



UNIVERSITÄT
LEIPZIG

Veterinärmedizinische Fakultät



14. Juli 2021

Programm & Abstracts

9. LEIPZIGER DOKTORAND:INNEN FORUM



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9. LEIPZIGER DOKTORAND:INNENFORUM

Der Veterinärmedizinischen Fakultät
der Universität Leipzig

Veranstaltungsdaten

14. Juli 2021
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Herbert-Gürtler-Haus
An den Tierkliniken 5
04103 Leipzig

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9. LEIPZIGER DOKTORAND:INNENFORUM

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ABLAUFPLAN

ABLAUFPLAN	UHRZEIT
Anmeldung	8.00
Begrüßung	8.30
1. Session Tierernährung	8.45
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2. Session Bewegung	10.15
Kaffeepause	11.35
3. Session Atemwegserkrankungen	11.45
Mittagspause	12.15
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Kaffeepause	14.00
4. Session Parasiten und Infektionen	14.15
Siegerehrung und ATF-Kontrolle	Ca. 16:30

**Liebe Doktorand:innen,**

wir freuen uns sehr, dass sich so viele entschieden haben, dem 9. Leipziger Doktorand:innenforum gemütlich von der Couch, dem Schreibtisch oder selbst dem Bett aus beizuwohnen, denn das „DokFo“ geht digital! Schluss mit weiten Fahrtwegen, Gedränge in den Hörsälen und Poster 3x drucken lassen, weil man immer wieder einen Fehler entdeckt hat... Spaß beiseite: wir hätten euch natürlich sehr gern persönlich in Leipzig begrüßt!

Doch auch dieses Jahr könnt ihr in ungezwungenem Rahmen eure Arbeiten vorstellen und mit Menschen in Kontakt kommen, die sich mit ähnlichen Problemen herum schlagen, wie ihr selbst. Einer regen Diskussion steht nichts im Wege – vielleicht gibt es sogar die ein oder andere Frage mehr, wenn man sie einfach in den Chat schreiben kann, statt sich in einem vollen Hörsaal zu Wort zu melden.

Zur Förderung der Internationalität des Doktorand:innenforums werden in diesem Jahr alle Vorträge in englischer Sprache gehalten. Passend dazu sind bereits alle folgenden Grußworte und Abstracts in englischer Sprache zu finden, um unser buntes Programm abzurunden.

Wir freuen uns auf viele spannende Vorträge, von denen die besten sechs mit tollen Preisen belohnt werden! Unser herzlicher Dank geht dabei an die Sponsoren, die das Doktorand:innenforum überhaupt erst möglich machen.

Also macht es euch gemütlich, holt euch einen Kaffee oder Tee und lasst uns den Tag gemeinsam genießen! Zumindest am Bildschirm können wir alle mal wieder enger zusammenrücken.

Dear doctoral students,

we are very happy that so many have decided to attend the 9th Leipzig Doctoral Students' Forum comfortably from the couch, the desk or even the bed, because the "DokFo" goes digital! No more long journeys, crowds in the lecture halls and having to print posters 3 times, because you repeatedly discovered a mistake... Fun aside: of course, we would have loved to welcome you personally in Leipzig!



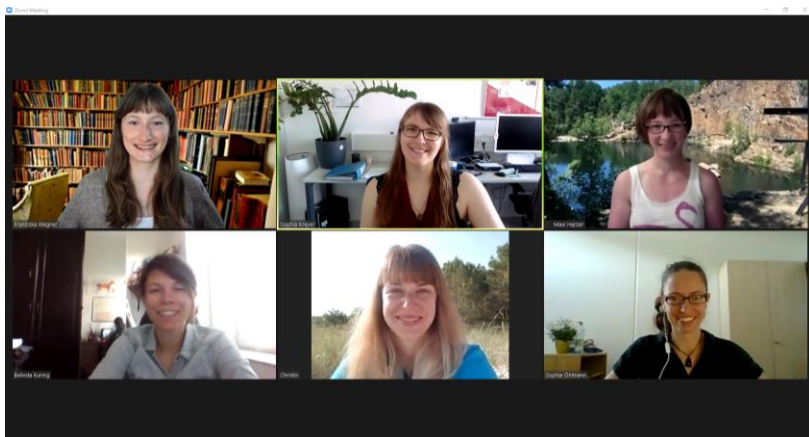
But also this year, you will be able to present your work in an informal setting and get in touch with people who are struggling with similar problems as yourself. There's nothing to stop a lively discussion - maybe there are even more questions if you can just write them in the chat instead of speaking in a crowded lecture hall.

In order to promote the internationality of the doctoral student forum, all lectures will be given in English this year. To round off our colourful programme, you can find the following greetings and abstracts in English, too.

We're looking forward to many exciting lectures, of which the best six will be rewarded with great prizes! Our heartfelt thanks go to the sponsors who make the doctoral forum possible in the first place.

So make yourselves comfortable, grab a coffee or tea and let's enjoy the day together! At least on the screen we can all get closer together again.

Your organization team of the 9th Leipzig Doctoral Forum



Von links oben nach rechts unten: **Franziska C. Wagner**, Institut für Anatomie, **Sophia Kreyer**, Institut für Pharmakologie, **Maxi Harzer**, Institut für Virologie, **Belinda Euring**, Institut für Virologie, **Christin Kamutzki**, Institut für Pharmakologie, **Sophie Öhlmann**, Institut für Bakteriologie und Mykologie



Dear doctoral candidates,

A warm welcome to all of you from the Doctoral Commission of our Faculty. I am very pleased that the organizers managed to enable the Leipziger Doktorand:innenforum this year in spite of the current pandemic situation. What could better show society than this pandemic the vital need for science, innovative ideas und thorough research to overcome a crisis like this one? And for good science we all need good scientists like you!

Although a virtual conference cannot totally replace the contacts and the exchange of ideas that occur in live meetings from face to face, I am convinced that you all will experience interesting talks, can participate in fruitful discussions and will meet new colleagues. I hope you will get valuable input and feedback for your own work, but that you will also use the chance to experience the wide variety of very different research projects that are so typical for veterinary science.

I hope that the next Doktorand:innenforum will take place again here in Leipzig as a traditional conference with the chance for „real“ (i.e. not only virtual) contacts. You should take the opportunity next year to catch up meeting your colleagues live here on our campus!

I wish the organizers a perfect conference without technical issues and for all of you an interesting day full of inspiration for your own projects!

Prof. Dr. Rainer Cermak

Head of the Doctoral Commission

Faculty of Veterinary Medicine, Leipzig University



Dear participants

Veterinary faculties are obliged to properly educate and train students so they are prepared to come in terms with any problems that may occur in their future professional career. However, it is equally important to provide opportunities to young fellows to gain insight into the fundamental basis of all we are doing as professional veterinarians: evidence based science.

