Posters
Phi Zeta Research Day
March 10, 2015, 1:00-5:00pm, Mara Conference Center, 4th Floor, Trotter Hall
(Posting from 1:00 – 5:00pm; Q&A for Judging 1:15 – 3:00pm)

Poster Number **Presenter – Title** **Abstract**

1. **Brittany Blattner** – Full Genomic Analysis of Two New Human Norovirus GII.4 Strains
2. **Mollie Burton** – Classical Swine Fever Virus E2 and E^{rns}-Specific Antibodies Neutralize Bovine Viral Diarrhea Virus in vitro
3. **Grace Chen** – The sodium-coupled monocarboxylate transporter (SLC5A8) associates with CFTR and the PDZ domain scaffolding protein, EBP-50, in the Fischer rat thyroid cell line, FRTL-5
4. **Sarah Guess** – The Effect of Concurrent and Delayed Sucralfate Administration on the Relative Bioavailability of Fluoroquinolones in Greyhounds
5. **Rui Guo** – Nucleocapsid protein of Porcine Epidemic Diarrhea Virus enhances viral replication in vitro
6. **Kaitlin Haukos** – The Detection of Antibodies to Vaccine and Field Strains of Rabies in Horses by a Multiplex Microsphere-Based Assay
7. **Sam Hocker** – Expression of Receptor Tyrosine Kinases in Canine Nasal Carcinomas
8. **Susumu Ishiguro** – A local chemotherapy with hyaluronan-cisplatin conjugate significantly attenuates growth of lung adenocarcinoma xenografts in mouse model
9. **Melissa Juby** – Viral dissemination in mosquitoes inoculated with Rift Valley fever virus MP-12 strain
10. **Ellen Kim** – Enumeration of Shiga Toxin-Producing Escherichia coli in Commercial Feedlot Cattle Feces Using the Spiral Plating Method
11. **Vien O** – Significance of animal feces and the associated microbial community in the oviposition and development of Culicoides sonorensis
12. **Kyle Pfeifer** – Expansion of Human Umbilical Cord Mesenchymal Stem Cells in Media Supplemented with Various Concentrations of Human Platelet Lysate.
13. **Michael Porta** – Efficacy of Fulvestrant in Canine Mammary Carcinoma Cells
14. **Izabela Ragan** – The DETECTION of Antibodies against Rift Valley Fever NUCLEOCAPSID Protein by Luminex bead-based multiplex Assay
15. **Stephanie Rainbolt** – Effects of different sources and levels of cobalt on in vitro fermentation
16. **Russell Ransburgh** – Development of a Multiplex Fluorescent Microsphere Immunoassay for Diagnosis of Porcine Disease Complex
17. **Chris Siepker** – Clinical Stage of Infection is Critical for the Antemortem Detection of Chronic Wasting Disease in White-Tailed Deer
18. **Ellen Unruh** – Associations between high rectal temperature and behavioral trends in beef cattle
19. **Yin Wang** – A fusion protein of Escherichia coli heat-labile toxoid(LTR192G) and spike protein epitopes of the porcine epidemic diarrhea virus induced neutralizing antibodies against PEDV
20. **Laura Constance** – Cochlear Pendrin Expression has a Protective Role in Noise-Induced Hearing Loss
21. **Fei Zhou** – The Prenatal Rise in the Utricular K^+ Concentration is Delayed in Mice Lacking Pendrin

5:00 – 6:00 **Reception and Awards Ceremony** Frick Auditorium and Foyer, 2nd Floor, Mosier Hall
- Initiation of New Members to Phi Zeta
- Announcement and Presentation of Awards Recognizing Research and Scholarship Accomplishments
- Closing Comments

Full Genomic Analysis of Two New Human Norovirus GII.4 Strains

Brittany Blattner

Author(s): Kyeong-Ok Chang, Brittany Blattner

Norovirus (NoV) is a non-enveloped, single-stranded RNA virus belonging to the Caliciviridae family. Accounting for around 19-21 million illnesses each year, NoV is a major cause of food-borne gastroenteritis worldwide. NoV Genogroup II, Genotype 4 (GII4) is known to be the cause of most adult gastroenteritis cases around the world today. The GII4 genotype reached predominance by the start of the 21st century, and its variants began sparking worldwide epidemics as of 2002. Contributing to the epidemic-inciting capacity of GII4 is its tendency to continually form new variants and recombinant strains. The goal of this study was to perform genetic characterization of two previously unknown strains of human NoV GII4 identified from stool samples. RNA was extracted from the stool samples of patients suffering from acute gastroenteritis. First-strand cDNA synthesis was carried out, and samples were amplified using 16 constructed primers by polymerase chain reaction. Samples were purified, sent out for sequencing, analyzed and compared to known GII4 strains using CLC Sequence Viewer and Finch TV. Results showed that the two strains are very similar (>99% identity) and the amino acid identities of ORF1, ORF2 and ORF3 of the strains to the counterparts of the Houston strain, a prototype strain of GII4, were ~98%, ~93% and ~ 89%, respectively. The greatest percentage of mutations were found to occur in the p22 non-structural region and in VP1 and VP2 structural regions. These new strains appear to be most closely related to known strains from East Asia.

