



The VSD committee is proud to present the abstracts of the presentations of the 2019 Veterinary Science Day of 2019. The theme of the Veterinary Science Day of 2019 is: “Synergy for Science”

We all will welcome new co-workers in our departments, when eight departments become three. The Veterinary Science Day of 2019 is a perfect moment to meet these new co-workers in your department as well as to explore opportunities for future collaborations with people in the other two departments.

We hope you will enjoy listening to the talks, viewing the posters and participating in the discussions.

Marijke Achterberg
Berend Jan Bosch
Manon Bouwmeester
Maaïke Scheenstra
Tijs Tobias
Aldert Zomer





PRESENTATIONS

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* Link to abstract

Regarding non available abstracts; the authors did not approve publishing of the abstract at time of submission, please contact authors for information

**Titel:**

The Menkes and Wilson disease genes counteract in copper toxicosis in Labrador retrievers: a new canine model for copper-metabolism disorders

Authors

H. Fieten, Y. Gil, A. Martin, M. Concilli, K. Dirksen, F. v Steenbeek, B. Spee, T. vd Ingh, E. Martens, P. Festa, G. Chesi, B. vd Sluis, R. Houwen, A. Watson, Y. Aulchenko, V. Hodgkinson, S. Zhu, M. Petris, R. Polishchuk, P. Leegwater, J. Rothuizen

Abstract:*Introduction*

The deleterious effects of a disrupted copper metabolism are illustrated by hereditary diseases caused by defects in copper transporters ATP7A and ATP7B. Menkes disease, involving ATP7A, is a fatal neurodegenerative disorder of copper deficiency. Mutations in ATP7B lead to Wilson disease, which is characterized by hepatic copper accumulation. The low incidence and the phenotypic variability of human copper toxicosis hamper identification of genes involved in the diseases. Purebred dogs have reduced genetic variability, which facilitates identification of genes involved in complex heritable traits that might influence phenotype in both humans and dogs.

The aim of this study was to unravel the hereditary background of copper toxicosis in the Labrador retriever.

Methods

We performed a genome wide association analysis in 235 Labrador retrievers that were phenotypically characterized by evaluation of liver biopsies and copper scoring. Coding regions of candidate genes in associated regions were investigated by Sanger sequencing. Significant variations in this group were replicated in an independent cohort. Identified mutations were further investigated using radio-active copper assays in dermal fibroblasts and by immunofluorescence studies in cells transfected with the identified mutations.

Results

Two chromosome regions containing the genes coding for the copper transporters ATP7A and ATP7B were respectively negatively and positively associated with hepatic copper levels. DNA sequence analysis identified missense mutations in each gene. Confocal microscopy indicated that aberrant copper metabolism upon expression of the ATP7B variant occurred because of mis-localization of the protein in the endoplasmic reticulum. Dermal fibroblasts derived from ATP7A mutants showed copper accumulation and delayed excretion.

Conclusions

The Labrador retriever is the first natural, non-rodent model for ATP7B-associated copper toxicosis. Attenuation of copper accumulation by the ATP7A mutation sheds an interesting light on the interplay of copper transporters in body copper homeostasis and warrants a thorough investigation of ATP7A as a modifier gene in copper-metabolism disorders.

**Titel:**

Novel in vitro strategies for poultry vaccine efficacy testing to secure vaccine quality and replace animal tests

Authors

Robin HGA van den Biggelaar, Larissa van der Maas, Hugo D Meiring, Victor PMG Rutten, Willem van Eden, Christine A Jansen / Department of Infectious Diseases and Immunology, Faculty of Veterinary Medicine, Utrecht University, the Netherlands

Abstract:

Many vaccines that were developed decades ago are still cost-effectively used today to prevent infectious disease outbreaks in the poultry industry. To secure efficacy, poultry vaccines are routinely tested using in vivo vaccination-challenge studies or serological assays. However, the large numbers of test animals required for these tests are an economical and ethical disadvantage. Therefore, the aim of this study was to develop cell-based assays to substitute current animal experiments for routine quality control of vaccines. These assays are based on acquired insights into the mechanisms by which inactivated poultry vaccines activate the innate immune system.

In vitro assays were set-up with chicken bone marrow-derived dendritic cells and the macrophage cell line HD11 to find novel vaccine-induced biomarkers associated with the innate immune system. To do so, cells were stimulated with a variety of mono- and multivalent whole-inactivated poultry vaccines formulated as water-in-oil emulsions. Subsequently, the stimulated cells were analyzed by mass spectrometry, flow cytometry, RT-qPCR, and functional assays.

Viral inactivated poultry vaccines were found to induce metabolic changes and enhanced phagocytosis capacity, but had only limited effects on co-stimulation or cytokine production. In contrast, a bacterial infectious coryza vaccine strongly induced immunological pathways including nitric oxide production and increased cytokine expression.

These results showed the potential of cell-based assays to perform a high quality immunological assessment of vaccines without the use of test animals. The next step will be to standardize and implement these assays to replace current animal tests used for routine quality control of vaccines.

This work has received support from the EU/EFPIA/Innovative Medicines Initiative 2 Joint Undertaking (VAC2VAC grant n° 115924).



Nr. Abstract
C

Titel:

Competition between Escherichia coli with and without Extended-Spectrum-Beta-Lactamase-carrying plasmids in the broiler chicken gut

Authors

Egil A.J. Fischer (IUU), Cindy M. Dierikx (RIVM), Alieda van Essen-Zandbergen (WBVR), Dik Mevius (UU, WBVR), Arjan Stegeman(UU), Francisca C. Velkers(UU), Don Klinkenberg(RIVM)

Abstract:

ESBL/AmpC-producing *Escherichia coli* are widely found in broiler faeces, much of which is due to the blaCTX-M-1 gene on IncI1-plasmids. Plasmid carriage is theorized to cause fitness loss, thus plasmid carriage should decrease when reducing antibiotic use. However, in vitro studies(1,2) showed plasmid carriage to increase in the absence of antimicrobials, due to plasmid conjugation. We investigate if this translates to plasmid increase in the gastrointestinal tracts of chickens, where conjugation rates may be different and subtle differences in growth rates may have a larger impact on colonization.

Eight groups of five chickens were orally inoculated at four days of age with 0.5 ml containing 10^6 CFU/ml *E. coli* of which 0%, 0.1%, 10% or 100% carried the IncI1-plasmid with the gene blaCTX-M-1. During 41 days, faecal samples were taken from each chicken. *E. coli* with and without plasmids were quantified. Bayesian regression and mathematical model fitting were performed to understand the ESBL-dynamics.

Trends in *E. coli* subpopulations were different between groups rather than individual chickens, suggesting substantial *E. coli* exchange between chickens in a group. The IncI1 plasmid carrying blaCTX-M-1 was transferred with conjugation coefficients larger than observed in vitro. Across groups, the plasmids disappeared or established independently of the initial fraction of plasmid-carrying *E. coli*, but no major increase occurred as observed in vitro. Differences in growth rates were observed, but competitive exclusion was counteracted by conjugation.

This presentation is based on our recent publication (3), and shows how the collaboration of mathematical modellers, veterinary microbiologists and poultry veterinarians increases insight in the spread of antimicrobial resistance. This was a collaboration with WBVR in Lelystad.

1 Fischer, E. A. J. et al. BMC Microbiol. 14, 77 (2014)

2 Lopatkin, A. J. et al. Nat. Commun. 8, (2017)

3 Fischer, E. A. J. et al. Appl. Environ. Microbiol. AEM.00892-19 (2019)

**Titel:**

*Evidence of high EEHV antibody seroprevalence and spatial variation among captive Asian elephants (*Elephas maximus*) in Thailand*

Authors

Taweepoke Angkawanish, Mirjam Nielen, Hans Vernooij, Janine Brown, Peter JS. van Kooten, Petra B. van den Doel, Willem Schaftenaar, Kannika Na Lampang, and Victor P.M.G Rutten

Abstract:

Elephant endotheliotropic herpesviruses (EEHV) can cause an acute highly fatal hemorrhagic disease in young Asian elephants (*Elephas maximus*), both ex situ and in situ. Amongst eight EEHV types described so far, type 1 (subtype 1A and 1B) is the most common and virulent. Little is known about routes of infection and pathogenesis. Likewise, knowledge of disease prevalence, especially in range countries, is poor. A large cross sectional serological survey using an EEHV1A glycoprotein B protein antigen specific ELISA, was conducted in camp elephants (n= 994) throughout Thailand. The results were combined with risk factors potentially related to EEHV infection, like elephant age, sex, and geographical location of camps, elephant density, and season. Results indicated a seroprevalence of 42.3%, with 420 of 994 elephants testing positive. Univariable regression analysis identified management system (extensive versus intensive) and region as potential risk factors for the presence of EEHV antibodies, with region being significant in the final multivariable regression model. Prevalence was highest in the North region of the country (49.4%). This study produced baseline serological data for captive elephants throughout Thailand, and found a potentially significant EEHV burden in the population. Thus, there is a high likelihood the disease will continue to be maintained in the captive population, which could potentially threaten wild elephants, although there is the possibility that they too have been exposed to EEHV.



Nr. Abstract

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Titel:

Lecithin: Retinol Acyl Transferase (LRAT) induces the formation of lipid droplets

Authors

Martijn R. Molenaar[§], Tsjerk A. Wassenaar[‡], Kamlesh K. Yadav[#], Alexandre Toulmay[#], Muriel C. Mari[&], Lucie Caillon[£], Aymeric Chorlay[£], Maya W. Haaker[§], Richard W. Wubbolts[§], Martin Houweling[§], A. Bas Vaandrager[§], Fulvio Reggiori[&], Abdou Rachid Thiam[£], William A. Prinz[#], J. Bernd Helms[§]

[§] Department of Biochemistry and Cell Biology, Faculty of Veterinary Medicine, Utrecht University, 3584 CM, Utrecht, The Netherlands

[‡] Groningen Biomolecular Sciences and Biotechnology Institute and Zernike Institute for Advanced Materials, University of Groningen, Nijenborgh 7, 9747 AG Groningen, The Netherlands

[#] National Institute of Diabetes and Digestive and Kidney Diseases, NIH, Bethesda, MD 20892, USA & Department of Cell Biology, University of Groningen, University Medical Center Groningen, Groningen, Netherlands [£] Laboratoire de Physique Statistique, Ecole Normale Supérieure, PSL Research University, Sorbonne Université, UPMC Université Paris 06, Université Paris Diderot, CNRS, Paris, France.

Abstract:

Liver is the primary reservoir of the body's vitamin A (retinol). The vitamin is predominantly found in a subpopulation of liver cells called hepatic stellate cells (HSCs). Within these cells, vitamin A is stored as retinyl esters in large lipid droplets (LDs), unique and nearly ubiquitous organelles that store neutral lipids in a hydrophobic core, surrounded by a monolayer of phospholipids. Hepatic stellate cell (HSC) activation plays a pivotal role in the development of chronic liver disease. In this process, HSCs lose their vitamin A-rich lipid droplets. We studied the molecular machinery involved in the formation and regulation of these remarkable lipid droplets to understand underlying mechanisms that lead to liver fibrosis.

The biosynthesis of LDs is most studied in model systems starts when triacylglycerols accumulate within the bilayer of biological membranes above a critical demixing concentration. It is not known whether other classes of neutral lipids, e.g. retinyl esters, can form lipid droplets by themselves.

We show that production of retinyl esters by lecithin:retinol acyl transferase (LRAT) in yeast cells, incapable of producing triacylglycerols and steryl esters, is sufficient for the formation of lipid droplets. By electron microscopy, these lipid droplets, that do not contain neutral lipids other than retinyl esters, are morphologically indistinguishable from those in wild-type cells.

In addition, we show that the hydrophobic N-terminus of LRAT displays preferential interactions with retinyl esters in membranes and promotes the formation of large retinyl ester-containing lipid droplets in mammalian cells. Our combined data indicate that the molecular design of LRAT is optimally suited to allow the formation of characteristic large lipid droplets in retinyl ester-storing cells.



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* Link to abstract

Regarding non available abstracts; the authors did not approve publishing of the abstract at time of submission, please contact authors for information

**Titel:**

Unraveling molecular mechanisms for the packaging and release of picornaviruses in extracellular vesicles

Authors

Susanne G. van der Grein ¹, Kyra A.Y. Defourny ¹, Huib H. Rabouw ², Frank J.M. van Kuppeveld ², Esther N.M. Nolte-'t Hoen ¹

(1) Department of Biochemistry & Cell Biology, Faculty of Veterinary Medicine, Utrecht University, Utrecht, The Netherlands

(2) Department of Infectious Diseases & Immunity, Division of Virology, Faculty of Veterinary Medicine, Utrecht University, Utrecht, The Netherlands

Abstract:

It was recently discovered that various naked viruses belonging to the picornaviridae family can be released from host cells via enclosure in extracellular vesicles (EV). These EV provide a host-derived membrane around the secreted virus particles, thereby altering virus-host interactions. Given the potential of EV to affect antiviral immune responses and disease severity, it is important to deepen our understanding of the formation and function of EV-enclosed viruses. This requires strong liaisons between the fields of Virology and EV biology. In a collaborative effort, we investigate the viral and host factors that regulate EV-mediated virus release. We have assessed the role of the Encephalomyocarditis virus (EMCV) Leader, a viral protein that is dispensable for replication but is required for symptomatic disease in vivo. Cells were infected with wildtype virus or a mutant carrying an inactive Leader, and EV produced during the infection were isolated. Interestingly, inactivation of the Leader strongly reduced the release of virus particles inside EV. Sorting of cargo into EV typically occurs at endosomal membrane compartments. However, picornaviruses are known to remodel intracellular membranes. These viruses often induce the formation of autophagosomes, organelles that engulf cytoplasmic content destined for degradation. Whereas autophagosomes normally fuse with lysosomes, we show that this process is blocked by the EMCV Leader. Instead, the autophagosomes fuse with the plasma membrane, as indicated by the secretion of autophagosome-related proteins during infection. Reactivation of degradative autophagy via drug treatment resulted in a strong reduction in EV and EV-enclosed virus release. These data indicate that autophagosomes contribute to the packaging and secretion of virus particles in host-derived membranes, and that this pathway is regulated by the EMCV Leader. Our work highlights an unconventional route for EV formation, which is located at the cross-section between virus and host-induced cellular pathways and contributes to viral pathogenesis.