The best way to get access to science is “learning by doing”, i.e. to work under supervision by an experienced colleague in a well-established science-oriented environment. To work on a research project does not only improve methodological skills and knowledge but, maybe even more important, stimulates critical thinking, communication and discussion. Your seminar today addresses these essential aspects of young researchers training perfectly, providing the opportunity to learn from each other and to look over the rim of your own, probably very focused project. I congratulate you and particular the organizers for not only keeping the idea of a young researchers seminar at the Faculty of Veterinary Medicine alive but also for further promoting the concept.

It is a pleasure and honor for the “Freundeskreis Tiermedizin e.V.” to support activities like your seminar as far as possible, and we are proud that we can provide at least some financial aid for the current seminar. This is absolutely in line with our intention to help by personal, idealistic or financial support where ever young colleagues strive for improvement of quality of graduate and post graduate education or conditions for qualification of young scientists, thus promoting the reputation of our faculty and graduates both on a national and international level.



The "Freundeskreis Tiermedizin e.V." would not be able to provide support for such activities without the many members that regularly pay their dues and also get directly involved in favor of the intentions and activities of our society. Starting with only few founding members in 1990 the "Freundeskreis Tiermedizin e.V." succeeded to attract more and more members, particularly students, and now, around 30 years later, we are proud to represent approximately 1300 members. This gives us the strength to collect funding and significant membership dues allowing us to financially support outstanding activities like the one today.

I am almost sure that most of you are already members of the "Freundeskreis Tiermedizin e.V.", but those who are not yet are cordially invited to join us and to thus contribute to and participate in our many activities in favor of teaching, learning and research at our faculty.

Prof. Dr. Arwid Dauschies

Vicepresident "Freundeskreis Tiermedizin e.V."

More information and application for membership:

<https://www.frk-leipzig.de>





PROGRAMM

	UHRZEIT
Einlass ins Meeting	8.00
Eröffnung durch das Organisationsteam	8.30
Grüßworte	8.35
1. Session Thema Tierernährung	8.45
Selection behaviour of horses related to hay contaminated with Meadow Saffron (<i>Colchicum autumnale</i> L.) C. Müller, <i>Institut für Tierernährung, VMF Leipzig</i>	
Are horses able to pick out tansy ragwort (<i>Senecio jacobaea</i> L.) from contaminated hay? L. Sroka, <i>Institut für Tierernährung, VMF Leipzig</i>	
Differences in the faecal microbiota phyla of horses and ponies during a two-year body weight gaining programme K. Langner, <i>Institut für Tierernährung, VMF Leipzig</i>	
5 min Pause	9.30
Performance and energy metabolism in fattening bulls exposed to glyphosate residues in the rations in combinations with varying amounts of supplemented concentrates A. Heymann, <i>Institut für Tierernährung, Friedrich-Loeffler-Institut, Braunschweig</i>	
Toxic effects of pyrrolizidine alkaloids on dairy cows K. Knoop, <i>Institut für Tierernährung, Friedrich-Loeffler-Institut, Braunschweig</i>	
Kaffeepause	10:05



UHRZEIT

10.15

2. Session Thema Bewegung

Combined gait analysis in cows, based on pressure sensors under the bovine claw and inertial measurement units

D. Fischer, *Veterinär-Anatomisches Institut, VMF Leipzig*

An attempt to reestablish functionality in damaged equine SDFT in vitro

J. Karoos, *Leibniz IOM Leipzig, Abteilung Biokompatible und bioaktive Oberflächen*

Influence of surgical intervention of the area of the dorsal spinous processes on the biomechanics of the equine thoracolumbar spine

N. Baudisch, *Klinik für Pferde, Freie Universität Berlin*

11.00

5 min Pause

Expression of c-Fos after short-term deep brain stimulation in the dystonic d¹⁹¹² hamster

A. Lüttig, *Institut für Pharmakologie, Pharmazie und Toxikologie, VMF Leipzig*

In vivo optogenetics: a method to examine the role of striatal GABAergic interneurons in the pathophysiology of dystonia

A. Schulz, *Institut für Pharmakologie, Pharmazie und Toxikologie, VMF Leipzig*

11.35

Kaffeepause

11.45

3. Session Thema Atemwegserkrankungen

The CLCA gene family – an exclusive entity of the mammals?

F. Bartenschlager, *Institut für Tierpathologie, Freie Universität Berlin*



PROGRAMM

**The CLCA proteins in respiratory disease -
Which homologs play a role in the horse?**
S. Zoeger, *Institut für Tierpathologie, Freie
Universität Berlin*

Mittagspause

**Equine β 2-adrenergic receptor expression in
HEK293T and COS-7 cell lines**
C. Kamutzki, *Institut für Pharmakologie,
Pharmazie und Toxikologie, VMF Leipzig*

**Identification of immunoreactive *Aspergillus
fumigatus* antigens in severe Equine Asthma**
M. Jentsch, *Institut für Immunologie, VMF
Leipzig*

**Deployment of suitcase lab for rapid
detection of SARS-CoV-2 in low resource
settings**

A. Ceruti, *Institut für Tierhygiene und
öffentliches Veterinärwesen, VMF Leipzig*

**Feline coronavirus as a surrogate virus for
SARS-CoV-2 in determining the efficacy of
various air purifiers**

R. Kobialka, *Institut für Tierhygiene und
öffentliches Veterinärwesen, VMF Leipzig*

Kaffeepause

4. Session Parasiten/Infektionen

**Effects of climate and land-use on rodents
and associated parasites**

P. Koch, *Institut für Tierhygiene und
öffentliches Veterinärwesen, VMF Leipzig*

UHRZEIT

12.15

13.00

14.00

14.15



UHRZEIT

Prevalence of *Leptospira* spp. in Rodents in Germany

E. Schmidt, *Institut für Tierhygiene und öffentliches Veterinärwesen, VMF Leipzig*

Does a nanoscale silicon dioxide coating affect the reduction of *Campylobacter jejuni* and *Salmonella* spp. contamination in poultry slaughter and processing

F. Muck, *Institut für Lebensmittelhygiene, VMF Leipzig*

Reducing paratuberculosis incidence in dairy goat herds by vaccination

C. Pickrodt, *Institut für molekulare Pathogenese, Friedrich-Loeffler-Institut, Jena*

15.15

Pause

15:30

Survival of *Streptococcus suis* in porcine blood

S. Öhlmann, *Institut für Bakteriologie und Mykologie, VMF Leipzig*

Survival of *Klebsiella pneumoniae* in porcine blood is putatively mediated by aerobactin generation and limited by IgM and complement

A. Krieger, *Institut für Bakteriologie und Mykologie, VMF Leipzig*

Pause

Ca 16:30

Siegerehrung & ATF Kontrolle



Selection behaviour of horses related to hay contaminated with Meadow Saffron (*Colchicum autumnale* L.)

