovitis.

Rana Akpinar

Investigating non-genetic regulators of mucosal melanoma plasticity and therapy tolerance

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Background: Human mucosal melanoma (MM) is a rare and aggressive malignancy, with surgery remaining the primary treatment for localized disease. For advanced disease, immunotherapy, targeted therapy and radiotherapy are commonly applied. However, responses are often limited. Comparable therapeutic resistance and poor clinical outcome are observed in canine MM. In both species, MM is characterized by pronounced molecular heterogeneity, with responses to surgical treatments, targeted therapy, immunotherapy and radiotherapy (RT) differing considerably between patients. Previous studies indicate that tumor plasticity and adaptive tumor cell states allow tumor cells to respond dynamically to therapy, enabling the tumor cells to survive and persist despite treatment. Cancer stem cell (CSC) markers represent genes associated with stem-like tumor states and therapy resistance, whereas differentiation markers reflect the melanocytic lineage and are typically reduced during tumor dedifferentiation. In this project, radiotherapy-induced cellular plasticity in mucosal melanoma will be investigated using both human and canine MM cell lines.
Aim: The aim of our study is to identify CSC-associated and differentiation genes differentially expressed before and after radiotherapy in human and canine MM, and to determine how these transcriptional changes are regulated at the chromatin level.
Materials and methods: Five canine and two human MM cell lines will be treated with RT. Cell survival will be evaluated and RNA will be isolated from untreated and treated cells at 24, 48 and 72 hours after RT. Quantitative PCR will be used to assess the expression of selected CSC markers and melanocytic differentiation markers before treatment and at the different timepoints. In addition, RNA-sequencing will be performed to investigate transcriptional changes induced by RT. Lastly, ATAC-sequencing will be used to assess the accessibility of genomic regions following RT, to provide insights that may contribute to improve therapeutic strategies for both human and canine MM.

Ennis Maiguashca Sorensen

The role of itaconate in carcinogenesis of fatty liver disease

Ennis Maiguashca Sorensen, Jung-Chin Chang

Metabolic dysfunction-associated steatotic liver disease (MASLD) affects approximately 25% of the global population and represents a major risk factor for the development of hepatocellular carcinoma (HCC). In the absence of therapeutic intervention, MASLD can progress to metabolic dysfunction-associated steatohepatitis (MASH), a more severe pathological state characterized by excessive lipid droplet accumulation in hepatocytes, chronic inflammation, and progressive liver injury. This cascade ultimately promotes fibrosis, cirrhosis, and ultimately, malignant transformation. Emerging evidence suggests that metabolite-driven signaling plays a critical role in mediating this disease progression.

One such signaling metabolite, itaconate, has been implicated in hepatic metabolic and inflammatory pathways. Itaconate is produced by activated Kupffer cells and subsequently taken up by hepatocytes, where it undergoes mitochondrial metabolism. Preliminary findings (JC Chang, unpublished data) indicate that intracellular itaconate is associated with increased oxidative stress and elevated markers of cellular damage in hepatocytes. However, the mechanistic basis of these effects remains poorly defined. In particular, it is unclear whether itaconate itself, or one or more of its metabolic intermediates, is responsible for inducing oxidative stress and DNA damage.

In this study, we aim to elucidate the contribution of itaconate metabolism to the pathogenesis of MASH, with a specific focus on its role in promoting oxidative stress, lipid burden and DNA damage in steatotic hepatocytes. To address this, we will employ dihydroethidium (DHE) oxidation assays to quantify production of reactive oxygen species, immunoblotting to assess DNA damage markers, and lipid quantification assays to characterize steatotic burden. By dissecting the role of itaconate and its downstream metabolites, this work seeks to provide mechanistic insight into metabolite-driven hepatocellular injury.

Michelle Smolenaers & Linde van Dijken

The role of risk-assessment in play and other contexts in shaping adaptive behaviour in Lister Hooded rats

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Abstract

Play is a widespread behaviour observed across species, with risky play representing a distinct subtype characterised by uncertainty. In recent years, children's engagement in risky play has declined compared to previous generations, raising concerns about its potential developmental consequences. Research has shown that rats exposed to risky play during childhood display enhanced cognitive flexibility in adulthood. However, it remains unclear whether this enhancement reflects a generalised improvement or is specific to the task in which it was originally observed. Moreover, rats deprived of risky play early in life show less cognitive flexibility but exhibit a partial recovery when only exposed to task-based risk exposure during young adulthood. This suggests that such risk exposure can mitigate, but not fully compensate for earlier risky play deprivation, and raises the question of whether such improvements result from risk exposure itself or from experience with a specific task. Therefore, the present study investigates whether early life risk exposure effects generalise to a novel context, and whether improvements following later risk exposure reflect risk experience or task-based learning. To this end, a modified risky decision-making task (RDT) will be performed in young adulthood. In addition, rats will also perform an inhibition task in adulthood. These experiments are executed to differentiate between different forms of risk exposure and repeated task experience. We hypothesize that (1) even task-based risk exposure enhances flexible and adaptive behaviour in adulthood, and (2) exposure to task-based risk in young adults leads to improved cognitive control and flexibility later in life. This suggests that experiencing risk in childhood and young adulthood may play a meaningful role in cognitive development throughout life.

Tessa van der Leek

The role of itaconate synthesis by Kupffer cells in metabolic dysfunction-associated steatohepatitis (MASH)

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The global burden of metabolic dysfunction-associated steatohepatitis (MASH, previously non-alcoholic steatohepatitis, [NASH]), a metabolic liver disease that is characterized by accumulation of fat accompanied by sterile inflammation and progressive fibrosis, has been rapidly increasing and has been predicted to continue this trend in the future. We hypothesized that metabolic interactions between liver cells plays a prominent role in MASH pathogenesis and disease progression. Using MASH-in-a-dish system, a transwell co-culture with hepatocyte organoids (HepOrgs), Kupffer cells (KCs), and hepatic stellate cells (HSCs), we identified itaconate as a MASH-associated interactive metabolite, which is also elevated in the livers of MASH patients.

In the liver, itaconate is synthesized in the mitochondria of KCs by *cis*-aconitate decarboxylase 1 (ACOD1) from the tricarboxylic acid (TCA) cycle metabolite *cis*-aconitate. Itaconate is an immunometabolite shown to modulate lipid metabolism and inflammation. The Liver is the main organ responsible for itaconate degradation. Therefore, we hypothesize that itaconate regulates MASH progression by affecting hepatocytes and HSCs.