**Titel:**

First steps towards a canine oviduct model for in vitro oocyte maturation

Authors

Lotte Meeuws¹, Jeffrey de Gier¹, Tom Stout², Karin Albers-Wolthers¹, Heiko Henning¹

¹ Clinical Sciences of Companion Animals,

² Department of Equine Sciences

Abstract:

Assisted reproduction techniques (ART) such as in vitro fertilization (IVF) are hampered in dogs by unique features of canine reproductive physiology. One of the main obstacles is that ovulated oocytes require two to four days of oviductal maturation before fertilization. Despite efforts to mimic the conditions of the canine post-ovulatory oviduct with chemically defined media, results of in vitro oocyte maturation (IVM) are contradictory and fertilization rates low. The aim of this study was to establish a canine oviduct model based on epithelial cells to support in vitro oocyte maturation. Oviducts were obtained from privately owned bitches that underwent elective ovariectomy during anestrus. Mechanical cell isolation of epithelial sheets and enzymatic digestion of the epithelial lining resulted in more viable cells per oviduct ($2.95 \pm 2.30 \times 10^6$ and $4.64 \pm 3.31 \times 10^6$) than flushing the oviduct lumen ($0.84 \pm 0.82 \times 10^6$; $P < 0.05$; $n = 7$ dogs). The epithelial origin of the isolated cells was confirmed by cytokeratin 18 staining. Isolated canine oviduct epithelial cells (COECs) were subjected to culture in Transwell® inserts with an air-liquid interface in the absence of hormones or with progesterone, estradiol, FSH and LH. Hormone concentrations during culture mimicked the cyclical change from late anestrus to ovulation in order to stimulate morphological and functional differentiation of the COECs ($n = 4$ dogs). Surprisingly, monolayers of ciliated and non-ciliated COECs were readily achieved in conditions without added hormones in 3 out of 4 samples. Mimicking the cyclic hormone changes had no added benefit on cell morphology. In conclusion, COEC monolayers cultured in Transwell® inserts provide a promising basis for developing a canine oviduct model to support IVM of canine oocytes in the future.

**Titel:**

Functional crosstalk between the receptor-binding properties of influenza A virus hemagglutinin and neuraminidase

Authors

Wenjuan Du ¹, Margreet A. Wolfert ², Ben Peeters ³, Frank J.M. van Kuppeveld ¹, Geert-Jan Boons ², Erik de Vries ¹, Cornelis A.M. de Haan ¹

1. Virology Division, Faculty of Veterinary Medicine, Utrecht University, Utrecht, The Netherlands,

2. Department of Chemical Biology and Drug Discovery, Utrecht University, Utrecht, the Netherlands

3. Department of Virology, Wageningen Bioveterinary Research, Lelystad, The Netherlands

Abstract:

Influenza A viruses (IAVs) pandemics are caused by viruses originating from animals that adapted to humans. IAVs contain two glycoproteins: the sialic acid (SIA) receptor-binding hemagglutinin (HA) and the SIA receptor-destroying neuraminidase (NA). The functional properties of HA are important for host tropism: avian and human viruses generally prefer binding to α 2,3- and α 2,6-linked SIAs, respectively. Mutations in HA resulting in increased binding to human-type receptors are occasionally, however, also selected in avian hosts. What drives the selection of such mutations is not known. Phylogenetic analysis indicates that debilitating mutations in the second sialic acid-binding site (2SBS) of NA precede mutations in HA resulting in increased binding to human-type receptors. This 2SBS, which contributes to NA catalytic activity, is highly conserved in most avian viruses, but not at all in human viruses. As the binding affinity of HA and the enzymatic activity of NA presumably need to be balanced for optimal virus fitness, we studied whether mutation of a functional 2SBS in NA drives the selection of altered HA receptor-binding properties. To this end, avian H5N1 viruses with or without a functional 2SBS were generated and passaged in cell culture followed by analysis of their HA and NA gene sequences and of their HA-NA balance by biolayer interferometry. Passaging of 2SBS-negative viruses rapidly selected viruses with a restored HA-NA balance resulting from a repaired 2SBS and/or mutations in HA that reduced receptor-binding avidity to avian-type receptors. Of note, one selected mutation in HA also resulted in increased binding to human-type receptors. Our study underscores the importance of the 2SBS for the HA-NA balance and virus replication, provides evidence for a functional crosstalk between the receptor-binding properties of HA and NA and shows that mutation of the 2SBS may select for viruses with increased zoonotic potential.

**Titel:**

Association of antimicrobial usage with faecal abundance of ermB, tetW, aph3 and sul2 resistance genes in veal calves in three European countries

Authors

Pim Sanders, Liese Van Gompel, Roosmarijn E.C. Luiken, Philip Joosten, Eri van Heijnsbergen, Inge Wouters, Peter Scherpenisse, Claire Chauvin, Katharina Wadepohl, Gerdit D Greve, Betty G.M. Jongerius-Gortemaker, Monique Tersteeg-Zijderveld, Christophe Soumet, Magdalena Skarzynska, Katharina Juraschek, Jennie Fischer, Dariusz Wasyl, [EFFORT group], Jaap A. Wagenaar, Jeroen Dewulf, Heike Schmitt, Dik J. Mevius, Dick J.J. Heederik, Lidwien A.M. Smit

Abstract:*Objectives*

High antimicrobial use (AMU) and antimicrobial resistance (AMR) in veal calves remains a source of concern. Therefore we examined the relationship between AMU and selected farm and management characteristics with the abundance of resistance genes in veal calf farms in three EU countries.

Methods

Faeces of calves close to slaughter was collected in Germany, France and the Netherlands (20 farms per country). Standardized questionnaires were used to record AMU in group treatment, and farm characteristics. Following DNA extraction, qPCR was performed in 407 individual samples to quantify the abundance of four AMR genes (ermB, tetW, aph3 and sul2) encoding for resistance to frequently used antimicrobial classes in calves. Multiple linear mixed models with a random effect for both country and farm were used to determine the association between AMU and AMR, adjusting for other farm characteristics.

Results

The relationship between AMU and AMR gene abundance varied for the different antimicrobial classes among the three countries. Mixed model showed a significant positive association between trimethoprim/sulphonamide combination (trim/sul) usage and the abundance of sul2 ($p < 0.01$), but not between AMU and the other three AMR genes ($p = 0.70$ for ermB, $p = 0.97$ for tetW, and $p = 0.18$ for aph3). Higher weight of calves at arrival was negatively associated with ermB ($p < 0.01$) and aph3 ($p = 0.04$). Farms with other animals present had a negative effect on the abundance of aph3 ($p = 0.05$). Farms only using water for cleaning showed a lower AMR abundance than other farms, which was significant for ermB ($p = 0.02$).

Conclusion

A positive association was found between trim/sul use and sul2 abundance in veal calves in three countries. Moreover, other independent risk factors influencing AMR in veal calves were identified, e.g. weight at arrival, and cleaning practices. Only using water in for cleaning stables showed a negative association with AMR abundance in veal calves.

**Titel:**

Campylobacter plasmids and source association

Authors

Linda Van Der Graaf-van Bloois^{1,2}, Lapo Mughini Gras³, Claudia Swart-Copain³, Birgitta Duim^{1,2}, Jaap Wagenaar^{1,2,4}, Miriam Koene⁴, Eelco Franz³, Hetty Blaak³, Ciska Schets³ and Aldert Zomer^{1,2}

1 Faculty of Veterinary Medicine, Department Infectious Diseases and Immunology, Utrecht University, Utrecht, Netherlands

2 WHO Collaborating Center for Campylobacter/OIE Reference Laboratory for Campylobacteriosis, Utrecht, the Netherlands

3 National Institute for Public Health and the Environment, Utrecht, the Netherlands

4 Wageningen Bioveterinary Institute, Lelystad, the Netherlands

Abstract:*Background:*

Campylobacter can spread from animal to human via different routes. The major source of human campylobacteriosis is chicken meat, however, only 20-30% of human cases can be traced back to the consumption of chicken meat. The environment, e.g. surface water, is possibly another infection route for human campylobacteriosis. In this study, we sequenced isolates from human campylobacteriosis cases, multiple animal species and surface water, and we associate plasmid prevalence and their genetic content with the different sources.

Methods and Results:

We sequenced in total 1418 *C. jejuni* and *C. coli* isolates; 280 from human cases, 253 surface water, 256 chickens, 38 turkey, 207 cattle, 110 pigs, 111 sheep/goat, 100 pets and 63 wild birds. Genomes were assembled using SPAdes and plasmid contigs were predicted using RFplasmid (github.com/aldertzomer/RFPlasmid). Plasmid and chromosome contigs of Campylobacter genomes can be identified from short read sequencing assemblies using RFplasmid with an error of only 0.37% in basecounts. Plasmid contigs were annotated using Prokka and gene presence-absence was determined using Roary. Plasmids were associated with source using PCA clustering of Jaccard distances and plasmid gene specific analysis was performed using Scoary.

Conclusions:

We observed that not all plasmids were found in all sources, e.g. Campylobacter strains isolated from sheep, goat and swine were lacking a group of plasmid genes and Campylobacter strains from wild birds did not contain plasmids with resistance genes. We observed a source association in plasmids from pigs, water and wild birds, and observed niche-adapted genes in plasmids from chickens, humans and wild birds

**Titel:**

Specific SCCmec types and clonal complexes are associated with low-level amoxicillin/clavulanic acid and cephalotin resistance in methicillin-resistant Staphylococcus pseudintermedius

Authors

Alice Wegener (1), Peter Damborg (2), Luca Guardabassi (2,3), Arshnee Moodley(2), Lapo Mughini-Gras (4,5), Birgitta Duim(1), Jaap A. Wagenaar (1,6), Els M. Broens (1).

1 Department of Infectious Diseases and Immunology, Faculty of Veterinary Medicine, Utrecht University, the Netherlands.

2 Department of Veterinary and Animal Sciences, Faculty of Health and Medical Sciences, University of Copenhagen, Frederiksberg, Denmark.

3 Department of Pathobiology and Population Sciences, The Royal Veterinary College, North Mymms, United Kingdom.

4 Center for Infectious Disease Control, National Institute for Public Health and the Environment (RIVM), Bilthoven, the Netherlands.

5 Institute for Risk Assessment Sciences, Utrecht University, Utrecht, the Netherlands.

6 Wageningen Bioveterinary Research, Lelystad, the Netherlands.

Abstract:

Staphylococcus pseudintermedius is a common opportunistic pathogen in dogs and a major cause of pyoderma and otitis externa. Methicillin resistance has emerged over the last decades and is carried on a mobile genetic element: the SCCmec. Screening for methicillin resistance in *S. pseudintermedius* is done by determining the minimum inhibitory concentration (MIC) for oxacillin. According to the current guidelines, *S. pseudintermedius* displaying resistance to oxacillin should be reported as resistant to all β -lactam antimicrobials.

This study aims to identify differences in resistance levels to β -lactams and possible association between clonal complexes (CC) and/or SCCmec types in methicillin-resistant *S. pseudintermedius* (MRSP).

MICs for five β -lactam antimicrobials: oxacillin (OXA), penicillin (PEN), ampicillin (AMP), amoxicillin/clavulanic acid (AMC) and cephalothin (CEP) were determined by broth microdilution for 86 clinical canine MRSP isolates from Denmark and the Netherlands. PCR as well as whole genome sequencing were used for SCCmec and multilocus sequence typing.

Isolates belonged to CC71 (n = 36), CC258 (n = 33), CC45 (n = 11), CC68 (n = 1) and five singleton sequence types. SCCmecII-III was exclusively found in CC71 and SCCmecIV was significantly associated with CC258. SCCmecV and untypeable SCCmec types occurred in 4 and 14 isolates, respectively.

SCCmecIV was associated with lower MICs for OXA, AMP, AMC and susceptibility to CEP. All isolates harbouring SCCmecV were susceptible to CEP as well.

In conclusion, SCCmec types were associated with different clonal complexes with either high or low-level resistance to different β -lactam antimicrobials. The finding of AMC (20%) and CEP (70%) in vitro susceptibility across all CCs might have clinical implications, since AMC and first generation cephalosporins are empiric choices in canine infections caused by staphylococci. Clinical outcome studies are warranted to evaluate the in vivo efficacy of these β -lactams for treatment of MRSP infections.

**Titel:**

Efficacy of needle-free iron injection, an on farm double blind randomized controlled clinical trial

Authors

T.J. Tobias¹ H. Vernooij¹ E. Teske² J. Vossen³ A. van Nes¹

1) Utrecht University, Faculty of Veterinary Medicine, Department of Farm Animal Health

2) Utrecht University, Faculty of Veterinary Medicine, Department of Clinical Sciences of Companion Animal Sciences

3) Utrecht University, Faculty of Veterinary Medicine, University Veterinary Diagnostic Laboratory (UVDL)

Abstract:*Introduction*

Recent introduction of needle-free injection devices are often used for routine administration of iron-dextran. However, the efficacy and safety are not yet well studied. This study aims to study the efficacy and safety of needle free injection of iron on D3 for the prevention of iron deficiency anaemia in 4 week old pigs.

Material and methods

A double blind randomized clinical trial was conducted with 72 pigs from 9 litters. From each litter 3 piglets were randomly allocated to the needle group, 3 to the needle-free group and 2 pigs to a non-treated control group. Pigs were weighed and blood (EDTA + Serum) was collected at D3, D14, D26 and D70. Blood was tested for haematological parameters as well as serum iron, iron binding etc.. A linear mixed effects model with random litter effect was used to compare the effect of needle-free injection on serum haemoglobin and haematocrit respectively on D26.

Results

Although variation of Hb on D26 was increased in the needle-free group compared to the needle group, no significant differences between needle-free and regular injection of iron dextran on Hb and Ht at D26 were found. In the control group, however, Hb and Ht at d26 was significantly lower. Interestingly, weight of the pigs between groups did not differ at any time point. Other haematological results showed almost equal results for both needle-free and needle injection group. No adverse signs were noted in all groups.

Discussion and conclusion

Needle-free iron injection is as effective and seems as safe as injection per needle to prevent anaemia at weaning.

**Titel:**

131-Iodine treatment of feline hyperthyroidism: development of radiation protection guidelines for Dutch legislation based on external dose rate and excretion of 131-Iodine in 10 cats

Authors

Sebastiaan van Nimwegen ¹, DVM, PhD, DECVS; Anke Wassink ¹; Hans Kooistra ¹, prof, DVM, PhD, DECVIM; Kees Vos ², Ing, RPE; Jan Willem Hesselink ¹, prof, DVM, PhD, DECAR;
1-Department of Clinical Sciences, Faculty of Veterinary Medicine, Utrecht University;
2-Corporate Real Estate & Campus, Safety and Environment, Utrecht University

Abstract:

Introduction – 131-Iodine (¹³¹I) is an effective treatment for feline hyperthyroidism with minimal morbidity and >95% cure rate. β -radiation of ¹³¹Iodine ($E_{\beta\max}=606$ keV), responsible for its therapeutic efficacy, poses an internal radiation exposure risk after excretion with urine/feces. γ -radiation of ¹³¹Iodine ($E_{\gamma}=364$ keV) poses an external exposure risk. Radiation protection regulations concerning time of discharge from hospital vary between countries. Tracing ¹³¹Iodine excretion in time has only been performed with limited number of measurements of radioactivity levels in excreta and external dose-rate in time, while excreted ¹³¹Iodine may be an important risk factor for unwanted public exposure.

Methods – 10 cats with hyperthyroidism were treated with 200 MBq ¹³¹Iodine and hospitalized for 14 days under constant monitoring of external dose-rate. All urine/feces were collected and radioactivity levels measured to trace total excreted radioactivity and radioactivity/gram. Data were analyzed and biological/effective $t_{1/2}$ determined.