C. Mueller¹, L. Sroka¹, M.-L. Hass², S. Aboling², A. These³, I. Vervuert¹

¹*Institute of Animal Nutrition, Nutrition Diseases and Dietetics, Faculty of Veterinary Medicine, University of Leipzig*

²*Institute for Animal Nutrition, University of Veterinary Medicine Hannover*

³*German Federal Institute for Risk Assessment*

Extensively used meadows may support the cultivation of toxic plants such as Meadow Saffron (MS) through reduced fertilisation and lower harvesting frequency. If such meadows are harvested, hay can be contaminated with MS. Symptoms like colic, coughing or death due to multiple organ failure in horses could be related retrospectively to the occurrence of MS in hay. On the other hand, farmers described an avoidance behaviour of horses for MS in hay if forage was available ad libitum. The ability of horses to avoid MS in hay has not been investigated until now.

Therefore, this study aims to assess the horse's ability to avoid MS in hay in an 18-day-experiment and an ad-libitum-feeding. Six adult (aged 11-17), clinical healthy Warmblood geldings with an average body mass (\pm SD) of 674 kilogram (kg) (\pm 85 kg) were repeatedly fed 1-1.5 kg of hay per horse with a level of contamination by 1 or 2% MS over one hour at different daytimes. Their selecting behaviour was observed and recorded (personally, protocol, video). If a horse ingested two plants of MS, the observation period was interrupted and repeated at another day. In case of a second intake, the horse was excluded from the trial. A clinical health examination of the horses was performed every two or three days including the monitoring of blood values at the beginning and the end of the trial.

None of the horses avoided MS in hay which was independent from MS-contamination level or daytime. Crude nutrients and fibre fractions in hay and MS as well as colchicine in MS were measured to identify the selection criteria.

Since all horses were unable to select MS, the feeding of hay contaminated with MS cannot be recommended.



Are horses able to pick out tansy ragwort (*Senecio jacobaea L.*) from contaminated hay?

L. Sroka¹, C. Mueller¹, L.-M. Hass², A. These³, S. Aboling², I. Vervuert¹

¹*Institute of Animal Nutrition, Nutrition Diseases and Dietetics, Faculty of Veterinary Medicine, University of Leipzig*

²*Institute for Animal Nutrition, University of Veterinary Medicine Hannover*

³*German Federal Institute for Risk Assessment, Unit mycotoxins and plant toxins, Department Safety in the Food Chain*

Tansy ragwort (TR) occurs as a toxic plant on extensive grasslands and in hay for horses. An intake of TR over a longer time can lead to severe chronic liver failures.¹

The aim of this study was to examine the ability of horses to reject TR out of hay under ad libitum feeding conditions. In a randomized study, six healthy warmblood geldings (11-17 years old, mean (\pm SD) body weight: 674 \pm 85 kg) were fed with defined amounts of hay (1-1.5 kg) contaminated with 5 or 10% TR at different daytimes over one hour. Sorting behaviour was monitored and documented. In case of poisonous plant intake during observation periods, session was stopped but repeated at further timepoints. If TR-ingestion was repeated a second time, the horse was excluded from the experiment. Toxin analyses (pyrrolizidine alkaloids) were performed in TR. Crude nutrients and fibre fractions were measured in both toxic plants and in hay. Horses were clinically examined every two to three days and blood samples were collected before and after the experiment.

Rejection behaviour of TR was individually variable in the horses. Two horses did not show any rejection of TR. Two horses showed an inconsistent sorting behaviour and had to be excluded in the last third of the study. Two horses were able to select TR throughout the whole feeding period.

Ingestion of TR cannot be ruled out even when hay was fed ad libitum. Therefore, feeding of contaminated hay with TR is not recommended.



Differences in the faecal microbiota phyla of horses and ponies during a two-year body weight gaining programme

K. Langner¹, D. Blaue¹, C. Schedlbauer¹, J. Starzonek¹, V. Julliand², I. Vervuert¹

¹Institute of Animal Nutrition, Nutrition Diseases and Dietetics, Faculty of Veterinary Medicine, University of Leipzig

²University Bourgogne Franche-Comté, AgroSup Dijon, France

Introduction Research in the last decade indicated that the intestinal microbiota may play an important role in the development of obesity. **Materials and methods** Ten Shetland ponies and ten Warmblood horses were fed a diet containing 200% of their maintenance metabolizable energy requirement. Body weight (BW), body condition score (BCS) and cresty neck score (CNS) were recorded weekly. Faecal samples were taken 5 (t1), 11 (t2) and 23 (t3) months after the start of the controlled feeding period. DNA was extracted, and a PCR was performed to amplify the V3-V4 region of the 16S rRNA. PCR products were sequenced using Illumina MiSeq. Community structure was analysed by calculation of observed richness, Simpson, and Shannon diversity index. Faecal concentrations of the short chain fatty acids (SCFAs) were analysed by gas liquid chromatography. For statistical analysis, a Friedman's ANOVA was performed factoring the effects of time and for breed related differences, a Mann-Whitney-U test was used. Significant levels were set at $p < 0.05$. **Results** BW, BCS and CNS increased significantly during the two-years of excessive energy intake. Between t1 and t2, a significant decrease in richness was recorded in the faecal microbiota of ponies. In ponies, an increase of the phylum Firmicutes ($p = 0.025$) was seen between t2 and t3 and in both breeds, a significant increase of the phyla Actinobacteria was recorded between t1 and t3. Between t1 and t2, a drop of the phylum Fibrobacteres ($p = 0.028$) followed by an increase between t2 and t3 was recorded in the faecal microbiota of horses. Between t1 and t2, the phylum Proteobacteria rose significantly in the faecal microbiota of ponies and showed a significant drop between t2 and t3. At t3, the phylum Fibrobacteres ($p = 0.026$) had a higher abundance in the microbiota of horses. Compared to ponies, horses had significantly higher faecal concentrations of some SCFA. **Conclusion** Whether the increase of the SCFA and the Phyla Fibrobacteres in the faecal microbiota of horses at t3 leads to a better fibre digestibility has to be addressed in further studies.



Performance and energy metabolism in fattening bulls exposed to glyphosate residues in the rations in combinations with varying amounts of supplemented concentrates

A. Heymann, K. Schnabel, F. Billenkamp, J. Frahm, S. Kersten, D. von Soosten, U. Meyer, S. Dänicke

Institute of Animal Nutrition, Friedrich-Loefer-Institut (FLI), Federal Research Institute for Animal Health, Brunswick