We have observed that itaconate causes oxidative stress in both hepatic stellate cells and hepatocyte organoids, suggesting that itaconate can drive DNA damage, potentially leading to tumour development in MASH patients. To further validate, we knocked out *Acod1* in KCs by lentivirus-mediated CRISPR-Cas9 knockout and assembled MASH-in-a-dish with Acod-deficient KCs. We found that KC-specific Acod1 deficiency did not affect KC immune function but reduced damage markers in MASH-in-a-dish, including aspartate transaminase and lactate dehydrogenase. Our results suggests that itaconate signalling is a novel, disease-modifying target for managing MASH progression. In the future, we plan to use 13C stable isotope tracing to understand the metabolic pathways leading to itaconate synthesis in MASH and examine whether these pathways can also be targeted to prevent MASH progression.

Edwin Veldhuizen

Synergistic antibacterial effect of CATH-2 and D-amino acids against mastitis causing Gram-positive bacteria.

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Abstract.
The overuse and misuse of antibiotics in veterinary medicine have contributed to the rise of antibiotic-resistant bacterial strains, creating a global issue that requires urgent action and innovative solutions. Mastitis, an inflammatory condition of the udder tissue, is one of the most significant diseases in dairy cows, leading to reduced milk production and economic losses for dairy farms. The growing prevalence of antibiotic resistance in the treatment of mastitis is becoming an increasing concern. Our research shows that a combination of the natural compounds, chicken cathelicidin-2 (CATH-2) and the positively charged D-amino acids, D-arginine and D-lysine synergistically inhibits Gram-positive mastitis-causing bacteria in both bacterial growth medium and raw cow milk. The fractional inhibitory concentration index (FICI) in raw milk was remarkably low (< 0,125), indicating strong synergy. Importantly the novel combination showed no detectable toxicity at the concentrations effective for antibacterial activity. This novel combination of antibacterial compounds offer promising potential for treating bacterial infections, particularly those caused by antibiotic-resistant strains.

Yvette de Geus

Individual goats can be super shedders of *Staphylococcus lugdunensis* and *Staphylococcus simulans*: from lab to farm practice

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Introduction The bulk milk total bacterial count (BMTBC) is a key milk quality indicator because bacterial byproducts reduce the shelf life of milk and dairy products and increase the risk of bacterial overgrowth before consumption. Exceeding BMTBC limits results in milk price penalties, motivating farmers to keep it under control. Factors influencing BMTBC include milking hygiene and milking technique, but also goats with intramammary infections (IMI) can shed bacteria into the milk.

Aim To investigate how many bacteria are shed during IMI, and to what extent individual goats can contribute to BMTBC.

Methods From January to October 2023, a Dutch dairy goat farm experienced persistently high geometric mean BMTBC and bulk milk somatic cell count (BMSCC). In a cross-sectional study of 98 goats on this farm, we evaluated whether goats with mastitis could significantly affect the BMTBC and BMSCC. Milk was cultured on blood agar plates and bacteria were identified using MALDI-TOF MS. Additionally, the numbers of bacteria in the milk samples were determined using both flow cytometry and total blood agar plate count with track dilution.

Conclusions Goats with IMI caused by S. lugdunensis and S. simulans are capable of shedding extremely high numbers of bacteria with their milk (~700 million cfu/mL), which has contributed to an increased BMTBC on a Dutch dairy goat farm.

Marit van den Berg

Whole genome sequencing analysis of canine pheochromocytoma

Abstract not available for publication

Inge van Geijlswijk

Pharmacokinetic investigation of orally administered GS-441524 in healthy cats following analytical development and validation of an HPLC-FD method

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Feline infectious peritonitis (FIP) is a fatal disease without treatment. GS-441524 has demonstrated promising efficacy in the treatment of FIP. However, in clinical practice, considerable interindividual variability in treatment response has been observed. Generating pharmacokinetic (PK) data is essential for understanding and addressing this issue. Therefore, this study aimed to: (1) develop and validate a robust high-performance liquid chromatography with fluorescence detection (HPLC-FD) method for the quantification of GS-441524 in feline serum; and (2) apply the validated method to characterise the pharmacokinetics of GS-441524 following oral administration in healthy cats under fed and fasted conditions. For HPLC-FD method 1,N6-Ethenoadenosine was selected as the internal standard (IS), chromatographic separation was achieved on a Luna Omega 5 µm Polar C18 100Å (150 x 4,6 mm) column using a gradient mobile phase consisting of 20 mM ammonium acetate (pH 4.5) with acetonitrile 5% or 70%. For the 24-hour PK study, a prospective, randomized, non-blinded crossover study was conducted to evaluate the pharmacokinetic parameters of orally administered GS-441524 (10 mg/kg and 20 mg/kg) in two healthy cats under both fed and fasted conditions. Results showed limited interindividual variability in drug exposure between the subjects. Administration under fed conditions was associated with increased maximum concentration (C_{max}) and systemic exposure. In conclusion, the developed HPLC-FD method is suitable for the quantification of GS-441524 in feline serum. The PK findings suggest that GS-441524 should preferably be administered with food and provide a basis for further PK modelling and the potential implementation of therapeutic drug monitoring.

Sem Harder

The impact of early-life cannabis exposure on social play behaviour and cognitive control in rats

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The global prevalence of substance use disorders is increasing. This rise is especially relevant during adolescence, which is a critical developmental period characterized by heightened risk of addiction due to ongoing brain development. Yet, it is also a period of remarkable resilience and potential for recovery. Cannabis, among the most commonly used substances by adolescents, has been linked in accumulating research to long-term effects on social and cognitive functioning. Furthermore, early-life disruptions in social play may impair cognitive control later in life. The present study aims to explore the impact of early-life cannabis exposure on adult social play behaviour and cognitive control, as well as the interrelation between these behavioural domains. The study consists of two experiments. In the first experiment, adolescent rats were administered high, low, or no cannabis. During adulthood, these animals were paired with untreated, unfamiliar conspecifics to assess social play behaviour. Subsequently, cognitive control was evaluated using an operant chamber task, in which reward-seeking behaviour had to be inhibited when associated with a risk of punishment. The second experiment was conducted in a similar manner, excluding the assessment of social play, to ensure that social play did not act as a confounding factor in the evaluation of cognitive control. The results showed that most couples, unexpectedly, displayed agonistic behaviour during the social play test. In the cognitive control task, there seemed to be a trend towards more behavioural flexibility in cannabis-exposed animals compared to controls. This study provides insight into the long-term neurobehavioral impact of early-life cannabis exposure, with implications for understanding mechanisms underlying addiction vulnerability and cognitive dysfunction.

