Junior Scientist – Symposium
19th – 22th August 2014
Mariensee, Germany

Institute of Farm Animal Genetics,
Mariensee

Institute of Animal Nutrition,
Braunschweig

Institute of Animal Welfare and Animal Husbandry,
Celle
Junior Scientist - Symposium
Friedrich-Loeffler-Institute
19th – 22th of August 2014
in Mariensee, Germany

Programme and abstracts

Organization team:
Birgit Burchardt
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Prof. Dr. Dr. h. c. Thomas C. Mettenleiter

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Programme

Tuesday, 19.08.2014

16:00 – 18:00 Arrival at the Hostel in Mardorf

18:00 Dinner (Hostel Mardorf)

19:00 Meet, greet and games at the Steinhuder Meer

Wednesday, 20.08.2014

09:00 Greeting

09:15 Institute presentation Braunschweig

09:30 **Melanie Schären**: Effects of pasture and ensiled feed on the health, production and rumen fermentation of dairy cows

09:45 **Jeanette Klüß**: The impact of deoxynivalenol and E.coli lipopolysaccharide on epithelial architecture and integrity along the porcine small intestinal axis

10:00 **Erik Bannert**: The oral deoxynivalenol exposition in pigs is modulating the pathophysiological effect of a downstream lipopolysaccharide stimulus

10:15 **Tanja Tesch**: The oral deoxynivalenol exposition in pigs is modulating the pathophysiological effect of a downstream LPS stimulus: methodological aspects

10:30 Coffee break

10:45 Institute presentation Mariensee

11:00 **Sandra Bernal**: Effects of cAMP regulators during oocyte *in vitro* maturation on bovine embryo development in prepubertal and adult donors

11:15 **Monika Entorf**: Phenotypic and genotypic analysis of tylosin resistance among staphylococci and streptococci from cases of bovine mastitis
11:30  **Stefanie Blodkamp:** Effects of cathelicidins against livestock-associated methicillin-resistant Staphylococcus aureus

11:45  **Daniela Tiedemann:** Oocyte maturation as a highly sensitive model to explore toxicity of metal and metal alloy nanoparticles

12:00  **Birgit Burchardt:** Preliminary experiments for the generation of porcine interspecies chimeras for xenotransplantation

12:15  Lunch (Hostel Mardorf)

**14:00**

**Expert speech**  
**Dr. Peter Hinsberger (IDT Biologika GmbH)**

15:00  Coffee break

15:15  Institute presentation Insel Riems

15:30  **Sebastian W. Böhm:** Structure-based analyses of pseudorabies virus glycoprotein H function

15:45  **Ute Wessels:** Virulence Determinants within the Hemagglutinin of Highly Pathogenic Avian Influenza Virus H5N1

16:00  **Lars Paßvogel:** Importance of nuclear trafficking signals in the pUL31 component of the herpesvirus nuclear egress complex

16:15  **Teresa Hellberg:** Molecular Analysis of vesicle-mediated Nuclear Egress of Herpesviruses

16:30  **Christina Schröter:** The important role of Proline in the conserved Serine-Proline-Cysteine motif in Pseudorabies Virus glycoprotein H

16:45  **Maya Gussmann:** A simulation model for testing surveillance systems for Bluetongue disease

17:00  Break

18:00  Walk through Mardorf to the restaurant

19:00  Dinner at the restaurant “Alte Schule” in Mardorf
Thursday, 21.08.2014

09:00     Institute presentation Jena

09:15     Gamal Wareth: Specific Immunogenic proteins of B. melitensis for the serodiagnosis of small ruminant brucellosis

09:30     Nadine Schmidt: Use of recombinant Escherichia coli Shiga toxoids as vaccines to reduce STEC transmission dynamics in cattle herds

09:45     Katharina Hamm: Experimental Infection of Calves with Escherichia coli O104:H4

10:00     Sarah Stalb: Adhesion and Shiga Toxin Production of Escherichia coli O104:H4 in Human and Bovine Intestinal Epithelial Cell Cultures

10:15     Coffee break

10:30     Institute presentation Celle

10:45     Anja Höhne: Effect of two housing systems on Ghrelin secretion in adult laying hens (Gallus gallus domesticus) at different times of life

11:00     Jana Sonnenburg: Validation of Harmonized Methods on Selected Wildlife Host-Pathogen Combinations

11:15     Yaqing Zhu: Interferon-response against lyssavirus in European bat species

11:30     Xiaocui He: Establishment of Myotis myotis cell lines for investigation of immune responses under lyssavirus infection

11:45     Christina Radtke: Characterisation of pestivirus glycoproteins E1 and E2

12:00     Joanna Jaros: Rainbow trout (Oncorhynchus mykiss) thrombocytes are involved in MHC II dependent antigen presentation

12:15     Lunch (Hostel Mardorf)

13:30     Bus transfer to the Institute of Farm Animal Genetics, Mariensee
14:00  Welcome address Prof. Dr. H. Niemann

14:15  **Expert speech**  
**Vicky Fachinger (MSD Tiergesundheit)**

15:15  Coffee break

15:30 – 17:30  Poster session (Institute Mariensee)

18:00  Barbecue (Institute Mariensee)

21:30  Transfer back to the hostel in Mardorf

Friday, 22.08.2014

09:00  **Tuan Nguyen:** Genotypic and phenotypic characterization of Campylobacter jejuni isolates from Vietnam

09:15  **Jan Schinköthe:** Experimental Infection of Goats with Mycobacterium avium subsp. paratuberculosis and Mycobacterium avium subsp. hominissuis: Lesions and Detection of Pathogens in Tissue 3 Months Post Inoculation

09:30  **Silke Hechinger:** Development of vaccine candidates for prevention of Schmallenberg virus infection

09:45  **Goshi Kato:** Vibrio anguillarum bacterin uptake via the gills of Japanese flounder and subsequent immune responses

10:00  **Charles Lyimo:** Regional Genetic contributions in Chicken populations from Europe, Asia and Africa

10:15  Coffee break

10:30-12:00  Final discussion with Prof. Mettenleiter
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Abstracts (oral presentations)

Effects of pasture and ensiled feed on the health, production and rumen fermentation of dairy cows
Melanie Schären, Ulrich Meyer, Johannes Isselstein, Gerhard Breves, Sven Dänicke

Institut für Tierernährung, FLI, Braunschweig
Physiologisches Institut, Stiftung Tierärztliche Hochschule Hannover
Institute of Grassland Science, Faculty of Agricultural Sciences, Georg-August Universität Göttingen

Our project is part of a larger project supported by the state of Lower Saxony initiated to compare pasture and confinement dairy cow housing. The aim of our study is to get a better understanding of how the conservation of feed influences a cow’s metabolism and health. The trial will be held this spring and will involve 60 dairy cows either receiving a common ration or fresh grass. The main interest lies on two aspects: 1. Feed and energy intake, production and efficiency. 2. Rumen health and metabolism. To measure the feed intakes of grazing dairy cows the n-alkane marker method will be used. To analyze rumen fermentation and other digestive processes we will make use of ten double fistulated cows. We will monitor the influence of the diet on the rumen microbiota by the use of single strand conformation polymorphism analysis (SSCP). Rumen boli will be employed to monitor rumen pH and temperature. Further, we will have the possibility to monitor the cow metabolism through analysis of milk, manure, urine, rumen fluid and blood sampling for different parameters.

The impact of deoxynivalenol and E.coli lipopolysaccharide on epithelial architecture and integrity along the porcine small intestinal axis.
Jeannette Kluess 1, Leslie Raja Klunker 1, Nicole Walk 1, Constanze Nossol 1, Stefan Kahlert 1, Bianca Brosig 2, Susanne Kersten 2, Sven Dänicke 2, Hermann-Josef Rothkötter 1

1 Institute of Anatomy, Medical Faculty, Otto-von-Guericke University, Magdeburg, Germany
2 Institute of Animal Nutrition, Federal Research Institute for Animal Health, Braunschweig, Germany

Deoxynivalenol (DON) is one of the most prevalent mycotoxins in temperate climates and occurs predominantly on cereal crops. Lipopolysaccharides (LPS) are part of the outer membrane of gram-negative bacteria. Both are thought to impair porcine intestinal morphology and epithelial barrier integrity. We investigated the effect of DON and LPS on crypt depth, cell proliferation and expression of tight junction protein (ZO-1) in the pig’s small intestine. 48 barrows (26 ± 4 kg BW) were fed a barley-based control or a diet containing 3.1 mg/kg DON for four weeks. Subsequently, control group was infused for an hour either with 100 µg/kg BW DON (CON-DON) or 7.5 µg/kg BW LPS (CON-LPS) or both treatments (CON-DON+LPS).
or 0.9 % NaCl (CON-CON) and the DON group with LPS (DON-LPS) or NaCl (DON-CON). Pigs were sacrificed 3.25 hours after start of infusion. An hour prior to sacrifice all pigs received an infusion of a nucleotide analogue (10 mg BrdU /kg BW) as a proliferation marker. Tissue was taken from duodenum, proximal jejunum, mid-jejunum, proximal ileum and terminal ileum. Crypt depth, BrdU positive cells and immunofluorescence of ZO-1 were analysed and data compared by ANOVA.

Duodenal crypts were deeper compared to the other gut sections irrespective of treatment (p<0.001). Proliferation was not highest in duodenum, but showed a bell-shaped distribution along the proximo-distal gut axis with the highest number of proliferating cells in proximal and mid-jejunum (p<0.001). There was no effect of treatment. ZO-1 was localised apical as well as cytosolic in the three upper gut sections whereas ileal sections showed only an apical signal. LPS markedly altered the spatial distribution of ZO-1: a strong apical ZO-1 signal was present whereas the cytosolic localisation disappeared in all gut sections. This effect was irrespective of DON presence. In conclusion, we could demonstrate that proliferation shows a distinct pattern along the small intestine and is not necessarily linked to crypt depth. Furthermore we showed that LPS modified ZO-1 distribution along the gut axis.