Results – External dose-rate data could be reliably fitted according to a 1st order exponential decay formula with 0.95

Discussion/conclusion – Considering the current H_{\max} (40 μ Sv), administered 200 MBq, and strict instructions for housing and care of patients during the first 2 weeks after discharge, a minimum of 6 days hospitalization is required to stay below H_{\max} . Furthermore, 20-22 MBq ¹³¹Iodine will be excreted following discharge, 95% of which will occur during the first two weeks at home. Safety instructions have to be obeyed to prevent unwanted radiation exposure in the public domain.

**Titel:**

Holmium-166 microspheres for image-guided intratumoral treatment of brain malignancies: a multi-institutional multi-disciplinary translational project

Authors

N.C. Morsink, CSCA-Surgery UU; N.J.M. Klaassen, Radiology/Nuclear Medicine Radboudumc; L.K. Ruven, CSCA-Surgery UU; B.P. Meij CSCA-Surgery UU; G.C.M. Grinwis, Pathobiology UU; I.A. Schaafsma, CSCA-Radiology UU; J.W. Hesselink, CSCA UU; J.F.W. Nijsen, Radiology/Nuclear Medicine Radboudumc; S.A. van Nimwegen, CSCA-Surgery UU.

Abstract:*Introduction*

- Radioactive holmium-166 microspheres (166HoMS) can deliver a high local radioactive dose with minimal collateral dose spread after intratumoral application. Multimodality imaging characteristics of 166HoMS fuel the development of image-guided delivery and dosimetry for controlled tumor treatment. Technique, safety, and efficacy of intratumoral 166HoMS injection was demonstrated in feline patients with oral squamous cell carcinoma (SCC) in a collaborative project of Veterinary Medicine, UMC Utrecht, and Quirem Medical. Results of this study led to translation of this treatment approach to humans with head&neck SCC at UMCU. Currently, image-guided treatment for brain malignancies is being developed in a multi-institutional project including injection device (TU Delft), biodistribution and dosimetry (Veterinary Medicine, Radboudumc, Quirem Medical), and treatment of veterinary patients as translational model.

Methods

- Proof of principle of intracranial 166HoMS treatment has been shown in a dog with a large pituitary adenocarcinoma treated by CT-guided 166HoMS injection. Serial holmium dilutions were scanned with different CT settings and MRI sequences to determine optimal quantification settings. Non-radioactive 165HoMS were injected in ex vivo porcine brains to develop HoMS quantification. To optimize and simulate the intratumoral injection approach, a brain tumor treatment model and imaging-compatible steerable needle were designed.

Results

- CT-guided injection of 1097 MBq (1583 Gy) in the center of a pituitary adenocarcinoma resulted in 42% tumor volume reduction after 4.5 months. Comparing different CT settings showed optimal holmium quantification using a soft tissue kernel, 400 mAs, 120 kV and 1.0 mm slice thickness. Detection limit in water was ≥ 2 mg/mL holmium. MRI-guided 165HoMS injection in brain tumor phantoms resulted in 1.2 mm artefact from the steerable nitinol needle.

Discussion

- The feasibility and clinical effectiveness of single HoMS injection was shown. Results may be improved using image-guided dosimetric feedback. Further optimization of image-guided quantification is ongoing and a multidisciplinary treatment trial of veterinary patients will start within the next months.

**Titel:**

Acute respiratory effects of livestock-related air pollution in a panel of COPD patients

Authors

Warner van Kersen/Institute for Risk Assessment Sciences, Marieke Oldenwening/Institute for Risk Assessment Sciences, Bernadette Aalders/Netherlands Expertise Centre for Occupational Respiratory Disorders, Lizan D. Bloemsma/Institute for Risk Assessment Sciences, Floor Borlée/Institute for Risk Assessment Sciences, Dick Heederik/Institute for Risk Assessment Sciences, Lidwien A.M. Smit/Institute for Risk Assessment Sciences

Abstract:

Living close to livestock farms has been associated with increased symptoms in patients with chronic obstructive pulmonary disease (COPD). The causes of these effects are still poorly understood. This panel study attempts to assess the acute effects of livestock-related air pollution in patients with COPD living in an area with intensive livestock farming in the Netherlands.

Between February 2015 and July 2016, 82 patients took spirometry measurements twice daily during a 3-month period, resulting in 12,672 FEV1 and PEF records. Patients also kept a diary on respiratory symptoms as well as livestock-related odor annoyance. Daily average ammonia (NH3) (a proxy for livestock-related air pollution) and fine particulate matter (PM10) levels were collected from monitoring stations in the area. Lung function was analyzed as decrements of >10% and >20% from their median as well as absolute values. Self-reported odor annoyance was analyzed as a dichotomous variable. All analyses were done using generalized estimated equations. We adjusted for age, gender, height, humidity, temperature, linear trend, and took multiple testing into account.

We found an odds ratio of 1.14 95%CI [1.05;1.25] for decrements >20% in morning FEV1 per interquartile range (12µg/m3) increase in NH3 concentration (lag 2). Odor annoyance was negatively associated with evening PEF (-4.46 l/min 95%CI [-7.59; -1.33]). Sensitivity analyses comparing these associations between participants with different COPD definitions yielded no significant differences. No associations with symptoms were found.

Our results show acute effects of livestock-related air pollution on lung function in COPD patients living in close proximity to farms.



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Titel:

The role of atypical E2Fs in cell cycle progression through regulating cohesin

Authors

Qingwu Liu, Bart Westendorp, Alain de Bruin

Abstract:

E2F7 and E2F8 are the latest discovered members of the E2F transcription factor family, consisting of eight members in mammals. They are able to form complexes with other factors, and we performed an unbiased screen search to find novel interaction partners of atypical E2Fs. In order to identify new binding partners. We used inducible HeLa cell lines that allow a controlled overexpression of E2F7-GFP or E2F8-GFP by addition of doxycyclin. We took advantage of the GFP-tag in the overexpression constructs to pull down E2F7-GFP, E2F8-GFP, or GFP, and elucidated the candidates by mass spectrometry. Using this approach, we found multiple novel E2F-binding partners including proteins off the cohesin-complex. We discovered that E2F7/8 can drive the release of the cohesin complex from the from DNA in G2-phase and during mitosis. Our findings suggest that E2F7 and E2F8 function as a docking spot on cohesin when it is bound to DNA, for a more efficient release of the cohesin ring

**Titel:**

Infrared thermography as a non-invasive method to measure stress in laboratory mice

Authors

José Lozeman van 't Klooster, Hetty Boleij, Loes Heeren

Abstract:

Laboratory animals frequently experience short or longer periods of stress during experiments. Next to procedures also transport from the breeder is a stressful event. To be able to compare and refine experimental procedures in animal experiments it is important to be able to reliably measure stress responses.

An increased body temperature is one of the physiological effects that occurs in response to an acute stressor, this is known as stress-induced hyperthermia (SIH). Infrared thermography (IRT) might be used as a non-invasive method to study SIH, its application contributes to refinement since other methods to measure stress often involve more invasive procedures (such as taking a blood sample to measure corticosterone). We did a pilot habituation experiment to investigate if basal temperatures in BALB/c and C57BL6 mice after transport and arrival in the lab change over time. The mice were tested with an IRT camera for 20 minutes in their home cage. These recordings were executed on day 1, 13 and 20 after they arrived from the breeder. From these recordings, temperature data was collected from the eye and the tail. An increase in basal eye temperature and decrease in basal tail temp and strain differences were found over time.

This shows that it is possible to pick up habituation of basal body temperature during acclimatization with the IRT camera as a non-invasive method. With more research we can validate this method further.

**Titel:**

Cathelicidins modulate the immune response of porcine M1/M2 macrophages by either inducing or inhibiting inflammation

Authors

RM van Harten MSc, Utrecht University; MR Scheenstra PhD, Utrecht University; Prof HP Haagsman PhD, Utrecht University

Abstract:

Cathelicidins are host defence peptides from the vertebrate innate immune system. Many have been tested for immune modulatory properties, but not yet extensively on porcine immune cells. Of these, macrophages are key effector cells of the immune system, inducing inflammation and subsequently restoring immune homeostasis. Previously, we developed a method to culture in vitro M1 (pro-inflammatory) and M2 (anti-inflammatory) macrophages from porcine bone marrow. The aim of this study is to assay immune modulation by various cathelicidins of porcine M1 and M2 macrophages. Porcine mononuclear bone marrow cells (BMDM) were used to culture M1 with GM-CSF, and M2 with M-CSF. Cells were activated with innate immune receptor ligands and/or host defence peptides. Cell polarization and immune activation state were analysed by flow cytometry and ELISA. chCATH-2 can inhibit LPS-induced activation of macrophages by reducing TNF α production in a dose-dependent manner. A scrambled CATH-2 control was unable to inhibit LPS-induced activation. Human LL-37 can also inhibit activation by TLR2 ligands. Porcine PMAP-23 inhibits IL-10 production but has no effect on TNF α , indicating a skewing effect towards a more inflammatory phenotype. Porcine PR-39 is directly inflammatory, as it induces TNF α production, while exacerbating inflammation in the presence of TLR ligands. Prolonged exposure of the cells to LL-37 seems to instill a more pro-inflammatory phenotype. PR-39, while inflammatory itself, leads after 3 days to a more tolerant macrophage. In conclusion, the immune response of porcine M1 and M2 macrophages can be modulated by host defence peptides to be pro- or anti-inflammatory, depending on the presence of other factors and timing of exposure. This offers opportunity to further develop cathelicidins as immune modulatory drugs.

**Titel:**

Research Support Office Veterinary Medicine

Authors

Faculty of Veterinary Medicine, Utrecht University

Abstract:*MISSION*

Our goal is to assist all scientists at the Faculty of Veterinary Medicine in pursuing their research to create innovation and meaningful impact for society. Therefore, we facilitate the use of funding opportunities provided by the EU and national governments, as well as international NGO's. We advise along the entire project development chain, from identifying the best fitting call and supporting the grant application process to business development and project management. This will enable scientists to spend more time on research, whilst increasing chances to get funded and to successfully finalise projects.

SERVICE

We 1) identify relevant EU and national funding opportunities, 2) advice on how to strategically position your project to optimally fit the call, 3) advise on the development of a first project plan, 4) assist with consortium building (e.g. finding partners), 5) review grant applications and advise on aspects such as impact and implementation, 6) advise on collaboration with consultants (for proposal writing, project management), 7) assist with relation management and contracts with third parties, 8) assist in exploring the options to start a spin off and 9) advise on project management.

CONNECT (intranet.uu.nl/RSO-DGK)

For questions regarding research funding acquisition, network building and post award activities, we have a grant advisor, a business developer, a project manager and a contract manager. Furthermore, we are connected with 1) fundraising/charity (Friends of VetMed) for everything related to philanthropy, and with 2) internationalization for all questions regarding funding education and staff/student mobility.



Nr. Abstract
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Titel:

Teaching Innovation: practicing the Scholarship for Teaching and Learning (SoTL) approach

Authors

Mieke Lumens (IRAS), Marianne Bol-Schoenmakers (IRAS)

Abstract:

With the formation of three departments within the Veterinary Faculty, new challenges lay ahead, not only for science but also in education. The new faculty structure provides educational opportunities including more interdisciplinary learning activities to better prepare students for their future careers. The innovative One Health master programme of the Veterinary and Medical faculties is an example of such interdisciplinary teaching. To achieve an interdisciplinary way of thinking in students different educational approaches are being used within UU. More knowledge on which approach is most effective is called for.

To ensure state-of-the-art education of good quality, constant innovation of teaching is essential. Various tools are developed over the years to help improving education. However, using something new does not guarantee better education and measuring the effects of innovation is equally important. The Scholarship of Teaching and Learning (SoTL) is a scientific approach to measure the effects of educational innovation. The Centre of Academic teaching (CAT) of the Utrecht University developed the 'Utrecht Roadmap for Teaching Innovation and Scholarship' as a guidance for the SoTL approach. We used this roadmap to design a USO project on identification of successful educational approaches for interdisciplinary learning. The step-by step approach of this roadmap helped us to identify the type of educational problem we were facing, to formulate a solid hypothesis and a smart research question.

Furthermore, the roadmap helped us to design a tiered approach enabling us to do measurements on approaches in interdisciplinary education. The USO project will be submitted this autumn. We highly recommend this SoTL roadmap when planning innovations in your education, it guarantees a systemic approach and can provide you with a valuable method to evaluate the success of the innovation.

**Titel:**

Improved flow cytometric detection of extra cellular vesicles by reduction of optical background signals

Authors

Ger J.A. Arkesteijn ^{1,2}, Sten F.W.M. Libregts ¹, Estefanía Lozano-Andrés ¹, Marca H.M. Wauben ¹.

1 Department of Biochemistry & Cell Biology, Faculty of Veterinary Medicine, Utrecht University, Utrecht, The Netherlands

2 Department of Immunology & Infectious Diseases, Faculty of Veterinary Medicine, Utrecht University, Utrecht, The Netherlands

Abstract:

Flow cytometry has become an indispensable tool in biological research. Besides multi-parameter analysis of cells in biological samples, flow cytometry is widely used to sort and purify sub-sets of cells from a heterogeneous population at high speed based on single cell characteristics. For decades, the design of flow cytometers fulfilled the demands to process cells or cell-sized particles. With the research field of extracellular vesicles (EVs) rapidly expanding, there is an increased demand to address heterogeneity of EV populations in biological samples. Although flow cytometry would be the ideal technique to do so, the available instruments are in general not equipped to optimally detect the dim light scatter signals generated by submicron-sized particles like EVs. Whereas sideward scattered light (SSC) and fluorescence are currently used as a trigger signal to identify EVs within samples, the forward scatter light (FSC) parameter is often neglected due to the lack of resolution to distinguish EV-related signals from noise. However, after optimization of FSC detection by adjusting the size of the obscuration bar, we recently showed that certain EV-subsets could only be identified based on FSC. This observation made us to further study the possibilities to reduce noise detection and enhance FSC-detection of submicron-sized particles. By testing differently sized obscuration bars and pinholes in front of the FSC detection lens, we generated a matrix that allowed us to determine which combination resulted in the best and most robust signal-to-noise ratio regarding FSC detection of submicron-sized particles. We found that a combination of an 8 mm obscuration bar and a 200 μ m pinhole reduced background noise in a reproducible manner to such extent that it allowed a robust separation of 100 nm polystyrene beads from noise within the FSC channel, and even allowed triggering on FSC without the presence of massive background noise signals when both beads and EVs were measured. These technical adaptations thus significantly improved FSC detection of submicron-sized particles and provide an important lead for the further development and design of flow cytometers that aid in detection of submicron-sized particles.

