PHI ZETA

The Honor Society of Veterinary Medicine
Epsilon Chapter

November 5, 2014

Research Emphasis Day

AUBURN UNIVERSITY
COLLEGE OF VETERINARY MEDICINE

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EPSILON CHAPTER
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welcomes you to our

PHI ZETA RESEARCH DAY FORUM
November 5, 2014

We want to thank all the presenters, their co-investigators and mentors for their participation in this annual event.

We also want to thank all sponsors for their generous support without which this event would not be possible:

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PHI ZETA RESEARCH DAY FORUM

NOVEMBER 5, 2014 – VETERINARY EDUCATION CENTER

8:30: BREAKFAST Buffet - VEC Lobby

9-11: MORNING Presentations - Overton Auditorium

Veterinary Students

9:00	Bonnie Coats	*Corynebacterium pseudotuberculosis* seroprevalence in horses in Alabama
9:15	Alison Emmert	Impaired Ketogenesis in $HCA_2^{-/-}$ Mice: The Role of FGF21

Graduate Students

9:30	Rosemary Cuming	Ocular Injection Characteristics and *in vitro* Release Profiles of Voriconazole from PLGA-PEG-PLGA Hydrogel
9:45	Patrick Flannery	PPM1A phosphatase regulates pregnane X receptor-mediated cytochrome p450 3A4 gene expression in liver hepatocytes
10:00	Alex Gillen	Aberdeen Knot Security in Large Gauge Suture
10:15	Amanda Hazi	Dual Selected Phage Ligands for Tumor Targeted Nanomedicines
10:30	Yen Chen Juan	The spike protein furin cleavage site in feline coronavirus is not the sole determinant of conversion to feline infectious peritonitis virus
10:45	Farruk Lutful Kabir	miRNA-141 Regulates INK4A Tumor Suppressor Gene that is Frequently Defective in Spontaneous Canine Breast Cancer Models

11–12:30 POSTER Presentations- VEC Lobby

11:00 - 12:30	Poster Session – Presenters are present from 11:00 – 12:30

11:30-1:00: LUNCH Buffet - VEC Lobby

1:00-2:45 AFTERNOON Presentations - Overton Auditorium

Graduate Students (continued)

1:00	Steven Kitchens	A one year study of factors associated with environmental *Salmonella* prevalence in a multi-species animal facility
1:15	Kh. Shamsur Rahman	Disordered and polymorphic, highly surface exposed protein regions preferentially harbor immunodominant B-cell epitopes of *Chlamydia* spp.
1:30	J. Forrest Shirley	Experimental Infection of Calves with *Salmonella* Enteritidis: Disease Variability and Peripheral Lymph Node Invasion
1:45	Zhao Yang	Functions of DPLIY motif and helix 8 of Human Melanocortin-3 Receptor

Post-graduate/Faculty

2:00	Payal Agarwal	Integrins: Co-conspirator of CAR mediated Adenovirus 5 Transduction in Cells
2:15	Tekla Lee-Fowler	Determining the influence of obesity on T regulatory lymphocytes in dogs
2:30	Thomas Passler	Real Time Detection of Bovine Viral Diarrhea Virus Using Detection Dogs

2:45-3:30: BREAK Buffet - VEC Lobby

3:30: Faculty Research Awards

4:00: KEYNOTE LECTURE

Encounters with Water Moulds: Characterization of a new group of Oomycete pathogens

Dr. Amy Grooters
Professor-Veterinary Medicine, Louisiana State University

Dr. Grooters is a Professor of Companion Animal Medicine in the Department of Veterinary Clinical Sciences at Louisiana State University, and provides clinical service as a small animal internist in the Veterinary Teaching Hospital. She earned her DVM degree from Iowa State University in 1989 and completed a rotating internship at the Virginia-Maryland College of Veterinary Medicine in 1990. She then completed residency training in small animal internal medicine at The Ohio State University in 1993, and taught small animal internal medicine at the University of Missouri College of Veterinary Medicine for two years before joining the faculty at Louisiana State University in 1995. Dr. Grooters is a Diplomate of the American College of Veterinary Internal Medicine, and has served as an Associate Editor for the Journal of Veterinary Internal Medicine since 2003. She was the recipient of the Thomas J. Walsh Award for the outstanding research abstract at the 10th annual Focus on Fungal Infections symposium, and has twice been awarded the Carl J. Norden Distinguished Teacher award at Louisiana State University.

Dr. Grooters' clinical and research interests include fungal and pseudofungal disease in small animals, with a focus on pythiosis, lagenidiosis, zygomycosis, and opportunistic fungal infections. She has developed a number of serologic and molecular tests for the diagnosis of pytiosis and lagenidiosis, and offers fungal-related diagnostic services to veterinarians through her research laboratory. Current studies focus on the identification and characterization of novel oomycete pathogens in the genera *Lagenidium* and *Paralagenidium*, and exploration of their taxonomic relationships with previously-described oomycetes.