The oral deoxynivalenol exposition in pigs is modulating the pathophysiological effect of a downstream lipopolysaccharide stimulus

Erik Bannert, Tanja Tesch, Jeannette Kluess, Jana Frahm, Susanne Kersten, Sven Dänicke

Institute of Animal Nutrition, FLI, Braunschweig

The Fusarium toxin deoxynivalenol (DON) is a common toxin in grain and pigs are known to be sensitive to DON. DON is a protein synthesis inhibitor and has an immune modulating effect. Lipopolysaccharides (LPS) are components of gram negative bacteria and induce inflammation in animals when entering the systemic circulation. The interaction of DON and LPS has been postulated before and will be investigated in this project. The main objective is to examine the role of the liver as mediator of the inflammatory reaction and whether a preexposure to DON has got an immune modulating effect. A total of 36 barrows pigs will be investigated of which 18 will be fed with DON contaminated feed (approx. 4mg DON/kg feed) and 18 with control feed. The animals are equipped with catheters at five different locations, facilitate pre- and posthepatic LPS application (7.5 µg/kgBW) and frequent blood sampling of the different locations. Besides the kinetic of DON and LPS, blood samples will be used to characterize the inflammatory response at cellular and metabolite level. In addition, the gut associated immune system will be characterized and tissue protein synthesis will be assessed to identify possible effects and interactions of the treatments.
The oral deoxynivalenol exposition in pigs is modulating the pathophysiological effect of a downstream LPS stimulus: methodological aspects
Erik Bannert, Jeannette Kluess, Jana Frahm, Susanne Kersten, Sven Dänicke, Tanja Tesch

Institute of Animal Nutrition, FLI, Braunschweig

The liver is a central metabolic organ facilitating systemic inflammatory reactions as well as processing and eliminating detrimental agents derived from the gastrointestinal tract (GIT). In order to investigate the pre- and posthepatic regions a catheterization method was developed. A total of 36 barrows are either exposed to deoxynivalenol-contaminated or a control feed for 29 days. Subsequently animals are systemically exposed to either 0.9%NaCl or lipopolysaccharide (7.5µg/kgBW/h). For systemic application and blood sampling animals are surgically equipped with catheters in five different vessels. After median laparotomy, one catheter is placed via Vena gastroepiploica sinistra into Vena lienalis for prehepatic application and another via Vena mesenterica cranialis into Vena portae hepatis for prehepatic sampling to investigate toxin drainage from GIT. Additionally a temperature logger is placed in the abdominal cavity for continuous measurement. Catheters are exteriorized into left flank and fixedated via suture on the skin. Thereafter left jugular groove is opened and a catheter is inserted into Vena jugularis interna for posthepatic sampling and Arteria carotis communis for assessing toxin influx to GIT. A third catheter is introduced into Vena jugularis externa for peripheral application. All catheters are subcutaneously exteriorized on the neck and fixed with stitches.

Effects of cAMP regulators during oocyte in vitro maturation on bovine embryo development in prepubertal and adult donors
S.M. Bernal¹,², J. Heinzmann¹, D. Herrmann¹, U. Baulain¹, K.G. Hadeler¹, P. Aldag¹, A. Lucas-Hahn¹, H. Niemann¹

¹Institute of Farm Animal Genetics, FLI, Mariensee  
²Facultad del Ciencias Agropecuarias, Universidad de Ciencias Aplicadas y Ambientales - U.D.C.A-, Bogotá, Colombia

Prepubertal bovine donors can be used to accelerate genetic gain and decrease the generation interval. However, prepuberal oocyte developmental competence is lower than that of their adult counterparts. In vitro maturation (IVM) using cyclic AMP regulators and an extended 30h culture period had been suggested to improve bovine blastocyst yields. This study evaluated the effects of the cAMP modulators forskolin, 3-Isobutyl-1-methylxanthine (IBMX) and cilostamide during IVM on oocyte and embryo developmental rates and gene expression in prepubertal and adult females. Oocytes from adult or prepubertal donors were used in the following groups:
TCM24 (24h IVM, control), TCM30 (2h pre-IVM (forskolin-IBMX) and 30h IVM adding cilostamide), DMSO30 (2h pre-IVM and 30h IVM with DMSO/vehicle control). After maturation, oocytes were fertilized in vitro and zygotes cultured in vitro to assess embryo development. Maturation, cleavage and blastocyst/zygote rates did not differ among in vitro treatments. Similar expression profiles were observed by RT-qPCR for SLC2A8, DNMT3B, BCL-XL and PRDX1 genes, in in vitro and in vivo derived blastocysts. EGR1 was down-regulated in all in vitro blastocysts, suggesting its usefulness as embryo quality marker. These results indicate that similar developmental capacity can be achieved in prepubertal compared to adult donors without addition of cAMP modulators.

Phenotypic and genotypic analysis of tylosin resistance among staphylococci and streptococci from cases of bovine mastitis
Monika Entorf1,2, Andrea T. Feßler1, Heike Kaspar3, Kristina Kadlec1, Thomas Peters2, Joachim Mankertz3, Stefan Schwarz1

1Institute of Farm Animal Genetics, FLI, Mariensee
2Milchtierherden-Betreuungs- und Forschungsgesellschaft mbH (MBFG), Wunstorf, Germany;
3Federal Office of Consumer Protection and Food Safety (BVL), Berlin, Germany

Tylosin is a 16-membered macrolide, which is commonly used for bovine mastitis therapy. The aim of the present study was to comparatively investigate staphylococci and streptococci for the correlation of their tylosin zone diameters and tylosin minimal inhibitory concentrations (MICs). In total, 525 bovine mastitis pathogens (112 Staphylococcus aureus, 110 coagulase-negative staphylococci (CoNS), 101 Streptococcus agalactiae, 100 Streptococcus dysgalactiae and 102 Streptococcus uberis) were included. For comparative reasons and for the detection of inducible macrolide resistance, the erythromycin susceptibility of the isolates was tested in parallel by both methods. The results were analysed by plotting the tylosin zone diameters against the tylosin MICs. Moreover, isolates with elevated erythromycin MICs were tested for the presence of macrolide resistance genes. In general, a good correlation between the two different testing methods was seen. In all five bacterial groups, isolates with elevated tylosin MICs were detected. All these 47 isolates were also resistant to erythromycin. Five additional erythromycin-resistant isolates with low tylosin MICs proved to be inducibly macrolide-resistant. Among the erythromycin-resistant isolates, the macrolide resistance genes erm(A), erm(B), erm(C), erm(T), mph(C), msr(A), msr(D) and/or mef(A) could be detected. The inducibly resistant isolates harboured the resistance genes erm(A), erm(B) and/or erm(C).
Effects of cathelicidins against livestock-associated methicillin-resistant Staphylococcus aureus
Stefanie Blodkamp$^{1,2}$, Kristina Kadlec$^2$, Hassan Y. Naim$^1$, Stefan Schwarz$^2$, Maren von Köckritz-Blickwede$^1$

$^1$Department of Physiological Chemistry, University for Veterinary Medicine, Hannover; $^2$Institute of Farm Animal Genetics, FLI, Mariensee

The treatment of infections with bacteria, such as Staphylococcus aureus, is hampered by their increasing antimicrobial resistance. Hence, alternatives to antimicrobial agents are urgently needed. Since cathelicidins are well-known for their broad spectrum bactericidal activity, the aim of this study was to characterize the antimicrobial activities of five different cathelicidins (LL-37, CRAMP, CAP18, BMAP-27 and BMAP-28) against livestock-associated methicillin-resistant S. aureus (LA-MRSA). For this purpose the minimal inhibitory concentrations (MICs) of 153 field isolates were determined. Moreover, the impact of 14 antimicrobial resistance genes, which specify different resistance mechanisms, on the MICs of cathelicidins was investigated. The results demonstrated that the lowest MIC values were obtained for the bovine cathelicidins, BMAP-27 and BMAP-28 (4-16 µg/ml and 2-16 µg/ml, respectively). The human and mouse cathelicidins, LL-37 and CRAMP, showed the highest MICs (both ≥ 128 µg/ml). These differences of the cathelicidin activities correlate with their hydrophobicity. An effect of antimicrobial resistance genes on the MICs could not be detected. Since bovine cathelicidins, as revealed in this study, exhibit lower MICs against LA-MRSA compared to cathelicidins of other species, they might be a promising target for pharmacological boosting, especially since none of the tested antimicrobial resistance genes altered the MIC values.

Oocyte maturation as a highly sensitive model to explore toxicity of metal and metal alloy nanoparticles
Daniela Tiedemann$^1$, Ulrike Taylor$^1$, Christoph Rehbock$^2$, Jurij Jakobi$^2$, Sabine Klein$^1$, Wilfried A. Kues$^1$, Stephan Barcikowski$^2$, Detlef Rath$^1$

$^1$Institute of Farm Animal Genetics, FLI, Mariensee $^2$Technical Chemistry I and Center for Nanointegration Duisburg-Essen (CENIDE), University of Duisburg-Essen, Essen, Germany

In the past two decades metal nanoparticles have been increasingly developed for industrial and biomedical as well as consumer products. However, the multitude of applications for metal and metal alloy nanoparticles, which are either already established or will be in due time, do not correspond to our knowledge about their toxicity. In particular their reprotoxicity has so far hardly been investigated, despite the potential to affect not only the exposed but also following generation. Therefore, the presented study aimed to develop a model for efficient reprotoxicological screening of nanoparticles. At the time of birth, oocytes are in the stage of meiotic
arrest and only mature to their fertilizing competence proximate to ovulation. This maturation process can be mimicked in vitro. This process is delicate and very susceptible to disturbances. It represents an excellent functional test for nanotoxicological examinations. Oocytes were exposed to either pure gold or silver nanoparticles, gold-silver alloy nanoparticles, nickel-titanium alloy nanoparticles or chromium-steel alloy nanoparticles. In agreement with related literature silver nanoparticles were detected to elicit a considerable reprotoxicity. For the first time it was shown that this also applies to silver alloy nanoparticles. Oocyte maturation proved to be a valuable and efficient tool to screen the reprotoxic potential of nanoparticles. Further studies will investigate the effect of various particle surface modifications as well as the mechanisms behind the observed toxicity.