**Titel:**

Usutu virus infection and plasmodium coinfection in blackbirds (turdus merula) in the Netherlands from 2016 to 2018

Authors

G. Giglia ^{1,2}, C.B.E.M. Reusken ^{3,4}, F. Schaafsma ¹, M.T. Mandara ², J. Rijks ⁵, R. Sikkema ³, A. Gröne ^{1,5}, M. Koopmans ³, M.H. Verheije ¹ and J.M.A. van den Brand ^{1,5}

1 Department of Pathobiology, Veterinary Faculty, Utrecht University, Utrecht, the Netherlands

2 Department of Veterinary Medicine, University of Perugia, Perugia, Italy

3 Department of Viroscience, Erasmus Medical Center, Rotterdam, the Netherlands

4 National Institute for Public Health and the Environment, de Bilt, the Netherlands

5 Dutch Wildlife Health Centre, Utrecht University, Utrecht, the Netherlands Utrecht University, Utrecht, the Netherlands

Abstract:*Introduction*

Usutu virus (USUV), a mosquito-borne flavivirus, has been identified as the cause of die offs in wild birds' species. In particular, high mortality has been observed in blackbirds (*Turdus merula*) in Europe. To monitor the circulation of USUV in blackbirds in the Netherlands and describe associated lesions, blackbirds that were found dead were investigated in a passive surveillance program from 2016 to 2018. Since *Plasmodium* sp. coinfection was previously reported in USUV infected birds, the co-existence of these agents was evaluated.

Materials and methods

From 2016 until 2018, 162 dead blackbirds were sent to the Dutch Wildlife Health Centre for necropsy. During necropsy various tissues were taken for virology, histopathology and immunohistochemistry (IHC).

Results

In qPCR, 72% of the examined blackbirds were positive for USUV (117/162). The most prominent gross lesions were spleno- and hepatomegaly. In histology, lymphoplasmacytic inflammation and necrosis were seen in the liver, spleen, heart and in the brain, there were foci of gliosis. IHC confirmed the presence of USUV associated with the described lesions. In a substantial number of the USUV positive birds there was microscopic evidence of exo-erythrocytic stages of *Plasmodium* sp. in lung, liver, spleen, brain and heart.

Conclusion

This research demonstrates the presence of USUV and associated lesions in blackbirds in the Netherlands from 2016 to 2018. Additionally, a coinfection with avian malaria, *Plasmodium* sp., is seen in a substantial proportion of the birds. Since the correlation between USUV and *Plasmodium* sp. in blackbirds is not known, further studies are needed.

**Titel:**

Synergy around equine chondrocyte pellets - Improving cartilaginous matrix formation in three-dimensional culture by the combination of BMP9 and TGFb1

Authors

Natalia Quiros ¹, Florencia Abinzano ², Saskia Plomp ¹, Emmie Giessen ¹, Riccardo Levato ², Marianna Tryfonidou ³, Nikae te Moller ¹

1 Department of Equine Sciences, Faculty of Veterinary Medicine, Utrecht University,

2 Regenerative Medicine Utrecht, Utrecht University,

3 Department of Clinical Sciences of Companion Animals, Faculty of Veterinary Medicine, Utrecht University

Abstract:*Background*

Articular cartilage degeneration is an important feature of osteoarthritis, a joint disease that is highly prevalent in horses. In vitro culture of chondrocytes is used to study disease processes under controlled conditions and the effects of (new) drugs at the tissue level. Bone morphogenetic protein 9 (BMP9) has been shown to be a potent modulator of cartilage development by bovine articular chondrocytes[1]. The hypertrophic effect of BMP9 could be antagonized by a low dose of TGFb-1 [2]. We compared the effects of BMP9 and TGFb-1 on cartilaginous matrix deposition by equine chondrocytes in pellet culture and hypothesized that BMP9 would synergize with a low dose of TGFb-1.

Methods

Pellets from equine chondrocytes were cultured for 28 days with chondrogenic medium only or with different combinations of growth factors: TGFb-1-low (0.1 ng/ml), TGFb-1-standard (10 ng/ml), BMP9 (50ng/ml), combination-low: BMP9 (50 ng/ml) + TGFb-1 (0.1ng/ml), combination-standard: BMP9 (50ng/ml) + TGFb-1 (10 ng/ml), and combination-low for one week, followed by TGFb-1-standard. Microscopic (Bern score) and biochemical analysis were done at days 7, 21 and 28 and qPCR at day 7 and 28. Statistical differences were evaluated between TGFb-1-standard and the other groups.

Results

Bern scores were higher (all time points) and collagen type-2 (COL2) staining was stronger (day 7) for groups that received BMP9. GAG content normalized per DNA was significantly higher for the BMP9 and combination-low groups at days 21 and 28. Expression levels of aggrecan were significantly higher in all groups that received BMP9 (day 7 and 28) but, COL2 expression was significantly higher for combination groups only. MMP13 was significantly downregulated in the BMP9 and combination-low groups at day 28.

Conclusions

BMP9 improved matrix deposition by equine chondrocyte pellets. The combination with low doses of TGFb-1 downregulated the expression of the late hypertrophy marker MMP13, while upregulating aggrecan and COL2 expression.



References

1. Blunk T, Sieminski AL, Appel B, et al. Bone morphogenetic protein 9: a potent modulator of cartilage development in vitro. *Growth Factors*. 2003;21(2):71-77.
2. van Caam A, Blaney Davidson E, Garcia de Vinuesa A, et al. The high affinity ALK1-ligand BMP9 induces a hypertrophy-like state in chondrocytes that is antagonized by TGF β 1. *Osteoarthritis Cartilage*. 2015;23(6):985-995.

**Titel:**

The influence of synovial fluid derived from equine joints with acute and chronic pathologies on in vitro chondrogenesis

Authors

Natalia Quirós¹, Emmie Giessen¹, Saskia Plomp¹, Florencia Abinzano², Marianna Tryfonidou³, Nikae te Moller¹

1 Department of Equine Sciences, Faculty of Veterinary Medicine, Utrecht University,

2 Regenerative Medicine Utrecht, Utrecht University,

3 Department of Clinical Sciences of Companion Animals, Faculty of Veterinary Medicine, Utrecht University

Abstract:*Background*

Articular cartilage lesions are highly prevalent in horses (and man) and potentially lead to post-traumatic osteoarthritis (OA). The horse is seen as a valuable model to study new regenerative strategies [1], employing healthy animals with normal joint homeostasis. In contrast, in patients suffering from cartilage injury there is (low grade) inflammation negating joint homeostasis. The latter significantly impairs chondrogenesis, affecting the outcome of tissue engineering [2,3]. We aimed to investigate the influence of equine synovial fluid (SF) derived from joints with various types of inflammation on chondrogenesis.

Methods

Equine chondrocytes were pelleted and after 14 days of preculture with BMP9 (50ng/ml) + TGF β -1 (0.1ng/ml) pellets were split into the following groups: 25% healthy-SF, acute-SF (LPS induced), 2 weeks post-joint-surgery-SF, chronic-OA-SF, 25%, Hanks Balanced Salt Solution (negative control), TNF α (5ng/ml), or preculture conditions (positive control). Microscopic (Bern score) and biochemical analysis were done at days 14, 21 and 28, qPCR at day 14 and 28.

Results

Only healthy-SF resulted in higher Bern scores and GAG content per DNA (GAG/DNA), the latter increased over time, comparable to the positive control group. The chronic-OA-SF group behaved similar to negative controls, showing decreased Bern scores and GAG/DNA from day 14 to 28. Collagen type-2 expression was significantly higher in the healthy-SF and chronic-OA-SF groups vs negative controls and presented no differences with positive controls. MMP13 and IL-6 expression was downregulated in healthy-SF vs. positive and negative controls and decreased over time, whereas in Chronic-OA-SF, MMP13 and IL-6 expression increased over time.



Conclusions

Healthy-SF stimulated matrix production by equine chondrocytes, whereas SF derived from chronic-OA joints had a deteriorating effect. The addition of chronic-OA-SF upregulated hypertrophy and inflammatory markers. These findings indicate that changes in joint homeostasis may affect the chondrogenic capacity of equine chondrocytes in vivo.

References

1. McIlwraith CW, Fortier LA, Frisbie DD, Nixon AJ. Equine Models of Articular Cartilage Repair. *Cartilage*. 2011;2(4):317-326.
2. Saris DBF, Dhert WJA, Verbout AJ. Joint homeostasis: the discrepancy between old and fresh defects in cartilage repair. *J Bone Jt Surg*. 2003;85(7):1067-1076.
3. Yang KGA, Saris DBF, Verbout AJ, Creemers LB, Dhert WJA. The effect of synovial fluid from injured knee joints on in vitro chondrogenesis. *Tissue Eng*. 2006;12(10):2957-2964.

**Titel:**

Leukoencephalomyelopathy in cats

Authors

Ted SGAM van den Ingh, Guy CM Grinwis, Ronald Jan Corbee

Abstract:

Four outbreaks of leukoencephalomyelopathy in colonies of SPF cats on a long term diet of irradiated dry cat food were observed in the Netherlands between 1989 and 2001. As a primary defect in myelin formation was suspected to be the cause of the disease and myelin consists mainly of lipids and their fatty acids, we investigated the fatty acid composition of the white matter of the spinal cord of affected and control cats and of irradiated and non-irradiated food. The white matter of the spinal cord showed low levels of linoleic acid and absence of alpha linolenic acid in affected cats. These abnormalities in fatty acid composition of the white matter of the spinal cord may reflect an increased need for alpha linolenic acid as a substrate for longer chain omega-3 fatty acids to compose myelin and thus indicate a particular species sensitivity to dietary deficiency in omega-3 polyunsaturated fatty acids, particularly alpha linolenic acid in cats. Our findings indicate that abnormalities in fatty acid metabolism in myelin play an essential role in the pathogenesis of this acquired form of leukoencephalomyelopathy in cats.

**Titel:**

Design of the TOLERANT trial: Tolerogenic DC vaccination for Rheumatoid Arthritis

Authors

Dr. Arie J. Stoppelenburg / UMCU, Dr. Marlies C. van der Goes / Meander Medical Centre, Dr. Irene S. Ludwig / UU, Prof. Dr. Femke Broere / UU, Prof. Dr. Willem van Eden / UU, Prof. Dr. Jaap van Laar / UMCU

Abstract:

In rheumatoid arthritis (RA), immune cells cause joint inflammation and destruction in response to auto-antigens. Immunosuppressive therapies offer relief, but fail to induce tolerance to auto-antigens. Injection of HSP70/B29 peptide-loaded tolerogenic dendritic cells induces immune tolerance and ameliorates disease in arthritis models. We hypothesize that dendritic cell therapy with HSP70/B29 TolDC is safe and induces immune tolerance in RA patients.

This Phase I/II clinical trial is designed to demonstrate the safety of intranodal HSP70/B29 TolDC administration. Our secondary objectives are the characterization of B29-peptide specific immune reactivity in response to HSP70/B29 TolDC treatment and the evaluation of the effect of the treatment on disease activity.

To this end, adult RA patients (>18 years) in remission or with low disease activity while on disease modifying anti-rheumatic drugs will receive two intranodal injections with the HSP70/B29 TolDC product with a four week interval. The study consists of a dose-escalation phase (n=9) and an extension study (n=9).

Primary outcome is the occurrence of (serious) adverse events including flares of disease activity.

Secondary outcomes are B29-specific T cell reactivity with analysis of phenotypic T cell markers, and change in clinical disease activity.

TOLERANT is expected to start in the first half of 2020.

**Titel:**

From catabolism to anabolism; the effect of joint distraction on osteoarthritis in dogs.

Authors

M.Teunissen¹, J. Popov-Celeketic², K.Coeleveld², B.P.Meij¹, F.P.J.G.Lafeber², M.A.Tryfonidou¹, S.C.Mastbergen²

1 Clinical Sciences of Companion Animals, Faculty of Veterinary Medicine, Utrecht University, Utrecht, The Netherlands,

2 Rheumatology & Clinical Immunology, University Medical Center (UMC) Utrecht, Utrecht University, Utrecht, The Netherlands

Abstract:

Knee joint distraction (KJD) is a joint-preserving treatment strategy for severe osteoarthritis (OA), providing long-term clinical and structural improvement [1]. Data from human trials and animal models indicate clear cartilage regeneration from 6 months onwards post-KJD [1, 2]. However, recent work showed that during distraction, there is a catabolic environment, as indicated by proteoglycan (PG) turnover and transcription profile [unpublished data]. This study aimed to elucidate the shift from a catabolic to an anabolic state by investigating the cartilage directly and 10 weeks after joint distraction. Knee OA was induced bilaterally in 8 dogs according to the groove model [2]. After 10 weeks of OA induction, the right knee received joint distraction, employing the left knee as an OA control. After 8 weeks of distraction, and after 10 weeks post-KJD, 4 dogs were euthanized. Respectively, macroscopic cartilage degeneration, PG content (Alcian Blue), and PG synthesis (35SO42-incorporation rate) were assessed [2].

Directly after KJD, macroscopic cartilage damage of the right tibial plateau was higher compared to the left OA control (OARSI score: 1.7 ± 0.2 vs 0.6 ± 0.3 ; $p < 0.001$). 10 weeks post-KJD this difference persisted (OARSI score: 1.4 ± 0.6 vs 0.6 ± 0.1 ; $p < 0.05$). Biochemical analysis of the tibia cartilage directly after KJD revealed a lower PG content (20.1 ± 10.3 mg/g vs 23.7 ± 11.7 mg/g). At 10 weeks post-KJD this difference in PG content was less (24.8 ± 6.8 mg/g vs 25.4 ± 7.8 mg/g). The PG synthesis rate directly after KJD appeared significantly lower vs. OA (1.4 ± 0.6 nmol/h.g vs 5.9 ± 4.4 nmol/h.g; $p < 0.001$). However, 10 weeks post-KJD this difference was not detected (3.7 ± 1.2 nmol/h.g vs 2.9 ± 0.8 nmol/h.g), with an increased synthesis rate in the distracted knee compared to directly after distraction ($p < 0.01$).

Further in-depth investigation is ongoing; these first results suggest that the shift from a catabolic to an anabolic state occurs within the first weeks after joint distraction, mostly reflected in the biochemical changes. Therefore, the post-distraction period seems to be essential in identifying key-players that support intrinsic cartilage repair.

References:

[1] Jansen MP et al. Osteoarthritis Cartilage. 2018 Dec;26(12):1604-1608

[2] Wiegant K et al. Arthritis Rheumatol. 2015 Feb;67(2):465-74

**Titel:**

The effect of cathelicidins on phagocytosis of E. coli by murine macrophages

Authors

S. Büchli, L.K. van Rooij, M. Vrielink, E.J.A. Veldhuizen, H.P. Haagsman, M.R. Scheenstra

Department of Infectious Diseases and Immunology, Division of Molecular Host Defence, Faculty of Veterinary Medicine, Utrecht University, The Netherlands

Abstract:

Due to extensive use of antibiotics, there is an increasing amount of antibiotic resistant bacterial strains and thus an urgent need for alternative methods to treat bacterial infections. Cathelicidins are small peptides of the innate immune system that have numerous functions, including direct antimicrobial properties as well as immunomodulatory activities, e.g. phagocytosis. Because of these properties, cathelicidins could be a promising alternative for the use of antibiotics. However, as a result of the antimicrobial function, it is challenging to find a suitable assay for the investigation of phagocytosis. Thus, the exact mechanism by which cathelicidins affect phagocytosis remains unclear. Hence, the aim of this research is to study the effect of chicken (ch)CATH-2, human LL-37, murine CRAMP and equine (e)CATH-2 on the phagocytosis of *E. coli*. To analyze this effect, first the minimal bactericidal concentration (MBC) of the cathelicidins LL-37, chCATH-2, eCATH-2 and CRAMP was determined by bacterial colony counts, which showed that chCATH-2 has the strongest bactericidal properties with an MBC of 5 μM . Next, to test the phagocytosis assay, different concentrations of *E. coli* were added to murine J774 macrophage-like cells, together with phagocytosis inhibitor CytoD. After 15, 60 or 120 minutes, external bacteria were killed using gentamycin for 15 min. After this, the J774 cells were lysed and the phagocytosed bacteria were plated out. The results showed a time-dependent uptake of bacteria. Addition of CytoD resulted in a decreased number of colony forming units. Our data suggest that the developed assay can be used to measure phagocytosis. It can be concluded that (1) the studied cathelicidins can kill bacteria directly and (2) phagocytosis is a time-dependent process. The next step will be to use this assay to test the effect of different cathelicidins on phagocytosis.