Ingrid de Boer

Characterizing airborne microbial diversity across a layer hen production system

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Biosecurity and biocontainment is important in livestock production, with implications for animal health, environmental emissions, and public health. Given limited understanding of the role of airborne transmission for disease spread between farms and their surroundings, research is needed to better characterize airborne microbial entry and emission from livestock farms.

In this study, we investigate the microbial diversity of air entering and exiting through a ventilation unit with heat exchanger in a commercial layer farm. Such heat exchanger systems may offer a practical point of intervention to mitigate microbial entry and exit from poultry systems. Air samples were collected from four locations: near the farm perimeter to represent incoming ambient air; within the ventilation system after filtration and passage through heat exchanger channels; at the barn exhaust; and after passage through the heat exchanger channels and water rinsing system. This sampling design allows for evaluation of microbial changes across key stages of air movement through the system. Total DNA and RNA were isolated from all air samples and total DNA and bacterial DNA were determined using fluorescence and qPCR. Shot-gun metagenomic sequencing was performed to characterize microbial community composition. Descriptive analyses involve comparing total and bacterial DNA load across sampling locations.

Preliminary results indicate highest total and bacterial DNA concentrations in air exiting the barn, while measurable concentrations are also observed in incoming air. Analysis of the ventilation unit's G2 filter indicates that biological materials are partially retained within the filter. Initial findings imply usability of modified air sampling techniques to asses entry and emission of pathogens via air. This work will contribute to a better understanding of bioaerosol dynamics in poultry production systems and may inform future monitoring, mitigation, and biosecurity strategies aimed at reducing environmental and public health risks.

Siem van Hensbergen

Pathogens of the Wolf in the Netherlands

Abstract not available for publication

Maxime Pals

Clinical mastitis incidence and etiology in dairy goats

Abstract not available for publication

Saskia Funk

A Dynamic Whole Knee Joint Culture that Preserves Cartilage and Synovium Viability

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Background: Small laboratory animals are often used for investigating potential therapeutic candidates for joint diseases such as osteoarthritis. This study aimed to establish a simple *ex-vivo* culture system for whole surplus rat knees that preserves viable articular cartilage and synovial membrane to be used for osteoarthritis research purposes.

Methods: Whole rat knee joints were harvested from 10-week-old surplus female and male Wistar rats and cultured for up to 7 days in custom 3D-printed culture inserts under dynamic culture conditions using gentle magnetic stirring. Static cultures in 6-well plates and 50mL vented tube served as comparators. Devitalized and fresh knees served as negative and positive controls, respectively. After culture, articular cartilage (AC) and synovial membrane (SM) explants were collected of each joint. Metabolic activity was assessed using the Alamar Blue assay, followed by enzymatic digestion and flow cytometry using calcein-AM and propidium iodide staining to distinguish live and dead cells.

Results: Dynamic culture conditions consistently outperformed static setups in maintaining AC and SM metabolic activity. However, metabolic activity declined within the first days of culture: reaching ~12% of the baseline by day 5, 8.4% (AC) and 30% (SM) by day 7. In contrast, flow cytometry demonstrated that cell viability remained high during the first 5 days (94-77% live cells), declining to 69% (AC) and 57% (SM) at day 7.

Conclusion: This minimalistic *ex-vivo* whole-knee dynamic culture model preserves AC and SM viability for at least 5 days. The observed discrepancy between metabolic activity and viability may reflect limited sensitivity of the Alamar Blue assay for small tissue samples. Future work will include histological analysis to evaluate microscopic changes during culture. This model reduces the need for animal experiments in early R&D stages for therapies for joint diseases while maintaining essential multi-tissue interactions that are absent in conventional *in vitro* models.

Alice Musi

Spatial Distribution of Targetable Molecules in Canine Melanoma Tissue Samples

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Background: Targeted therapy represents a promising therapeutic option for canine patients with malignant melanoma (cMM). In particular, “cold-immune” melanomas are considered poorly responsive to immunotherapy, but potentially susceptible to targeted treatments based on specific oncogenic mutations. However, cMM is a highly heterogeneous tumor, and this may significantly affect therapeutic response.
Aim: To investigate the expression patterns and genomic alterations of molecular targets associated with potentially effective therapies in cMM.
Materials and methods: *MDM2* and *CDK4* alterations were evaluated in formalin-fixed paraffin-embedded tissue samples from 47 cases of cMM (28 cutaneous and 19 oral). Gene amplification was assessed by fluorescence in situ hybridization (FISH), while protein expression patterns were analyzed by immunohistochemistry. Clinical follow up data, histopathological features and the Ki-67 proliferation index were collected and evaluated.
Results: Diffuse and strong MDM2 immunoreactivity was observed in the majority of the analyzed cMM samples, whereas CDK4 showed a patchy distribution, predominantly localized at the lateral and deep tumor margins. FISH analysis confirmed *MDM2* gene amplification in 50% of the evaluated cases. Notably, gene amplification was also detected at the lateral margins and in intraepithelial growth areas, suggesting a role in more differentiated components and potentially early stages of neoplastic lesions.
Conclusions: No statistically significant associations were observed between molecular alterations and clinicopathological parameters. However, the tissue expression of MDM2 and CDK4, together with the presence of gene amplification in the majority of tumor cells, including intraepithelial melanoma cells, supports the potential application of targeted therapy using MDM2- or CDK4-inhibitors in cMM patients harboring these alterations.

Nena Spyksma

Oncolytic virotherapy: a One Medicine approach on treating Osteosarcoma

Nena Spyksma, Vera Kemp, Sebastiaan van Nimwegen

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Introduction - Osteosarcoma (OSA) is an aggressive form of cancer with very high metastatic rate affecting both humans and dogs. Despite standard of care treatment (surgery and chemotherapy) 90% of dogs die of this disease, denoting the relevance of novel therapeutic strategies. Dogs may act as a large animal model and can help to develop a new therapy approach for OSA treatment in humans (and dogs). Oncolytic virus therapy has the potential to directly infect and destroy tumor cells and to indirectly elicit an immune response against tumor cells. In this research, the oncolytic potential of existing live canine vaccines was tested in vitro in cell lines and in vivo in canine patients with OSA with the primary aim to detect viral replication in canine OSA tissue in a treat-and-resect clinical trial. Secondary aims were to detect possible side effects and long-term efficacy.

Methods - Dogs with osteosarcoma were referred by their primary veterinarian, and if no metastases were found on CT-scan, the dogs were included and received an intra- or peritumoral injection of canine parainfluenzavirus (CPIV). After approximately 7 days, the affected limb was amputated, and the tumor was tested for CPIV using qPCR. Additionally dogs received chemotherapy and frequent metastasis follow ups.