**Preliminary experiments for the generation of porcine interspecies chimeras for xenotransplantation**

Birgit Burchardt, Andrea Lucas-Hahn, Thirumala Talluri, Maren Ziegler, Heiner Niemann

*Institute of Farm Animal Genetics, FLI, Mariensee*

Today the need for organ transplantation is much higher than the number of available human organs. A possible solution for this shortage is the use of organs from other species, e.g. the pig. The biggest barrier for xenotransplantation are problems with the immune tolerance for the transplanted xenografts. One answer to this problem is the generation of transgenic pigs with several knock-out or knock-ins of immune-relevant surface proteins and complement factors. Another approach is the generation of interspecies chimeras, where the required organ would consist of mostly human cells in an organ niche in a pig (humanized pigs). In order to assess feasibility and safety of these interspecies chimeras several pretests need to be performed. One of which is the integration of non-porcine induced pluripotent stem cells (iPS cells) in a porcine embryo. Here the preliminary results with porcine parthenogenetic and murine embryos were presented to show the feasibility to build interspecies chimeras by aggregation of embryos and iPS cells. For establishment of the aggregation method, mouse-mouse chimeras were generated. Then the aggregation method was optimized for porcine parthenogenetic embryos and the integration of murine iPS cells in porcine parthenogenetic and murine embryos was tested.
Structure-based analyses of pseudorabies virus glycoprotein H function
Sebastian W. Böhm¹, Marija Backovic², Elisa Eckroth¹, Walter Fuchs¹, Barbara G. Klupp¹, Felix A. Rey², Thomas C. Mettenleiter¹

¹Institute of Molecular Virology and Cell Biology, FLI, Insel Riems
²Unité de Virologie Structurale, Institut Pasteur, Paris, France

Fusion of viral and cellular membranes is essential for entry of herpesviruses into host cells, direct viral cell-to-cell spread and virus-induced syncytia formation. In the Herpesviridae, three conserved envelope glycoproteins are required for these events: the core fusion protein gB, and a heterodimer formed by gH and gL. The crystal structures of three gH homologues, including that of pseudorabies virus (PrV), revealed a protein that consists of four conserved domains.

The present study focuses on domain II, consisting of a planar β-sheet (fence) and a syntaxin-like-bundle (SLB) of α-helices, similar to those found in cellular fusion proteins. These structures were targeted by site-directed mutagenesis to disrupt α-helical regions by proline insertions, or to prevent possible structural changes during fusion by introducing disulfide bonds between artificially introduced cysteine residues. Processing and transport of mutated gH were tested by Western blot and immunofluorescence, whereas fusion activity was investigated by in vitro assays in cells cotransfected with expression plasmids for PrV gB, gD, gL and mutated gH. Furthermore, replication competence, penetration kinetics, and cell-to-cell spread of PrV recombinants expressing mutated gH were investigated. The results demonstrate that the helical structure and flexibility of the SLB are relevant for function of PrV gH.

Virulence Determinants within the Hemagglutinin of Highly Pathogenic Avian Influenza Virus H5N1
Ute Wessels, Jutta Veits, Sayed Abdel-Whab, Thomas C. Mettenleiter, Olga Stech, Juergen Stech

Institute of molecular virology and cell biology, FLI, Insel Riems

Highly pathogenic avian influenza viruses (HPAIV) cause rapid and severe course of disease accompanied with extreme mortality in gallinaceous poultry. Prime virulence marker of HPAIV is the polybasic cleavage site of the hemagglutinin (HA). Previously, we demonstrated the existence of virulence determinants within the HA in addition to the polybasic cleavage site. Here, we aimed to investigate those yet unknown virulence determinants and their functional consequences regarding the activation pH of HA-mediated fusion. By reverse genetics, we constructed different reassortants, HA chimeras and mutants from HPAIV A/Swan/Germany/R65/2006 (H5N1) (R65) and the low-pathogenic A/Teal/Germany/Wv632/2005 (H5N1) with an engineered polybasic cleavage site (TG05poly): R65-HA1TG05poly, R65-HA(R123S), R65-HA(I124T), R65-HA(R123S/I124T), R65-HA(R156N), R65-HA2TG05poly, R65-
Importance of nuclear trafficking signals in the pUL31 component of the herpesvirus nuclear egress complex

Lars Paßvogel\textsuperscript{1}, Barbara G. Klupp\textsuperscript{1}, Harald Granzow\textsuperscript{2}, Thomas C. Mettenleiter\textsuperscript{1}

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Herpesvirus capsid formation and DNA packaging occur in the host cell nucleus while further maturation takes place in the cytosol. Translocation of nucleocapsids through the nuclear envelope is accomplished by budding at the inner nuclear membrane (INM) resulting in primary enveloped virions in the perinuclear space. Primary envelopes then fuse with the outer nuclear membrane releasing the nucleocapsids into the cytosol. This process is mediated by the heterodimeric nuclear egress complex (NEC) consisting of viral proteins pUL31 and pUL34. pUL31 enters the nucleus presumably via a predicted N-terminally located nuclear localization signal (NLS), where it is recruited by the nuclear membrane targeted pUL34 to the INM. Disruption of the NLS resulted in exclusive cytoplasmic localization of pUL31 proving its functionality in nuclear translocation. Moreover, since the protein should be small enough to reach the nucleus also by passive diffusion, active pUL31 export was assumed. Computer analysis predicted a nuclear export signal (NES) in the C-terminus of pUL31. To study their functional importance, both signals were deleted/mutated. Localization and interaction with pUL34 was investigated by confocal microscopy and pUL31-expressing cells were tested for functional complementation. The results show that both, the NLS and NES, are important for NEC-mediated nuclear egress.

Molecular Analysis of vesicle-mediated Nuclear Egress of Herpesviruses

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Herpesviruses use a vesicle-mediated transport for translocation of nucleocapsids from the nucleus to the cytoplasm for final virus maturation. To cross the nuclear envelope, newly synthesized nucleocapsids acquire a primary envelope by budding at the inner nuclear membrane (INM) resulting in the formation of primary enveloped
virions residing in the perinuclear space. This primary envelope then fuses with the outer nuclear membrane (ONM) thereby releasing the nucleocapsid into the cytosol. Two conserved herpesviral proteins, designated as pUL34 and pUL31, build the nuclear egress complex, which is required for efficient nuclear egress. In absence of either protein nucleocapsids remain trapped in the nucleus. However, expression of both proteins results in formation of primary envelopes from the INM indicating that no other viral protein is involved in vesicle formation. Fusion with the ONM seems to require additional proteins. To identify these viral and/or cellular proteins primary enveloped virions will be isolated from the perinuclear space. For this, we tagged the membrane-anchored pUL34 with different affinity-tags and established stably expressing cell lines. After infection, affinity purification and mass spectrometry will be performed to analyse the protein content of primary virions. These results should shed light on the molecular mechanism orchestrating vesicle-mediated nuclear transport.

The important role of Proline in the conserved Serine-Proline-Cysteine motif in Pseudorabies Virus glycoprotein H
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Fusion of the viral envelope with the cell membrane is an essential step during infection. While in most viruses one or two proteins are sufficient to mediate entry herpesviruses require at least four proteins. Receptor binding triggers fusion which is mediated by the conserved core fusion machinery consisting of the conserved glycoproteins gB and gH/gL. gB probably is the actual fusion protein, but unable to function without gH/gL.

The crystal structures of several gH/gL homologs have been elucidated. Despite moderate sequence conservation they all exhibit four distinct domains. Domain III contains a conserved serine-proline-cysteine motif. Proline was suggested to be necessary for protein bending so that cysteine can form an essential disulphide bridge. Surprisingly, pseudorabies virus strain Bartha, which is used as a live vaccine against Aujeszky’s disease, carries a serine in this position.

To investigate the influence of this alteration we generated gH mutants carrying either a serine or a proline. All serine mutants showed low fusion activity in transient assays. Corresponding virus mutants exhibited delayed penetration kinetics and formed only small plaques, pointing to an important role of proline in this motif for gH function.
A simulation model for testing surveillance systems for Bluetongue disease
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In August 2006 Bluetongue disease (serotype 8) occurred in Belgium, Germany and the Netherlands for the first time. In response to the outbreaks, a commission regulation containing implementing rules for monitoring and surveillance of animals was passed (No 1266/2007). These systems were supposed to ensure early detection and to show freedom of disease in the respective European countries. Within the EMIDA ERA-Net project VICE different surveillance systems are compared concerning their cost-effectiveness. These systems have spatial and temporal components that have to be represented in a model. Our spatial-temporal model simulates the dispersal of Bluetongue disease over two years (2006 and 2007) in Belgium, Germany and the Netherlands. To describe the outbreaks in the first months properly, a Random-Walk module will be used. With the help of these simulations, different surveillance systems and scenarios are tested for efficiency and cost-effectiveness.

Specific Immunogenic proteins of B. melitensis for the serodiagnosis of small ruminant brucellosis
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Brucella melitensis is the most virulent species of Brucella affecting a wide range of livestock and humans. Sheep and goats are the classical and preferred hosts for B. melitensis. False positive reactions in serodiagnosis reduce the efficiency of diagnosis, and hamper monitoring and eradication programs to brucellosis. Thus, there is a pressing need to identify immunogenic proteins which can be used as antigen in highly specific serological tests to detect Brucella infection in sheep and goats. In the present study nine immunodominant proteins from whole cell lysate of a Brucella melitensis field strain isolated from sheep were detected by immunoblotting and identified by MALDI-TOF MS/MS. The identified proteins are: superoxide dismutase, copper/zinc binding protein; chain A, Cu/ZN superoxide dismutase; 19 kDa periplasmic protein; transaldolase; fructose-6-phosphate aldolase; rhizopine-binding protein; hydrolase; 31 kDa cell surface protein and a hypothetical protein. These proteins showed immunoreactivity only against positive antisera from sheep and goats naturally infected with Brucella, but failed to have immunogenicity towards...
negative antisera. Therefore, it would be of great value to further validate these proteins as novel specific candidates for serodiagnosis of brucellosis in small ruminants.