PLEASE JOIN US FOR THE INDUCTION AND AWARDS BANQUET! Veterinary Education Center, College of Veterinary Medicine, Auburn University.

Everybody is invited! PLEASE NOTE – Inductees and Presenters (and a guest) are invited to attend at no cost as the guests of the Epsilon Chapter.
Tickets $30/person - Reserve ticket with Dr. Josephson (josepem@auburn.edu, 334-844-5423) - Deposit check for ticket with Dr. Eleanor Josephson, 109 Greene Hall, or pay at the banquet in the **Veterinary Education Center**. For the purposes of catering, please let Dr. Josephson know that you plan to attend even if you will pay at the door.

5:30 Doors open

6:00 BANQUET – Veterinary Education Center, College of Veterinary Medicine, Auburn University.

7:00 INDUCTION of new Phi Zeta Members

AWARD CEREMONY to honor winners of the Platform and Poster Competitions

Posters

Undergraduate Students

Paulina Platten	Validation of Canine TRAIL in Canine Tumor Cells

Veterinary Students

Morgan Adams	The Effect of N-butylscopolammonium Bromide (Buscopan®) on Equine Intra-abdominal and Abdominal Perfusion Pressures
Jordan Ayers	Validation and Genetic Profiling of Canine Mammary Tumors
Bonnie Coats	*Corynebacterium pseudotuberculosis* seroprevalence in horses in Alabama
Whitney Dettmer	Establishing reference intervals in dogs using the Multiplate® platelet function analyzer
Lyndsey Hayden	Validation of Equine Distal Metacarpal III Cartilage Thickness Through Multiple Imaging Modalities
D. Andrew Hestad	Pharmacokinetics of altrenogest (Regu-Mate®) in lactating mares and suckling foals: Potential impacts on foal performance and fertility
Sarah Larosche	The Herbicide, Atrazine, Mimics Restraint Stress in Adrenal Morphological Changes but Potentiates Stimulation of Aldosterone Synthesis
Anne Maguire	Effects of intracranial gene therapy on peripheral pathology in cats with inherited neurologic disease
Rachel Maloney	Evaluation of Vetericyn Plus™ Pinkeye Spray as a Healing Aid for Infectious Bovine Keratoconjunctivitis caused by *Moraxella bovis*
Brandon Mason	Plant-based Omega-3 stearidonic acid (SDA) enhances anti-proliferative activity of docetaxel in human prostate cancer cells
Matthew Miller	Effects of Vaccination Age on IBV-Specific Antibody Production and Avidity in Chickens
Dominique Peel	Characterization of the Ocular and Neuromuscular Pharmacodynamic Effects of Rocuronium in Raptors
Anna Richburg	Impact of Cyclophosphamide and Doxorubicin on Antimicrobial Minimum Inhibitory Concentration and Resistance in Escherichia coli

Veterinary Student (continued)

Ashley Sharpe	Investigation of alternate sources for the isolation of endothelial progenitor cells from horses
Matthew Vogel	Microgliosis in Tay-Sachs Jacob Sheep
Elizabeth Yanchak	Evaluation of the Ability of Adenovirus Vectors to Infect Canine Mast Cell Tumors
Niloofar Yeganeh	Effects of bisphenol A (BPA) and diethyl (hexyl) phthalate (DEHP) on epididymal development in the rat

Graduate Students

Rosemary Cuming	Subconjunctival Space Temperatures in Horses
Patrick Flannery	Diindolylmethane, a naturally occurring compound, induces cytochrome p450 3A4 and multidrug resistance protein 1 gene expression by activating human pregnane X receptor
Alex Gillen	The Ideal Square and Surgeon's Knots Evaluated in Large Gauge Suture
Shan He	Defect in MAPK Signaling as a Cause for Monogenic Obesity Caused by Inactivating Mutations in the Melanocortin-4 Receptor Gene
Michelle Hoffman	Neuroprotective mechanisms of carnitinoid antioxidants in rodent models of mitochondrial dysfunction
Jennifer Koziol	*In vitro* efficacy of anti-protozoal compounds against *Tritrichomonas foetus*
Taya Marquardt	Evaluation of Mitoxantrone in CHOP-like Chemotherapy for Canine Lymphoma
Mattie McMaster	Modified Equine Teno-Fix Tenorrhaphy Repair
Abdul Mohin Sajib	Towards Multilevel Targeting of Adenoviral Vectors to Malignant Cells of Lymphocyte Origin
Marike Visser	Population Pharmacokinetics and Pharmacodynamics of Itraconazole in Panamanian Golden Frogs (*Atelopus zeteki*)