Classical Swine Fever Virus E2 and E^{rns}-Specific Antibodies Neutralize Bovine Viral Diarrhea Virus *in vitro*

Mollie Burton

Author(s): Mollie Burton, Ben Trible, Raymond RR Rowland

Classical Swine Fever (CSF) is an economically important foreign animal disease affecting both domestic swine and wild boar. CSFV, which belongs to the genus *Pestivirus*, is antigenically related to Bovine Viral Diarrhea Virus (BVDV). For example, we previously demonstrated that antibodies resulting from immunization with a subunit replicon based vaccine containing CSFV E2 or E^{rns} can stain BVDV infected cells in culture. The purpose of this study was to determine whether CSFV E2 or E^{rns} derived antibodies possessed neutralizing activity (NA) to BVDV. Sera samples specific for BVDV E2, CSFV E2, and CSFV E^{rns} were obtained from a previous study. NA assays were performed by incubating serial Log2 dilutions of sera with a constant amount of virus, then plating on replicating bovine turbinate cells in a 96 well plate. Following a 4 day incubation, each well was examined for virus induced cytopathic effects. Results were reported as the neutralizing antibody titer, the highest sera dilution showing no CPE. NA assays showed the highest neutralizing antibody titers were from pigs immunized with BVDV E2. However, immunization with CSFV E2 or E^{rns} resulted in neutralizing antibody titers ranging from 1:8 to 1:16. Overall, these results have major implications for diagnostic assays and points to the necessity for serological assays that can differentiate CSFV from other *Pestiviruses*. Future efforts are directed towards identifying antibody epitopes that can differentiate CSFV from BVDV.

The sodium-coupled monocarboxylate transporter (SLC5A8) associates with CFTR and the PDZ domain scaffolding protein, EBP-50, in the Fischer rat thyroid cell line, FRTL-5

Grace Chen

Author(s): Grace Chen, Suhasini Ganta, Yonghai Li, Peying Fong

SLC5A8 is a sodium-coupled monocarboxylate transporter and tumor suppressor that is expressed in the apical membrane of epithelial cells of many tissues including colon, breast and thyroid. Methylation of the *SLC5A8* gene leads to a loss of apoptosis and associates with different human cancers, including those affecting the thyroid. The present studies aim to determine whether SLC5A8 interacts with a scaffolding protein, EBP-50, thereby forming a complex with the cystic fibrosis transmembrane conductance regulator (CFTR). CFTR is an ATP cassette binding transport protein whose function is to conduct halide anions. *CFTR* mutations and subsequent dysfunction cause Cystic Fibrosis, the most common autosomal-recessive disease in Caucasians. In epithelial cells, EBP-50 mediates apical CFTR-CFTR interactions thus potentiating the CFTR-mediated chloride conductance. Whereas CFTR may also mediate iodide efflux into the thyroid follicular lumen, EBP-50's role in the thyroid remains elusive. FRTL-5 cells, a cell line derived from Fischer rat thyroids that retains specialized thyroid characteristics, such as secretion of thyroglobulin and iodide accumulation, were cultured for these experiments. Total FRTL-5 cell lysates were prepared and immunoprecipitated using an antibody targeted against SLC5A8. The immunoprecipitated fraction was probed with antibodies specific for CFTR and EBP-50 by immunoblot detection. Concurrently, immunoprecipitation of CFTR was performed and the isolated complex was probed using antibodies targeted against SLC5A8 and EBP-50. Results of our experiments indicate that SLC5A8 complexes with CFTR and possibly EBP-50, but CFTR does not interact with EBP-50. These findings provide new insights into how SLC5A8, CFTR and EBP-50 interact at the cell membrane and allow us to better understand the function of iodide transport in the thyroid.

The Effect of Concurrent and Delayed Sucralfate Administration on the Relative Bioavailability of Fluoroquinolones in Greyhounds

Sarah Guess

Author(s): Kate KuKanich, Butch KuKanich, Sarah Guess, Ellen Heinrich

Insert abstract (250 words or less): Sucralfate is a gastroprotectant that has been reported to impair absorption of certain antimicrobials. Our objective was to determine whether co-administration of sucralfate and a fluoroquinolone impairs fluoroquinolone bioavailability in dogs and to determine the effect of a 2-hour delay of sucralfate on fluoroquinolone pharmacokinetics. Five Greyhounds were incorporated into a randomized crossover design and administered either ciprofloxacin (25mg/kg PO) or enrofloxacin (5mg/kg PO) alone, concurrent sucralfate (1 g oral suspension PO) with ciprofloxacin or enrofloxacin, or sucralfate administered two hours after either fluoroquinolone. Fluoroquinolone concentrations were evaluated with liquid chromatography/mass spectrometry. Area under the curve (AUC), time to maximum plasma concentration (T_{MAX}), and maximum plasma concentration (C_{MAX}) were compared between groups. Dogs had variable absorption of ciprofloxacin (AUC range: 5.52-22.47 hr*µg/mL) compared with enrofloxacin (3.86-7.50 hr*µg/mL). Relative bioavailability (%F) of ciprofloxacin was 48% when administered concurrently with sucralfate (with one dog at 8%), and improved to 87% when sucralfate was delayed by 2 hours. In contrast, no significant difference in absorption was demonstrated based on AUC or C_{MAX} when enrofloxacin was administered concurrently with sucralfate as compared with enrofloxacin alone, and %F for concurrent administration was 104%. This study confirmed that oral bioavailability of ciprofloxacin is variable, which could lead to treatment failures or drug toxicity in some treated dogs. When sucralfate and ciprofloxacin are both indicated, a 2-hour delay should be considered to allow improved bioavailability. On the contrary, no drug interaction was documented for concurrent sucralfate and enrofloxacin, and concurrent administration may improve compliance.