**Use of recombinant Escherichia coli Shiga toxoids as vaccines to reduce STEC transmission dynamics in cattle herds**

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Cattle are the primary reservoir for Shiga toxin-producing *Escherichia coli* (STEC), bacteria that can cause human life-threatening diseases. The principal STEC virulence factor is the eponymous Shiga toxin (Stx), which modulates the local intestinal immune response in cattle and thereby supports persistent carriage and shedding. Aim of the study is to assess whether immunization with genetically inactivated recombinant Shiga toxoids (rStx1\(_{MUT}/rStx2_{MUT}\)) (a) confers protection against Stx’s immunosuppressive effects and (b) influences STEC shedding and transmission in a calf cohort. Therefore 24 calves were passively (colostrum) and actively (5\(^{th}\) and 8\(^{th}\) week of life) vaccinated with rStx\(_{MUT}\), further 24 calves served as unvaccinated controls. From birth to 34\(^{th}\) week of life blood, serum, and feces were repeatedly collected. Complete blood cell, CD4 and CD8 T cells counts were assessed by flow cytometry in order to determine calves’ health state at each sampling. Calves were quantitatively monitored for humoral (Stx-neutralizing antibodies) and cellular (STEC-specific T cells) immune responses and fecal STEC shedding. A reduction of fecal STEC shedding in cattle by vaccination would be a milestone for the protection of humans against STEC infections and an eminently suitable method from scientific, ethic, and economic point of view.

**Experimental Infection of Calves with Escherichia coli O104:H4**

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A major human HUS outbreak in Germany 2011 could be traced back to an unusual EHEC strain of serotype O104:H4, that was never detected in cattle, the primary STEC/EHEC reservoir, before. To assess if EHEC O104:H4 can utilize ruminants as reservoir, we determined the clinical appearance, the pattern and magnitude of fecal shedding and the site of colonization in a bovine infection model. Fifteen (five per
strain) 100-day-old calves were inoculated with 1010 CFU of EHEC O104:H4, of EHEC O157:H7 (positive control) or of non-pathogenic E. coli O43:H28 (negative control) and necropsied 4 dpi. EHEC O157 and O104 were recovered in equal numbers (approx. 5 x 105 CFU/g feces) from feces 4 dpi whereas the non-pathogenic E. coli was detected in much lower numbers (approx. 5 x 102 CFU/g feces). EHEC O104 was recovered from intestinal content as well as from mucosal tissue samples. Fifteen other calves were inoculated the same way and necropsied 28 dpi. EHEC O104:H4 was directly detectable until day 24, whereas only EHEC O157:H7 were recovered from the feces, intestinal content and associated with intestinal tissue on day 28 pi. These results are the first evidence that cattle can carry EHEC O104:H4 at least transiently.

**Adhesion and Shiga Toxin Production of Escherichia coli O104:H4 in Human and Bovine Intestinal Epithelial Cell Cultures**

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The German 2011’s HUS outbreak strain LB226692 (Escherichia coli O104:H4), exhibits virulence patterns of human adapted enteroaggregative E. coli and of bovine adapted Shiga toxin (Stx) producing E. coli.

Aim of this study was to compare the ability of LB226692 to adhere to and to invade into bovine (FKD-R 971) and human intestinal epithelial cells (CaCo-2, INT407) in vitro and the capability to release Stx upon host cell contact.

We performed adhesion and invasion assays in order to quantitatively and qualitatively assess bacterial adherence (Giemsa, Fluorescence actin staining, colony counts). Amounts of Stx were measured by ELISA.

LB226692 was able to adhere to any of the epithelial cell lines. In particular FKD-R 971 and INT407 cells were heavily colonized, while only a minor portion of CaCo-2 cells permitted bacterial adherence. Although colonizing a similar or reduced number of human intestinal cells, classical EHEC strains secreted significantly higher amounts of Stx upon host cell contact as compared to LB226692. However, LB226692 secreted notably higher levels of Stx when attached to bovine cells as compared to human cells.

The results point to a milieu-dependent control of virulence gene expression by LB226692 independent of bacterial attachment to epithelial cells.
Effect of two housing systems on Ghrelin secretion in adult laying hens (Gallus gallus domesticus) at different times of life
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Ghrelin is important for food intake regulation and energy metabolism. To test whether Ghrelin titers change during ontogeny in relation to energetic demand we analyzed Ghrelin titers before and during laying period in four different lines of layers differently selected for laying performance (4x16 animals): high performing white and brown lines (Lohmann Tierzucht GmbH) and low performing lines (White Leghorn and New Hampshire). Additionally effects of housing condition were tested by keeping hens from all lines in single cages (N=32) and a floor housing system (N=32). At 16-19, 33-35 and 49-51 weeks of life plasma samples were analyzed with a chicken Ghrelin ELISA Kit and data were analysed using Glimmix procedure of SAS 9.2. Ghrelin titers did not differ between genetic lines but were affected by housing condition x sampling time (p<0.0001). Before start of laying period, hens from floor housing showed higher Ghrelin titers compared to hens from single cages. In addition, Ghrelin concentrations were highest in the first sampling period, and lowest in the second period (p<0.0001). Results suggest that Ghrelin titers in fowl seem not to be related to laying performance and energy demand but change during ontogeny and are affected by housing condition.

Validation of Harmonized Methods on Selected Wildlife Host-Pathogen Combinations
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APHAEA (harmonized Approaches in monitoring wildlife Population Health, And Ecology and Abundance, www.aphaea.org) aims to establish a European wildlife disease surveillance network that is capable of providing reliable estimates on abundance of wildlife species and pathogen distribution and occurrence in key wildlife species in order to improve wildlife health surveillance in general. To test proposed harmonized protocols for practical feasibility and to demonstrate the advantages of harmonization, three host-pathogen combinations have been selected: Wild boar and Aujeszky’s disease virus
Red fox and Echinococcus multilocularis
Common vole and Francisella tularensis
Project and external partners were invited to share existing data or contribute to collecting new ones for each host-pathogen combination. Therefore, questionnaires were developed to scan data concerning population and disease related questions. Partners provided information on the considered region and time period, existing data sources, hunting or collecting strategies and the possibility of collecting new population related data following the proposed harmonized protocols. Moreover, questions on disease occurrence, ongoing, finished or planned investigations, the numbers of collected samples and the possibility of participating in future studies were asked. The feedback on the respective questionnaires was analyzed to identify strengths and weaknesses of the proposed surveillance schemes.

**Interferon-response against lyssavirus in European bat species**

Yaqing Zhu, Xiaocui He, Bernd Köllner

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Rabies virus (RABV) is a viral pathogen of rabies disease, which infects nervous system in human and other animals. Bats are the natural hosts of RABV and relative lyssaviruses. They can live with infection of RABV without the clinical symptom. However, the mechanisms of disease resistance of bats are not well understood. Interferons (IFNs) are glycoproteins. They release by host cells in response to the presence of pathogens such as viruses, bacteria, and play a role in the innate immunity. IFN α and β are members of the type I IFNs, which are mainly involved in the response against viral infection. Thereby an alternative signaling pathway of IFN α or β might contribute to the bat’s survival with lyssavirus infection.

In this case we clone and characterize the IFN α and β from European bat, Myotis myotis. The recombinant IFN α and β are expressed from the different established cell lines and their biological activities are investigated by induction of signaling pathway. And their antiviral activities against lyssaviruses (EBLV-1, EBLV-2 and RABV) in different cell lines are further analyzed. The obtained results will provide an insight into the interactions between bat IFN-system and lyssaviruses in the natural reservoir species.

**Establishment of Myotis myotis cell lines for investigation of immune responses under lyssavirus infection**

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Bats are natural reservoirs for many neurotropic viruses like lyssaviruses. In contrast to the lethal encephalitis in other animals caused by lyssavirus infection, clinical symptoms in bats are normally not seen. This indicates differences in the lyssavirus-host interactions and underlines the necessity to develop natural host related models to study these phenomena. Here, we report about the establishment of Myotis myotis derived cell lines from neural and immune relative tissues: brain (MmBr), tonsil (MmTo), peritoneal cavity (MmPca), nasal epithelium (MmNep) and nervus olfactorius (MmNol) after immortalization by SV 40 large T antigen. The usefulness of these cell lines to study antiviral responses has been confirmed by analysis of their susceptibility to lyssavirus infection and the mRNA patterns of immune-relevant genes after poly I:C stimulation. Performed experiments indicated varying susceptibility to lyssavirus infection with MmBr being considerably less susceptible than the other cell lines. Further investigation demonstrated a strong activation of interferon-mediated antiviral response in MmBr contributing to its resistance. Overall, the established cell lines are important tools to analyze antiviral innate immunity in M. myotis against neurotropic virus infections and represent a valuable tool for a broad spectrum of future investigations in cellular biology of M. myotis.