**Titel:**

The presence of lipoprotein particles in plasma interferes with labeling and fluorescence-based flow cytometric analysis of extracellular vesicles

Authors

Estefanía Lozano-Andrés ^{1,#}, Sten F.W.M. Libregts ¹, Cláudio Pinheiro ^{2,3,#}, Guillaume Van Niel ⁴, An Hendrix ^{2,3,#}, Ger. J.A. Arkesteijn ^{1,5}, Marca H.M. Wauben ^{1,#}

1 Department of Biochemistry and Cell Biology, Faculty of Veterinary Medicine, Utrecht University

2 Laboratory of Experimental Cancer Research, Department of Human Structure and Repair Ghent University, Ghent, Belgium.

3 Cancer Research Institute Ghent, Ghent, Belgium.

4 Institute for Psychiatry and Neuroscience of Paris, Hopital Saint-Anne, Université Descartes, INSERM U1266, Paris 75014, France.

5 Department of Immunology & Infectious Diseases, Faculty of Veterinary Medicine, Utrecht University, Utrecht, The Netherlands

TRAIN-EV Marie Skłodowska-Curie Action-Innovative Training Network

Abstract:

Introduction: Blood contains extracellular vesicles (EV), which are submicron-sized signaling structures secreted by cells that reflect unique features of the cell of origin, which makes them attractive liquid biopsy biomarkers. Detection of single EV by fluorescence-based high resolution flow cytometry (FC) allows for high throughput analysis of EV. Besides EV, blood plasma contains many lipoprotein particles (LPP) that overlap with size, molecular make-up or density of EV. Therefore, we investigated whether labeling and detection of single EV with the widely used PKH67 dye is hampered in the presence of LPP.

Methods: EV were obtained from conditioned cell culture media of the 4T1 mammary carcinoma cell line by a combination of density-gradient ultracentrifugation and size exclusion chromatography.

Commercially available human LPP were characterized by Dot Blot, ELISA, Electron Microscopy and FC. Next, LPP were spiked with EV and stained with PKH67 and a CD9-PE antibody. Samples were then fractionated by density gradient floatation and analyzed by FC using a BD Influx that was optimized for the detection of submicron-sized particles.

Results: We found that besides EV also LPP can be labeled with PKH67 and that their light scatter and fluorescent signals cannot be discriminated from those generated by purified PKH67-stained EV. In addition, the presence of LPP negatively affected the fluorescent PKH67 labeling of EV and hampered the detection of EV labeled with CD9-specific antibodies. These data illustrate that for fluorescence-based flow cytometric characterization of EV in plasma there is a need to separate EV from contaminating LPP before fluorescent labeling.

Conclusion: In order to perform reliable fluorescent-based FC-analysis of single EV in blood plasma, samples should be cleared from particles that are prone to incorporate generic dyes.

Funding: European Union's Horizon 2020 research and innovation programme under the Marie Skłodowska-Curie grant agreement No [722148] and STW-Perspectief Cancer-ID grant [14,191].

**Titel:**

Plasmid transfer is more efficient between phylogenetically closer related bacteria: a literature review

Authors

Jesse B. Alderliesten, Egil A.J. Fischer, Arjan Stegeman (Utrecht University, Faculty of Veterinary Medicine), Arjan de Visser, Sarah Duxbury (Wageningen University & Research, Laboratory of Genetics), Mark Zwart (NIOO-KNAW, Department of Microbial Ecology)

Abstract:

Antimicrobial resistance genes that are located on plasmids can be transferred between bacteria. In bacterial communities, this can lead to the transfer of resistance genes from one species to another. We want to quantify the influence of phylogenetic relatedness between donor and recipient bacteria on the efficiency of plasmid transfer between species. We performed a systematic literature review using PubMed, CAB Abstracts, and a backward search based on a previous literature review to search for quantitative data on plasmid transfer from *E. coli* to other bacteria in liquid medium. Eighteen studies were included in the analysis with a total of 284 conjugation frequencies expressed as the number of transconjugants per donor. Characteristics of the bacteria (phylogenetic distance to *E. coli* based on 16S rRNA sequences, growth phase), plasmids (incompatibility type), and experimental conditions (medium, culture type, pH, temperature, duration, donor to recipient ratio) were recorded. We used linear mixed effect models with “study” as random effect to estimate the relation between phylogenetic distance and the logarithm of the conjugation frequency. Model selection was performed after excluding variables that were not significant (p -value > 0.2) in univariable analyses or contained more than 15% missing data. Akaike information criterion was used to select the best multivariable model using the remaining variables. The final model contained the following variables: phylogenetic distance to *E. coli*, origin of the donor bacterium, incompatibility type of the plasmid, and the used temperature. Phylogenetic distances ranged from 0.00 to 0.30 units (*E. coli* or *Bacteroidetes* as recipients). The log of the plasmid transfer frequency decreased with 13.90 units per unit of phylogenetic distance (95% CI: -20.12, -6.54), meaning that the efficiency of plasmid transfer is higher for phylogenetic closer related species in liquid medium culture.



Nr. Abstract
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Titel:

Congenital deafness in Dalmatian dogs. The effect of the NCDH breeding program

Authors

Vera Pleijsier (Master student), Hans Vernooij (Farm Animal Health), Peter Leegwater (Companion Animal Health)

Abstract:

Hereditary deafness in Dalmatian dogs has been a problem for many years, and has been studied for years too. Nowadays, the hereditary deafness in Dalmatians is known to be a form of Canine Congenital Sensorineural Deafness (CCSD). Several phenotypical factors seem to be involved in the development of this disease. A breeding program for Dalmatian dogs has been set up in several countries, to help prevent deafness. In the Netherlands, the breeding program was set up by the Dutch Club for Dalmatian Dogs (NCDH). However, the effectiveness of the breeding program by NCDH on the incidence of CCSD is unclear and subject of this research.

A Logistic regression analysis has been performed on data collected by the NCDH from 1995 to 2018: 4948 BAER-tested Dutch pups have been analysed on the degree of association between hearing status and birth year period, head spot, coat colour, blue eyes, litter size and data of the parents. The effects of the sire and dam were considered as random effects.

The incidence of deafness has decreased since the breeding program was implemented. The incidence of deafness was proven to be significant decreasing for the birth periods 2001-2006 and 2013-2018. The decrease was also found for unilateral and bilateral deafness separately. It was also found that females have significantly higher odds on deafness than males. Furthermore, presence of head spot significantly decreases the odds on deafness, whereas blue eyes do not seem to have a significant effect. The incidence of deafness might have decreased more, had dogs with presence of a head spot been allowed for breeding.

**Titel:**

STOC free: An innovative framework to compare probability of freedom from disease in heterogeneous control programmes

Authors

I. Santman/Utrecht University and GD Animal Health, A. Madouasse/ONIRIS France, M. Mercat/ONIRIS France, C. Fourichon/ONIRIS France, S. More/University College Dublin, D. Graham/Animal Health Ireland, J. Frössling/Swedish National Veterinary Institute, A. Lindberg/Swedish National Veterinary Institute, J. Gethmann/FLI Germany, C. Sauter/FLI Germany, G. Gunn/SRUC Scotland, C. Gomes/SRUC Scotland, M. Henry/SRUC Scotland, A. van Roon/Utrecht University, M. Nielen/Utrecht University, L. van Duijn/GD Animal Health, G. van Schaik/Utrecht University and GD Animal Health

Abstract:

Countries differ in existence, stage of eradication and design of control programmes (CPs) for non-regulated diseases. When freedom from infection is reached or being pursued, safe trade is essential to protect that status. The aim of the STOC free project, a collaboration between six countries, is to develop and validate a framework that enables a transparent and standardized comparison of confidence of disease freedom for CPs across herds, regions or countries. The STOC free framework consists of a model combined with a tool to facilitate the collection of the necessary parameters. All relevant actions taken in a CP will be included in a Bayesian model, which allows prior distributions for most parameters. Data for the distributions can be obtained from databases of CPs, demographic data and contact structures between herds. In addition, frequency of occurrence and risk estimates for factors that influence either the probability of introduction or delayed detection of the infection in an animal or herd will be included in the model. Bovine viral diarrhoea virus (BVDV) is used as an example disease. Many countries have CPs in place for BVDV and although elements of the CPs are similar, the place of vaccination, the combination of diagnostic tests, frequency and target groups differ widely between countries and regions. Although the initially developed framework is based on BVDV, the aim is to make it sufficiently generic to be adaptable to CPs for other diseases and possibly other species and enhance the safety of trade.



Nr. Abstract
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Titel:

Chronical stress as an indicator of the welfare of deer

Authors

Judith Hendriks, Susanne Kirchhoff, José Lozeman-van het Klooster, Anne-Marie Baars, Susanne Kirchhoff, Rory Putman, Heidi Lesscher

Abstract:

Wildlife welfare management is a matter of societal concern, and there is an increasing need to objectively measure the welfare status of wildlife.

Positive welfare is defined as the possibility for an individual to adapt to the prevailing environmental circumstances. This requires a healthy stress response, mediated by the hypothalamus-pituitary-adrenal (HPA) axis. However, prolonged stress, arising from challenging conditions, can compromise the individual's adaptive capacity, due to down-regulation of glucocorticoid receptors (GR) in the brain, resulting in elevated circulating cortisol levels and impaired negative feedback of the HPA-axis, thereby compromising an individual's adaptive capacity and welfare status.

This project aimed to use physiological markers for the HPA-axis to measure chronic stress in deer as a model system. For that purpose, we have collected fur and brain samples from deer from different parks in the UK over the past few years. These parks differ in population density, human interference, climate etc. Moreover, since the onset of culling schemes in the Amsterdamse Waterleiding Duinen (AWD), we gathered fur and brain samples from this area on a yearly basis. All samples were processed for cortisol analysis in fur, and measurement of GR levels in brain.

The integrated analysis of brain GR and hair cortisol in different populations in the UK, in relation to diverging management factors, revealed that hair cortisol levels were related to population density, particularly in females. However, we found no significant relation between brain GR levels and management factors, and hair cortisol did not correlate with brain GR. In AWD samples, a gradual decline in hair cortisol levels was observed paralleling the reduction in population size. This was again more prominent in females, except for the last year. These findings show that population density is a major determinant for prolonged stress, and hence an important factor to safeguard deer welfare.

**Titel:**

Collection and composition of saliva from suckling piglets

Authors

E.M. de Goede (BSc)¹ F. Bikker (PhD)² T.J. Tobias (PhD, DVM, MSc, Dipl ECPHM)¹

1) Utrecht University, Department of Farm Animal Health

2) Academisch Centrum Tandheelkunde Amsterdam, Department of Oral biochemistry

Abstract:*Background and objectives*

The potential composition of saliva and the easy way in which it can be collected, makes it an important matrix for the diagnosis of several diseases or biomarkers. Therefore, the aim of this study was twofold: to develop best practices for saliva collection of individual suckling piglets and determine age- related protein patterns in saliva.

Material and methods

A randomized block design was used with 5 groups of 32 suckling piglets from 18 litters of different ages (1-2 days, 1, 2, 3 and 4 weeks old) which had to chew for 0.5, 1, 2 and 4 minutes on a Salivette for saliva collection. The quantity, protein concentration, protease activity and protein composition in the saliva samples were determined with a protein assay, protease assay and SDS-page.

Results

It was found that a minimal sampling time of 2 minutes is necessary to collect sufficient saliva in individual suckling piglets from ~2 weeks and older to perform the abovementioned analyses. In contrast, in younger piglets, saliva collection was hardly possible, even at 4 min collection time. Both amount of saliva collected and protein concentration increased with sampling time and both parameters were dependent on age. Protease activity was associated with saliva quantity, protein concentration and a random litter effect. Moreover, the protease activity was higher in piglets from 3 and 4 weeks old than in younger piglets. Finally, the longer the chewing time, the higher the probability that proteins with a mass of 110 and 20 kDa were detected in saliva samples. So far the identity of these proteins has not been revealed.

Conclusion

To obtain sufficient saliva from each piglet, individual saliva sampling is possible in pigs \pm 2 weeks old and requires 2 minutes of chewing time per piglet. With these samples various biochemical analysis can be performed.

**Titel:**

Dynamic micro-physiological in vitro models of articular joint tissues - modular multi-organ-on-chip approach

Authors

L. Moreira Teixeira (UT/UU), S. Piluso (UT/UMU), J. Leijten (UT), J. Malda (UMC/UU), R. van Weeren (UMC/UU), M. Karperien (UT)

Abstract:

Osteoarthritis (OA) affects circa 300 million people globally, being the main cause of permanent disability. Erroneously, it has been considered a distant pathology, associated with old age. Despite its prevalence, it remains incurable and unknown to the majority of the population. This degenerative disease affects all tissues that compose the joints, including cartilage, bone and soft tissue; notably, the disease onset can be triggered in any of these tissues. For this reason, current in vitro models fail to reproduce critical interactions between the joint tissues at various disease stages. Furthermore, animal models rarely reflect the complexity of its etiology. To overcome these limitations, we are currently developing micro-engineered biomimetic models that reconstitute the complexity, dynamic microenvironment, and physiological function of the joint tissues, using a modular design approach. The individual joint tissues, such as cartilage, subchondral bone, and synovium, are micro-engineered in microfluidic platforms, which can be actuated to mimic both the compression and shear stress, emulating multi-axial joint movement and load bearing functions. This multi-organ-on-a-chip technology is enabled by tunable interconnectivity via microfluidic channels mirroring blood vessels and/or intra-articular space. A toolbox of in situ and ex situ analysis options is available to characterize cell behavior, including inflammation, remodeling and mechano-transduction responses to stimuli. The versatility of each individual platform and joint modular features offers the potential for applications in healthy/disease modelling, therapy screening, and personalized medicine. The ultimate goal is to use this technology to understand and prevent OA onset or contribute to efficient treatment.