Results - 5 out of 7 tumors tested positive on PCR, with Cq values of <4.09.

Discussion/conclusion - The positive PCR results obtained are promising for future research into the therapy's efficacy and human clinical trials. Furthermore, the limited incidence of side effects underscores the therapy's safety profile.

Sponsored by Leids Universitair Fonds

Kirsten van Bokhorst

Evaluation of long-term follow-up after adrenalectomy and tumor characteristics in silent adrenocortical tumors in dogs

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Hormonally silent adrenocortical tumors (SATs) are typically diagnosed incidentally during diagnostic imaging in dogs without overt clinical features suggestive of adrenal disease. Despite absence of clinical signs and without endocrine test results suggestive of hormone excess, SATs in dogs might be malignant and carry risks of local invasion or metastasis. Limited information is currently available regarding survival of affected dogs and the proliferative activity of SATs. The aim of this study was therefore to analyze long-term follow-up after adrenalectomy of dogs with a SAT and evaluate tumor characteristics including Ki-67 proliferation index (PI) and Utrecht scores.

Diagnosis of SAT was based on finding an adrenal mass using diagnostic imaging, absence of clinical signs suggestive of adrenal hormone excess, negative endocrine function tests and histopathological and immunohistochemical confirmation of adrenocortical origin. Additionally, the Ki-67 PI was quantified, and Utrecht score assigned. Survival data was obtained via referring veterinarians and owners. Tumor-associated survival was evaluated using Kaplan-Meier analysis.

Twenty-seven dogs with a SAT (24 unilateral, 3 bilateral) were included. Median follow-up was 708 days (range 13-2094). One postoperative death occurred after 13 days. Two dogs experienced possible tumor-associated death after 371 and 1898 days. Due to extensive extramedullary hematopoiesis, the Ki-67 PI could not be reliably assessed in 9/30 SATs. In the remaining SATs, the median Ki-67 PI was 3.6% (range 0.3-16.8%) and median Utrecht score 6.6 (range 0.9-21.9).

Results of this study demonstrate excellent long-term prognosis after adrenalectomy in dogs with a SAT. Ki-67 PI values were however observed in ranges associated with poor outcome in other adrenal tumor types, as well as high Utrecht scores exceeding prognostic thresholds established for cortisol-secreting adrenocortical tumors in dogs. Therefore, these scores provide insufficient information on prognostication in SATs.

Rosalie Lotstra

ABSTRACT PRELIMINARY RESEARCH

Comparing CT and contrast-enhanced ultrasound features in adrenal tumors in dogs

R.J. Lotstra, D.H.N. van den Broek, S. Galac, S. Veraa.

Department of Clinical Sciences, Faculty of Veterinary Medicine, Utrecht University. 2026

INTRODUCTION

Hormonally silent or -active adrenal gland tumors are rare in cats and dogs, and diagnosing them requires a combination of clinical information, endocrine testing, ultrasound evaluation and CT imaging. Often hormonally silent tumors are incidental findings in dogs due to their lack of clinical symptoms, frequently called “incidentaloma’s”. After further evaluation around 50% of these incidentaloma’s appear to be malignant, which affects treatment and prognosis. Diagnostic imaging plays a crucial role in differentiating malignant and benign adrenal neoplasia. In veterinary medicine CT imaging is well implemented diagnosing adrenal neoplasia, but clearly defined comprehensive and well proven diagnostic criteria are lacking. Contrast patterns as seen on multiphase CT can help differentiate tumors based on perfusion patterns and attenuation, although more information is needed on the specific characterization of incidentaloma’s. Additionally, contrast-enhanced ultrasonography (CEUS) is seen as a promising non-invasive, low cost diagnostic modality which also has the potential to visualize and characterize perfusion patterns in real time. This study aims to compare and quantify CEUS and multiphase dynamic CT characteristics of adrenal tumors, with special regard to incidentaloma’s, for future use in the work up of adrenal masses in veterinary clinical settings, and to potentially simplify the characterization of incidentaloma’s for clinician and patient.

METHODS

All patients that are eligible for adrenalectomy will have standardized multiphase dynamic CT performed, after which qualitative and quantitative CEUS is performed. Comparative measurements of enhancement pattern, intra-lesional microcirculation, wash-in and wash-out times will be recorded. Lesions will be characterized post-operatively by means of histopathology. Dynamic CT characteristics, CEUS characteristics and histopathology results will be evaluated.

PRELIMINARY RESULTS

Commencing early 2026, patients will be selected and procedures will be commenced and preliminary results are expected to be available within these months.

Anna Tellegen

Normal development and anatomical variation of the canine acromion on computed tomography and radiography.

A.R. Tellegen, A. M. T. van Beek, S. Veraa

Utrecht University, Faculty of Veterinary Medicine, Division of Diagnostic Imaging, The Netherlands.

Introduction:

This retrospective study aims to define the age and pattern of complete ossification of the canine acromion and characterizes acromion contour variations using radiographs and CT scans. Moreover, the radiological appearance and variations of the adult canine acromion are described. Irregularities along the acromial contour have been observed anecdotally, but the age of complete ossification has not yet been reported in dogs.

Materials and Methods:

Imaging studies from 1,208 dogs (902 CT scans, 424 radiographs) at a university referral hospital were analyzed. Dogs were categorized into two age groups: <2 years and ≥2 years. On radiographs, the acromia were classified as regular or irregular. On CT scans, the acromia were assigned a score ranging from regular (score 0), irregular (score 1), osseous fragments (score 2), growth plate-like structures (score 3) to separate ossification centers (score 4). The cranial and lateral acromial margins were scored separately on CT. The study also considered the factors age, sex, breed, castration status, and body weight.

Results:

The maximum observed age for complete acromial fusion was 478 days on CT and 295 days on radiographs, with significant variation in individual closure timelines. In the ≥2 years group, 24% had irregular acromion contours on radiographs. On CT, 45% of the acromia showed irregularities. In dogs >2 years old, higher body weight was associated with irregularities of the acromion.

Conclusion:

Irregular acromion contours in younger dogs are likely developmental (i.e., separate ossification centres). In older dogs, irregularities may be normal anatomical variations or signs of pathology, such as fractures or enthesophytes, as the acromion serves as the attachment site of the acromial part of the deltoid muscle. Further studies, including histopathological analysis, are needed to confirm the results and clarify the clinical significance of these findings.