Nucleocapsid protein of Porcine Epidemic Diarrhea Virus enhances viral replication in vitro

Rui Guo

Author(s): R. Guo*, E. Poulsen, Y. Wang, R. Ransburgh, J.F. Bai, W. Zhang, Y. Fang

The recent outbreak of porcine epidemic diarrhea virus (PEDV) has caused significant challenge to US swine industry. The isolation and propagation of PEDV in cell culture is the first step toward development of vaccines and diagnostic tests. However, the natural characteristics of the virus pose certain difficulties on the adaptation and passage of PEDV in cell cultures. In this study, we demonstrated that nucleocapsid (N) protein of PEDV has the ability to enhance infectivity of PEDV. In transfected cells expressing N protein, the PEDV grows to 10-fold higher peak viral titer, in comparison to the virus titer in untransfected cells. Further analysis showed that N protein interacts with nonstructural protein 3 (nsp3) and localized to the viral replication-transcription complex (RTC). The nsp3-N interaction was mapped to the N-terminal 160 amino acids region containing ubiquitin-like domain of nsp3. These results support a previously established model of murine coronavirus, in which the N-nsp3 interaction serves to tether the viral genome to the newly translated RTC at early stage of infection, which could be a mechanism for N-protein dependent enhancement of PEDV infectivity.

The Detection of Antibodies to Vaccine and Field Strains of Rabies in Horses by a Multiplex Microsphere-Based Assay

Kaitlin Haukos

Author(s): Kaitlin Haukos, Susan Moore, Elizabeth Davis, Chris Blevins, Melinda Wilkerson

Rabies is a fatal neurological disease caused by a RNA virus in the family Rhabdoviridae. Prevalence among horses in North America is steadily increasing. The majority of rabies viruses isolated from positive horses in Kansas are skunk strains; however several bat and raccoon strains have also been identified.

We hypothesize that horses vaccinated with laboratory adapted rabies strains have weak antibody titers to virus variants occurring in nature. We developed a multiplex-bead-based indirect immunoassay to screen sera obtained from vaccinated horses against rabies antigens isolated from 7 rabies virus isolates. We designed a 7-plex bead-based capture antibody assay to quantify equine IgG using known concentrations of equine IgG; whereby a standard curve was established. This multi-analyte technology (xMap) is designed to quantify equine IgG that binds viral antigens derived from 7 different rabies virus strains simultaneously.

We confirmed the coupling of a capture antibody to the standard 7-plex and established a standard curve that quantifies equine IgG. We characterized the dominant viral proteins by silver stain of SDS-PAGE. We confirmed the coupling of $_{viral}$ proteins G and N derived from three laboratory rabies strains to three sets of xMag beads.

A 7-plex set of rabies antigen coated xMap beads were tested against seven horses before and after rabies booster vaccination. The results indicated that all horses were able to increase their vaccine response after booster, but a variety of responses were recorded to the rabies strains that differ from the vaccine strain.

Expression of Receptor Tyrosine Kinases in Canine Nasal Carcinomas

Sam Hocker, DVM

Author(s):Sam Hocker, DVM
Mary Lynn Higginbotham, DVM, MS, DACVIM (Oncology)
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Jamie Henningson, DVM, PhD, DACVP

Introduction:

Toceranib phosphate has a reported 71.4% clinical benefit rate in canine nasal carcinomas. The objective of this study was to assess for the expression of multiple receptor tyrosine kinases (RTKs) and evaluate their potential role in the biologic activity of toceranib in canine nasal carcinomas.

Methods:

Archived canine nasal carcinoma tissue samples were identified. Antibodies for vascular endothelial growth factor receptor-2 (VEGFR2), platelet derived growth factor receptor alpha and beta (PDGFR-α; PDGFR- β), stem cell factor receptor (KIT) and epidermal growth factor receptor (EGFR) were obtained for immunohistochemical analysis. Immunoreactivity was determined to be positive or negative based on tumor tissue uptake. Normal canine nasal epithelium was used as a baseline for RTK expression.

Results:

Twenty-four nasal carcinoma samples were identified. Cytoplasmic expression of VEGFR-2 was present in 22/24 (91.7%). Positivity for PDGFR-α was present in 23/24 samples; 12/22 (54%) cytoplasmic, 8/22 (36%) nuclear and cytoplasmic, and 2/22 (9.0%) only nuclear. Only 4/24 samples expressed PDGFR-β; however, all showed significant stromal staining of PDGFR-β. EGFR staining was present in 14/24 (58.3%) with the majority being membranous. KIT immunoreactivity was positive in 12/24 (50%) samples. Labeling was cytoplasmic in 6/12 (50%), membranous in 3/12 (25%), and a combination in 3/12 (50%). Normal nasal tissue expressed all receptors in varying locations.

Conclusion:

Known targets of toceranib are expressed in canine nasal carcinomas. These RTKs merit further investigation into their roles in the biology of nasal carcinomas and their contribution to toceranib's perceived biologic activity.