Glyphosate (GLY), an active substance in broadband herbicides, is a common contaminant in ruminant feed. This study investigated effects of GLY-contaminated feed combined with varying amounts of concentrates © on performance and energy metabolism in fattening bulls. In a 15-week feeding trial, 47 German Holstein bulls (body weight (BW) 392±60kg; mean±SD) were grouped by low (1kg/animal/day; LC) or high (2.5-5kg/animal/day; HC) amounts of supplemented C with (GLYLC, GLYHC) or without GLY-contaminations (CONLC, CONHC) in the rations. Daily dry matter intake (DMI), water intake and BW on weekly basis were documented throughout the trial. Blood samples were collected at the beginning of the trial, after seven and 15 weeks. Serum concentrations of glucose, non-esterified fatty acids (NEFA), β -hydroxybutyrate (BHB), triglycerides (TG) and cholesterol were determined photometrically. Statistical analyses were conducted using PROC MIXED (SAS v9.4) and two-way ANOVA (R v3.6.0). Average daily GLY exposure was 54.4 (GLYHC), 80.6 (GLYLC), 0.6 (CONHC) and 0.8 mg/d (CONLC). During the course of trial, BW and DMI was increasing significantly stronger in HC groups ($p_{C \times T} < 0.05$), while water intake varied irrespective of treatment ($p_T < 0.01$). Daily BW gain ($p_C < 0.01$) and feed conversion ($p > 0.05$) showed opposite patterns. In HC groups, NEFA concentrations ($p_{C \times T} < 0.05$) were lower and glucose levels mostly higher over time compared to LC ($p_{C \times T} < 0.01$). BHB, TG ($p_{C \times T, GLY \times T} < 0.05$) and cholesterol ($p_{C \times T, GLY \times T} < 0.05$) levels were affected by C, GLY and time in an interactive manner. Under applied conditions, GLY did not induce adverse effects on performance agreeing with results in dairy cows. The GLY effects observed for some biochemical traits resulted from interactions in an inconsistent manner hampering a stringent discussion. Moreover, the magnitude of these effects was lower relative to the marked and consistent C-effects. Further analyses are needed.



Toxic effects of pyrrolizidine alkaloids on dairy cows

K. Knoop, S. Dänicke

Institute of Animal Nutrition, Friedrich-Loefer-Institut (FLI), Federal Research Institute for Animal Health, Brunswick

The increasing spread of ragwort (*Senecio* spp.) is observed with concern by farmers and institutions concerned with risk assessment and risk management. Ragwort like common ragwort (*Jacobea vulgaris*) or water ragwort (*J. aquatica*) contain hepatotoxic and potential cancerogenic effective pyrrolizidine alkaloids (PA) and their N-oxides (PANO). These compounds could harm animals directly through oral exposure and humans via consumption of PA/PANO transferred from feed to animal derived food. Particularly extensive managed pastureland and farmed grassland are increasingly infested with PA/PANO-containing ragwort in several regions of Germany. Therefore, exposure of farm animals through pasturing or due to the feeding with contaminated preserved feed (silage, hay) is expected to play an increasing role. In order to assess the hazard posed by a PA/PANO exposure, a trial with 20 German Holstein cows was performed. They were divided in two control groups (CONWater, CONMolasses) and three groups of increasing daily PA exposure (PA1 0.47 mg PA/d/kg body weight [BW]; PA2 0.95 mg PA/d/kg BW; PA3 1.91 mg PA/d/kg BW) (n=4). PA were administered once daily in form of a sugary extract directly into the reticulorumen over 28 days while the control groups received equivalent volumes of water or molasses. Increasing PA exposure neither affected feed intake nor milking performance. However, individual cows of groups exposed with 0.95 and 1.91 mg PA/d/kg BW responded with an increase of enzyme activities in peripheral blood indicative for hepatocellular lesions. Analyses of samples collected from this experiment are in progress in order to assess the health-related effects of PA/PANO more comprehensively.



Combined gait analysis in cows, based on pressure sensors under the bovine claw and inertial measurement units

D. Fischer, S. Grund, L. Friebel, W. Winter, C. K. W. Mülling
*Institute of Anatomy, Histology and Embryology, Faculty of Veterinary Medicine,
University of Leipzig*

Claw lesions and lameness are one of the biggest welfare concerns in dairy cows. Often, they are unappreciated and have a big impact on welfare of affected animals. The aetiology of many claw lesions is multifactorial with housing conditions such as hard flooring being among the major risk factors for lesion development. Therefore, pressure distributions between claws and acting vertical forces need to be better understood. Pressure measurements with mobile claw sensors on animals in their housing environment will contribute to a better understanding of mechanical impacts, enable objective evaluation of claw trimming methods and to analyse the impact of the claw-floor-interaction while locomotion. Locomotion is a complex, dynamic interaction of forces and movements. Kinetic gait analysis may be completed by additional kinematic data, as a broader approach for understanding bovine biomechanics. Kinematic gait analysis can be performed by video analysis or inertial measurement units (IMUs). In this study, preliminary tests were undertaken to investigate and evaluate the feasibility and potential of the simultaneous application of an innovative pressure sensor system and IMUs, attached to the hind limbs of 10 dairy cows. The cows walked on a concrete and rubber mat walkway. Sequences in walk and trot were recorded. An innovative software was used for pressure analysis. A MATLAB-algorithm was written for step analysis based on accelerometer and gyroscope data, obtained by two IMUs. Parameters like average pressure, maximum pressure, loaded area, step duration, duration of stance and swing phase and maximum acceleration were evaluated. Results show the general suitability of both systems, although further adjustments need to be made. The combination of different gait analysis systems is a promising tool for further studies concerning risk factors of claw lesions and lameness, like the evaluation of different flooring systems.



An attempt to reestablish functionality in damaged equine SDFT in vitro

J. Karoos¹, A. Müller¹, S. Friebe¹, F. C. Wagner², C. K. W. Mülling², S. G. Mayr¹

¹*Leibniz Institute for surface engineering, Leipzig*

²*Institute of Anatomy, Histology and Embryology, Faculty of Veterinary Medicine, University of Leipzig*

The horse is a truly loyal fellow for the human for more than a bimillenary now. No matter if it served as a pack animal, mount or in racing, it has always been crucial for humankind. However, due to increased physical stress and unnatural husbandry many horses suffer from overload injuries which often occur to tendons in the limb area. Quite frequently, these injuries lead to foundering of the horse and require a time-consuming healing process with insecure outcome. Our work investigates the impact of irradiation and decellularization on the mechanical properties of equine superficial digital flexor tendon (SDFT) and tries to combine these methods to regain original tendon properties after damage.

In order to do so, we split the tendons alongside the fibers and modify the obtained samples before testing their properties with a tensile stage. Modifications include:

- Decellularization to eliminate cellular impact on mechanical properties
- Irradiation and chemical treatment to alter crosslinking
- Increased collagen supply for structural rehabilitation

Our overall aim is to restore the collagen structure once the tendon has been damaged, thus regaining its immense strength without losing valuable time or the possibility to never heal properly at all.



Influence of surgical intervention of the area of the dorsal spinous processes on the biomechanics of the equine thoracolumbar spine

N. Baudisch, A. Ehrle, H. Meyer, C. Lischer
Equine Clinic, Faculty of Veterinary Medicine, Free University of Berlin

The term “kissing spines” describes the narrowing of spinous processes of the thoracolumbar spine of the horse. Horses with kissing spines showed alterations of structure and innervation of the interspinous ligament. With a prevalence of 34 - 92 %, many authors cite kissing spines as the most common cause of back problems in horses. Successful therapy relies on understanding the normal anatomic relationships and movement of the thoracolumbar spine. Conservative management aims to reduce pain and facilitate movement, whereas surgical management alters the normal anatomy. The biomechanical effect, either positive or negative, of surgical alteration is unknown. We constructed a mechanical frame, which allows quantified manipulation of the thoracolumbar spine in three planes, to study the biomechanics of the thoracolumbar spine of the horse in a cadaver model. To investigate the biomechanical role of the interspinous ligament, a pressure transducer is used to measure the forces on the ligament during spine motion. The three-dimensional bone motion is measured using computed tomography. Once the normal motion has been studied, the effect of different surgical procedures will be assessed by pre- and post-surgery biomechanical testing of the range of motion.