Post-graduate/Faculty

Fatma Abdel-Maksoud	Effect of prenatal exposure to di (2-ethylhexyl) phthalate (DEHP) and bisphenol A (BPA) on sexual differentiation and steroid hormone secretion capacity in male rats

Benjamin Newcomer	Vaccinal prevention of reproductive disease due to bovine viral diarrhea virus: a meta-analysis
Paul Walz	Impact of passive immunity induced by maternal vaccination on subsequent immunization and disease-sparing in early-weaned beef calves challenged with highly virulent BVDV

Veterinary Student Platform Presentations

Corynebacterium pseudotuberculosis seroprevalence in horses in Alabama

B Coats,[1] B Bradford,[2] AJ Stewart,[1] M Barba,[1] T Passler,[1] AA Wooldridge,[1]

[1]College of Veterinary Medicine, Auburn University, Auburn, AL [2]School of Veterinary Medicine, Tuskegee University, Tuskegee, AL

Introduction. *Corynebacterium pseudotuberculosis* infection in horses, also called pigeon fever, has not been reported in the equine population of Alabama. This study is investigating the seroprevalance of *C. pseudotuberculosis* to determine the significance of detectable titers, which may result from cross-reaction with the ruminant strain of *C. pseudotuberculosis*; residual titers from previous exposure; or false positive reaction due to soil *Corynebacterium* species.

Methods. We intend to obtain samples from 450 horses, with 5-15 samples from each of the 50 Alabama counties. Sixty-one veterinarians practicing in the state were contacted to collect blood from client horses for SHI (synergistic hemolysis inhibition) titer testing. Each owner was asked to complete a comprehensive questionnaire detailing history of previous disease, exposure to ruminants, and travel history.

Results. 217 horses (29 counties) are currently represented. Of these, 95/217 (43.8%) had titers <1:8 (negative), 38/217 (17.5%) at 1:8, 44/217 (20.3%) at 1:16, 28/217 (12.9%) at 1:32, 6/217 (2.8%) at 1:64, 4/217 (1.8%) at 1:128, 1/217 (0.4%) at 1:256 and 1/217 (0.4%) at 1:512. No correlation between exposure to small ruminants and a higher antibody titer was observed. Similarly, no significant correlation between traveling to or having lived in an endemic state and having a higher antibody titer was noted.

Conclusions. With 44% of our samples collected and analyzed, positive titers that would indicate active infection to *C. pseudotuberculosis* are rare in Alabama. However, the incidence of positive titers in a negative population questions the accuracy of the SHI antibody titer test.

Acknowledgments. Thank you to our mentor, Dr. Allison Stewart; also, thanks to Dr. Tara Riddick, Dr. Tim Stewart, Dr. Hal Noble, Dr. Barbara Benhart, Dr. Justin Mims, Dr. Lynn Hall, Dr. Misty Edmondson, Dr. Aime Johnson, Dr. Robyn Wilborn, Dr. Rosemary Cuming, Dr. Robert Carson, Dr. J.R. Crum, Dr. Jonathan Whitley, Dr. Lauren Marks, Dr. April Andrews, Dr. Randy Plaisance, Dr. William Bledsoe, Dr. John Sudduth, Dr. Justin Howard, Dr. Carrie Wright, Dr. William Maddox and Dr. Judd Easterwood for collecting serum samples. Funding was provided by:
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Impaired Ketogenesis in $HCA_2^{-/-}$ Mice: The Role of FGF21

Alison R Emmert, Olga Norris, Emily Graff[1,2], Robert Judd[2]

Dept. of Pathobiology[1] and Dept. Of Anatomy, Physiology & Pharmacology[2], College of Veterinary Medicine, Auburn University

Introduction. Hydroxycarboxylic acid (HCA) receptors, primarily found in adipocytes, are a family of G-protein coupled receptors capable of sensing and responding to changes in nutrient availability. The HCA_2 receptor binds beta-hydroxybutyrate (βOHB), the primary ketone body found in circulation during times of fasting or starvation. Ketone bodies act as an alternative fuel source once the body has depleted its glycogen and started to break down adipose tissue reserves through lipolysis. Previous studies have demonstrated that in the presence of elevated βOHB, HCA_2 inhibits lipolysis and regulates the release of free fatty acids. This suggests that mice lacking the HCA_2 receptor would experience increased lipolysis and ketogenesis during a prolonged fast. Contrary to proposed theories, work from our laboratory discovered that prolonged fasting does not result in significantly different body weight, glucose, insulin or NEFA concentrations in wild type mice compared to $HCA_2^{-/-}$ mice. Instead, $HCA_2^{-/-}$ mice displayed a diminished increase in βOHB rather than the dramatic increase anticipated. Given these findings, we propose that $HCA_2^{-/-}$ mice have alternative mechanisms to regulate lipolysis, preserve fat stores, and prevent ketogenesis during a prolonged fast. A likely candidate is fibroblast growth factor 21 (FGF21), a critical regulator of lipolysis, triglyceride metabolism, and ketogenesis during fasting.