A local chemotherapy with hyaluronan-cisplatin conjugate significantly attenuates growth of lung adenocarcinoma xenografts in mouse model

Susumu Ishiguro

Author(s): **Susumu Ishiguro**[1], Deepthi Uppalapati[1], Shuang Cai[2], Jacob Hodge[1], Laird Forest[2], Masaaki Tamura.[1]

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Nanoparticle-based chemotherapy, while promising, remains clinically unsuccessful, mainly due to a lack of targeted delivery methods of the therapeutics into cancer tissues and the side effect of chemotherapeutics. We have demonstrated that the nanoparticle formulation with hyaluronan cisplatin conjugates (HA-Pt) is suggested to be an effective chemotherapeutic delivery method in breast cancer mouse models. The objectives of the present study are to examine the therapeutic efficacy of HA-Pt formulation on lung adenocarcinoma cells in a cell culture and of the local administration of HA-Pt on lung adenocarcinoma in mice. Cell culture studies clarified that the HA-Pt effectively attenuated cell growth in 2D and 3D cultures significantly more than control cisplatin. In the 3D spheroid study, the treatment with HA-Pt induced apoptosis in the cells located in the central area of the spheroid. A single intratracheal administration of 7.5mg/kg HA-Pt (1mg cisplatin equivalent/kg) seven days after LLC cell inoculation almost completely inhibited growth of LLC allografts in the mouse lungs without any severe side effects. Histological analysis of dissected lungs revealed that a small number of microscopic tumor nodules were detected in the treated mouse lung, whereas several large tumors were detected in the untreated control mouse lung. Apoptotic index was significantly higher in the treated tumors than untreated control tumors, suggesting that cisplatin was successfully delivered to the tumor tissues by HA-Pt and caused apoptosis of tumor cells. Taken together, the current study suggests that an intratracheal administration of HA-Pt offers an effective strategy for lung cancer treatment.

Viral dissemination in mosquitoes inoculated with Rift Valley fever virus MP-12 strain

Melissa Juby

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Arthropod-borne viruses (arboviruses) comprise 70% of emerging or re-emerging zoonotic disease agents globally. Rift Valley fever virus (RVFV) is an arbovirus endemic to sub-Saharan Africa that can induce severe disease or death in newborns, abortion in pregnant ruminants, and hemorrhagic complications in humans. RVFV is transmitted by mosquitoes to animals and humans, however, the handling of infectious animal tissue is the highest risk factor for human disease. An outbreak of RVFV in Kenya in 2007 is reported to have had an economic impact of more than $72.5 million, indicating an acute need for preventative measures to control the spread of the virus. Although it is cited that RVFV has been isolated from at least forty species in eight genera of mosquitoes, *Culex tarsalis* has been shown to be a competent vector species of North America. For transmission to occur, the virus must disseminate from the midgut to the salivary glands of the mosquito, whereupon it is introduced to the host during a blood-meal. The objective of the current study was two-fold: using female *Culex tarsalis* mosquitoes, determine the optimal day post-inoculation (dpi) with RVFV MP-12 strain in which a disseminated infection was observed (time-course study); using the optimal dpi as described previously, collect saliva from female *Culex tarsalis* mosquitoes and perform plaque assays to determine virus titers. Viral dissemination was monitored by RVFV-specific qRT-PCR of extracted RNA from the bodies, legs, and heads of inoculated mosquitoes. Optimal viral dissemination was observed and subsequent saliva collections were performed on 7 dpi. Infected saliva collected in the current study will be used for downstream experimental applications to evaluate immune responses and viral pathogenesis using *in vitro* and *in vivo* host models.

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Enumeration of Shiga Toxin-Producing *Escherichia coli* in Commercial Feedlot Cattle Feces Using the Spiral Plating Method

Ellen Kim

Author(s): Ellen Kim, Pragathi B. Shridhar, T.G. Nagaraja, Natalia Cernicchiaro, and David G. Renter

Shiga toxin-producing *Escherichia coli* (STEC) bacteria are a major food safety concern. Cattle shed these organisms in their feces contaminating the environment through effluents, ground meat, and other beef products via cross-contamination at the processing plant. To better identify the risk of human illness, concentration data is needed to populate quantitative microbial risk assessment models. The objective of the study was to assess the performance of the spiral plating method on quantifying colonies of STEC in feces of commercial feedlot cattle. From June to August 2014, 8 feedlots in Nebraska and Texas (4/state) were visited monthly. At each visit, pen-floor fecal samples from cattle pens within 2-3 weeks of harvest were collected. 100µl suspensions of fecal samples were inoculated onto agar plates using a spiral plater, from which colonies were counted to estimate the total CFU/g of the sample. DNA was extracted and tested individually by an 11-gene multiplex PCR assay to detect O157, O26, O45, O103, O111, O121, and O145 serogroups and four major virulence genes (*stx*1, *stx*2, *eae*A, and *ehx*A). First week results indicate that of a total of 96 fecal samples processed, 7 samples were enumerable: 1 (1.0%) sample was positive for O157 (concentration=3.90×10^4 CFU/g) and 6 (6.3%) samples were positive for non-O157 serogroups (range=3.31×10^4 to 6.20×10^5 CFU/g). Upon further data analyses, the resulting data will be used to further assess the performance of this technique as well as to characterize the distribution of non-O157 STEC bacteria present in feces of cattle entering the beef chain.