Characterisation of pestivirus glycoproteins E1 and E2
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The family Flaviviridae comprises 4 genera, Pestivirus (e.g. bovine viral diarrhea virus (BVDV)), Hepacivirus, Flavivirus and Pegivirus. BVDV is an enveloped virus with a single stranded RNA genome of positive polarity. The polyprotein encoded by the genomic RNA is cleaved by viral and host cell proteases into structural- and non-structural proteins.
The glycoproteins Erns, E2 and E1 are associated with the envelope of BVDV particles. E2 is the receptor-binding protein and main target of neutralizing antibodies in BVDV-infected hosts. E1 is known to represent an essential component of the virion. Both E1 and E2 are integral membrane proteins with a C-terminal transmembrane anchor.
In our studies we are analyzing the membrane topology as well as the localization and retention of E1 and E2. We want to have a closer look at the position where the glycoproteins are found inside the cell and which signal is necessary for their specific localization. The results obtained so far show a localisation predominantly in the ER for both glycoproteins. Furthermore, we identified several mutants of the envelope proteins that show a change in retention behaviour.
Rainbow trout (Oncorhynchus mykiss) thrombocytes are involved in MHC II dependent antigen presentation
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Antigen presentation involves highly specialized cells (myeloid cells, B-cells, dendritic cells) that take up antigen, process it and activate the helper T-cells by presenting digested foreign peptides. In higher vertebrates like birds the thrombocytes are also considered as effective phagocytic cells. Unfortunately, the information whether thrombocytes in lower vertebrates may belong also to true phagocytic cells is incomplete. They may contribute to the stimulation process by antigen uptake and presentation in carp, turbot, snakes or turtle. However, it has never been clearly investigated in trout. Thus, neither any functional and molecular data related to MHC II restricted presentation nor the ability to engulf, kill and digest the antigen has been shown. In this study we prove that by in vitro and in vivo BSA stimulation, bearing acidified vesicles and lysis of the antigen, trout thrombocytes may act similarly to antigen presenting cells. Production of antigen specific antibodies after the adoptive transfer of stimulated thrombocytes confirm that antigen presentation is probably “second” function of trout thrombocytes. Nevertheless, the involvement of thrombocytes in presentation of antigenic peptides via expressed MHC class II molecule to circulating B or T lymphocyte subsets remains to be investigated in further studies.

Genotypic and phenotypic characterization of Campylobacter jejuni isolates from Vietnam
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Thermophilic Campylobacter species, especially C. jejuni and C. coli are the major cause of bacterial foodborne diseases in humans and are the most common bacteria that cause gastroenteritis worldwide (Acheson and Allos, 2001). In Vietnam, only a few reports were published which give information about prevalence of Campylobacter in animal population and meat products. Campylobacter were detected in 26-31% of chicken meat samples in Hanoi (Luu et al., 2006, Ha and Pham, 2006), in 31-76% of poultry and pig farms (Schwan, 2010, Carrique-Mas et al., 2013) and in 15.3% of HCMC market samples (Garin et al., 2012) and represent a potential risk to human health.

The aim of this study is to characterize C. jejuni isolates which were recovered from chicken Meat in Vietnam. For the first time to our knowledge, Vietnamese Campylobacter isolates were characterized in detail. Flagellin gene typing, MLST
analysis and investigations concerning presence and absence of genes responsible for colonization, invasion, toxin production etc. were carried out. Also the susceptibility against antimicrobial agents was subject of this study.

Experimental Infection of Goats with Mycobacterium avium subsp. paratuberculosis and Mycobacterium avium subsp. hominissuis: Lesions and Detection of Pathogens in Tissue 3 Months Post Inoculation
Jan Schinköthe, H.Köhler, E.Liebler-Tenorio

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Mycobacterium avium subsp. paratuberculosis (MAP) and Mycobacterium avium subsp. hominissuis (MAH) are both subspecies of M. avium complex. While MAP causes chronic granulomatous enteritis (Johne`s diseases or paratuberculosis) in ruminants, MAH is known as opportunistic pathogen in several species, including man and other mammals. The purpose of this study was to compare the course of infection with MAP and MAH during the first 3 month after last inoculation (mpi). Twenty-one goat kids each were orally inoculated with either MAP or MAH, while 10 goat kids were sham inoculated. MAP infected goats were sacrificed at 3 mpi. The same date was scheduled for MAH-infected goats, however 41-60 days after last inoculation (dpi) nine of twenty-one goats died spontaneously or were euthanized in extremis. Macroscopic lesions were recorded and tissue specimens were taken for histology and immunohistochemistry. All MAP-infected goats remain clinically healthy, but had granulomatous lesions in JPP and mesenteric lymph nodes at 3 mpi. MAH-infected goats developed severe weight loss or cachexia with granulomatous lesions frequently in highly ulcerated IPP and severely enlarged mesenteric lymph nodes with extensive necrosis and calcification. In conclusion both pathogens were able to establish infection, however with marked differences in distribution and severity of lesions.

Developement of vaccine candidates for prevention of Schmallenberg virus infection
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Schmallenberg virus (SBV), genus Orthobunyavirus, family Bunyaviridae emerged in Europe in summer 2011. Clinical cases have since been reported from all over the continent. The arthropod-borne pathogen mainly infects ruminants. Similar to closely related orthobunyaviruses, e.g. Akabane virus, Aino virus or Sathuperi virus, malformation and death of the fetus after transplacental infection during early to mid pregnancy cause considerable financial losses. Non-infected animals without immune protection remain even in regions severely affected by the virus. Together
with a predominantly naïve host population at the fringes of the affected area they provide a potential basis for renewed epidemics. To encounter these challenges a number of inactivated SBV candidate vaccines have been developed. Cattle were effectively protected after two applications, sheep also after a single injection. Additionally an inactivated vaccine against Akabane virus and Aino virus was evaluated for its potential to protect cattle from SBV infection. Finally, an IFNAR-/- mouse model could be established for infection studies and efficacy testing of vaccines.

**Vibrio anguillarum** bacterin uptake via the gills of Japanese flounder and subsequent immune responses
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The mucosal surfaces of fish allow for the introduction of foreign substances, including antigens, from the surrounding environment. In this study, uptake of *Vibrio anguillarum* J-O-3 serotype bacterin by Japanese flounder, and the subsequent immune responses were investigated. Immunohistochemistry revealed that the bacterin was taken up through the epithelial cells of gills. The transcription levels of inflammatory cytokines such as interleukin (IL)-1β, IL-6 and tumor necrosis factor α were significantly up-regulated in the gills at 3 days following exposure to the bacterin. There was also a corresponding increase in IL-8 receptor, CD4-1, CD4-2 and CD8α transcript levels in the gills. Our findings suggest that the gills play a major role in the uptake of *V. anguillarum* bacterin and induction of inflammation, which results in an activation of the adaptive immune response in teleost fish.

**Regional Genetic contributions in Chicken populations from Europe, Asia and Africa**
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Genetic variations of 113 chicken populations from Africa, Asia, and Europe were studied using 29 microsatellite markers. These populations included three wild chicken populations (RJF) and nine commercial purebred lines for comparison. Allele frequencies, mean number of alleles, heterozygosity, and Wright’s fixation indices were estimated to investigate the extent of genetic variability between and within
chicken populations from different geographical regions. Phylogenetic network and the degrees of relatedness between chicken populations were judged from molecular data by estimating marker-estimated kinship (MEK). Ranking of regional chicken subpopulations were evaluated by computing their contribution to the optimal core set and sequential safe set analyses. High heterozygosity and lower genetic differentiation ($F_{ST}$) were observed in RJF, African and Asian chickens relative to European and Commercial breeds. Higher populations kinship coefficients were corresponded to lower genetic diversity. Asian chicken populations contributed most, followed by African chickens and European breeds contributing least in a core set analysis. Although European breeds have lower genetic contribution to the sequential safe set, yet many populations are required from Europe to the total genetic diversity contribution as they have higher range of genetic differentiation. Attention should be drawn to conservation of some European chicken breeds in maintaining the entire genetic diversity threshold.
Abstracts (poster presentations)

Different freezing methods of small volumes of bull semen and their effects on post freezing quality  
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Genomic selection was introduced in cattle breeding programs in 2010. Since then it is possible to use young bulls as sire. However, young bulls produce smaller ejaculates of lower sperm concentration. The purpose of this work is to develop an effective freezing method for small volumes of bovine semen with competitive post thaw results to conventionally cryopreserved semen. Four different volumes and freezing methods were tested. Straw (250 µl) served as control. A so-called Nanostraw® (20 µl) was used as second treatment. Both were diluted in a TRIS-egg yolk based extender and frozen in nitrogen vapour. Two different pellet sizes of 100 µl and 30 µl were produced from an egg yolk based lactose extender and were frozen on dry ice. All samples comprise 1x10⁶ sperm stored at -196°C in LN2. Motility characteristics were compared during a thermo-tolerance test employing a CASA system. There were no significant differences (P>0.05) between Nanostraw®, control straw and 30 µl pellet. However, the 100 µl pellet showed significantly lower motility (P>0.05). The results indicate the possibility to freeze small semen volumes in pellet and Nanostraws® with acceptable sperm viability after thawing.

Establishing of biocide susceptibility testing of field isolates of Staphylococcus aureus and Escherichia coli  
Andrea T. Feßler, Vivian Hensel, Marita Meurer, Geovana Brenner Michael, Stefan Schwarz

Institute of Farm Animal Genetics, FLI, Mariensee

Biocides are commonly used in the human hospital setting, in livestock holdings and veterinary clinics. Compared to antimicrobial agents, little is known about resistance to disinfectants and biocides. Therefore, Staphylococcus aureus and Escherichia coli isolates from various sources are tested for their biocide resistance in the BMBF projects MedVet-Staph2 and RESET2, respectively. Biocide susceptibility testing is commonly used to test the activity of new biocides in comparison to so-called “reference substances”. Using such a protocol of the German Veterinary Medical Society, minimal inhibitory concentrations (MICs) of field isolates are determined. For this, bacterial suspensions ranging from 1 x 10⁸ to 1 x 10⁹ CFU/ml are prepared and further diluted 1:10. Volumes of 100 µl of this suspension are added to each of the tubes containing 5 ml tryptic soy broth with different concentrations of the respective
biocides. The results are read after 72 h of incubation at 37°C. Plate counts of the bacterial suspensions are performed as quality control. The results of these investigations can provide guidance on the biocides which should be used in stables and veterinary clinics to prevent the spread of S. aureus and E. coli most effectively.