Methods. Male and female mice (14 weeks of age) were used for this study: WT fed (male, n = 7; female, n = 7), WT fasted (male, n = 7; female, n = 7), $HCA_2^{-/-}$ fed (male, n = 6; female, n=6) and $HCA_2^{-/-}$ fasted (male, n = 7; female, n = 6). Mice were separated into individual housing and allowed to acclimate for 1 week, after which mice were either continued *ad libitum* on standard laboratory mouse chow or were fasted. Free access to water was provided at all times. For the fasted mice, food was removed at 5am to ensure the initial time point for all groups represented a fed sample. Mice were weighed and approximately 3-4 drops of blood were collected from the submandibular vein at 0, 5, 12, 24, and 36 hours. Fecal material was removed from the cage during each sample collection. After the final blood sample was obtained, mice were euthanized, and tissues (adipose and liver) were divided for fixation and flash freezing. Liver lipid deposition was evaluated on H&E stained sections of formalin fixed tissue as well as Oil Red O stained sections of frozen tissue. In addition, liver triglyceride content was quantified. Serum and hepatic mRNA expression of FGF21 were also measured.

Results. While there was a significant increase in liver triglycerides due to fasting in WT mice, $HCA_2^{-/-}$ mice had increased liver triglyceride content in both the fed and fasted state. As expected, WT mice displayed a significant increase in both gene expression and serum protein concentrations of FGF21 during fasting. However, altered to absent regulation of FGF21 was observed in $HCA_2^{-/-}$ mice in response to fasting.

Conclusions. These findings suggests that mice lacking the HCA_2 receptor have increased liver triglyceride content and altered FGF21 regulation. This data supports possible mechanisms for the previous findings of impaired ketogenesis and reduced lipolysis during fasting in $HCA_2^{-/-\ mice}$.

Acknowledgements. Merial Veterinary Scholars Program, Boshell Diabetes and Metabolic Disease Research Program, Diabetes Action Research and Education Foundation, National Institute of Health, Auburn College of Veterinary Medicine.

Graduate Student Platform Presentations

Subconjunctival Space Temperatures in Horses

Rosemary Cuming, Sue Duran, Allison Stewart, Anne Wooldridge and Eva Abarca

Department of Clinical Sciences, College of Veterinary Medicine, Auburn University, AL.

Introduction. An ideal eye drug delivery technique should be minimally invasive with a drug depot that delivers a steady drug concentration over an extended period of time. Thermosensitive hydrogels injected into the subconjunctival space (SCS) show promise as a sustained delivery system targeting the cornea and anterior chamber in horses. The aim of this study was to describe the subconjunctival space temperature in normal horse eyes.

Methods. Temperatures measurements were obtained from five adult horses (10 eyes) within 10 minutes of induction of general anesthesia. SCS temperatures were measured under the dorsal and ventral bulbar conjunctiva using a type T thermocouple and rectal temperatures were measured using an electronic rectal thermometer. Results were analysed for range, mean and standard deviation. A student's paired t–test was used to determine if there was a significant difference between rectal and subconjunctival temperatures, with $P < 0.05$ considered significant.

Results. SCS temperatures ranged from 33.3°C-35.3°C (mean ± SD; 34.29°C ± 0.69°C) in the dorsal, and 33.5°C-35°C (34.33°C ± 0.50°C) in the ventral, bulbar SCS. The mean rectal temperature (37.39°C ± 0.93°C) was within reference ranges. Subconjunctival temperatures were significantly lower than rectal temperatures ($P = 0.0004$).

Conclusions. The temperature range in the bulbar SCS of horses in this study was 33.3°C-35.3°C i.e. 2.5°C-4.1°C below recorded rectal temperatures. These findings were consistent with previously reported findings in human and rabbit eyes, and support use of a thermosensitive hydrogel with a gelation setpoint of 32°C-36°C for subconjunctival injection in horses.

Acknowledgments. Dr. Jennifer Taintor, Dr. Hui-Chu Lin, Glen Sellers and Shelby Whitman for facilitating horse availability and temperature collection.