Significance of animal feces and the associated microbial community in the oviposition and development of *Culicoides sonorensis*

Vien O

Author(s): Vien O, Dinesh Erram, Bob Pfannenstiel, Mark Ruder, and Ludek Zurek

The biting midge, *Culicoides sonorensis*, is an important vector of Orbiviruses, including bluetongue virus (BTV) and epizootic hemorrhagic disease virus (EHDV) that infect domestic and wild ruminants. Better understanding of developmental requirements and behavior of *C. sonorensis* will help with designing novel strategies for management of the midge vector and reduction of BTV and EHDV infections. This project aims to investigate the oviposition preference of *C. sonorensis* for feces of different animal species as well as in different concentrations of fecal content. Additionally, four field sites, two at Konza Prairie Biological Station and two at Kansas State University Large Animal Research Facility, were selected for the comparative analysis of *Culicoides* populations and the microbial community composition. For oviposition, laboratory reared blood-fed females were exposed to autoclaved soil with 25% fresh fecal content of different animals, including dairy and feedlot cattle, swine, chicken, sheep, goat, horse, and deer in four-choice assays. In addition, different concentrations (0.0, 3.1, 6.2, 12.4, 25.0, 50.0, and 100.0%) of dairy feces in autoclaved soil were also tested. The larval substrate (shoreline mud) at four field sites was sampled weekly for adult midge emergence, moisture measurement, and analysis of the microbial community using a culturing approach (plate count agar with cycloheximide, modified fecal coliform agar, and potato dextrose agar with chloramphenicol and gentamicin). Oviposition behavior of *C. sonorensis* showed consistent aversion to 100% fecal concentration of dairy feces and variable preference in lower concentrations. No clear oviposition preference for feces of different animal species was detected. The analysis of the data from the four field sites is currently in progress and will be presented.

Expansion of Human Umbilical Cord Mesenchymal Stem Cells in Media Supplemented with Various Concentrations of Human Platelet Lysate

Kyle Pfeifer

Author(s): Kyle Pfeifer, J. Robert Smith, Pavan Rajanahalli, Shoshana Levshin, Mark L. Weiss

Xenogen-free media formulation is important for clinical translation of human mesenchymal stromal cells (MSCs). Since human platelet lysate (HPL) has been shown to be an effective media supplement for expanding MSCs, we tested 2, 5, and 10% supplementation to determine which was optimal for reaching a target of > 10^10 cells for banking for clinical use. MSCs were isolated from human umbilical cords and expanded in Dulbecco's Modified Essential Medium supplemented with 2%, 5%, or 10% pooled HPL for 5 consecutive passages. The cells were expanded in 6 well plates and grown as a monolayer reaching ~80% confluency before passing. Cell number, size, viability and days till passage were recorded at each passage. Preliminary data collected from four MSC isolates show that the cells grow faster while maintaining a small size and high viability with 10% HPL, compared to 2% or 5% HPL. In contrast, 2% HPL supplementation had slow growth and fewer cells were counted at each passage. Cells grown in 5% HPL grew faster than 2% HPL, but not as fast as in 10% HPL. Using 10% HPL supplementation will permit us to achieve our target of 10^10 cells in 2 to 3 passages. Therefore, it appears to be the optimal media supplement for achieving high cell numbers in fewer passages as well as maintaining a small cell size and high viability with each passage. Further work will be needed to confirm these preliminary findings, and to confirm that the cells expanded are MSCs per the ISCT definition.

Efficacy of Fulvestrant in Canine Mammary Carcinoma Cells

Michael Porta

Author(s): Michael Porta and Annelise Nguyen

The most common neoplasms found in female intact canines are mammary cell tumors with approximately half of these tumors being malignant and having metastasized by the time of their diagnosis. Canine mammary cell carcinomas have been poorly studied and little is known in regards to differential patterns for hormonal receptors, kinases and intercellular communication. The preliminary data showed that CF41.Mg cells, derived from canine mammary carcinoma, express estrogen receptor alpha (ERα), progesterone receptor (PR), human epidermal growth factor receptor 2 (HER2). The goal of this study was to determine the response of CF41.Mg cells to the anti-cancer drug fulvestrant, an ER antagonist that down-regulates and degrades the estrogen receptor ERα. Western blot analysis was used to examine the expression of the proliferating and anti-proliferating biomarkers cyclin D1, caspase 8, and caspase 3, in response to fulvestrant. Results showed a 42.7% decrease in cyclin D1 expression after 48 hour fulvestrant exposure at 500 nM. Immunofluorescent imaging shows a decrease in ERα expression in the presence of fulvestrant compared to controls. In addition, cell viability assay was performed to determine the cytotoxicity of fulvestrant on CF41. Mg cells. Despite the changes found in protein expression, no significant changes in cell viability were observed after 24 or 48 hours at any concentration of fulvestrant. Overall, the findings demonstrate for the first time that fulvestrant modulates cyclin D1, ER and PR in CF41.Mg canine mammary carcinoma cells.