ABSTRACTS 2. SESSION

Expression of c-Fos after short-term deep brain stimulation in the dystonic dt^{sz} hamster

A. Lüttig, S. Perl, M. Paap, D. Franz*, M. Heerdegen*, R. Köhling*, A. Richter
*Institute of Pharmacology, Pharmacy and Toxicology, Faculty of Veterinary
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Deep brain stimulation (DBS) is an important therapeutic option for patients with dystonia, Parkinson disease and other neurological disorders. While the molecular effects of DBS are nearly unknown, it has been hypothesized that beneficial effects of DBS of the globus pallidus internus (entopeduncular nucleus, EPN, in rodents) in patients with dystonia could be based on slow normalization of synaptic plasticity or re-organization of the basal ganglia network in addition to immediate effects on neuronal activity. In the dt^{sz} mutant hamster, a model of paroxysmal dystonia, we recently found that the severity of dystonia was significantly reduced during short-term (3 h, 50 μ A, 60 μ s) EPN-DBS with 130 Hz EPN-DBS, while 40 Hz were less effective. 130 Hz stimulations of the subthalamic nucleus (STN) failed to exert beneficial effects as also observed in sham-stimulated groups with implanted electrodes into the EPN or STN (Paap et al., 2020, *Neurobiol. Dis.*). Immediately (15 min) after DBS or sham-stimulation, the hamsters were transcardially perfused and brains were collected for fluorescence immunohistochemistry (IHC). In order to examine the neuronal activity, we used c-Fos as an indirect marker for neuronal activity. C-Fos-reactive cells were counted in regions of the cortico-basal ganglia network (striatum, STN, EPN, substantia nigra, ventromedial thalamic nuclei and motor cortex) as well as in the lateral habenula and deep cerebellar nuclei. The number of c-Fos reactive cells in stimulated groups was compared with those in age-matched sham-stimulated as well as naïve dt^{sz} hamsters. Furthermore, we performed intensity measurement 100 μ m around the electrode location. As expected, the intensity measurement revealed a higher c-Fos expression around the electrodes in stimulated groups in comparison to sham groups (EPN 130 Hz vs. sham, $p < 0.05$). We found a tendency towards decreased neuronal activity in the deep cerebellar nuclei after 130 Hz EPN DBS, but there were no significant differences within all investigated brain regions between EPN stimulated groups (130 Hz, 40 Hz) or in STN (130 Hz) stimulated hamsters vs. control groups. This unexpected finding does not exclude changes of specific cell types. With regard to recent electrophysiological data after DBS in mutant hamsters, double labeling of c-Fos and GABAergic neurons are currently performed. Furthermore, ongoing long-term DBS over ten days by novel implantable stimulators should clarify the mechanisms of slow normalization of synaptic plasticity. This study was supported by the German Research Foundation (Deutsche Forschungsgemeinschaft, DFG) within the Collaborative Research Centre (SFB 1270/1 ELAINE 299150580).



In vivo optogenetics: a method to examine the role of striatal GABAergic interneurons in the pathophysiology of dystonia

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Circuit dysfunction in the striatum, the input structure of the basal ganglia, is involved in causing hyperkinetic and hypokinetic disorders, including dystonia - a movement disorder characterized by sustained or intermittent muscle contractions causing abnormal postures and movements. The most common form of inherited generalized dystonia is the DYT1 dystonia caused by a 3-base pair deletion leading to a dysfunction of the protein torsin A. Striatal fast-spiking, parvalbumin-positive GABAergic interneurons (FSI) are the major source of inhibition onto striatal projection neurons (medium spiny neurons, MSN) and exert powerful regulation on MSN activity. Modulating of FSI leads to altered MSN inhibition and subsequently to changes in the motor control. Previous studies in the *dt^{SZ}* mutant hamster, a phenotypic model of dystonia, indicated abnormal FSI function, shown by a retarded maturation of FSI. In our project, we investigate the hypothesis that FSI dysfunction plays a crucial role in the pathophysiology of dystonia and the development of dystonic symptoms. We use in vivo optogenetics in a DYT1 knock-in (KI) mouse model, which does not exhibit dystonia (as 70% of human carriers). For optogenetic silencing of FSI, we created mice (Cre-LoxP) expressing the yellow light-sensitive ion pump Halorhodopsin (NpHr). During optogenetic stimulations, effects on the behavior are measured in 6-month-old DYT1 KI mice and wildtype mice to identify genotype differences. Striatal sections from stimulated and naive mice were stereologically quantified for neuronal activity (c-Fos) and NpHr. Behavioral tests did not detect motor and non-motor dysfunctions and optogenetic manipulations were not sufficient to induce overt dystonic symptoms. Cell counts revealed a decreased number of c-Fos+ cells in the striatum of stimulated DYT1 KI, not in stimulated wildtype. The number of double-labelled c-Fos+/NpHr+ cells of stimulated DYT1 KI was reduced, compared to stimulated wildtype and respective naive. To further determine how neuronal activity was altered by optogenetic silencing, ongoing studies on potential changes in neuronal activity of cholinergic interneurons and quantification of gene expression (c-Fos, parvalbumin) might provide more insights into the role of FSI in the pathophysiology of dystonia.



The CLCA gene family – an exclusive entity of the mammals?

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Introduction: Chloride channel regulators, Calcium-Activated (CLCA) are important biomolecules, which are associated with cancer and chronic inflammatory airway diseases. These genes are highly diverse in mammals, which possess up to eight copies in four distinct genetic clusters with a wide tissue and cell specific expression pattern. Hitherto, little is known about their functional mechanisms and evolution. However, the genomic diversity of mammal CLCAs suggests an interesting evolutionary background of these genes and resolving this might further help to elucidate functional mechanisms. Therefore, the presence, expression and protein-biochemical properties of selected avian species as representatives of the sauropsid lineage were analyzed.

Material and Methods: The genomes of chicken, turkey, quail, and ostrich were screened for the presence of CLCA homologues and identified members were analyzed *in silico*. The chicken homologues were cloned and protein-biochemical properties subsequently characterized using immunoblot and immunocytochemistry. The tissue expression pattern was analyzed using RT-qPCR, immunofluorescence, and immunohistochemistry.

Results: Only two CLCA homologues were found in the genomes of all birds investigated. Avian CLCA1 showed a close genetic distance to mammalian clusters 1, 3, and 4, however, CLCA2 forms a monophyletic group with mammalian cluster 2. The cellular expression pattern and the protein domain architecture of the galline CLCA1 was similar to mammalian cluster 4, while the avian CLCA2 had similar properties as mammalian cluster 2 members.