Diindolylmethane, a naturally occurring compound, induces cytochrome p450 3A4 and multidrug resistance protein 1 gene expression by activating human pregnane X receptor

Patrick C Flannery[1,2], Kodye L Abbott[1,2], Elaine S Coleman[1], Sridhar Mani[3], Samuel Temesgen[4], Wen Xie[5], Satyanarayana R Pondugula[1,2]

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[3]Albert Einstein Cancer Center, Albert Einstein College of Medicine, New York, NY.
[4]Department of Pathobiology, College of Veterinary Medicine, Nursing and Allied Health, Tuskegee University, AL.
[5]Center for Pharmacogenetics and Department of Pharmaceutical Sciences, University of Pittsburgh, Pittsburgh, PA.

Introduction. Activation of human pregnane X receptor (hPXR)-regulated expression of cytochrome p450 3A4 (CYP3A4) and multidrug resistance protein 1 (MDR1) plays an important role in mediating adverse drug interactions. Given the common use of natural products as part of adjunct human health behavior, there is a growing concern about natural products for their potential to induce undesired drug interactions through the activation of hPXR-regulated CYP3A4 and MDR1. Here, we studied whether 3,3'-Diindolylmethane (DIM), a natural health supplement, could induce hPXR-mediated regulation of CYP3A4 and MDR1 in human hepatocytes and intestinal cells.

Methods. PXR transactivation and Rhodamine 123 accumulation assays were performed to determine the function of PXR and MDR1, respectively. Quantitative RT-PCR and western blot analyses were conducted to study gene and protein expression. RNAi experiments were performed for gene knockdown studies.

Results. DIM, at its physiologically relevant concentrations, not only induced hPXR transactivation of CYP3A4 promoter activity but also induced gene expression of CYP3A4 and MDR1. DIM decreased intracellular accumulation of MDR1 substrate rhodamine 123, suggesting that DIM induces the functional expression of MDR1. Pharmacologic inhibition or genetic knockdown of hPXR resulted in attenuation of DIM induced CYP3A4 and MDR1 gene expression, suggesting that DIM induces CYP3A4 and MDR1 in an hPXR-dependent manner.

Conclusions. The results support our conclusion that DIM induces hPXR-regulated CYP3A4 and MDR1 gene expression. The inductive effects of DIM on CYP3A4 and MDR1 expression caution the use of DIM in conjunction with other medications metabolized and transported via CYP3A4 and MDR1, respectively.

Acknowledgments. We thank Drs. Tao and Pinkert for sharing their microplate readers. This work was supported by the Animal Health and Disease Research Grant, Auburn University Research Initiative in Cancer and Auburn University Startup Funds to Pondugula SR.

The Ideal Square and Surgeon's Knots Evaluated in Large Gauge Suture

Alex Gillen[1], Amelia Munsterman[1], Fred Caldwell[1], Ramsis Farag[2], Reid Hanson[1]

[1]Department of Clinical Sciences, Auburn University, AL
[2]Department of Engineering, Samuel Ginn College of Engineering, Auburn University, AL

Introduction. This study investigated optimum knot configuration using large gauge suture (2USP and 3USP polyglactin 910; 2USP polydioxanone). We hypothesized surgeon's knots would: 1) have higher relative knot security (RKS) and knot holding capacity (KHC) than square knots; 2) have higher volume and weight than square knots; 3) require an additional throw to provide a secure knot at the end of a suture line, compared to the start.

Methods. Surgeon's and square knots were tested under linear tension on a Universal testing machine, recording failure mode and KHC. A digital micrometer measured knot size. An ANOVA and Tukey's post hoc test compared strength between number of throws, suture, suture size, and knot type. Significance was set at $p \leq 0.05$.

Results. Comparing knots with the same number of throws, there was no significant difference in KHC (p=0.299) and RKS (p=0.317) between square and surgeon's knots. There was no significant different between volume (p=0.128) and weight (p=0.310) of square and surgeon's knots. Overall, there was no significant difference in KHC (p=0.255) and RKS (p=0.267) between knots at the start and end of a suture line, however, seven throws were required to form a secure knot ending a suture line, compared to six throws at the start.

Conclusions. Comparing knots tested, there was no significant difference between strength and size of square and surgeon's knots with the same number of throws. An additional throw was required to form a secure knot at the end, compared to the start, of a continuous suture line.

Acknowledgements. The investigation was funded, in part, by the Birmingham Racing Commission. Suture was generously donated by Ethicon US, LLC.