THE DETECTION OF ANTIBODIES AGAINST RIFT VALLEY FEVER NUCLEOCAPSID PROTEIN BY LUMINEX BEAD-BASED MULTIPLEX ASSAY

Izabela Ragan

Author(s): **Izabela K. Ragan**[1], Rachel M. Palinksi[1], Mohammad M. Hossain[1], William C. Wilson[2], David S. McVey[2], and Raymond R. Rowland[1]

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Rift Valley Fever Virus (RVFV) is a zoonotic viral disease that infects ruminants including cattle, sheep, goats, camels and buffalo. A multiplex bead based assay (Luminex) was developed for the detection of RVFV antibodies towards the immunodominant nucleocapsid (N) protein in cattle and sheep. The purpose of this study was to validate the assay for the detection of IgG antibodies against the N protein and apply it to the screening of animals for positive or negative disease status. Well-characterized experimentally infected calf sera from a MP12 vaccine study were used for the validation of the assay. Viral proteins were expressed using *E. coli* and baculovirus systems and then coupled to polysystrene magnetic bead sets for analysis using Luminex xMAP technology. Median Fluorescence Intensity (MFI) results were converted to Sample/Positive ratios to standardize test results. The experimental validation of the assay identified several false positive samples that arose from non-specific binding of antibodies to antigen-coupled beads. A viral antigen (PCV2) coupled bead set was added as a non-specific binding bead control. Results showed MFI values as high as 40,000 for the detection of IgG antibodies against the N protein in positive samples. Compared to the serum neutralization test, Luminex showed earlier detection of antibodies in experimentally infected calves. Our results demonstrate that the Luminex assay provides a rapid and robust diagnostic screening tool for the detection of antibodies against RVFV. The work from this study will be applied to a multiplex assay that can simultaneously detect and screen several ruminant diseases.

Effects of different sources and levels of cobalt on in vitro fermentation

Stephanie Rainbolt

Author(s): Stephanie Rainbolt, Fabian Vargas, Gail Carpenter, Barry Bradford

In ruminant animals, the dietary needs of the animal must be understood to maintain the animal's health, production, and nutrition status. Another important factor to consider in ruminant nutrition is the dietary needs of ruminal bacteria. Although animal requirements of cobalt are well-defined, the requirement for unhindered microbial growth and activity is not known. To determine the requirement of cobalt for rumen microbes, an in vitro fermentation study was carried out to assess microbial activity. Two different sources of cobalt were evaluated: Co carbonate and Co glucoheptonate. Both sources were evaluated at Co concentrations of 0, 0.1, 0.5, 1, 2, 5, 10, and 15 mg/kg, with glucose added to the Co carbonate treatment to equal the mass of glucoheptonate in the other treatment. Ruminal contents were collected from 3 Holstein heifers fed high-forage diets, and after straining out feed particles and removing protozoa, the ruminal inoculates were mixed. During the 24-hour batch culture fermentation, microbial activity was assessed by measurement of gas production, and pH and dry matter disappearance were assessed at the end of the incubation period. We observed no significant effect of Co concentration on final pH or on dry matter disappearance over 24 h. However, Co carbonate decreased final pH compared to Co glucoheptonate (6.32 vs. 6.35 ± 0.008, $P < 0.03$); Co source did not influence dry matter disappearance. Results thus far do not support a specific Co requirement for growth and activity of ruminal bacteria, but nevertheless suggest that Co source may modify fermentation.

Development of a Multiplex Fluorescent Microsphere Immunoassay for Diagnosis of Porcine Disease Complex

Russell Ransburgh

Author(s): Russell Ransburgh, Longchao Zhu, Rui Guo, and Ying Fang

Porcine Disease Complex (PDC) is a significant economic problem for the swine industry. The recent outbreak of porcine epidemic diarrhea virus (PEDV) in the US swine herd emphasizes the need for diagnostic tests that are able to rapidly, simultaneously detect multiple pathogens for disease surveillance and control measurements. In this study, we developed a multiplexed fluorescent microsphere immunoassay (FMIA) for the simultaneous detection of specific antibodies in serum samples from animals infected with PEDV, porcine reproductive and respiratory syndrome virus (PRRSV), swine influenza virus (SwIV), and porcine circovirus (PCV2). Recombinant nucleocapsid proteins of PEDV, PRRSV, SwIV, PCV2 were generated and used as antigens to covalently couple to Luminex fluorescent microspheres with a distinct spectral address. The FMIA was developed based on testing experimentally derived standard positive and negative control sera, and the diagnostic specificity and sensitivity were compared to that generated from the classical enzyme-linked immunosorbent assay (ELISA) or hemagglutination inhibition (HI) test. Based on an evaluation of 2655 serum samples with known serostatus, the multiplex FMIAs reached greater than 95.5% sensitivity and 96.5 % specificity. To test the robustness of the multiplex FMIA, we obtained 332 field serum samples that were seropositive for PEDV, PRRSV, PCV2 or SwIV. Results showed that multiplexing did not affect the diagnostic sensitivity or specificity of each individual assay. This study provides a platform for the development of multiplex assays for detecting various swine pathogens simultaneously in preventing and controlling the PDC.