Ronette Gehring

Tk Plate 2.0: A Generic Multi-Species Physiologically Based Toxicokinetic Model for Regulatory Application

BACKGROUND
TK Plate was developed as a generic physiologically based toxicokinetic (PBTK) modelling platform to support regulatory risk assessment using literature-derived system and chemical-specific parameters. TK Plate 2.0 extends the framework to additional food-producing species, wildlife mammals, and plant protection products in birds, and strengthens the parameter databases underpinning model application.

HYPOTHESIS
A generic PBTK model parameterised a priori with species-specific physiological and anatomical values, combined with curated and predicted chemical-specific parameters, can support robust cross-species toxicokinetic predictions for regulatory decision-making in data-limited situations.

METHODS
The model structure was expanded to incorporate additional species and chemical domains. Physiological and anatomical parameters were compiled from published literature and implemented as fixed system parameters. Emphasis was placed on systematic population of databases for chemical-specific inputs, including clearance, absorption rate constant (ka), oral bioavailability (F), and tissue:blood partition coefficients. Parameter gaps were identified and in silico approaches, including machine learning, were developed to predict missing values from curated physicochemical and biological descriptors.

RESULTS
TK Plate 2.0 provides a broadened multi-species platform supported by an extensive database of metabolic enzyme and transporter expression and activity across species. Machine learning models showed good predictive performance for clearance and partition coefficients, substantially reducing missing parameter space and enabling model use in data-poor scenarios relevant to regulatory assessment.

CONCLUSION
TK Plate 2.0 is a scalable, regulator-oriented, literature-driven PBTK platform integrating curated databases with predictive in silico methods. The framework supports cross-species extrapolation, strengthens evidence-based risk assessment, and contributes to reduced reliance on experimental animal data while maintaining mechanistic biological relevance.

Esther Winter

A Population Pharmacokinetic Model of Gentamicin In Hospitalized Neonatel Foals

Astrid J. van den Brom-Spierenburg[1] & Esther A. Winter[2], Mathijs J.P. Theelen[1], Ingeborg M. van Geijlswijk[2], Cornélie M. Westermann[1], Marianne M. Sloet van Oldruitenborgh-Oosterbaan[1], Tim Florschütz[1], Johannes C. Vendrig[2], Esther W. Siegers[1], Kim de Graaf[3], Marc Cherlet [4], Mathias Devreese[4], Ronette Gehring[2]

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Background: Gentamicin is frequently used in critically ill neonatal foals. As a concentration-dependent antimicrobial, dosing must ensure sufficiently high peak plasma concentrations for efficacy while maintaining low trough concentrations to minimize nephro- and ototoxicity. Currently, there is limited information on pharmacokinetics of gentamicin in critically ill neonatal foals to inform optimal dosing regimens.

Aim: This study aimed to provide guidance regarding optimal dosing regimens by means of a population pharmacokinetic (popPK) model.

Methods: Gentamicin plasma concentrations were measured in foals (≤ 14 days old) admitted to two clinics in the Netherlands, and treated with intravenous gentamicin as part of their treatment. A population pharmacokinetic (popPK) model was developed using non-linear mixed effect modeling. Patient characteristics and selected hematological and biochemical parameters were evaluated as potential covariates on pharmacokinetic parameters. The final popPK model was used to simulate clinically relevant dosing regimens in hospitalized neonatal foals, specifically 6.6mg/kg q24h and 12mg/kg q36h.

Results: Seventy-two foals were included, contributing to 291 samples. A 2-compartment model with combined proportional and multiplicative error best described the data. Age was identified as a significant covariate on clearance. Following administration of 6.6mg/kg q24h, Cmax (1 hour post-administration) range is 4.8-16.3mg/L in 1-day-old foals and 3.5-15.5mg/L in 14-day-old-foals. With 12mg/kg q36h, Cmax range is 8.7-29.7mg/L in 1-day-old foals and 6.3-28.2mg/L in 14-day-old foals. Mean trough concentrations were <1mg/L for both dosing regimens, irrespective of age.

Conclusion: A dose of 6.6mg/kg q24h appears appropriate for pathogens with a minimum inhibitory concentration (MIC) ≤ 1mg/L when targeting a pharmacodynamic index of Cmax/MIC >8. The 12mg/kg q36h regimen may achieve adequate exposure for pathogens with an MIC of up to 2mg/L. The model provides a rational basis for selecting an initial dosing regimen in neonatal foals. However, the substantial interindividual variability observed supports the use of therapeutic drug monitoring to further individualize therapy.

Zheng Yen Ng

Inclusive play and sports for children with disability or chronic health conditions in urban physical spaces: a protocol of the SPACES project

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This project investigates inclusivity in play and sports in urban physical spaces for children with a disability or chronic health condition. Urban physical spaces, where children gather, play, and do sports together, can be made more inclusive for all children. Children with a disability often participate less in physical and social activities, due to an inaccessible design of physical spaces or a lack of understanding about their abilities, strengths, and needs. Playing and doing sports together can give children self-confidence and connection with community, which becomes increasingly important as they grow and participate in society. Limitations in opportunities to engage in play may hamper children's development. This project therefore aims to first determine the barriers and facilitating factors that affect inclusive play for children with a disability, with a particular focus on the physical outdoor space. This knowledge will be taken as the starting point for co-design of solutions to encourage inclusive play for all children. For this project we will use participatory methods and co-creation with children with and without disability, their families, care professionals, lived experience experts, designers, architects, city council, and other stakeholders. Ultimately this work will contribute to inclusive participation of all children, empowering them to thrive as they grow up into adulthood.

Ziqiong Wang

Zinc regulates CRISP2 condensation in sperm

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CRISP2 (cysteine-rich secretory protein 2) is a member of the CAP protein superfamily and is associated with multiple stages of sperm maturation. It is synthesized during spermatogenesis and localizes to both the sperm tail and the post-acrosomal region of the sperm head. Previous work demonstrated that CRISP2 forms condensates in sperm, which are released from the sperm nucleus after fertilization, suggesting a potential role in early reproductive processes.

Zinc is a key regulator of sperm function and displays marked changes across the reproductive context, being abundant in seminal plasma and reduced during capacitation-associated transitions in the female tract. Given that zinc also regulates protein condensation, these dynamics suggest that zinc may control the formation and stability of CRISP2 condensates. Consistent with this, our previous studies showed that zinc regulates amyloid-like protein condensation. The parallel behaviour of CRISP2 assemblies and zinc availability during fertilization led us to hypothesize that zinc may regulate CRISP2 condensation.