Discussion: Unlike in birds, CLCA members in mammals that are closely related to avian CLCA1 show a high evolutionary dynamic, which led to the formation of three distinct gene clusters with incompletely differing protein and expressional properties. In contrast to that, CLCA2 seems to be conserved among mammals and the avian species investigated in this study. These results suggest that CLCA is an evolutionary old gene family, which already existed in early members of the amniotic lineage.



The CLCA proteins in respiratory disease - Which homologs play a role in the horse?

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Introduction: Members of the CLCA (chloride channel regulators, calcium-activated) protein family are modulators of respiratory diseases such as human asthma and are discussed as biomarkers and therapeutic targets in this condition. The mouse and the cat are important animal models for this disease. However, important species-specific differences regarding the expression pattern of CLCA-orthologues were revealed which must be respected when translating results from animal models to humans. Equine asthma is a significant disease and discussed as a model for human asthma. The CLCA family is unknown in horses, except for CLCA1. With regard to the horse as an additional animal model for human asthma, the aim of this study was to identify the equine CLCA family at the genome level and initially to analyze their physiological expression in the equine respiratory tract. **Material and Methods:** The equine genome was analyzed for representatives of the equine CLCA gene family. The identified CLCA members were phylogenetically screened, and their amino acid sequences were analyzed in silico. Using RT-qPCR and specific primer systems for the equine CLCA members and four reference genes, the CLCA expression profile was determined in lung-healthy horses (n=3) from the dissection material of the Institute of Veterinary Pathology of the Freie Universität Berlin. All data were compared with findings from other species. **Results:** In contrast to other species, five, putatively functional CLCA homologs were identified in the equine genome. The encoded proteins showed a typical CLCA domain architecture. Of the five homologs, CLCA1 was predominately expressed in the equine respiratory tract. Additionally, CLCA3 and CLCA2 were detectable, however, CLCA4 representatives were not expressed in the respiratory tract. **Discussion:** The horse showed its own respiratory CLCA expression profile with differences to other species, especially to humans. CLCA1 also appears to be the most significant CLCA homolog in the respiratory tract in the horse with possibly similar function to other species. Species-specific similarities, as well as differences, should be considered when translating data between models.



Equine β 2-adrenergic receptor expression in HEK293T and COS-7 cell lines

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The β 2-adrenoreceptors (β 2AR) play an important role in a variety of cell types in the lung, thus being the main target in the treatment of human and equine asthma. For further functional and receptor activation studies under the influence of β 2-agonists and antagonist, we established a stable in vitro inducible expression model of equine *ADRB2* in HEK293T and COS-7 cells. In these *ADRB2*-cell lines, the gene expression is under the control of a tetracycline regulated tet-On system and can be induced with doxycycline. However, it is not known so far what is the optimal dosage and time exposure for inducing a consistently high expression level of equine *ADRB2* in the different cell lines. Therefore, transfected cells were treated with increasing concentrations (0 – 8.0 μ g/ml) of doxycycline for 24, 48 and 72 hours. In qPCR experiments (N=3, n=2), the 48 hours dox treatment with concentrations of 4.0 μ g/ml (COS) and 8.0 μ g/ml (HEK) revealed the highest mRNA expression levels. Hence, further experiments were performed with this incubation time. In contrast, the maximum cAMP response in the AlphaScreen cAMP accumulation assay (N=3, n=3) was induced with 2.0 μ g/ml (COS) and 0.5 μ g/ml (HEK) doxycycline. Thus, there is a discrepancy in dose-dependent mRNA expression and receptor function. To further investigate the dose-effect of dox on the exact number of membrane receptors, (–)-[125I]-iodocyanopindolol (ICYP) binding studies are planned. Furthermore, in a second step the influence of selective and non-selective β -agonists on mRNA expression, protein level and cAMP production will be examined. In conclusion, from the present point of view, our systems are precise and reproducible and seem to be highly suitable to study signaling and pharmacological properties of *ADRB2*. At this moment, we recommend an induction with 1.0 μ g/ml of doxycycline for 48 hours.



Identification of immunoreactive *Aspergillus fumigatus* antigens in severe Equine Asthma

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Equine Asthma affects up to 20% of adult horses with recurrent respiratory symptoms. Exposure to hay dust induces exacerbation, whereas a remission of symptoms can be achieved with a dust-free environment. Nevertheless, the inducing agents of severe Equine Asthma have hardly been identified on a molecular basis. *Aspergillus fumigatus* (*A. fumigatus*), a mould species in hay dust, is explored as a main extrinsic cause of severe Equine Asthma. In this study we analysed *A. fumigatus* as a source of candidate antigens in an immune proteomics approach. *A. fumigatus* mycelia were lysed by sonication and proteins from the lysate were precipitated with acetone. Using two-dimensional (2D) western blots the resulting *A. fumigatus* proteins were probed with serum from asthmatic or healthy control horses from the same environments (n=5 each). Serum antibodies bound on immunogenic protein spots on 2D western blot membranes were detected by fluorescently-labelled detection antibodies. Detection was performed for pan-Ig, and for the isotypes IgG3/5 and IgG4/7. The isotypes IgG3/5 and IgG4/7 seem associated with equine T helper 2 or T helper 1 responses, respectively. Differences between asthmatic and control sera in quantity and quality (antibody isotype) of the spot patterns on the western blots were identified and indicated several proteins as relevant antigen candidates. These antigens were selected for further analysis by mass spectrometry to identify the proteins. Cloning of the proteins of interest, recombinant expression and serological testing will follow to confirm the antigens as important immunogens for serum antibody responses specifically provoked in severe Equine Asthma. Antigen identification will help to elucidate the pathogenesis of sEA and potentially enable future specific immunotherapy.



ABSTRACTS 3. SESSION

Deployment of suitcase lab for rapid detection of SARS-CoV-2 in low resource settings

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SARS-CoV-2 causes the severe acute respiratory disease named COVID-19. In March 2020, the disease was declared as a global pandemic. Key measures to control viral spread are rapid identification of infected cases as well as vaccination. Despite the availability of the vaccine, fast diagnostics remain one of the effective interventions especially with the current vaccine distribution rate and the emerging of new variants. Currently, real-time RT-PCR is the standard detection method. However, the need of rapid and simple point-of-care diagnostics that maintain the performance of the gold standard method is essential. Recombinase polymerase amplification (RPA) has shown to be a promising method in this regard. Thus, in this project, a multicountry study is ongoing to determine the assays accuracy. Sample inactivation and nucleic acid extraction is conducted in a secured glove box to ensure the safety of the technician. To ease the use at point of need, a suitcase lab is used for master mix preparation and amplification steps. Total sample size is 3500 from seven African countries. Each sample is tested for the presence of SARS-CoV-2 RNA-dependent RNA polymerase (RdRP) as well as envelope (E) and nucleocapsid protein (N) genes. Positive and negative predictive values are calculated. All results are compared to the real-time RT-PCR tests. Partial testing of samples was performed. Hitherto, E gene dependent assay produced many false negative results (37% specificity), while the RT-RPA assay's clinical sensitivity and specificity in comparison to real-time RT-PCR were 81 and 100% for RdRP and 63 and 96% for the N RT-RPA assay. Our preliminary results revealed the better performance of RdRP based molecular assay. On top of that, the mobile set up is an ideal platform for rapid detection of SARS-CoV-2 at point of need.