Production of chimeric pigs with humanized hepatocytes
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Using porcine organs for pig-to-human transplantation is considered a promising solution to overcome the growing shortage of human donor organs. Goal of this study is to develop chimeric pigs harboring hepatocytes derived from human induced pluripotent stem cells (hiPSC). The study consists of two parts: (1) induction of a genetic knockout of the human forkhead box transcription factor G1 (Foxg1) gene to prevent neuronal development and (2) induction of a genetic knockout of the porcine fumarylacetoacetat hydrolase (FAH) gene, which leads to a loss of hepatocytes, allowing a repopulation with human iPSC. Porcine FAH knockout embryos and human Foxg1 knockout iPSCs will be used for chimera formation, but the basic procedures will be established in mice and a non-human primate model before being transferred to humans. Currently, the Foxg1 knockout is established in iPSC of a Venus-fluorescent mouse. For this, the CRISPR/Cas9 (clustered regularly interspaced short palindromic repeats/CRISPR-associated protein 9) system is used, which confers precise genome editing by small RNAs that guide the Cas9 endonuclease to a complementary genomic target site. If the mouse experiments show that a functional knockout can be established, the results will be transferred to Cynomolgus (Macaca fascicularis) iPSC.

Distribution of antimicrobial resistance genes via integrative and conjugative elements in Pasteurellaceae, the ICEMh1
Christopher Eidam\(^1\), Anja Poehlein\(^2\), Andreas Leimbach\(^2\), Geovana Brenner Michael\(^1\), Heiko Liesegang\(^2\), Kristina Kadlec\(^1\), Rolf Daniel\(^2\), Michael T. Sweeney\(^3\), Robert W. Murray\(^3\), Jeffrey L. Watts\(^3\), Stefan Schwarz\(^1\)

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Every year, the global cattle industry suffers losses of approximately three billion U.S. dollars due to the multifactorial bovine respiratory disease (BRD) complex, for which Mannheimia haemolytica is considered the major bacterial agent. If preventive vaccination fails, BRD is commonly treated using antimicrobial agents. Unfortunately,
a growing number of isolates showed resistance to one or more antimicrobial agents. One possible reservoir and distributor of antimicrobial resistance genes are integrative and conjugative elements (ICEs).

The M. haemolytica strain 42548 was subjected to whole genome sequencing, followed by sequence analysis and comparative genomics. This revealed the presence of an ICE, which was designated ICEMh1.

ICEMh1 harbours five resistance genes within two resistance regions, which confer resistance to streptomycin (strA and strB), kanamycin/neomycin (aphA1), tetracycline [tetR-tet(H)] and sulphonamides (sul2). Furthermore, it is closely related to ICEPmu1 and seems to have evolved from a common ancestor. A region of ICEMh1 that is not present in ICEPmu1, can be found in putative ICE regions of other M. haemolytica genomes, suggesting a recombination event of two ICES. These findings support the observation that ICES can easily spread, even across genus borders, allowing for the acquisition of multidrug resistance via a single horizontal gene transfer event.

**Generation of transgenic pigs carrying an siRNA vector directed against tissue factor expression**

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Porcine organs are considered as potential solution for the shortage of human donor organs. However, pig-to-primate xenotransplantation results in a severe immune rejection. Since the hyperacute rejection can be overcome, the prevailing cause for xenograft failure is now the acute vascular rejection (AVR) which is characterised by microvascular thrombosis. Our strategy is to prevent AVR by siRNA-mediated downregulation of tissue factor (TF), initiator of the extrinsic coagulation pathway. Porcine fibroblasts were transfected with a TF knockdown vector (kindly provided by Dr. Denner, Robert Koch Institute). After antibiotic selection, cell clones served as donor cells for somatic cell nuclear transfer. Live born offspring (n=7) were analysed for siRNA expression and TF mRNA expression levels. Three TF knockdown pigs were sacrificed to isolate aortic endothelial cells (PAECs). PAECs were seeded into a flow chamber micowell plate and perfused with heparinized human platelet rich plasma. Preliminary data indicate reduced thrombus coverage compared to wild type controls (data by Rataj/Tiede, MHH). Western blot and immunofluorescence analysis are planned for the near future. These promising results underline that TF knockdown would be an important component in the generation of multi-transgenic pigs and could drive xenotransplantation closer to the pre-clinical level.
Examination of prion protein genotypes in German goats
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Transmissible Spongiforme Encephalopathies (TSEs) are fatal, neurodegenerative diseases of the central nervous system. Like in sheep the susceptibility of goats to TSE depends on the genotype of prion protein gene (PRNP). Many polymorphisms of this gene are known, but only a few are associated with resistance (i.e. Q222K, N146S/D).

Whereas breeding programs for scrapie resistance in sheep are quite successful, data on the prion polymorphisms and their distribution in the German goat population are not available at present. Therefore, within the context of the European project “Goat-TSE-Free”, a statistically representative number of blood samples from goats, belonging to the large variety of different goat breeds in Germany, will be collected and genotyped.

For this purpose DNA is isolated, amplified and the whole ORF of PRNP is sequenced and analysed to reveal polymorphic carriers. Preliminary results indicate the presence of 11 of the generally described 42 known polymorphisms in the German goat population, including those which are associated with resistance.

In particular goats carrying the resistant genotypes (Q222K, N146S/D) are potential candidates for breeding programs aiming at the eradication of classical TSEs in this species.

Host association and spillover of bank vole hepacivirus, Germany
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Hepatitis C is a human disease, caused by the hepatitis C virus, frequently resulting in chronic infections leading to cirrhosis and hepatocellular carcinoma. An opportunistic virus hunting approach in 4770 rodents resulted in the identification and genome characterization of a novel hepacivirus, associated to the bank vole Myodes glareolus (Drexler et al., 2013).

Within the network “Rodent-borne pathogens” a monitoring of rodents and other small mammals is performed since spring 2010 at selected sites in Baden-Wuerttemberg, North Rhine Westphalia, Thuringia and Mecklenburg-Western Pomerania. As expected, bank voles and yellow-necked mouse were mainly trapped in forest habitats. In contrast, trapping in grassland habitats resulted mainly in
trapping of common voles. In a pilot study, the novel hepacivirus was almost exclusively detected in bank voles suggesting a corresponding host specificity. In addition, hepacivirus RNA was detected in a few, most likely spillover-infected individuals of other rodent species.
In conclusion, the previously identified novel hepacivirus might have a host specificity for the bank vole with only few spillover infections. Future investigations will have to prove the molecular basis of the host specificity of this hepacivirus. The bank vole-associated hepacivirus may allow the development of a novel animal model for human hepatitis C.

In vitro Characterization of the Cellular Immune Response of the Porcine Host to Nipah Virus Infection
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Nipah virus (NiV), a BSL-4 classified zoonotic paramyxovirus, causes a severe respiratory disease in pigs and fatal encephalitis in humans. Several studies have shown that NiV is able to evade the innate immune response by an interferon antagonistic activity. However, how the adaptive cellular immune system of different hosts responds to NiV infection remains poorly understood. Aim of this work is to study the impact and modulation of NiV infection on different porcine immune cells in vitro.
For this purpose, porcine PBMC will be isolated and separated into the individual subpopulations (dendritic cells, monocytes, CD4+ and CD8+ T lymphocytes) by cell sorting. Subsequently, they will be stimulated either artificially by synthetic activators, by virus-like particles (VLPs) composed of the surface glycoproteins or infectious VLPs under BSL-2-, or by NiV under BSL-4-conditions. Maturation of the immune cells will be monitored by analysis of cell-specific cytokine- and surface activation marker proteins by qRT-PCR, Western Blotting, flow cytometry and immunofluorescence assays as well as by detection of secreted cytokines by ELISA. Altogether, our findings will improve our knowledge on how the immune system of swine responds to NiV infections and moreover, whether this response has beneficial or harmful effects for pigs.

CCHFV – Development of Serological and Molecular Diagnostic Tests and Epidemiological Studies in Subsaharan Countries
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Crimean-Congo Hemorrhagic Fever (CCHF) is caused by a tick-borne virus in many countries of Asia, Africa and South-Eastern Europe. Infected animals do not show clinical signs, but infected humans can suffer from a severe hemorrhagic fever with high lethality rates.
The knowledge of the distribution of CCHFV in African countries is limited. Therefore, the prevalence of CCHFV infections will be investigated in Mauretania, Sierra Leone, Cameroon and the Democratic Republic of Congo over a period of 3 years as part of a project of the German Ministry of Foreign Affairs. For this purpose serum samples of different ruminants will be collected and tested for CCHFV-specific antibodies in in-house ELISAs and other diagnostic methods. African colleagues will be trained at FLI and in their home laboratories in using these assays. A Luminex assay will be developed for the simultaneous detection of antibodies against CCHFV and other highly pathogenic viruses. Viremic animals will be detected by qPCR as well as by a newly developed isothermal PCR (LAMP).

The ultimate aim of this project is to establish diagnostic capacities in the partner laboratories, to identify risk areas in the different countries and to raise awareness for high pathogenic viruses.

**Virulence of Toxoplasma gondii: genetic characterization of different clonal type II/III cross-products.**

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Toxoplasma gondii is an obligate intracellular protozoan parasite whose definitive hosts are felines while nearly all warm-blooded animals can serve as intermediate hosts. The parasite shows a clonal population structure. In Europe, the clonal lineages I, II, and III prevail. While type I T. gondii has a high virulence for mice, the remaining T. gondii types II and III are less virulent.

Recently, clonal products from a sexual type II/III cross of T. gondii, which differ in their genotypes, were isolated. Interestingly, these clones elicited remarkable differences in mouse and chicken virulence and showed variation in the distribution of type II and III alleles as determined by PCR-RFLP. Genome sequence data were obtained for these different T. gondii clonal type II/III cross-products by Next Generation Sequencing. The sequence data will be bioinformatically analyzed for potential associations between the genetic composition and differences in virulence for mice and chicken.