Dual Selected Phage Ligands for Tumor Targeted Nanomedicines

Amanda Hazi, James Gillespie, and Valery Petrenko

Department of Pathobiology, College of Veterinary Medicine, Auburn University, AL

Introduction. Targeting nanomedicines can greatly increase the amount of drug delivered to tumors, allowing for greater cytotoxic effect with the same dosage in comparison with untargeted nanomedicines. Using phage ligands for targeting is advantageous because of the low cost to produce the phage libraries in addition to the ease of screening whole libraries for potential targets. The cell population of a patient's tumor is immensely diverse, unlike cell cultures maintained in the laboratory. Within each population of tissues, including cancers, there are receptors which allow for ligands to selectively bind to the tissue of interest. It was hypothesized that phage ligands derived from two cell cultures would have a greater variability in their binding capabilities through a sub-library of phage ligands that have peptides to both cancer lines.

Methods. Isolation of landscape phage clones that bind specifically to lung (CALU-3) and pancreatic cancer (PANC-1) cell lines was achieved using standard biopanning procedures. Here, biopanning was modified to include a second target cell line, PANC-1, that was exchanged for CALU-3 cells following the first round of selection. Unique clones were analyzed for their relative affinity to both target cell lines, Small Airway Epithelial (SAE) cells, and medium containing 10% FBS. Top clones, as identified based on their selectivity to the desired targets, were stripped of their pVIII major coat protein by isopropanol precipitation of phage DNA and mixed with liposomal doxorubicin (LipoDox) to produce cancer-specific, doxorubicin-loaded liposomes. Finally, the efficacy of cancer-specific LipoDox was determined by incubation with target cell lines for 24 hours followed by cell viability assessment using a 3-(4,5-Dimethylthiazol-2-yl)-2,5-Diphenyltrazolium Bromide (MTT) assay.

Results. The inclusion of a second target cell line during our selection scheme produced 87 unique phage clones that specifically bind with both PANC-1 and CALU-3 cells. We identified the top five phage ligands based on their selectivity towards target cells and their specificity to discriminate between normal and cancer cells Cell viability studies show that LipoDox modified with these selected cancer-specific phage major coat proteins can produce a decrease in the percentage of viable cancer cells *in vitro* as compared to unmodified LipoDox or non-specifically targeted LipoDox under identical conditions.

Conclusions. We have shown that ligands derived from phage display peptides can be introduced into pre-formed liposomal nanomedicines with minor modifications and increase the therapeutic index of existing chemotherapies. By modifying the selection scheme, we were able to identify phage clones from two very different cell lines with high selectivity to both target cell lines. Several of the phage ligands identified from this selection scheme were also identified following selection against breast cancer and prostate cancer cells. These conclusions suggest that universal cancer specific receptors may be present on the surface of many types cancer cells that can be utilized for optimized and possibly universal cancer treatment.

Acknowledgments. We would like to thank the Petrenko and Torchillin lab for their collaboration and assistance during this project. This work was funded by an NIH grant to VAP 1U54CA151881-0 and by Auburn University Research Initiative in Cancer (AURIC).

The spike protein furin cleavage site in feline coronavirus is not the sole determinant of conversion to feline infectious peritonitis virus

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Department Pathobiology, College of Veterinary Medicine, Auburn University, Auburn, AL, USA

Introduction. Feline coronavirus (FCoV) infection is ubiquitous in domestic cat populations. As yet unknown mutations result in extra-intestinal replication of the virus, causing feline infectious peritonitis (FIP), a highly fatal immune-mediated disease. Recently, it was proposed that mutations within the furin cleavage site of FCoV spike protein that reduce the ability of the cellular proteinase, furin, to cleave the spike protein, convert FCoV to FIP.

Methods. A quantitative fluorescence resonance energy transfer (FRET)-PCR was developed that amplifies a fragment of the spike gene sequence encoding the furin cleavage site. As an FIPV population, we tested nucleic acids extracted from peritoneal effusions of cats that had clinical signs of FIP and were positive for coronavirus M protein mRNA in their peritoneal effusions. M protein mRNA indicates extraintestinal replication of feline coronavirus in FIP lesions, the functional correlate of the FIP pathogenic mechanism. Amplification products were sequenced, and the amino acid sequence of the furin cleavage site was determined. We also tested 26 samples of FCoV-positive juvenile shelter cats as a non-FIPV population with this PCR. The translated furin cleavage sites of the FIPV and non-FIPV populations of sequences were then comparatively evaluated for mutations that affected furin cleavage efficiency.

Results. In the FIPV population, 26 (56.5%) out of 46 amplification products had critical mutations within the furin cleavage site that are known to reduce cleavage efficiency. The sequence of the canonical core motif of the FCoV furin cleavage site is R-S/A-R-R-S. These 26 feline coronavirus spike protein amplification products variably showed mutations at each of the positions of the core motif. Twenty-four out of 26 sequences harbored only one mutation within the core motif, whereas two samples harbored two mutations. The 4^{th} position had the highest mutation frequency (30.8%), with changes from arginine (R) to serine (S). In the non-FIPV population, one out of 26 samples harbored a single mutation within the core motif, with the 2^{nd} position changed from serine (S) to threonine (T). Other samples harbored no mutations within the core motif and shared identical sequences of the furin cleavage site.