Feline coronavirus as a surrogate virus for SARS-CoV-2 in determining the efficacy of various air purifiers

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Introduction. The spread of viruses through air poses a high risk for health e.g. influenza and coronaviruses. Reducing the burden of airborne pathogens is therefore an effective measure to minimize the exposure. The potency of the air purifier is greatly affected by the ambient condition as well as differences in the biophysical properties of viruses. Moreover, with new emerging viruses, e.g. SARS-CoV-2, a high level of biosafety lab is necessary (BSL-3). This, however, is not widely available and of high maintenance cost. Therefore, in this study, feline coronavirus (FCoV) was used as a surrogate virus for SARS-CoV-2 to test the effectiveness of seven different air disinfection devices which are based on different disinfection principles.

Methods. The efficacy of ionization system, Ultraviolet C (UVC), electro-, hepa- and moss-based filters as well as UVC/electrofilter with or without hepafilter on FCoV were examined. Using an aerosol generator with 5 bars, FCoV (approx. 1×10^7 TCID₅₀/m³ air) was nebulized in a closed container. Air was drawn through a gelatin membrane filter at two sampling points, at the virus entry site and after filtration and/or ionization. The reduction in infectivity (TCID₅₀) of the FCoV was determined on cell culture according to the Spearman-Kärber method.

Results. Around 2 log₁₀ was the reduction in FCoV infectivity due to handling and nebulization as measured at the point of virus entry. The most potent systems (approx. 100% reduction) were the ones including hepafilter and/or UVC. The efficacy of air ionization system varied greatly depending on the number of emitters and the point of measurement (0-78%). With electro- or the moss-based filter, virus reductions of 80 and 20% respectively, were reached.

Conclusion. The use of hepafilter as well as UVC can be considered the best method to inactivate infectious coronavirus particles in air.



Effects of climate and land-use on rodents and associated parasites

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Small rodents play a key role in ecosystems, are of central importance for a variety of agricultural processes and can be major pests when overabundant. Underlying reproduction and population dynamics are strongly affected by biotic and abiotic factors like climate and land-use. To determine the influence and importance of these two parameters on population outbreaks of common voles (*Microtus arvalis*) and other abundant rodents, a large-scale field study was conducted at the Global Change Experimental Facility at the Helmholtz-Zentrum for ecological research in Bad Lauchstädt, Germany.

A two ha experimental area was subdivided into ten replicates of five land-use-types. Half of them were experimental controls without manipulation. In the other replicates, climate conditions were created similar to conditions expected in Central Germany for 2050 – 2080, marked by increased night temperature and adjusted precipitation patterns.

During 2019 and 2020, live trapping of rodents was performed monthly during the reproductive period. Handling involved the identification of species, sex and reproductive activity as well as marking individuals with RFID-transponder and collecting parasites. Individuals were examined once per month and released at place of capture after treatment.

First results indicate that species specific effects may exist on the levels of land-use and climate. Assessments of climate change impact on biological systems in the relevant habitat are essential to judge potential consequences for the protection of plants and human health.



Prevalence of *Leptospira* spp. in Rodents in Germany

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Leptospirosis is a worldwide emerging zoonotic disease with more than 1 million human cases annually. Infections are associated with direct contact to infected animals or indirect contact to contaminated water or soil. Highest case numbers occur in regions with tropical climate and poor hygiene but also in countries with a moderate climate. Because rodents are the main reservoir hosts, this study was conducted to evaluate the prevalence of *Leptospira* spp. in rodents from 39 locations in west, north, south and central Germany from 2010 to 2020 which were available from former studies. Rodents belonged to the genera *Clethrionomys* (n=2197), *Apodemus* (n=29), *Microtus* (n=2), *Sorex* (n=1) and *Mus* (n=1). DNA of kidney samples was analyzed by real-time PCR targeting the *lipL32* gene. Positive samples were further analyzed by targeting the *secY* gene to determine *Leptospira* genomospecies and multilocus sequence typing (MLST) to determine the sequence type (ST). The mean prevalence was 9% (n=195). Rodents were mostly infected with *L. interrogans* (74%) represented by ST 24, followed by *L. kirschneri* (22%) ST 110 and *L. borgpetersenii* (4%) ST 197. Rodents of the genera *Clethrionomys* and *Apodemus* were infected with *L. interrogans* and *L. kirschnerii*. *L. borgpetersenii* was only found in *Clethrionomys*. Highest prevalence was found in south Germany (17%, n=330), followed by west Germany (8%, n=1008). The study shows that pathogenic *Leptospira* species are widely spread in rodents in Germany and should be on the differential diagnoses list of every physician and veterinarian.



ABSTRACTS 4. SESSION

Does a nanoscale silicon dioxide coating affect the reduction of *Campylobacter jejuni* and *Salmonella* spp. contamination in poultry slaughter and processing?

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Campylobacter jejuni and *Salmonella* spp. cause the most common bacterial enteric diseases in humans for years. In Germany, between 60 000 and 70 000 cases of campylobacteriosis are recorded annually, while 13 693 cases of salmonellosis were most recently reported to the Robert-Koch-Institute in 2019. The main cause is consumption of contaminated food of animal origin like raw or inadequately heated poultry meat and eggs. Possible causes of poultry meat contaminated with both foodborne pathogens are cross-contaminations of carcasses via equipment during slaughter and processing. Modification of contact surfaces like nanostructure coating could contribute to decrease bacterial attachment, proliferation and increase detachment. Such coated surfaces are already used in the food industry to reduce cross-contamination on food preparation surfaces and could also be a promising tool for the intended use on other food contact materials. Therefore, the aim of the study is to investigate whether the nanostructured silica coating on stainless steel and thermoplastic elastomer as common materials in the poultry slaughter and processing is a tool to reduce the presence of *Campylobacter jejuni* and *Salmonella* spp. in poultry meat.