**Targeted attenuation of CSFV - the crucial role of Erns homodimer formation for CSFV virulence**

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Erns is a remarkable glycoprotein of Pestiviruses with several distinct functions. It is well known to suppress the cellular innate immune responses of its host through the RNase activity and partial secretion from infected cells. Another feature of Erns is its
ability to form disulfide linked homodimers. Cysteine 171 of Erns was identified to be crucial for the intermolecular disulfide bond formation. Mutation or deletion of Codon 171 leads to the loss of the proteins ability to form homodimers. Classical swine fever virus Alfort/Tübingen exhibiting such mutations proved to be attenuated in pigs while inducing a neutralizing antibody response (Tews, Schuermann et al. 2009). During animal studies pseudorevertants were identified that had regained the ability to form dimers, through a second site mutation of Serine 209 to Cysteine while retaining the mutations of codon 171. The virulence of such pseudorevertants was characterized in animal studies. These viruses induced clear clinical signs of CSF in pigs. To confirm our results, we conducted further work to assess the pathogenicity of dimerization negative mutants and their pseudorevertant relatives in pigs to further proof the importance of Erns dimerization for CSFV virulence.

Structural determinants of Erns for membrane association, partial secretion and proteolytic release
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Classical swine fever (CSF) is a highly contagious disease which is caused by the classical swine fever virus (CSFV). CSFV is a member of the genus Pestivirus within the family Flaviviridae. Its genome encodes a single polyprotein which is proteolytically processed into various structural and non-structural proteins. The glycoprotein Erns, a structural protein, is of special interest because it possesses an intrinsic RNase activity. This RNase activity as well as the generally occurring dimerization of Erns are known to be important virulence factors of CSFV. Furthermore, it is presumed that partial secretion of Erns supports inhibition of a host’s innate immune response. This secretion is expected to be caused by the unusual membrane association of Erns via an amphipathic helix.

The studies at hand have revealed the functional importance of specifically charged amino acids within the amphipathic helix, most prevalent R194, for the proteolytic release of Erns from its precursor as well as for the secretion level of Erns. For a deeper insight into the importance of these specifically charged amino acids for virus replication and viability, various mutations were introduced into full-length constructs to allow testing of virus mutant recovery and growth parameter.

Functional analyses of Erns dimerization and the role of the carboxy-terminal part of the protein
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The family Flaviviridae consists of four genera including Pestivirus, Flavivirus, Pegivirus and Hepacivirus. The genus Pestivirus comprises pathogens like Classical Swine Fever Virus (CSFV) or Bovine Viral Diarrhea Virus (BVDV) which cause
economically important animal diseases worldwide. Pestiviruses are RNA viruses with a single-stranded RNA genome of approximately 12.3 kb in size. The one single open reading frame encodes for a large polyprotein which is then co- and posttranscriptionally processed into at least 12 viral proteins. In this study the unique viral glycoprotein Erns is of special interest. Besides of its essential role as a structural glycoprotein it also functions as an RNase which is exceptional within the RNA-virus family. It is also known that Erns monomers form disulfide-linked homodimers (90 kDa) with the cysteine residue at position 171 in Erns sequence. Changes of cysteine residue 171 (deletion and/or mutation) had different effects, namely loss of dimerization and attenuation within the natural host. Besides Cys 171, a contact surface has to be present to allow homodimerization. Future work will concentrate on identification of the protein region necessary for dimer formation and its importance for membrane association and secretion of the protein.

**Virus transmission and infection dynamics in indigenous bat colonies in Germany**

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In recent years, bats have been identified to be the natural host of several zoonotic viral agents. Although sometimes even carrying highly pathogenic viruses, these viruses mostly do not seem to have any health impact on the bats. In a pre-study over the last three years, prevalence rates of different viruses in European bat species were analysed. As a result, Astroviruses were identified as a potential target to study the infection dynamics in bats, due to their sufficiently high prevalence rate and sequence variability. Human Astroviruses have been identified to cause gastrointestinal symptoms in young children. In our study, we sampled indigenous bat colonies of different species in Bavaria and Mecklenburg-Western Pomerania. Equipped with transponders, these bats have been individually monitored for many years. In 2013 more than 400 fecal and urine samples have been investigated by PCR for the detection of Astrovirus sequences, which were phylogenetically analysed. Correlating these various sequences with the social and genetic structures of the sampled bats will provide an invaluable insight into the virus ecology in bats and reveal mechanisms of intra- and inter-species virus transmission and transmissibility.
Interaction of the Foot-and-Mouth Disease Virus with the Innate Immune System
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The Foot-and-Mouth Disease is a highly contagious disease of cloven-hoofed animals. It is characterized by lameness and vesicular lesions on tongue, snout and between cloves. The aetiologic agent, the Foot-and-Mouth Disease Virus (FMDV), belongs within the Picornaviridae family to the genus Aphthovirus. Its genome is composed of a single-stranded positive-sense RNA and codes for one polyprotein. The first translated viral protein is the Leaderpro tease (LPro) which autocatalytically cleaves itself from the nascent polyprotein. Another protease encoded by the viral genome is the 3C protease (3CPro). It cleaves the nascent polyprotein in precursor and mature viral proteins. In addition both proteases have indicated impact on the transcription and translation machinery. To circumvent the host innate immune system FMDV has evolved different strategies. Both LPro and 3CPro target the Interferon-I pathways and lead to a decreased Interferon α/β secretion. LPro reduces the level of the transcription factors IRF-3, IRF-7 and NFκB and 3CPro cleaves the adapterprotein NEMO but the detailed mechanisms of interactions are to date unclear. The present study analyses the influence of different viral proteins on differentially activated IFN-I pathways. For both LPro and 3CPro the direct interacting cellular factors of the IFN-I signaling will be identified.

Echinococcus multilocularis: Prevalence and intensity of infection in juvenile and adult red foxes
Mandy Herzig, Kirsten Tackmann, Roswitha Mattis, Astrid Sutor, Sabine Schwarz, Andreas Fröhlich, Franz J. Conraths

FLI, Insel Riems

Alveolar echinococcosis is a serious zoonosis caused by the tapeworm Echinococcus multilocularis. If left untreated, the disease may take a lethal course in humans. Infection occurs by ingestion of E. multilocularis eggs. In Europe, the red fox is the main definitive host of the parasite and rodents serve as intermediate hosts. Other carnivores such as raccoon dogs, dogs and cats also represent definitive hosts of the parasite.

In this study, data on 18,518 foxes from Brandenburg, which were examined for E. multilocularis between 1993 and 2013, were further analysed. A total of 1,774 (9.58 %) of the examined foxes were found infected with E. multilocularis. There was a statistically significant difference between juvenile and adult red foxes regarding the prevalence and the intensity of infection. Adult foxes were found more often infected with E. multilocularis than juvenile animals. The intensity of infection was higher in juvenile foxes as compared to adult foxes. This finding may indicate that foxes are able to develop at least a partially protective immune response.
In further analyses, potential spatial and temporal associations will be investigated for E. multilocularis and similar studies conducted for other intestinal helminths in red foxes using this large data set.

**Development of ultrasensitive biochemical methods for the detection of Bovine Spongiform Encephalopathy prions in peripheral organs**
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Bovine Spongiform Encephalopathy (BSE) in cattle belongs to the Transmissible Spongiform Encephalopathies, a group of incurable neurodegenerative diseases. The causative agents are prions, the misfolded pathological isoform (PrPSc) of the cellular protein (PrPC). PrPC is expressed primarily on neuronal cells in the brain and on other nervous tissues. The exposure with BSE contaminated food has caused variant Creutzfeldt-Jakob-Disease in humans. Organs with assumed highest infectivity concentrations in bovine slaughter-carcasses are declared as specified risk material and have to be excluded from the human and animal food/feed. This categorization is based on data obtained for classical BSE but not for atypical BSE. The aim of this approach is to investigate peripheral organs from an atypical BSE challenge study using highly sensitive biochemical methods. Firstly, the Protein Misfolding Cyclic Amplification (PMCA) reaction is used, which multiplies minuscule amounts of PrPSc in an excess of PrPC to an extent where it can be detected with western blotting. Secondly, the Quaking Induced Conversion (QuIC) will be employed, which measures the amplification of thioflavin-linked PrPSc via the increase of fluorescence emission. Moreover, the suitability of these methods to replace the mouse bioassay as the only reliable method to detect BSE prions will be examined.

**Identification and functional characterization of cellular regulators mediating the exocytic transport of Newcastle Disease Virus proteins**
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Newcastle Disease Virus (NDV) is one of the major pathogens affecting poultry worldwide. It belongs to the genus Avulavirus in the Paramyxoviridae family and possesses a single-stranded, non-segmented ribonucleic acid (RNA) genome of negative polarity. The genome encodes for six viral proteins: the nucleoprotein (NP), phosphoprotein (P), matrix protein (M), fusion protein (F), hemagglutinin-neuraminidase protein (HN) and the RNA-dependent RNA-polymerase or large protein (L). While several steps of the viral replication cycle such as attachment, entry, cytosolic replication, transcription and translation are well described, later phases like the transport of NDV proteins to specific sites at the plasma membrane to allow assembly and release are poorly investigated. Thus, the aim of this project is to
characterize the exocytic transport route, to identify cellular interaction partners and to clarify whether there are differences in transport between viral proteins. Therefore, we constructed plasmids for expression of Strep-tagged viral proteins as well as recombinant NDV that expresses Strep-tagged proteins from an additional gene to perform pulldown experiments and mass spectrometric analyses. Systematic colocalization studies already point out a role for the exocyst complex and ESCRT machinery, even though it turned out to be slightly different among viral proteins.