Conclusions. Mutated sequences versus non-mutated sequences within the furin cleavage site of the FIPV population are approximately equally distributed, whereas in the non-FIPV population only one out of 26 sequences was mutated. This result indicates a highly significant ($p<10^{-4}$; two-tailed Fisher Exact test) difference in mutation frequency of the furin cleavage site between FIPV and non-FIPV feline coronavirus spike protein sequences. This strongly suggests that the presence of a mutation within the furin cleavage site converts FCoV to FIPV. However, the absence of the mutation in 43.5% of FIPV spike proteins indicates that such a mutation is not essential for the conversion of feline coronavirus to feline infectious peritonitis virus, and that this conversion may also be mediated by other mutations different from the furin cleavage site.

Acknowledgments. This investigation was funded by the Molecular Diagnostics Laboratory and an Auburn University Presidential Fellowship to Y.-C. Juan.

miRNA-141 Regulates INK4A Tumor Suppressor Gene that is Frequently Defective in Spontaneous Canine Breast Cancer Models

Farruk M Lutful Kabir[1,2], Patricia DeInnocentes[1], and R. Curtis Bird[1,2]

[1]Department of Pathobiology, College of Veterinary Medicine
[2]Auburn University Research Initiative in Cancer (AURIC), College of Veterinary Medicine, Auburn, AL

Introduction. miRNAs are evolutionarily conserved and endogenous small structural RNAs of 20-22 nucleotides that post-transcriptionally suppress gene expression in a sequence specific manner. Expression of these small RNAs is tightly regulated during development and in normal tissues and is frequently altered in cancers. The cyclin dependent kinase inhibitors (CKIs) act as powerful cell cycle regulators and endogenous tumor suppressors. The INK4A gene encodes alternatively spliced p16 and p14 CKI tumor suppressors defects in which have been associated with a number of cancers including human and canine breast cancers. The objective of this study was to experimentally evaluate the post-transcriptional regulation of the INK4A gene by miRNA-141 in spontaneous canine mammary tumor (CMT) cell lines.

Methods. Comprehensive miRNA expression profiles have been evaluated by miRNA QPCR arrays. The miRNA-141 binding to the 3'-untranslated region (3'-UTR) of the target INK4A mRNA was validated by functional 3'-UTR luciferase reporter assays.

Results. For the first time, the 277 most abundantly expressed and highly characterized miRNAs from the canine genome have been screened in three CMT cell lines (CMT12, CMT27 and CMT28) that were characterized for leading INK4 gene (p16, p14 and p15) defects. Several miRNAs that were altered in the CMT cell lines could potentially target these INK4 genes. Particularly, miRNA-141, identified as one of the members of the altered miRNA panel, has been validated for binding the 3'-UTR target sequence of the INK4A mRNA in the CMT cell lines. This experimental evidence demonstrated that the miRNA-141 endogenously down-regulates INK4A mRNA because it is frequently overexpressed in the CMT cell lines.

Conclusions. The very first evidence of comprehensive expression profiles of the 277 canine miRNAs in CMT cells revealed a large number of altered miRNAs (up-/down-regulated) that might serve as potential oncogenic and tumor suppressor targets for advancing the development of future therapeutic reagents. The miRNA-141 has been experimentally validated for the INK4A 3'-UTR target binding in the CMT cell lines providing an essential mechanism for the post-transcriptional regulation of the INK4A tumor suppressor gene that is frequently mutated in canine breast cancer. These findings strengthen the validity and use of canine breast cancers as appropriate models for the study of human breast cancers.

Acknowledgments. Auburn University Research Initiative in Cancer (AURIC).

A one year study of factors associated with environmental *Salmonella* prevalence in a multi-species animal facility

Steven R. Kitchens[1], James C. Wright[1], Sue H. Duran[2] and Stuart B. Price[1]

[1]Department of Pathobiology, College of Veterinary Medicine, Auburn University, AL
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Introduction. Diseases caused by serotypes from the bacterial genus *Salmonella* can have a major impact on animal and human health. Studies have been conducted to assess factors that contribute to the prevalence of certain *Salmonella* serotypes on single-species food animal production operations. Little research has been performed to examine the factors contributing to *Salmonella* serotype prevalence in multi-species animal production facilities such as veterinary teaching hospitals. We hypothesize that *Salmonella* can move between proximally located animal facilities and pastures and that specific factors increase the likelihood of isolating environmental *Salmonella* serotypes in these locations.