Reducing paratuberculosis incidence in dairy goat herds by vaccination

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Observing a four hundred head Thuringian dairy goat herd over a period of three years, this project aims at reducing the number of new infections with *Mycobacterium avium* subspecies *paratuberculosis* (MAP). MAP infections lead to a chronic enteritis known as Johne's Disease or paratuberculosis which is widely spread in ruminants worldwide. Clinical symptoms include decreasing milk yield, weight loss and diarrhea. To reduce the number of new cases, vaccination of newly born kids as well as immunization of adult goats using a commercial vaccine (Gudair®, CZ vaccines, Spain) was implemented. During the course of the project, faecal samples from each adult goat shall be tested twice a year to determine the individual infection status and the current faecal shedding of MAP, respectively. Based on the results, the goats will be divided in groups of MAP positive and MAP negative animals. Differences between these two categories, concerning antibody titer, metabolic parameters and milk yield, will be studied to highlight the economic impact of MAP infections. In addition, environmental samples (bedding, dust, water, feed) will be collected to investigate which barn areas are highly contaminated with MAP and therefore may serve as a source of infection. The faecal-oral route is known to be the most common route of infection for paratuberculosis but transmission through milk or colostrum must be considered as well. Lactogenic shedding of MAP was shown in previous studies but mostly with a low number of bacteria. Colostrum samples will be collected to determine whether this is an important transmission route in the goat herd or if other hygienic measures should be focused to prohibit infection events. The results of the project are intended to develop a guideline for farmers on managing MAP infections in goat or sheep herds.



Infection of intestinal organoids with *C. parvum* sporozoites without microinjection

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Introduction Organoids are three-dimensional structures that resemble their organ of origin in anatomy and function and can be cultured *in vitro*. Unlike two-dimensional cell culture systems, which are limited by a lack of complexity regarding structures, cell organization and cell signalling networks, organoids are able to mimic *in vivo* conditions. This is of special interest studying parasite-host interactions, but also for all other kinds of infectious organisms. A downside of working with organoids is the dependence on the rather complicated and fragile technique of microinjection, which is required for most infection systems. In this experiment, *Cryptosporidium parvum* was used as a model organism to establish a method for infecting organoids using simple mechanical disruption. Developing an easy and accessible way of infecting organoids can help improving research on various infectious organisms *in vitro*.

Animals, Materials and Methods Organoids (kindly provided by Dr. Christian Klotz, RKI Berlin) were generated from the small intestine of C57/Bl6 mice according to a protocol by Sato and Clevers. They were grown on Matrigel covered with AdvDMEM medium and incubated at 37 °C and 5 % CO₂. Prior to infection with *C. parvum* sporozoites, the organoids were washed with AdvDMEM and centrifuged down at 300 x g. The supernatant was removed and replaced with cold AdvDMEM. The organoids were physically broken open via simple pipetting and incubated with AdvDMEM medium containing freshly excysted *C. parvum* sporozoites. This suspension was incubated for 1 h at 37 °C, then the suspension was mixed with Matrigel, seeded in 24 well plates and incubated for 48 h. For immunofluorescence assays the infected organoids were fixed with 4 % PFA. Permeabilization was reached using 0.25 % Triton X in 1 x PBS and blocking was induced adding 1 % BSA to the solution. The organoids were stained with Phalloidin-iFluor 488, Sporo-Glo 594 and Hoechst 33342. They were then placed on slides, let dry and mounted using mounting medium. The samples were examined with a TCS SP8 laser scanning microscope (Leica).

Results and Conclusions Infected organoids were analysed 48 hpi, since former studies in the cell culture revealed a high density of meront stages at this point, proving a successful infiltration of the host cells. Examining the infected organoids at this time resulted in the detection of many trophozoites and different types of meronts, both representing intracellular stages of the parasite (Fig. 1). This outcome was observed in all three iterations of the experiment. Those findings suggest that mechanical disruption may serve as a stable method to infect organoids without depending on microinjection. This thesis is supported by recent findings of Delgado Betancourt et al. [5] who successfully infected intestinal organoids with the apicomplexan parasite *Toxoplasma gondii* using this very technique. Facilitating the work with organoids might help increasing the availability of this promising *in vitro* culture system for laboratories without access to high-end devices, such as microinjectors



Survival of *Streptococcus suis* in porcine blood

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Streptococcus suis (*S. suis*) is a major porcine pathogen associated with diseases like meningitis, septicemia, arthritis and endocarditis but it can also colonize pigs without clinical signs. As a zoonotic pathogen, it poses a threat to human health and has already caused outbreaks in Asia with partly lethal outcome due to streptococcal toxic shock like syndrome.

As bacteremia is a hallmark of invasive infection, the objective of this study was to understand how the immune system counteracts *S. suis* in porcine blood. An important defense mechanism of neutrophils is the generation of reactive oxygen species (ROS). Therefore, we measured the susceptibility of *S. suis* strains to the oxidative burst intermediate hydrogen peroxide and evaluated the differences in bacterial survival depending on the induction of ROS in *ex vivo*-infected porcine blood reconstituted with sera containing different pathogen-specific antibody levels.

The usage of NADPH oxidase inhibitor apocynin highlighted the importance of the ROS production for bacterial killing. Also the inhibition of complement and cleavage of IgM reduced the oxidative burst activity in granulocytes and increased bacterial survival in accordance with an IgM-complement-oxidative burst axis.

In conclusion, this study highlights an important control mechanism of *S. suis* bacteremia in the natural host: the induction of ROS in blood granulocytes via specific immunoglobulins such as IgM.



Survival of *Klebsiella pneumoniae* in porcine blood is putatively mediated by aerobactin generation and limited by IgM and complement

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Background: In humans, diseases caused by hypervirulent *Klebsiella pneumoniae* strains are an emerging major healthcare concern worldwide. In pigs, *K. pneumoniae* is a neglected pathogen though it causes different diseases such as septicemia in piglets and mastitis in sows. The objective of this study was to characterize recent veterinary *K. pneumoniae* isolates regarding hypervirulence-associated genotypes, siderophore generation and survival in porcine blood. Furthermore, the working hypothesis that adaptive IgM and complement are crucial for killing invasive *K. pneumoniae* strains in blood of older weaning piglets was investigated.

Results: The investigated veterinary *K. pneumoniae* isolates did not carry *rpmA* (regulator of the mucoid phenotype). However, *K. pneumoniae* isolates associated with diseases in pigs were positive for the aerobactin siderophore gene *iucA* (iron uptake chelat), which is also a hallmark of invasive human *K. pneumoniae* strains. Differences in siderophore production were confirmed phenotypically. Porcine *iucA*+*rpmA*- *K. pneumoniae* strains proliferated efficiently in blood of suckling piglets in contrast to *iucA*- isolates. At an age of 8 weeks, piglets showed a prominent peak of *K. pneumoniae* specific IgM and an invasive porcine *K. pneumoniae* strain was killed in their blood. As shown by specific degradation, adaptive IgM was crucial for bactericidal immunity at this age. Furthermore, complement dependent killing was demonstrated through addition of the complement inhibitor vaccinia virus complement control protein. Serum of the same piglets did not mediate killing of invasive *K. pneumoniae* strains in contrast to an *E. coli* strain.


Conclusions: Our results suggest that an *iucA* positive genotype is associated with *K. pneumoniae* diseases in pigs and that aerobactin expression might be important for survival in porcine blood. Adaptive IgM, complement and immune cells are crucial for killing of invasive *K. pneumoniae* strains in porcine blood of older weaning piglets, most likely by opsonophagocytosis.



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