To test this, we employ a biochemical approach using a novel inducible yeast-based system developed in our laboratory. Fluorescently tagged CRISP2 is overexpressed in yeast, enabling visualization of condensate formation and its modulation by external factors such as zinc. In addition, metal-binding site prediction combined with targeted mutagenesis is used to investigate the molecular basis of zinc-dependent CRISP2 condensation.

Currently we are studying the zinc dependent condensation dynamics of sperm CRISP2 prior to and after fertilization. Together, these findings aim to provide insights into the role of protein condensation as well as decondensation in fertilization and contribute to a broader understanding of dynamic protein assemblies and the dissociation of them in reproductive biology.

Thijs Messing

An Affordable Smoke Delivery System for the Inhaled Administration of Combusted or Vaporized Cannabis to Rodents

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The legalization of cannabis has led to increased cannabis use across the world. Yet, much of the effects of cannabis on the development of the brain and cognitive functions remains unclear. Current preclinical in vivo studies typically rely on injections of THC, the main psychoactive component, to study the effects of cannabis. This differs substantially from human cannabis use in both pharmacokinetics and chemical composition, limiting the translational validity of these models. Inhalation of combusted or vaporized cannabis addresses these limitations, but methods of delivering the resulting smoke can be costly and difficult to implement.
Here, we present a custom-built smoke delivery system that is affordable, easy to construct, and allows for safe administration of combusted and vaporized cannabis to small rodents. It consists of two airtight chambers connected in a tube system with controlled airflow and manually powered by a pump. For combustion, an electronic vape controlled by an Arduino system heats up a coil holding the cannabis and the smoke is directly pulled inside the smoke delivery system. Vaporization of cannabis is achieved with a volcano device where the vapor is collected inside a balloon, that is subsequently attached to the smoke delivery system.
Adult rats were exposed to either combusted or vaporized cannais before blood and brain samples were collected and analyzed to confirm effective intake of THC and its metabolites with both methods. These findings demonstrate that cannabis vapor can be administered to rodents, substantially increasing the validity of cannabis research.

Effrosyni Kritsi

Paired CT and Standard X-ray Workflow for AI-Assisted Post-Mortem Broiler Carcass Health Assessment

Effrosyni Kritsi, Andrea Basso, Kacper Libera, Alex Bossers, Lidwien A. M. Smit, Lapo Mughini-Gras, Lourens Heres

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Modern poultry slaughterhouses process large numbers of carcasses under time pressure, making thorough post-mortem assessment challenging when relying only on visual inspection. This creates an opportunity for imaging approaches that are meaningful, repeatable, and relevant to post-mortem inspection practice. In this work, we build links between controlled laboratory imaging of carcasses and isolated organs, and the conditions that matter in daily veterinary and food safety inspection.

To address this transition from laboratory work to practical application, we develop 3D model concepts that link whole-carcass imaging, isolated-organ imaging, and projection-based imaging. These include whole-carcass three-dimensional computed tomography (CT) imaging, isolated healthy and diseased hearts and livers, and specially designed 3D positioning structures that mimic body-cavity context and slaughter-line orientation. In addition, tray- and hanger-based concepts were developed to standardize organ placement and carcass positioning, enabling matched acquisition of CT volumes and standard X-ray images in anteroposterior (AP) and lateral view in a controlled yet practice-oriented way.

Together, these 3D designs form a workflow in which CT serves as the detailed three-dimensional anatomical reference and standard X-ray represents the more practical route toward future inspection applications. This work therefore lays the foundation for subsequent AI tasks such as organ localization, segmentation, and healthy-versus-diseased differentiation, with the broader aim of moving imaging innovation from the laboratory toward application in food safety inspection.

VetPhD council

Emel Souiki , Population Health Sciences, Animals in Science and Society
Iram Gladan, Population Health Sciences, Farm Animal Health
Maxime Pals, Population Health Sciences, Farm Animal Health
Amitabha Govande, Population Health Sciences, Institute for Risk Assessment Sciences
Esmée Janssen, Population Health Sciences, Institute for Risk Assessment Sciences (IRAS)

The Veterinary PhD Council is concerned with the interests and issues of the PhD students at the Veterinary Faculty of Utrecht University. Moreover, we organize several PhD-related activities such as lectures concerning PhD matters, but we also organize social activities to bring PhD's together, such as tastings and escape rooms. To receive invites for our activities, please register yourself to our email list via VetPhDC@uu.nl

The Council consists of PhD representatives from the research programs within our faculty. The council meets every month to discuss issues and problems relevant to veterinary medicine PhD students. We are looking for new members. Would you be interested in joining? You are more than welcome, please get in touch via VetPhDC@uu.nl

Do you have any questions or concerns regarding PhD policy or PhD related matters, feel free to contact us via VetPhDC@uu.nl or approach one of our representatives:

- Emel Souiki , Population Health Sciences, Animals in Science and Society
- Iram Gladan, Population Health Sciences, Farm Animal Health
- Maxime Pals, Population Health Sciences, Farm Animal Health
- Amitabha Govande, Population Health Sciences, Institute for Risk Assessment Sciences
- Esmée Janssen, Population Health Sciences, Institute for Risk Assessment Sciences (IRAS)

Graduate School of Life Sciences (GSLS) PhD council

June Kim[1], Anne Rittscher-Fogg[1]

1. IRAS, Utrecht University

The interests of PhD candidates enrolled with the graduate school of life sciences are represented by the PhD council. The GSLS PhD Council is made up of representatives from all GSLS PhD programmes and the three Life Sciences faculties and main institutes. The PhD Council advises the GSLS management team and the Board of Studies on the quality of doctoral education. Activities include evaluating the PhD research environment and PhD programmes through an annual survey, organizing an annual PhD Day, the Supervisor of the Year Awards, and other PhD events. The Council meets monthly with the GSLS coordinator of doctoral education.(Text from the GSLS website)

Meet Team Safety

Ruud Eerland, Karen Loots, Judith Oymans, Femke Schelling, Astrid van Velden, Anke Wassink, Hans van Woudenberg

Hi, we are Team Safety, a team consisting of colleagues in biosafety, occupational health, radiation protection, and emergency response, all dedicated to supporting everyone at the Faculty of Veterinary Medicine.

You may know us as the team to call during emergencies or incidents, but our work goes much further than that. We manage permits for research involving genetically modified organisms and provide guidance on the safe handling of chemicals and biological agents. Workplace wellbeing is also central to what we do: we conduct ergonomic assessments and supply the tools and protective equipment needed to keep you healthy on the job. Beyond day-to-day safety, we serve as expert advisors on the construction of the new faculty building and the Veeducatorium, helping to ensure both spaces are designed with safety at their core from the very start. We also develop e-learning programs and contribute to the faculty's sustainability and environmental efforts. In short, we are your go-to team for all the things you didn't know you needed to ask.