Clinical Stage of Infection is Critical for the Antemortem Detection of Chronic Wasting Disease in White-Tailed Deer

Chris Siepker

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Chronic wasting disease (CWD) is an efficiently transmitted spongiform encephalopathy of cervids (e.g. deer, elk, and moose), and is the only known prion disease affecting both free-ranging wildlife and captive animals. Complications in tissue acquisition and test sensitivity has made antemortem detection of CWD difficult. At present, biopsies of the recto-anal mucosal-associated lymphoid tissues (RAMALT) and nasal brush sampling have shown to have good sensitivity and are not impractical to collect in live animals. In this study, we evaluated both RAMALT and nasal brush sample collections from 356 captive white-tailed deer (*Odocoileus virginianus*) under quarantine for CWD by real time quaking-induced conversion (RT-QuIC). In order to assess diagnostic sensitivity and specificity, our RT-QuIC findings were compared to that of currently accepted diagnostic RAMALT, obex and retropharyngeal lymph node immunohistochemistry (IHC) staining. We correlated our results with stage of clinical infection as determined by obex scoring, PrP genotype, age, and sex. While the sensitivity of RAMALT RT-QuIC analyses exceeded that of RAMALT IHC (69% vs. 44%) and nasal brush collections (15%), the sensitivity of both biopsies and nasal brushes was dependent primarily on clinical stage of disease. Interestingly, PrP genotype played an important role in whether antemortem detection was possible, with overall diagnostic sensitivity of 96S animals reduced. RT-QuIC had a higher sensitivity in detecting prion seeding in these peripheral, antemortem sampled tissues compared to that sampled by IHC. Our findings further demonstrate the potential and limitations of antemortem sample analyses by RT-QuIC in the identification and management of prion diseases.

Associations between high rectal temperature and behavioral trends in beef cattle

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Behavioral trends are commonly used to identify cattle thought to have respiratory illness in the feedlot. Rectal temperature is used to assess health and determine if treatment is needed. Increased understanding of the relationship between behavioral trends and rectal temperature can influence effective disease identification. This cross-sectional study was conducted to determine association of rectal temperature with time spent at specific locations (feed bunk, waterer, hay, shed), distance traveled, postural changes (transitioning from lying to standing), and the percent time spent lying down. Rectal temperatures were recorded on forty-seven crossbred male beef calves (mean weight: 517 pounds) in two replicates, one week apart. Behavior activity was continuously monitored with accelerometers and a real time location system for 48 hours, with the temperature being taken at the midpoint of that period. Cattle with a rectal temperature greater than or equal to 103.5° F (39.7° C) (replicate 1 n=7; replicate 2 n=10) were considered high temperatures and less than 103.5° F were considered normal (replicate 1 n=40; replicate 2 n=37). Calves with high temperatures (>103.5° F) spent more ($P=0.05$) time lying (52.1% ±4.2) compared to calves with normal temperatures (48.7% ±4.0). Calves with high temperatures had more ($P<0.05$) postural changes (28.6 ±4) over the 48 hour period compared to calves with normal temperatures (24.7 ±3.7). No statistical differences were identified in the distance traveled or locational variables. Data from this trial identified associations between postural behavior and rectal temperature although these differences may not be easily observed clinically.

A fusion protein of Escherichia coli heat-labile toxoid(LTR192G) and spike protein epitopes of the porcine epidemic diarrhea virus induced neutralizing antibodies against PEDV

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Porcine epidemic diarrhea virus (PEDV), a highly contagious porcine enteric pathogen, recently emerged in the US, and caused significant losses to the US swine industry. Currently, there's no effective prevention against PEDV in the US. Vaccination would be the most practical and effective approach to control PED. However, no vaccine has been developed in the US, and an effective vaccine is in great need. In this study, the chimeric gene of heat-labile toxoid(LTR192G) of Escherichia coli which embedding B-cell epitopes of the spike (S) protein of PEDV were constructed and expressed as a fusion protein, which could be detected by anti-PEDV S2 mAb and anti-LT pAb, respectively. The antigenicity of the fusion protein was examined in mouse IP immunization, which showed high antibody titer against PEDV S proteins and LT toxin. Also, the result of the oral immunization of live E.coli with mice, which could secrete holotoxin LT bearing with PEDV epitopes, showed higher antibody titer against PEDV S1 protein and neutralizing antibody against PEDV in immunized mouse serum.

Cochlear Pendrin Expression has a Protective Role in Noise-Induced Hearing Loss

Laura Constance

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Mutations of *SLC26A4* lead to progressive hearing loss in humans. The human gene *SLC26A4* and the mouse ortholog *Slc26a4* encode the chloride bicarbonate exchanger pendrin. Pendrin is expressed in the cochlea, the vestibular labyrinth and the endolymphatic sac of the inner ear. A conventional mouse model, *Slc26a4*$^{\Delta/\Delta}$ that completely lacks pendrin expression, fails to develop hearing, which is a phenotype that is more severe than what is observed in most human patients. Hearing in *Slc26a4*$^{\Delta/\Delta}$ mice can be fully restored by a transgene that drives pendrin expression in the endolymphatic sac (Li et al 2013). Similar hearing thresholds were found in Tg(+);*Slc26a4*$^{\Delta/\Delta}$ and Tg(-);*Slc26a4*$^{\Delta/+}$ mice. Both mice expressed pendrin in the endolymphatic sac but differed in the expression of pendrin in the cochlea. These observations suggested that cochlear pendrin expression is not needed for the development of hearing, however, the possibility remained that cochlear pendrin expression provides an advantage in stress situations such as noise. Here we test the hypothesis that cochlear pendrin expression has a protective role in noise-induced hearing loss.