**Impact of nutrition and metabolic effects on in vivo VOC profiles in breath and faeces of young, healthy goats**

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Physiological changes in metabolism can generate variances in VOC (volatile organic compounds) analysis that act as confounding factors. This study aimed to assess variability of VOCs exhaled or emitted by faeces, resulting from the effects of growth and changing nutrition. A standardized caprine animal model was chosen because significant and well defined changes occur with respect to digestion and metabolism when developing from milk suckling kids to ruminating goats. Fifteen clinically healthy animals were included and were kept under standardized conditions. Within their first year of life, VOCs were analyzed repeatedly in breath samples and the headspace of faecal samples by gas chromatography and mass spectrometry. In parallel, composition of venous blood was analyzed. Within the first few months of life a significantly larger intra-subject and inter-subject variability in VOC profiles occurred compared to data obtained above 6 months of life. Observations in VOCs were accompanied by significant changes in blood composition that occurred with increasing age. This study revealed significant alterations of VOC profiles due to nutrition regimes and metabolic development in growing subjects. Results stress the importance of a profound knowledge about physiological influences on VOC composition before defining reliable and accurate marker sets for diagnostic purposes.

**Local and systemic response of immunomodulatory cytokines and eicosanoids after experimental infection of goats with Mycobacterium avium ssp. paratuberculosis (MAP)**

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BACKGROUND: Paratuberculosis is an infectious disease of domestic and wild ruminants. The identification of infected young stock is inadequate with available testing methods because of insufficient sensitivity and specificity.

OBJECTIVE: We aim to identify specific patterns of immune and inflammatory mediators which could be used as diagnostic markers for an early identification of the infection.

METHOD: Twenty-one Thuringian goats were inoculated with MAP. Ten untreated animals served as controls. Study 1: In order to detect gamma-interferon (IFN-γ) induction of different T lymphocyte subsets, peripheral blood mononuclear cells (PBMC) were isolated from every goat in four-weekly intervals, stimulated with johnin purified protein derivative (jPPD) for 18h and IFN-γ expression studied by FACS analysis.

RESULTS: PBMC consisted of more CD4+ T cells than CD8+ T cells in MAP+ and control goats. The proportion of γδ T cells increased until 4-5 month p.p. but increase was less prominent in MAP+ goats. Antigen-specific IFN-γ induction in these animals was restricted to CD8+ T cells and was not noticed in CD4+ T cells and γδ-TcR+ T cells.

PROSPECT: Next local and systemic gene expression of selected cytokines will be examined by realtime-RT-PCR and responses of regulatory eicosanoids will be analysed locally and systemically.

Analysis of the host proteome during infection of bovine epithelial cells with Coxiella burnetii
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Ruminants are the main source of human infections with Coxiella burnetii (C. b.), the causative agent of Q fever. Aims of the study are to analyze the interactions of C. b. with the bovine host at the entry site (lung epithelium) which imprints on the initiated host immune response and in epithelial cells of gut, udder and placenta that determine the quantity of pathogen excretion. Epithelial origin of several bovine cell lines (PS [udder], FKD-R 971 [small intestine], BCEC12/T2 [maternal placenta], F3 [fetal placenta], BEL-26 [lung]) was confirmed by immunofluorescence studies. Cells were inoculated with C. b. strain “Nine Mile phase II clone 4” at different cultivation conditions. Cell viability was evaluated by LDH (lactate dehydrogenase) and MTT (methyl-thiazolyl tetrazolium) assays. Invasion and replication of C. b. were analyzed by quantitative real-time PCR and microscopic studies. Epithelial cells exhibited different permissiveness to C. b. while maintaining cell viability. Udder cells allowed for the highest invasion rate. Subsequent host cell proteome analyses will aim at identifying molecules and metabolic pathways of host cells determining C. b. replication at the cellular and duration of infection at the animal level to unveil the immunobiology of this zoonotic pathogen in its host.
Influence of niacin supplementation on red and white blood cells and immune status of dairy cows during the periparturient period
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Niacin as precursor for NAD and NADP, is involved in major biochemical processes. Niacin supplementation leads to an increased intracellular NAD pool which prevents oxidative stress and results in enhanced genomic stability and higher cell viability [1]. To test the effects of niacin on hematological, immune and biochemical parameters 47 cows were used. Experiment started on day 42 ante partum (a.p.) and ended on day 100 post partum (p.p.). Niacin was supplemented from day 42 a.p. until day 24 p.p. in a dose of 24 g per animal and day. Blood samples for biochemical and hematological parameters were taken on 15 consecutive time points in relation to calving. Serum samples for clinical chemistry and EDTA blood samples for hematology were analyzed by using an automatic analyzer system. To test the functionality of peripheral blood mononuclear cells (PBMC) heparinized blood was taken on days -42, -14, 3, 7, 14, 28, 42 and 100 and PBMC were separated by gradient centrifugation. Proliferative capacity and metabolic activity were determined using Concanavalin A as mitogen and the Alamar Blue assay.


Kinetic studies of deoxynivalenol (DON) and its metabolites, DON sulfonates (DONS) 1, 2 and 3 with sodium sulfite treated DON contaminated maize in the pig
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DON intoxications might cause serious problems in pig nutrition when critical dietary toxin concentrations are exceeded. Therefore detoxification measures are needed. In a previous experiment a wet preservation method with sodium sulfite resulted in a significant DON reduction in contaminated maize. Furthermore, the preserved material had a characteristic DONS pattern.

In order to investigate the toxicokinetics and bioavailability of DON and DONS in pigs, different variants of oral administration and i.v. application were tested. The calculation of the area under the curves of the substance concentrations vs. time
curves should enable to evaluate the systemic absorption of the individual compounds.

The study was carried out with 16 male castrated pigs. For serial blood sampling pigs underwent surgery to be equipped with two permanent intravenous catheters in the external jugular veins. The plasma concentrations of DON and DONS were determined with a (U)HPLC-LC-MS/MS method. The preliminary analysis showed decreased DON concentrations in plasma of pigs fed with sodium sulfite treated DON diet compared to DON diet without addition. The low DON and high DONS concentrations of sodium sulfite preserved variants were partially confirmed in plasma but no DONS could be detected in plasma so far. Therefore, further investigations are essential.

Simultaneous determination of zearalenone, deoxynivalenol and their metabolites in follicular fluid with LC-MS/MS

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Mycotoxins like zearalenone (ZEN) and deoxynivalenol (DON) are natural contaminants especially in grain and represent a risk for human and animal health. Due to its strong affinity to oestrogen receptors, ZEN plays an important role in reproductive status. Even DON affects oocyte maturation and follicles. Therefore, a selective and sensitive LC-MS/MS method combined with an economic sample preparation for determining of ZEN, DON and their metabolites in follicular fluid was developed. The method comprises solid phase extraction clean-up on Oasis HLB cartridges followed by LC-MS/MS measurement. Recoveries were in the range of 89-112% and limit of detection was calculated as 0.02 - 0.26 ng/ml. The applicability was confirmed with bovine follicular fluid samples obtained from a dose-response-study with practically relevant dietary ZEN and DON concentrations. Therefore, follicular fluid of 26 dairy-cows which were divided into three feeding groups was obtained via ultrasonic guided follicle puncture one week after second ovulation post partum. ZEN, DON and de-epoxy-DON could be detected in the follicular samples collected during the feeding trial. Due to the results of the in-house validation and the analysis of the life-samples, the method can be regarded as suitable for evaluation of the exposure of developing follicles.
Relevance of stocking density for the occurrence of tail tip necrosis in fattening bulls
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Tail tip necrosis (TTN) is a serious problem especially by intensively housed beef cattle with economic losses and reduction of animal welfare. The aetiology of this multifactorial disease is still not completely understood. The purpose of this study was to determine the relevance of stocking density for the occurrence of tail tip necrosis in fattening bulls.

The investigation consisted of two parts and took place on a beef cattle farm in Germany. The first part of the study was comprised of a cohort which included 96 bulls belonging to two breeds (n=48) Holstein Frisian and (n=48) German Simmental. The breeds were split into two groups, exposed and non-exposed, 30 male calves were allocated to the exposed groups, and held in a 60m2 pen with rubber slatted floors and 18 calves were allocated to the non-exposed groups, increasing the space allowance. During the course of one year blood samples were taken and tail tips were scored three times. At the end of our investigation the bulls were slaughtered and the pH-value of the ruminal fluid was measured. In the second part of our work, tail tips of bulls (n=836) of different weights and housing conditions were scored. Furthermore the amputated tail tips of bulls with tail tip necrosis, were scored and the feed was examined for the presence of mycotoxines.

There is a significant (p≤0.01) correlation between stocking density and the incidence of tail tip necrosis, supporting the hypothesis of this research project, that stocking density has a positive effect on the occurrence of tail tip necrosis. This study also revealed that tail tip necrosis should be divided into a traumatic and a metabolic form. The chi square test showed a highly significant (p≤0.001) correlation between low pH values of ruminal fluid and the incidence of tail tip necrosis, which makes this division necessary. In only 1.2% cases of TTN were traumatic. It could be determined, that breed or flooring type is not significant to the occurrence of TTN. Earlier assumed correlations between mycotoxine contamination of feed and the occurrence of TTN could not be verified in this study.

In conclusion the results allow a better understanding of tail tip necrosis in fattening bulls, the prophylaxis with consistent implementation of earlier mentioned parameters seems possible. Furthermore a synergism of etiological complexes, housing conditions and feeding is very important.
The Rift Valley Fever, a mosquito – borne disease in ruminants, camels and humans is caused by the Rift Valley Fever virus (RVFV), a phlebovirus in the family bunyaviridae. First identified in 1930 in Kenya the virus causes high neonatal mortality in livestock and may cause deadly hemorrhagic fever in humans. RVFV is considered to have the greatest potential of all zoonotic arboviral diseases to spread from Africa to Europe since this virus amplifies productively in numerous arthropod vectors. Therefore there is an urgent need for highly reliable molecular and serological assays to diagnose RVF virus infections. Earlier studies have been carried out to develop an indirect enzyme-linked immunosorbant assay (ELISA) for antibodies against RVFV Gn-protein in small ruminants. Future activities will be to adapt this assay to other species (e.g. cattle) and use it in seroepidemiosurveillance studies. Moreover, the suitability of other virus antigens will be examined. Monoclonal antibodies (mAbs) against RVF Gn-, Gc- and N-protein are already available and mAbs against the nonstructural proteins NSm and NSs are in progress, which may allow the development of a species independent competitive ELISA.