UU Library Support Team

The library offers support for staff and students on data, publishing, education and collections. So if you need just that one book or want to find all relevant articles on a topic, there's support! Also if you're planning a research project, we'll review your DMP and think about how and where to publish. And if you can't find the full text of a publication - Want to give a training or workshop on information literacy or data management (for students or colleagues) – Or, have a question about (almost) anything else, contact the library!

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The Research Support Office (RSO) of the Faculty of Veterinary Medicine provides comprehensive advice and guidance on policy and quality assurance, external collaboration, legal advice, pre- and post-award management, research data management and data protection. Our goal is to create and sustain relationships. We combine our strengths and expertise to enhance the quality and impact of your research. We build bridges between faculty, external partners and donors (public and private). We advise - proactively and pragmatically - throughout the project cycle: from identifying the best grant/donation options, navigating your application to business development, legal advice and project management.

Parallel session 4: From Bioengineering to Regenerative Medicine

Paulina Nunez Bernal

Volumetric bioprinting of functional iPSC-derived cardiac constructs using dynamic hydrogels for *in vitro* modelling

Bernal PN*[1,2,3], Janssen J*[3,4], Größbacher G[1,3], Chirico N[3,4], Falandt M[1,3], Cervera i Barea A[3,4], Brice C[1,3], Florczak S[1,3], Mesfin J[5], Dokter I[3,4], Snijders Blok CJB[3,4], Christman K[5], Sluijter J[3,4], Malda J[1,2,3], van Mil A[#3,4], Levato R[#1,2,3]
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Introduction: Cardiovascular disease is the leading cause of mortality worldwide, yet existing *in vitro* cardiac models do not fully capture the structural and functional complexity of human heart tissue. Conventional 2D cultures and engineered heart tissues often fail to capture physiologically relevant cell-cell interactions and anatomical organization. Here, we developed an iPSC-derived, anatomically-relevant *in vitro* cardiac model using high-speed, contactless volumetric bioprinting (VBP)[1] and HybriGel, a dynamic, viscoelastic hydrogel designed to support cell organization and coordinated beating.

Methodology: Bi-chamber heart models were fabricated via VBP using human induced pluripotent stem cell-derived cardiomyocytes (iPSC-CMs) and cardiac fibroblasts (10:1, $10x10^6$cells/mL) embedded in HybriGel. his hydrogel combines covalent crosslinking with reversible supramolecular interactions, creating a dynamic environment that promotes cellular organization. Cell viability, network formation, and structural organization were assessed via LIVE/DEAD and immunofluorescence imaging. Functional characterization included beating rate analysis and calcium handling imaging. To evaluate disease modelling capabilities, myocardial infarction was simulated via cryoinjury, followed by histological, functional, and RNA sequencing analysis.

Results: Heart models (~165mm^3, printing time=21s) showed high shape fidelity and enabled the generation of complex geometries, including asymmetric models and a four-chamber heart replica. iPSC-CMs in HybriGel maintained >75% viability and formed more extensive networks than in fully covalent GelMA controls over 14 days. Printed hearts displayed coordinated contractions and directional calcium waves within one week of culture. Immunofluorescence confirmed cardiomyocyte alignment along chamber walls and septal regions. Cryoinjury disrupted local calcium signaling and and induced asynchronous activity across the heart wall. RNA sequencing of cryoinjured hearts revealed upregulation of genes associated with fibrotic remodeling and ECM deposition, mirroring clinical patterns observed post-myocardial infarction.

Conclusion: VBP combined with dynamic biomaterials enables rapid fabrication of anatomically relevant cardiac tissues that support functional organization and injury responses. This scalable platform has potential for disease modelling, drug screening, and personalized therapies.

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Camille Bonhomme

AddGraft: a biocompatible and versatile additive for light-based 3D patterning within crosslinked hydrogel networks.

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Replicating the time-dependent changes in the physicochemical microenvironment of native tissues remains a key challenge in biofabrication. While 3D bioprinting enables the creation of complex cell-laden architectures, the resulting constructs are mostly static. New strategies, like photo-grafting, can introduce spatiotemporal modifications in a contact-less manner, but often rely on specific chemistries, requiring complex synthetic steps and optimization for new materials. In this study, we propose an off-the-shelf biocompatible additive, AddGraft, enabling post-gelation modification via thiol-ene click chemistry into any acrylated network. AddGraft's compatibility with volumetric bioprinting allows for tomographic light patterns-driven freedom of design for distributing grafted molecules across a wide range of biomaterials.

The incorporation of the additive into GelMA did not affect bulk stiffness, crosslinking kinetics, or physical properties, and maintained a high hMSCs viability (>95%). Up to a 3-time fold increase in the stiffness of AddGraft-containing GelMA hydrogels was observed upon grafting different thiol crosslinkers. Volumetric photografting enabled high-resolution patterning of fluorescent molecules (60.8±13.4 µm features), including grafting of multiple compounds. Grafting density is light-dose dependent, enabling the formation of gradients upon greyscale illumination. Comparable results were achieved when using AlgMA and PEGDA with incorporated AddGraft. Finally, encapsulated hMSCs exhibited high viability post-stiffening (>85%) and significant changes in morphology and circularity.

AddGraft provides a versatile and biocompatible strategy for on-demand spatiotemporal modifications of acrylated hydrogels. This control over the hydrogels' properties over time allows for studying specific cells' behavior. Its compatibility with light-based biofabrication techniques, such as volumetric printing, and widely used acrylate-based hydrogels, positions AddGraft as a powerful tool for 4D bioprinting strategies, with a strong potential in regenerative medicine and relevant *in vitro* modelling.

Charlotte Brice

Volumetric bioprinting of iPSC-derived pancreatic islets into perfusable constructs for diabetes drug testing

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Functional islets from induced pluripotent stem cells (iPSCs) hold promise as a renewable cell source for pancreatic tissue engineering. Despite advances in differentiation protocols, iPSC-derived islets remain immature and lack the microenvironmental cues necessary for full functionality. This limits development of robust *in vitro* platforms, impeding deeper insights into islet biology and function. Therefore, the aim of this work is to develop an engineered pancreatic construct integrating iPSC-derived islets within gelatine methacryloyl (GelMA) using light-based volumetric bioprinting (VBP), creating endocrine units that better replicate the pancreatic microenvironment.

iPSC-derived pancreatic islets were generated using an established protocol. Upon differentiation, islets were suspended in GelMA and bioprinted using VBP. Constructs were analysed by immunofluorescence (IF) imaging, single-cell transcriptomics, and glucose-stimulated insulin secretion (GSIS) assays under static/dynamic conditions. Dynamic GSIS assays were performed in the presence of a GLP-1 analogue to assess the potentiation of insulin secretion, as well as following streptozotocin treatment to evaluate β-cell susceptibility within the constructs.