Noise-induced hearing loss was evaluated in Tg(+);*Slc26a4*$^{\Delta/\Delta}$ mice which lack cochlear pendrin expression and Tg(-);*Slc26a4*$^{\Delta/+}$ mice which express pendrin in the cochlea. Hearing thresholds at 8, 16, and 32 kHz were determined from auditory brainstem responses before and two weeks after noise exposure (Gaussian, 14-18 kHz, 109 dB, 2 hrs). Tg(+);*Slc26a4*$^{\Delta/\Delta}$ and Tg(-);*Slc26a4*$^{\Delta/+}$ mice were raised in a mixed background containing alleles from CBA, C57BL6, and 129S6 strains. Tg(+);*Slc26a4*$^{\Delta/\Delta}$ mice were backcrossed into the 129S6 strain for two generations to reduce differences in the genetic background.

Prior to noise exposure, hearing thresholds of Tg(+);*Slc26a4*$^{\Delta/\Delta}$ (n=58) and Tg(-);*Slc26a4*$^{\Delta/+}$ (n=49) mice were similar. After noise exposure, thresholds at all frequencies were elevated in both types of mice. Thresholds at 8 and 16 kHz were higher in Tg(+);*Slc26a4*$^{\Delta/\Delta}$ mice compared to Tg(-);*Slc26a4*$^{\Delta/+}$ mice.

The data demonstrate that hearing in Tg(-);*Slc26a4*$^{\Delta/+}$ mice that express pendrin in the cochlea is more robust compared to Tg(+);*Slc26a4*$^{\Delta/\Delta}$ mice that lack pendrin expression in the cochlea. Although differences between the genotypes could be due to a segregation of background alleles, the data suggest that the greater robustness of hearing in Tg(-);*Slc26a4*$^{\Delta/+}$ mice is due to a beneficial effect of cochlear pendrin expression. This opens the prospect that restoration of pendrin function in the cochlea may slow the progression of hearing loss in humans carrying mutations of *SLC26A4*.

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The Prenatal Rise in the Utricular K^+ Concentration is Delayed in Mice Lacking Pendrin.

Fei Zhou

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SLC26A4 and the murine ortholog *Slc26a4* encode the anion-exchanger pendrin that is expressed in the inner ear. Mutations of *SLC26A4* cause syndromic or non-syndromic hearing loss associated with a prenatal enlargement of the membranous labyrinth. This enlargement is replicated in the mouse model *Slc26a4*$^{\Delta/\Delta}$. *Slc26a4*$^{\Delta/\Delta}$ mice fail to acquire hearing and thereby model a severe phenotype. Recent studies have shown that cochlear pendrin expression is not required for the acquisition of normal hearing in mouse models that do not develop an enlargement of the membranous labyrinth (Choi et al 2011; Li et al 2013). These findings suggest that prevention of the enlargement is a strategy to preserve hearing in the absence of functional pendrin expression. A recent study implicated the vestibular labyrinth as the origin for fluid secretion that leads to the enlargement of the entire membranous labyrinth (Kim et al 2010). The goal of the present study was to measure K^+ concentrations ($[K^+]$) in utricular endolymph of *Slc26a4*$^{\Delta/+}$ and *Slc26a4*$^{\Delta/\Delta}$ mice during pre- and postnatal development as a first step toward a mechanistic understanding of fluid secretion.

$[K^+]$ and transepithelial potentials (V_{te}) were measured with double-barreled ion-selective electrodes in isolated *in vitro* superfused temporal bones. Although tissues likely experienced anoxia, measurements of $[K^+]$ were assumed to resemble normoxic levels since stable $[K^+]$ have been recorded for as long as 50 min of anoxia (Mori et al 1987). Temporal bones were obtained from perinatal and adult *Slc26a4*$^{\Delta/+}$ and *Slc26a4*$^{\Delta/\Delta}$ mice.

At embryonic (E) day 16.5, $[K^+]$ was ~10 mM in both genotypes and V_{te} was -10 mV in *Slc26a4*$^{\Delta/+}$ mice and -2 mV in *Slc26a4*$^{\Delta/\Delta}$ mice. $[K^+]$ rose in *Slc26a4*$^{\Delta/+}$ mice at E17.5. This rise appeared delayed by ~1 day in *Slc26a4*$^{\Delta/\Delta}$ mice. In adult mice, $[K^+]$ and V_{te} were 150 mM and -22 mV in *Slc26a4*$^{\Delta/+}$ and 132 mM and -5 mV in *Slc26a4*$^{\Delta/\Delta}$ mice.

The observations in adult mice of a negative V_{te} in *Slc26a4*$^{\Delta/+}$ and normal high [K+] in *Slc26a4*$^{\Delta/+}$ and *Slc26a4*$^{\Delta/\Delta}$ mice is consistent with the assumptions that measurements at anoxic conditions resemble normoxic $[K^+]$. Utricular $[K^+]$ were similar to cochlear $[K^+]$ at E16.5. The rise of utricular $[K^+]$ at E17.5 suggests an earlier onset of K^+ secretion and the delay in the rise of $[K^+]$ in *Slc26a4*$^{\Delta/\Delta}$ mice is consistent with the larger fluid volume.

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