**Titel:**

Studying liver disease in a cell culture dish

Authors

Maya W. Haaker¹, Hedwig S. Kruitwagen², Danielle ter Braake³, Arie B. Vaandrager¹, Louis C. Penning², Bart Spee², Femke Broere^{3,4}, J. Bernd Helms¹

1 Department of Biochemistry and Cell Biology, Faculty of Veterinary Medicine, Utrecht University

2 Department of Clinical Sciences of Companion Animals, Faculty of Veterinary Medicine, Utrecht University

3 Department of Infectious Diseases & Immunology, Faculty of Veterinary Sciences, Utrecht University

4 Department of Clinical Sciences of Companion Animals, Faculty of Veterinary Sciences, Utrecht University

Abstract:

Both humans and animals suffer from liver diseases with causes ranging from viral infections to steatohepatitis. Pathologies frequently observed in patient livers are lipid accumulation and fibrosis. We aim to understand the cellular mechanisms behind both pathologies by studying the different cell types involved in cell culture systems, with the ultimate goal to develop treatments for patients suffering from liver disease.

Hepatic lipidosis is a problem frequently seen in cats during periods of anorexia. To study this, feline liver organoids were used, creating a species specific system. By adding additional fatty acids to the cell culture medium lipid accumulation can be induced, and the effect of drugs that aim at decreasing the lipid accumulation can be measured. Using this system we identified two drugs, T863 (a diacylglycerol O-acyltransferase 1 (DGAT1) inhibitor) and 5-aminoimidazole-4-carboxamide 1- β -D-ribofuranoside (AICAR) (an AMP kinase activator), potentially able to treat feline hepatic lipidosis.

The main cell type responsible for liver fibrosis is the hepatic stellate cell (HSC). HSCs are professional lipid storing cells and in a healthy liver they have a quiescent phenotype, serving as the storage site of vitamin A (which is a fat-soluble vitamin). Upon liver damage they activate, releasing their vitamin A and becoming myofibroblasts producing extracellular matrix proteins like collagen. When the HSCs remain activated after they have lost their vitamin A storage the large amounts of ECM production can lead to liver fibrosis. We investigate the interaction between HSCs and their surroundings, both the matrix they are in and the other cell types they interact with. The matrix and hepatic adult stem cells (organoids) both appear to be able to influence the activation of HSCs. Recently we started to investigate the potential of HSCs to function as antigen presenting cells and their interaction with professional antigen presenting cells like dendritic cells.

**Titel:**

Optimization of a biobanking and isolation protocol for robust analysis of synovial fluid-derived extracellular vesicles from healthy and diseased subjects.

Authors

Laura Varela*[1], Sanne Mol [2,3], Filipe Bragança [1], Sander Tas [3,4], René van Weeren [1], Chris van de Lest [1,2], Marca Wauben [2].

* Presenting author,

1: Department of Equine Sciences, Utrecht University,

2: Department of Biochemistry & Cell Biology, Utrecht University,

3: Department of Experimental Immunology, Amsterdam Infection & Immunity Institute,

4: Department of Rheumatology and Clinical Immunology, Amsterdam Rheumatology and Immunology Center.

Abstract:

Introduction: Extracellular vesicles (EV) are known to play a role in intercellular communication between cells in both health and disease states and increasing evidence accumulates that EVs could play a role in joint diseases. We are interested to disclose the (patho)physiological role of EV in synovial fluid.

Formerly, we defined a storage and isolation protocol for EV from healthy equine derived synovial fluid. However, in the processing of synovial fluid samples from inflamed joints, we encountered in some samples aggregation and or clotting problems, despite lack of blood contamination by visual inspection. We found that these aggregates interfered in quantitative and qualitative EV analysis for both samples derived from inflamed human and horse synovia. We here explored a strategy to optimize the protocol based on the hypothesis that the aggregate structures are caused by a combination of fibrin clots and DNA, which could be formed after neutrophil activation.

Methods: SF was obtained from human psoriatic arthritis patients and equine LPS-induced acute synovitis. Thawed cell-free SF samples were treated with hyaluronidase, DNase I and diluted in EDTA/PBS at different ratios. EV were isolated by differential (ultra)centrifugation followed by density gradient ultracentrifugation. Collected fractions were assessed for protein and lipid contents and EV markers were evaluated.

Results: SF-derived from inflamed synovia displayed variable clot-like aggregates after thawing. Despite these aggregates, EVs were isolated, but we also observed a loss of EV in high-density fractions. Currently, we are investigating whether the loss of EV could be prevented by the addition of DNase I and EDTA immediately after SF collection before biobanking.

Conclusion: There is a critical need to design a protocol for SF biobanking and EV isolation that allows a robust and reliable analysis of EV from SF of healthy and diseased subjects.

**Titel:**

Histological aspects of corneal cross linking (CXL) in equines

Authors

Eva Kammergruber¹, Inge Slenter², Hanneke Hermans³, Guy Grinwis¹

1 Department of Pathobiology, Faculty of Veterinary Medicine, Utrecht University,

2 Ophthalmology Section, Department of Clinical Sciences of Companion Animals, Utrecht University,

3 Department of Equine Sciences, Faculty of Veterinary Medicine, Utrecht University,

Abstract:*Introduction*

Despite advances in medical treatment of corneal diseases in horses, keratectomy is frequently required. Corneal crosslinking (CXL), a recently introduced method of treatment in veterinary ophthalmology, induces new chemical bridges between proteins and other molecules within the corneal stroma, after exposure to riboflavin and ultraviolet light. In this study, histopathology of the treated corneas is used to evaluate the efficacy of the CXL procedure and is a co-operation of the departments of pathobiology, clinical sciences of companion animals, and equine sciences.

Material and Methods

Fixation of the entire globe versus corneal tissue only, and formaldehyde versus Davidson's fixative were compared in H&E-stained paraffin-embedded tissue sections of eyes from 8 horses. Furthermore, PAS and Masson's trichrome staining, as well as immunohistochemistry using antibodies against laminin were analyzed.

Results

Corneal tissue collapsed once removed from the globe, and fixation in 10% neutral buffered formalin created microfolds, both hampering histologic evaluation. PAS and Masson's trichrome -stained crosslinked corneas showed a change in density within the superficial stroma, indicating a possible change in composition of extra-cellular matrix. However, somewhat similar, though far less prominent, change was seen in the in PAS stain of the negative control eye in which only the epithelium was removed. The basement of corneal epithelium was still present in the area of the removed epithelium as visualized in the immunohistochemistry for laminin.

Summary

Preliminary results of establishing a practical protocol to assess the effect of CXL indicate that entire globe fixation, improved histomorphological evaluation compared to fixation of a removed cornea. Furthermore, Davidson's fixative, improved histomorphological evaluation compared to formaldehyde fixation. There appears to be an effect on the cornea after CXL treatment as seen with histochemical methods. However, only removal of the epithelium also resulted in similar though less prominent changes as visualized in PAS-stained slides.

Future perspectives

The preliminary findings need to be confirmed in a larger number of equine eyes. Furthermore, quantitative assessment of composition of extracellular matrix and measurement of collagen fiber diameters might be considered in future studies to support presented preliminary findings. Also a method to assess stromal cell necrosis needs to be involved to determine the penetration depth of CXL protocols.

**Titel:**

Epigenetic and transcriptomic profiling of canine adult vascular endothelial cells.

Authors

Riemers FM¹, Mokry M², Oosterhoff LA¹, Tryfonidou MA¹, van Steenbeek FG^{1,2}

1 Department of Clinical Sciences of Companion Animals, Faculty of Veterinary Medicine,

2 Department of Cardiology, Division Heart & Lungs, UMC Utrecht

Abstract:

Vascular endothelial cells (ECs) are essential in studying vascular characteristics and behavior. The emerging field of bioprinting tissues is challenged by the lack of vascularization which results in ischemia. Therefore, the inclusion of vasculature and its communication with the surrounding tissue is essential. These challenges are typically addressed by studying EC functionality based on cells derived from the human umbilical vein of the new-born (HUVECs). This gold standard fails to recapitulate nature given that ECs have a distinct molecular signature depending on their location. Within this context adult canine ECs allow us to obtain fundamental knowledge about regulation of expression in adult vessels. Therefore, the aim of this study was to identify the vessel type specific patterns of expression and open chromatin regions, and to integrate the expression and the open chromatin in order to gain insight into expression regulation and the transcriptional programs of the vessel types studied. Canine primary ECs were isolated from the aorta, vena cava and vena porta of three donors and ATAC-seq and RNA-seq was performed, followed by combining the data of the two methods. Interestingly the ECs maintain their locational specific epigenetic program in culture, making this culture system very useful for research into adult vascular endothelial cells.



Nr. Abstract
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Titel:

Chemically Defined Synthetic Hydrogel as Matrigel Alternative for Human Liver Organoid Culture

Authors

Shicheng Ye, Jochem W.B. Boeter, Louis C. Penning, Bart Spee and Kerstin Schneeberger*
Department of Clinical Sciences of Companion Animals, Faculty of Veterinary Medicine, Utrecht University, Utrecht 3584 CT, The Netherlands

Abstract:

Adult stem cell (ASC) derived organoids can be infinitely expanded without any genetic modifications, making it a promising cell source for regenerative medicine. However, current organoid culture relies on Matrigel that has disadvantages such as batch-to-batch variability, and the fact that it is an ill-defined, animal-derived matrix, which implies ethical issues and significantly compromises the translation of organoid-derived cells for clinical applications. To overcome this limitation, a chemically defined synthetic hydrogel (SH) was used to replace Matrigel for the expansion of human ASC derived liver organoids. Here, the hydrogel was functionalized with extracellular matrix (ECM) components and mechanical properties were measured. Single cells were seeded in the hydrogel and their organoid formation capacity and proliferation were verified. Remarkably, cells formed mini-organoids on the second day after seeding, and they grew in the functionalized synthetic hydrogel with a dose dependent effect of ECM proteins. After more than 6 weeks of expansion, organoids in SH are still comparable to those cultured in Matrigel and keep proliferating without obvious differences. To conclude, this chemically defined synthetic hydrogel is an adequate Matrigel alternative for long term expansion of human liver organoids.

**Titel:**

A potential role for Hepatic Stellate Cells in the induction of Immune Tolerance

Authors

D. ter Braake^a, M.W. Haaker^b, I.S. Ludwig^a, A.B. Vaandrager^b, J.B. Helms^b, F. Broere^{a,c}

a Department of Infectious Diseases & Immunology, Faculty of Veterinary Sciences, Utrecht University,

b Department of Biochemistry & Cell Biology, Faculty of Veterinary Sciences, Utrecht University,

c Department of Clinical Sciences of Companion Animals, Faculty of Veterinary Sciences, Utrecht University

Abstract:

Tolerogenic dendritic cells (toIDCs) are key players in the induction and maintenance of immune tolerance. ToIDC are characterized by a semi-mature surface marker phenotype and the ability to induce antigen specific regulatory T cells, inhibit proinflammatory responses or both. In vitro, toIDCs can be induced by stimulation of DCs with tolerogenic compounds, including retinoic acid (RA) and TGF- β^2 . Hepatic Stellate Cells (HSCs) store 80% of the body's retinoids, and have the ability to secrete TGF- β^2 . Therefore, we hypothesize that HSCs might play a role in the natural induction and maintenance of immune tolerance.

In order to gain further understanding of the role of HSCs in the induction of immune tolerance, HSCs were isolated from C57Bl/6 mice and cultured for 7 days, after which medium was removed and added to a culture of DCs obtained from the same mice. Flow cytometry analysis revealed that HSC conditioned medium contributes to the induction of toIDCs in vitro, as shown by the semimature CD11c+MHCIIhiCD40lowCD86low phenotype characteristic for toIDCs. Stimulation with HSC conditioned medium and TGF- β^2 caused even lower expression of co-stimulatory molecules. Our preliminary data therefore suggest a role for HSCs in toIDC induction.



Nr. Abstract
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Titel:

Interdependence: Making Sense of One Health

Authors

Joachim Nieuwland & Franck Meijboom, Department of Animals in Science and Society

Abstract:

In response to the rise of emerging infectious diseases at the beginning of the 21st century, the One Health initiative gained traction. The idea of One Health includes a push for cross-disciplinary collaboration as well as a recognition of the myriad relations between the health of humans, nonhuman animals, and the environment. In other words, in order to strengthen human health policy, we'll have to make it multidisciplinary and multispecies. One Health could provide both a conceptual understanding as well a practical roadmap of how to work together across disciplinary boundaries so to best address the complexities that underlie health policy in a more-than-human world. In what way does One Health relate to veterinary ethics as part of the broader curriculum of educating future veterinary professionals?

An answer to that question assumes knowledge as to the meaning and scope of One Health. Providing a specific interpretation and / or implementation of One Health is however an evaluative matter itself, as such delineation reflects one's values. What One Health implies for veterinary medicine thereby becomes a central question and addition to the field of veterinary ethics itself, as it requires students and veterinarians to engage with interdependence.

We explore three interpretations of interdependence: (1) embedded in ecosystems, (2) being ecosystems, and (3) interbeing, concluding that all of these require an enrichment of veterinary ethics and its pedagogies. In addition to rational and sentimental pedagogies, engaging with interdependence within veterinary ethics can benefit from additional contemplative pedagogies.

**Titel:**

Biochemical characterization of CRISP2 protein interactions and localization in boar sperm

Authors

Min Zhang, R. Zenezini Chiozzi, Dora V. Kaloyanova, Nick K. Orlachs, J. Bernd Helms, Bart Gadella

Abstract:

Background: Members of cysteine-rich secretory proteins (CRISPs) belong to CAP (CRISPs, Antigen 5 and Pathogenesis related protein 1) superfamily. Three paralogues of four CRISP members have been described in mammals and are highly expressed in male reproductive tracts. Of them, CRISP2, is the only testicular expressed protein and is proposed to be involved in sperm-egg fusion. It is expressed in the early stage of spermiogenesis and is later incorporated into sperm head and tail. The present study focuses on the localization and organization of CRISP2 in boar sperm cells and its involvement in amyloid/complex formation which might be necessary for normal fertilization.

Methods: Freshly ejaculated sperm cells from highly fertile boars were used in this study. Sperm cells were washed through a discontinuous Percoll® gradient followed by sonication to detach sperm heads from tails. Perinuclear theca (PT) proteins were extracted from purified sonicated sperm heads. Protein lysates were prepared in RIPA or HEPES (1% Triton) for Western blots or native blots, respectively. Indirect immunofluorescence was performed to assess the presence of CRISP2 in boar sperm. PT extracts were further analyzed by mass spectrometry to establish the presence of PT proteins from boar sperm.

Results: Three bands (~100kDa, ~50kDa, ~35kDa) showed up in whole sperm extractions while an additional ~25kDa emerged after sonication/Triton treatment. Interestingly, the three higher MW CRISP2 were present in sperm tail fraction while the ~25kDa was specific for the sperm head fraction. However, whole cell and tail isolates with the urea treatment caused a complete dissociation of CRISP2 from the β -Mercapto-ethanol and SDS-resistant high MW complexes and as a result only the un-complexed ~25kDa form of the protein was detected on Western blot. This suggests that CRISP2 was incorporated into different detergent and reduced condition resistant protein complexes. Native lysates from sonicated heads and tails showed that CRISP2 contributed to a ~140 kDa complex. Immunostaining on whole sperm showed that CRISP2 was present in the post acrosome region of the sperm head as well as in the connecting piece. At both locations CRISP-2 was localized intracellularly as it could only be immunolabelled after detergent treatment. Membrane fractionation experiments showed that CRISP2 was an inner acrosomal membrane associated (IAM) protein. In the sonicated sperm head CRISP2 was present in the PT alkaline extracts demonstrated that it was a member of post acrosome sheath (PAS)-PT proteins, which was further confirmed by proteomic mass spectrometry identification.