VBP enabled rapid fabrication of centimetre-scale constructs (t<30s), preserving viability and metabolic activity for at least 21 days. Islets bioprinted and matured within GelMA exhibited increased insulin secretory capacity compared to non-printed controls. These constructs contained mature, single-hormone producing cells, confirmed by IF and single-cell transcriptomics. Mathematically-defined structures containing iPSC-islets were perfused for up to 21 days and functionally validated using dynamic GSIS assays. The perfusable constructs supported anti-diabetic drug screening as shown by their significant increase in insulin secretion following administration of a GLP-1 analogue. Additionally, streptozotocin-treated constructs showed no insulin release capacity, with selective β-cell toxicity confirmed through IF analysis.

This study established VBP as a rapid, versatile strategy for fabricating perfusable pancreatic constructs. The engineered architectures support high cell viability, preserve the endocrine phenotype, and enable dynamic functional assessment of insulin secretion in response to physiological and pharmacological stimuli.

Parallel session 5: From Omics to Molecular Pathways

Marit van den Berg

Plasma proteomic profiling in dogs with pheochromocytoma

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Pheochromocytomas (PCCs) in dogs are challenging to diagnose and treat owing to nonspecific clinical signs, the occurrence of biochemically negative tumors, and limited medical options. Plasma proteomics offers a minimally invasive approach to identify circulating biomarkers and disease-relevant pathways to guide diagnosis and therapy.

This study aimed to compare the plasma proteome of dogs with PCC and controls to identify differentially abundant proteins, characterize altered pathways, and nominate candidate circulating biomarkers and therapeutic targets.

Plasma from 10 client-owned dogs with PCC and 10 healthy controls was analyzed in a multicenter, retrospective, observational, exploratory study using label-free liquid chromatography-mass spectrometry.

Principal component analysis demonstrated clear separation between PCC and control dogs. Of 261 reliably quantified proteins, 51 were differentially abundant (FDR-adjusted $P < .05$). Of these, 33 had a $\log_2$ fold change of >1 or <−1, with 15 showing higher and 18 lower plasma abundance in PCC. Compared with controls, PCC dogs showed increased abundance of proteins linked to cell adhesion/migration and metastatic potential, hemostasis, and regulation of apoptotic signaling, alongside alterations in oxidative stress and metabolic processes. Several proteins with higher abundance, including CD44, peroxiredoxin-2 (PRDX2), and peptidyl-prolyl cis-trans isomerase (PPIA), emerged as promising candidates for therapeutic exploration.

In conclusion, dogs with PCC exhibit a distinct circulating protein signature versus healthy controls, providing clues to PCC pathogenesis and nominating proteins as diagnostic biomarker candidates and potential therapeutic targets.

Marnix van Soest

Characterizing Yolk Sac–Associated Malignancy through Tumoroid Modeling and Single-Cell Transcriptomics to Enhance Understanding of Pluripotent Stem Cell Safety

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Concern about the safety of pluripotent stem cell (PSC)-based products, particularly their potential malignant transformation, hinders their clinical translation. The teratoma assay, where PSCs are xenografted into immunocompromised mice, remains the standard for PSC safety assessment. Histologically, a PSC line is considered malignant when embryonal carcinoma-like cells are present in the resulting xenograft. Yolk sac elements may also appear, yet their significance for PSC product safety is unclear. Yolk sac elements resemble yolk sac tumor (YST), which is a clinically malignant subtype of human germ cell tumors with poor prognosis. However, the mechanism underlying YST malignancy remains largely unexplored.

We hypothesize that yolk sac elements observed in PSC-derived xenografts and YSTs share a similar developmental origin and differentiation pathway, potentially hinting at a shared malignant behavior. To investigate this, we performed single-cell RNA sequencing on historical paraffin-embedded human pure YSTs, revealing intratumoral heterogeneity and distinct developmental subpopulations. In parallel, we have characterized available YST cell lines and established a novel patient-derived YST tumoroid, to validate our transcriptomic findings and study yolk sac-related malignancy *in vitro*.

Together, these findings may clarify the developmental and molecular pathways driving yolk sac–related malignancy, informing both clinical understanding of YST and safety evaluation of PSC–based products.

Ruoshui Guo

Chemical mixture identification and characterization using GC-HRMS data from blood samples of the general EU population

Abstract not available for publication

Parallel session 6: From Animal and Environment to Human Health

Ana-Maria Pirvulescu

Quantifying The Economic Burden Of Zoonotic *Salmonella* In Poultry Using A Stochastic Modelling Approach For The Netherlands, Belgium, And Denmark

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Zoonotic *Salmonella* remains a major target of control programmes in European poultry production, yet its economic burden at the animal production level is not well quantified across countries. This study aimed to develop an animal cost-of-illness (a-COI) framework and apply it to estimate the economic burden of zoonotic *Salmonella* infections in poultry populations under surveillance in the Netherlands, Belgium, and Denmark. The framework combined publicly available epidemiological data, economic inputs, and production-impact parameters to estimate direct tangible costs associated with surveillance, control, and eradication, as well as indirect tangible costs from production losses and revenues foregone due to excess mortality or culling. Uncertainty was propagated using Monte Carlo simulation, and results were additionally expressed as purchasing power parity (PPP)-adjusted estimates and per 100,000 birds. Median total tangible a-COI was estimated at €14.61 million/year (95% UI: €10.53-20.15 million) in the Netherlands, €4.11 million/year (€3.07-5.61 million) in Belgium, and €0.78 million/year (€0.69-0.89 million) in Denmark. Direct tangible costs accounted for the largest share of the burden in all three countries. PPP-adjusted results and size-standardised metrics showed the same cross-country ranking. Sensitivity analysis indicated that uncertainty was driven mainly by broiler excess mortality and production-performance parameters. This study provides a novel and transparent framework for quantifying the animal-sector economic burden of zoonotic *Salmonella* in poultry and offers a useful basis for future One Health economic assessments.

Sade Adetunji

A Cross-Sectional Study of Staphylococcus aureus Reservoirs in Dairy Goats, their Environment and Farm Workers

Abstract not available for publication

Jolyn Oosters

Mapping the landscape of small-scale and backyard poultry flocks in the Netherlands: a nationwide survey for One Health surveillance

Abstract not available for publication