Conclusions: These data indicated that CRISP2 is a PAS-PT protein in boar sperm and is present in a ~140 kDa protein complex in native state as well as an ~25 kDa form interacting with the IAM of the sperm head. Further studies will focus on what is present in the native ~140 kDa CRISP2 containing native protein complex and how such protein aggregates are involved in sperm physiology. Special attention will be paid to the redox- as well as the divalent cation- dependent generation or opening of disulfide bridges in the cysteine rich regions of this protein and their role in protein complex formation and dissociation.

**Titel:**

The high amount lysophospholipids found in Campylobacter jejuni are produced by PldA enzyme are needed for motility and can cause cell to lyse

Authors

Xuefeng Cao, Chris H. van der Lest, Jos P. M. van Putten and Marc M.M.S.M. Wösten

Abstract:

Aim: Cell membranes are composed primarily of phospholipids and proteins. The activity of the membrane proteins is affected by the structure of the surrounding phospholipids. In response to changes in their environment bacteria need to change not only their protein repertoire, but also their lipid composition in the membrane. Therefore, bacteria need to modify or replace existing phospholipids to adjust their membrane viscosity to match environmental requirements. Most bacterial phospholipids are composed of two fatty acids, a glycerol moiety, a phosphate group and a variable head group. Previous we have shown that the human pathogen *Campylobacter jejuni* is able to produce 203 different phospholipid species belonging to nine different phospholipid classes.

Method/Results: Here we report that by LC-MS/MS analyses revealed that a large part up to 40% of the *C. jejuni* phospholipids are cleaved by the enzyme phospholipase A (PldA) resulting in the lysolipids lysophosphatidylethanolamine and lysophosphatidylglycerol. Both acyl chains present on phospholipids can be cleaved by the PldA enzyme.

Conclusion: Genetic inactivation of *pldA* largely prevented lysolipid formation and resulted in a non-motile bacterial phenotype at low oxygen conditions, suggesting that lysophospholipids are necessary for *C. jejuni* motility and thus human *Campylobacteriosis*.

**Titel:**

Recognizing lameness in pigs: challenges and opportunities

Authors

Ellen Meijer/Behaviour and Welfare group, Department of Farm Animal Health, Joost J. Uilenreef/Animal Research Institute AMC, Franz Josef van der Staay/Behaviour and Welfare group, Department of Farm Animal Health

Abstract:

Lameness is a common problem in swine husbandry. Affected pigs experience welfare issues such as pain and impaired locomotion. For farmers, it causes financial losses due to slower growth, treatment costs or early culling. To mitigate these consequences, early detection and treatment is important. However, identifying lame pigs is difficult since, as prey animals, they tend to hide signs of weakness. We assessed if visual scoring (the method used in practice) can identify lame pigs when compared to an objective measure for weight bearing.

Lameness was induced in 8 seven-week-old piglets by injecting monosodium-iodoacetate (MIA), an inhibitor of chondrocyte glycolysis, into the left intercarpal joint. Ten control animals received intra-articular physiological saline solution. On the day before injection and day 1, 3 and 28 after injection lameness was scored by two experienced pig veterinarians. Objective measures for weight-bearing were obtained by pressure mat analysis. An asymmetry index (ASI) of Peak Vertical Force of the front limbs was calculated, resulting in a dimensionless number between -200 and +200.

On day 1, ASI of all pigs was significantly lower than baseline. On day 3 and 28 only pigs that received MIA had significantly lower ASI's, -33.1 and -51.8 respectively. At cutoff ASI of -25, sensitivity of visual scoring was 0.02 and specificity was 0.94. Positive predictive value for visual scoring was 0.14 and negative predictive value was 0.68.

Although lameness was scored by trained observers under favorable conditions, only a small proportion of pigs with decreased weight-bearing was detected. In practical conditions, with large groups and limited time available this number may be even lower. Pigs that are visually lame can therefore be considered as the "tip of the iceberg". Considering that weight redistribution is usually a behavioral adaptation to pain, many animals with impaired welfare are not identified on farm.

**Titel:**

Transport of 18-day old hatching eggs -- effects on behavior and physiology of broiler chicken?

Authors

Vivian C. Goerlich-Jansson ¹, Celeste Dull ¹, Ashley Pascual ¹, Gerrit van der Linde ², Rebecca Nordquist ³

1= Animals in Science and Society, FVM, UU,

2= Heering Holland,

3 = Farm Animal Health, FVM, UU

Abstract:

Incubation, hatching, and raising of broiler chicken commonly takes places at separate facilities. Recently, hatching at the grow-out facility is tested, avoiding the transport of day-old chicks. Instead, eggs are transported on embryonic day 18 from the breeding to the grow-out facility, where they hatch on day 21.

Transport is a known stressor to animals. As the hypothalamic–pituitary–adrenal (HPA)-axis becomes functional in chicken around embryonic day 14-16, it is conceivable that transport of eggs may lead to a stress response and increased production of corticosterone in the embryo. Exposure to prenatal stress may program the HPA axis in the long term, negatively impacting the further development of the chick. Moreover, malpositioning of late-stage embryo seems to occur more often after transport of long durations, leading to hatching failure.

We investigated whether prolonged transport on day 18 has effects on the development of a slow growing broiler chicken strain (Hubbard JA257). We compared a short transport (ST) and long transport (LT) group of eggs and measured the heart rate of embryos during transport, hatching success and growth of the chicks. We performed two established behavioural tests related to stress susceptibility, tonic immobility and open field. Moreover, we determined the concentration of corticosterone in feathers at the end of the experiment. We expected LT chicks to grow less, show stronger behavioural responses to stressful challenges, and higher levels of feather corticosterone compared to ST chicks. Embryonic heart indeed increased with prolonged transport, confirming at least a temporary effect of transport on the physiology of the chicks. On several occasions, LT birds were slightly lighter than ST birds. The tonic immobility test showed no significant differences between the ST and LT group. The open field test as well as the feather corticosterone concentrations are currently being analysed.

**Titel:**

Interrogating humoral immune responses to find key protective antibodies against coronaviruses

Authors

Chunyan Wang¹, Ivy Widjaja¹, Rien van Haperen^{2,3}, Javier Gutiérrez-Álvarez⁴, Brenda van Dieren¹, Nisreen M.A. Okba², V. Stalin Raj², Wentao Li¹, Raul Fernandez-Delgado⁴, Frank Grosveld^{2,3}, Frank J.M. van Kuppeveld¹, Bart L. Haagmans², Luis Enjuanes⁴, Dubravka Drabek^{2,3} and Berend-Jan Bosch¹

1 Utrecht University - Veterinary Medicine, Utrecht, the Netherlands

2 Erasmus Medical Centre, Rotterdam, the Netherlands

3 Harbour Antibodies B.V., Rotterdam, the Netherlands

4 National Center for Biotechnology - Spanish National Research Council, Madrid, Spain

Abstract:

The Middle-East respiratory syndrome coronavirus (MERS-CoV) is a zoonotic virus that causes severe and often fatal respiratory disease in humans. Efforts to develop antibody-based therapies have mainly focused on neutralizing antibodies that target the receptor binding domain of the viral spike protein thereby blocking receptor binding. We developed a set of human monoclonal antibodies that target functionally distinct domains of the MERS-CoV spike protein. These antibodies belong to six distinct epitope groups and interfere with the three critical entry functions of the MERS-CoV spike protein: sialic acid binding, receptor binding and membrane fusion. Passive immunization with potently as well as with poorly neutralizing antibodies protected mice from lethal MERS-CoV challenge. Collectively, these antibodies offer new ways to gain humoral protection in humans against the emerging MERS-CoV by targeting different spike protein epitopes and functions.

**Titel:**

Congenital malformation of the front claws of a pig

Authors

A.van Nes, L. Macri, L. Boessen, T.J. Tobias,
Utrecht University, Faculty of Veterinary Medicine, Department of Farm Animal Health

Abstract:*Introduction*

During a Farm visit, a lame piglet was noticed with a malformation of both claws of both front legs. Other pigs in the litter and in the compartment showed no abnormalities. The pig was transported to the Farm Animal Health clinic for further investigations.

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After arrival, the pig was examined clinically and thereafter X-ray examination was performed. After four days the pig was euthanized and post mortem analysis was performed.

Results

Clinically, breathing frequency, and heartrate were slightly elevated during the 4 days, whereas body temperature was only elevated on day 1. All four hoofs in the front legs were absent and the dew claws were absent or distorted. X-ray examination revealed that left front leg and the right front leg missed distal phalanx of the second toe, the middle and distal phalanx of the third toe. The fifth toe right missed the middle and distal phalanx, the firth toe left front missed the proximal, middle and distal phalanx and also the middle phalanx of the second toe had signs of osteomyelitis. At necropsy, in addition to the missing bones in the left front leg an abscess was observed, originating from a pododermatitis as well as signs of an osteomyelitis.

Discussion

This case study describes a malformation of the claws of both front legs. The malformation is probably due to a defect of the dermal ossification of the phalanges. As far as the authors know, this anomaly has been described for foals, but not for pigs.

**Titel:**

A modified density gradient centrifugation protocol to select stallion spermatozoa with intact DNA

Authors

Muhammad Umair, Tom Stout, Anthony Claes, Heiko Henning
Department of Equine sciences, Faculty of Veterinary Medicine

Abstract:

Selecting spermatozoa with intact DNA is important for producing viable embryos after intracytoplasmic sperm injection because spermatozoa lack the machinery to repair DNA damage. This study aimed to optimize a method of density gradient centrifugation to enrich for spermatozoa with intact DNA. Semen was collected from 6 stallions, diluted (1:1) in INRA96, and then mixed (1:1) with a 60% solution of the colloid Opti-Prep™ (OP). This mix was layered onto 40% OP in three 15mL tubes to which, respectively, a 20%, 25% or 30% OP top layer was added before centrifugation (1000g, 20 min). Sperm recovery, motility, morphology, viability, acrosome integrity and DNA fragmentation index (%DFI) were determined in the uncentrifuged sample (UC) and in the 3 bands per tube resulting after centrifugation, and compared using the Wilcoxon signed-rank test. Semen quality was better in the 2nd band (interface between top layer and the mix) and, therefore, this band was used for subsequent analysis. Average sperm recovery was 53%, 36% and 21% for the 20%, 25% and 30%OP top layers, respectively. Percentages of viable and acrosome intact spermatozoa were higher ($P<0.05$) after centrifugation with a 20%OP top layer (88%) than in UC (79%), 25%OP (82%) or 30%OP (83%). Total motility was higher ($P<0.05$) for the 20%OP (84%) and 25%OP (82%) top layer than in UC (69%), and progressive motility was higher ($P<0.05$) with the 25%OP (71%) and 30%OP (74%) than in the UC (53%). Percentage of spermatozoa with normal morphology did not change ($P=0.2$) after centrifugation. Compared to the UC (12 ± 5), %DFI decreased ($P<0.05$) for 20%OP (4 ± 1) and tended ($P<0.07$) to decrease for the 25%OP top layer (5 ± 4). In conclusion, using a modified density gradient centrifugation protocol with a 20%OP top layer, a sperm population with higher percentages of motile, viable, acrosome intact spermatozoa with intact DNA could be selected.

**Titel:**

Infectious bronchitis virus QX field progenitor loses nephropathogenicity after attenuation into a live viral vaccine

Authors

A. Laconi(a), E.A.W.S. Weerts (a), J.C.G. Bloodgood (a), J.P. Deniz Marrero (a), A.J. Berends (a), G. Cocciolo (b), J.J. de Wit(c,d), M.H. Verheije (a)

(a) Department of Pathobiology, Faculty of Veterinary Medicine, Utrecht University, Utrecht,

(b) Department of Veterinary Medicine, University of Bari, Valenzano, Italy

(c) Department of Farm Animal Health, Faculty of Veterinary Medicine, Utrecht University, Utrecht,

(d) GD Animal Health, Deventer

Abstract:*Introduction:*

Infectious bronchitis (IB) is a gammacoronavirus-induced respiratory disease in chickens. Of the several IB virus (IBV) subtypes, IBV QX is one of the strains additionally known for its marked nephropathogenicity. In this study we compared the phenotypic differences between an IB QX vaccine virus and its direct field progenitor with main focus on viral replication and the potential to disseminate through the host.

Materials and methods:

Specific pathogen free chickens were inoculated intratracheally with either 103 EID₅₀ vaccine strain Nobilis IB Primo QX (MSD/Animal Health) or its progenitor field strain IBV-D388 and euthanized daily over an eight-day period. Histopathologic tissue changes were studied over time and compared in the trachea, kidney, cloaca and gastrointestinal tract. Immunohistochemistry was used to demonstrate viral protein expression in these tissues. Additionally, viral RNA loads were defined with qPCR on swabs from trachea and cloaca and in kidney samples.

Results:

IB Primo QX and IBV-D388 induced comparable tracheal lesions, characterized by epithelial cell death and desquamation and mainly heterophilic and lymphohistiocytic infiltration. Changes were seen earlier and more severe in IBV-D388 infected birds, while tracheal viral RNA loads were comparable over time for both viruses. Contrastingly, in the kidneys degenerative and inflammatory lesions and viral protein and RNA were only found after IBV-D388 infection. Interestingly, viral presence in the kidneys was preceded by viral RNA detection in the cloaca and viral protein expression in the gastrointestinal (GI) tract, albeit without associated lesions in these tissues.

Conclusion:

IB Primo QX vaccine strain induced delayed, though morphologically comparable tracheal damage in comparison with its field progenitor. Unlike IBV-D388 though, IB Primo QX was unable to cause renal infection, which likely majorly contributes to attenuation of its phenotype. Furthermore, dissemination to the kidneys by IBV-D388 might result from cloacal ascending infection.



Nr. Abstract
65

Titel:

Good care for animals, from the beginning until the end

Authors

E.Deelen, F.Meijboom, T.Tobias, J.W.Hesselink, B.Rodenburg

Abstract:

Good care for animals is not limited to the care during their lives. Likewise, the end of an animal's life is a significant dimension of caring for animals. More than once the end of life decision making process leads to questions and discussion among veterinarians and the wider public. End of animal life decisions turn out to be complex on different levels. First, context suggests to play an important role in the way veterinarians deal with these decisions. Furthermore, next to more technical and veterinary aspects, several societal and ethical dimensions play a role. This leads to questions, such as 'what considerations are relevant for making the decisions?', 'should context play a role?' and 'who is involved and what is their responsibility in the decision making process?'. The aim of the poster is to present this problem and the approach we use in a recently started project to address questions at the end of animal life in a more integrated way. This approach entails integrating data, gained via qualitative research among various (veterinary) stakeholders in different contexts with literature study in the societal and ethical and veterinary dimensions. In this way, we aim to contribute to more transparency and knowledge about the end of life decision making process to enable vets to better deal with these problems.

**Titel:**

A repeated spatial Delayed Non-Matching-to-Sample task in pigs

Authors

Amparo Koot, Sander Klerk, Franz Josef van der Staay, Ellen Meijer
Behaviour and Welfare group, department of Farm Animal Health

Abstract:

Recently, there has been an increased interest in studying pig cognition. A better understanding of the cognitive abilities of pigs can be used to improve their welfare on pig farms and can be useful for biomedical research since pigs are also used as a model animal species. A valid method to assess learning and memory in animals is the Delayed Non-Matching-to-Sample (DNMS) task. The current study aimed to investigate the effect of varying delays on the performance of pigs in a spatial DNMS task. A second aim was to assess long-term memory in pigs by retesting the same task after a retention interval of eight weeks. They performed the task in a Y-maze, and during test trials, the pigs were rewarded for choosing the opposite direction of the sample arm. The pigs started testing with a delay of 30 seconds and then gradually moved on to longer delays (60, 120, 240, 480 seconds) once they reached criterion performance (8 out of 10 consecutive trials correct). They reached the criterion for all delays. However, the pigs needed more sessions to reach criterion performance with a delay of 120 seconds compared to a delay of 30 seconds. Furthermore, the pigs had a preserved performance when they were retested after the retention interval. Our results show that pigs can successfully perform a spatial DNMS task with delays up to 480 seconds. Contrary to earlier studies in rats and pigs, a delay-dependent impairment of performance was found for an intermediate delay (120 seconds) and not for the longest delays (240 and 480 seconds). This is the first study to show that pigs remember a spatial DNMS task after a retention interval of eight weeks.

**Titel:**

Clinical mastitis detection, antimicrobial usage and antimicrobial resistance profiles on Dutch dairy farms using automatic and conventional milking systems

Authors

Z. Deng - Department of Farm Animal Health

Abstract:

Mastitis is one of the major causes for antimicrobial usage (AMU) on dairy farms. On dairy farms with conventional milking systems (CMS), mastitis detection is mainly performed by farmers, but on farms using automatic milking systems (AMS), mastitis detection relies on AMS. This difference in mastitis detection might result in differences in treatment of mastitis and AMU between these two types of farms. The aims of this study were (1) to compare AMU between AMS and CMS farms, (2) to determine the factors associated with AMU in both types of herds.

The AMU parameters, which were transformed into animal defined daily dose (ADDD), including total usage (Total-ADDD), intramammary usage (IMM-ADDD), usage for dry cow therapy (DCT-ADDD) and usage by injection (Inject-ADDD), together with the proportions of each usage were compared between AMS (n = 42) and CMS (n = 254) farms.

To investigate the determinants of AMU on both types of farms, 18 AMS and 24 CMS farms participated in a survey about definition, detection and treatment of mastitis. Questionnaire data combined with AMU parameters were subjected to nonlinear principal component analysis to investigate the potential determinants of AMU.

The Total-ADDD and DCT-ADDD were comparable on AMS and CMS farms. While IMI-ADDD was lower and Inject-ADDD was non-significantly higher on AMS than on CMS farms. Significantly lower proportion of IMI-ADDD and higher proportion of Inject-ADDD were found for AMS compared to CMS farms. The Total-ADDD was negatively associated with udder health status, while IMI-ADDD was related to farmers' decisions on treatment of mastitis.

In conclusion, our study shows that AMS farms used equivalent amounts of total antimicrobials compared to CMS farms, but, AMS farms used a lower proportion of antimicrobials for intramammary use. Udder health status and decisions on treatment for mastitis are related to the antimicrobial usage.

**Titel:**

Identification of a novel protein:lipid binding motif involved in the recognition of phosphoinositides by an alpha-helical structures

Authors

Ziyang Shen, Martijn Molenaar, Nick K. Olrichs, Dora V. Kaloyanova, and J. Bernd Helms
Department of Biochemistry and Cell Biology, Faculty of Veterinary Medicine, Utrecht University

Abstract:*Introduction*

The CAP superfamily of proteins is characterized by the presence of a CAP domain and are found in thousands of species across the entire biological kingdom. In addition, CAP proteins are involved in a wide variety of functions, including fertilization, tumor suppression and immune modulation. We previously proposed that oligomerization and amyloid formation/modulation is a common functionality of the CAP domain, allowing regulation of a broad range of physiological functions.

GAPR-1 (Golgi-Associated Plant Pathogenesis-Related protein 1) might well be the first mammalian CAP protein in evolution and is well conserved among most vertebrates. GAPR-1 acts as a negative regulator of autophagy via interaction with the essential autophagy effector Beclin 1. GAPR-1 binds to lipid bilayers containing negatively charged lipids and forms amyloid fibrils upon prolonged incubation with liposomes.

Results

To identify specificity of binding to negatively charged lipids, GAPR-1 was incubated with lipids immobilized on nitrocellulose membranes (lipid strips). GAPR-1 showed preferential binding to phosphoinositides and PA, but not to PI and PS. These results were validated in liposome binding assays. The N-terminus of GAPR-1 contains a potential lipid binding motif with 4 lysine residues (K3, K7, K15, and K23) in an amphipathic helix configuration. Molecular dynamics simulations suggested that 3 of these lysines contribute to the specific binding of PIP of the amphipathic helix. Systematic mutation of these lysines in the GAPR-1 protein showed that K3 strongly affects the binding of GAPR-1 to phosphoinositide-containing liposomes.

Conclusion

We identified a novel three-lysine-finger sensor in the N-terminus of GAPR-1 to recognize phosphoinositides in biological membranes. Study on the interaction between phosphoinositides and GAPR-1 may shed a light on the regulation of membrane association of GAPR-1 and its involvement in autophagy. We speculate that this interaction may provide a biological switch regulating GAPR-1 oligomerization.

**Titel:**

Are cells "talking" via extracellular vesicles during different cell cycle phases? A comparison of cancerous versus non-cancerous cells

Authors

Bongiovanni L.¹, Schoonbeek M.¹, Andriessen A.¹, Arkesteijn G.², Westendorp B.¹, Driedonks T.A.P.², Wauben M.H.M.², Nolte-'t Hoen E.N.M.², de Bruin A.¹

1. Department of Pathobiology, Faculty of Veterinary Medicine, Utrecht University, Utrecht.

2. Department of Biochemistry & Cell Biology; Department of Infectious Diseases & Immunology, Faculty of Veterinary Medicine, Utrecht University, Utrecht.

3. Department of Biochemistry & Cell Biology, Faculty of Veterinary Medicine, Utrecht University, Utrecht.

Abstract:

Both normal and cancer cells actively communicate via extracellular vesicles (EVs) in order to maintain tissue homeostasis or favour tumour development and progression. During cell cycle, cells undergo different phases in which they replicate their DNA and grow (G1, S, G2 phases, interphase) and divide the nucleus (M phase, mitosis). Known as one of the hallmarks of cancer, cell cycle deregulation is a key event during neoplastic transformation and progression.

In order to understand how the specific cell cycle phases can influence EVs release, cancerous (canine melanoma cells, LMCK) and non-cancerous (human RPE) cells were synchronized into the same cell cycle phase. Vesicles released in the G1- or S-phase were harvested and isolated by differential centrifugation followed by sucrose density gradient centrifugation of the 100.000g pellet, and quantified using high-resolution flow cytometry.

Fascinatingly, different results were obtained from normal and cancer cells. Normal cells release more vesicles during the G1-phase compared to the S-phase. In contrast, tumour cells shed more vesicles compared to normal cells and they release more vesicles during the S-phase compared to the G1-phase. A marked drop in EV release is observed when RPE cells are in the S-phase of the cell cycle, suggesting that during active replication of their DNA, cells are less committed to communicating with other cells. During tumour development, cells evidently increase the level of EV-mediated communication, as indicated by the increased EV release and the acquired capacity of shedding vesicles during DNA replication.

Our results would corroborate the idea that actively proliferating cancer cells potentiate their ability to send messages to neighbour cells, in order to favour tumour growth and progression. The next step of our research will be to understand what cancer cells are "saying" during proliferation, by characterizing the cargo of EVs released during the different cell cycle phases.

**Titel:**

Milk-derived extracellular vesicles modulate Toll like receptor 3 activation at different levels

Authors

Alberta Giovanazzi^{1*}, Martijn van Herwijnen¹, Marije Kleinjan¹, Marcela Fernandez-Gutierrez², Tom Driedonks¹, Esther Nolte-'t Hoen¹, Michiel Kleerebezem², Marca Wauben^{1*}

¹Department of Biochemistry & Cell Biology, Faculty of Veterinary Medicine, Utrecht University, Utrecht, The Netherlands

² Host-Microbe Interactomics Group, Wageningen University & Research, Wageningen, The Netherlands

* TRAIN-EV Marie Skłodowska-Curie Action-Innovative Training Network, train-ev.eu

Abstract:

In mammals, milk supports the post-natal innate immune responses of the newborn by providing maternal components. Besides activating maternal components, we previously found that milk-derived extracellular vesicles (EV) can suppress innate immune responses initiated by endosomal Toll-like receptors (TLR). One such intracellular receptor is TLR3, which recognizes virus-derived double stranded RNA. This study aimed to unravel the molecular mechanism of direct effects of milk EV on TLR3 expression and function.

Oral cavity-derived epithelial cells, endogenously expressing TLR3, were co-cultured with physiological concentrations of purified milk EV or EV-depleted milk control in presence of a TLR3 agonist, after which TLR3 mRNA and protein were determined. A comprehensive genomic and proteomic analysis performed on milk EV linked miRNAs and proteins to the TLR3 signaling pathway.

Upon co-culture with milk EV, the expression of TLR3 gene transcript and other TLR-related genes was downregulated. Genomic analysis identified several miRNAs with known associations with these genes. Furthermore, proteomic analysis revealed the presence of several inhibitory proteins capable of modulating TLR3 signaling, including TLR3 proteolytic cleavage. Since interference with TLR cleavage affects optimal signal transduction, we analyzed the presence of TLR3 isoforms and found that both full length and cleaved TLR3 isoform levels were altered.

Milk EV-mediated modulation of TLR3 responses in epithelial cells can occur both during and after TLR3 biosynthesis. This indicates that milk EV can have direct effects on TLR3 expression and function by interfering via multiple molecular regulatory mechanisms. The physiological role of milk-EV in the development of a robust and resilient immune system is unexplored. Understanding this role is the major goal of our milk-EV research in mammals.

**Titel:**

Isolation and characterisation of canine synovial membrane-derived mesenchymal progenitor cells

Authors

Nora S. Ahrens (1), Michelle Teunissen (1), Kavitha Sivasubramaniyan (2), Marianna A. Tryfonidou (1)

(1) Department of Clinical Sciences of Companion Animals, Faculty of Veterinary Medicine, Utrecht University, Utrecht, the Netherlands.

(2) Department of Orthopaedics, Erasmus MC University Medical Center, Rotterdam, the Netherlands.

Abstract:

Isolation and characterisation of canine synovial membrane-derived mesenchymal progenitor cells

Osteoarthritis (OA) affects both humans and canines. It is characterised by progressive articular cartilage degeneration, osteophyte formation, subchondral bone changes and synovitis. Current treatment options consist of symptomatic approaches, which fail to restore the original structure and function of the joint. Therefore, the development of new regenerative therapies is needed. Synovial membrane-derived mesenchymal progenitor cells (SM-MPCs) are considered a promising cell-based treatment strategy that might induce cartilage regeneration due to their superior chondrogenic potential *in vitro*. However, in contrast with their human counterparts, canine SM-MPCs have not been investigated in detail yet.

This study investigated the SM-MPC population of the healthy and diseased (OA) canine joint. SM-MPCs were characterised by colony forming unit potential, proliferation rate, surface marker profile and their capacity to differentiate towards the osteogenic, adipogenic and chondrogenic lineage.

Both healthy and OA synovial membranes contained a cell population displaying MPC-like properties, including colony forming potential, MPC surface marker expression and multilineage capacity. Native synovial membrane cells derived from OA joints displayed a lower percentage of CD90+ and CD34+ cells compared to healthy joints. Furthermore, OA SM-MPCs possessed a reduced chondrogenic, but enhanced osteogenic potential in comparison to healthy SM-MPCs.

In conclusion, the canine synovial membrane proved to be a source of MPCs with chondrogenic properties. However, these preliminary data show that joint environment seems to have an important influence on their characteristics. This may have important consequences when using these cells in the treatment of OA, but also may provide possibilities to study OA *in vitro*.

**Titel:**

Viral and hypersensitivity causes of mild respiratory disease in weaning piglets?

Authors

K. Monpellier¹, L. Swinkels¹, M. Derkx¹, W. Bergmann², S. Wijburg³, T.J. Tobias¹

1) UU, FVM, Farm Animal Health;

2) UU, FVM, dep. Pathobiology, Veterinary Pathologic Diagnostic Centre,

3) UU, FVM, Institute for Risk Assessment Sciences

Abstract:**Introduction**

At a 530 head multiplier pig farm, since some years, mild respiratory disease in weaned pigs is extensive. Repeated laboratory diagnostic investigations by the herd veterinarian have not yet revealed a cause. The objective of this project was to investigate possible causes systematically.

Material and methods

1. A cross sectional study was performed to estimate prevalence of sneezing and coughing and describe course of clinical signs with age. Subsequently a problem definition was set and
2. 4 Clinical representative pigs were selected and submitted for post mortem examination at VPDC and samples obtained for histologic and bacteriologic examination.
3. A PCR for SIV and PRCV was performed
4. A cross sectional serologic examination
5. A climate evaluation was performed
6. An additional evaluation of respiratory dust and endotoxin exposure was performed by IRAS

Results

1. 50% of the suckling pigs of 4 weeks old and weaned pigs, 1 to 2 weeks post weaning, experience mild respiratory disease, characterized by sneezing and in approx. 20% of pigs a productive cough, most remarkable during the morning and less over the day. Pigs do not show conjunctivitis, tearing or fever.
2. In all pigs a multifocal to coalescent, milde, subacute, eosinofiele rhinitis was found next to a very mild conchae atrophy, an interstitial pneumonia in 3/4 pigs as well as an eosinophilic bronchitis in 2 pigs. Bacteriologic examinations were non-conclusive; in ¼ S suis and in another ¼ B. bronchiseptica were found in the nose.
3. Influenza and PRCV PCR were negative
4. No antibodies were found to PRRSv, App, H Parasuis and PCV2 and but positive for Influenza in 7 week old pigs.
5. No aberrant climatic factors were found
6. Results are still pending

Discussion

Based on the temporal pattern of signs over the day as well as over time in combination with histopathologic results, the most probable cause of sneezing is due to eosinophilic rhinitis (and bronchitis) due to hypersensitivity due to exposure to substances of yet unknown origin. The coughing is likely due to the interstitial pneumonia due to a viral cause, either Influenza or another virus. Results of endotoxin and dust exposure are still pending.