Methods. Over a one year period, 349 samples were collected from various large animal facilities and pastures within a veterinary school. Data such as season, rainfall within 24 hours of sampling, resident species, facility, environment, and sample type was recorded to assess factors that contribute to increased prevalence of environmental *Salmonella* contamination. Samples were processed for *Salmonella* isolation as described in the USDA FSIS *Microbiology Laboratory Guidebook's* procedure for "Isolation and Identification of *Salmonella* from Meat, Poultry, Pasteurized Egg, and Catfish Products and Carcass and Environmental Sponges". *Salmonella* isolates were submitted to the National Veterinary Services Laboratories, Ames, IA (NVSL), or Biovet, Inc., for serotyping. Data was analyzed with Statistical Analysis System (SAS)(version 9.3; SAS Institute Inc., Cary, NC) and JMP Pro (version 11.0.0, SAS Institute Inc., Cary, NC).

Results. Of the 349 samples obtained, 137 (39%) samples were positive for at least one *Salmonella* serotype. *Salmonella* was recovered from the majority of facilities and areas sampled regardless of animal species exposure. Two serotypes, *S.* Muenster and *S.* Cerro, were isolated from multiple sites across seasons. Serotypes *S.* Typhimurium and *S.* Anatum were found during the summer season. A Chi-Square for independence ($p \leq 0.05$) was used for univariate analysis of factors associated with *Salmonella* isolation. The factors shown to be significant were season, resident species, recent rainfall, and environment. The highest number of *Salmonella*-containing samples was recovered during the summer season and from areas exposed to dairy cattle. *Salmonella* isolates were recovered more frequently during rainy weather and from man-made animal facilities compared to other variables.

Conclusions. The significantly increased frequency of *Salmonella* isolation from environmental samples exposed to dairy cattle indicates that this species is either the source of this pathogen, or is serving as an amplifying host for *Salmonella*. The recovery of cattle-associated serotypes supports this conclusion. This preliminary study is part of a larger investigation aimed at examining the dynamics of *Salmonella* serotype movement among different animal species raised in proximity to one another.

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Disordered and polymorphic, highly surface exposed protein regions preferentially harbor immunodominant B-cell epitopes of *Chlamydia* spp.

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Introduction. Prediction of B cell epitopes by standard prediction algorithms is unsatisfactory. Using mono-specific mouse hyperimmune sera against all 9 chlamydial species, we identified immunodominant B-cell epitopes and developed peptide immune-assays for detection of species-specific anti-chlamydial antibodies with high specificity and sensitivity. Using data from the large number of peptides tested, we developed bioinformatics approaches to predict immunodominant B-cell epitopes with high accuracy. Additionally, we also investigated the optimum length of peptides containing B-cell epitopes for capturing antibodies in serological assays.

Methods. We selected the 18 most immunodominant proteins that showed reactivity with antisera, and which contained at least one peptide sequence with high antibody reactivity as well as one peptide with no antibody reactivity. For ranking of 77 algorithms of protein physicochemical properties and evolutionary information, scores of 21 amino acid (AA) sliding windows of the annotated proteins were used in discriminant analysis. The performance of these algorithms as binary classifiers (discriminants) of epitope versus non-epitope regions were also evaluated with receiver operating characteristic (ROC) curves. To determine optimum peptide length for ELISAs, reactivities of peptides of different lengths were compared.

Results. Functional B cell epitopes were 1.27- to 1.87-fold enriched for amino acids E, D, T, A, P, and 0.20- to 0.79-fold depleted of K, H, M, L, Y, W ($p < 0.001$). B-cell epitopes had 87% increased intrinsic disorder tendency, i.e. undefined 3-dimensional structure in the natively folded protein, than non-epitope regions ($p < 10^{-5}$). Backbone torsion angle fluctuations of the epitopes were 78% higher, and AA mobility was 77% higher ($p < 10^{-5}$). The second best parameter was the mutation rate expressed as relative sequence polymorphism which was 80% higher in the epitope regions ($p < 10^{-5}$). Increased relative solvent accessibility (surface exposed tendency) was the third best parameter to predict B-cell epitopes. Epitopes were also enriched for predicted coils/loops (64%) and had high hydrophilicity scores (57%). Disorder tendency combined with sequence polymorphism achieved B cell epitope prediction with 80% sensitivity and 80% specificity. Sixteen AA-long peptides produced a 20-fold higher signal than 8-11 AA-long peptides, and 20-40 AA-long peptides produced a 3-fold increased signal over 16 AA peptides ($p \leq 0.01$).

Conclusions. Hydrophilic, surface exposed B cell epitope regions with high disorder tendency accommodate mutations more frequently compared to the remaining protein regions. Such regions are preferentially recognized by antibodies, and our finding of immunodominant B cell epitopes in protein regions with the highest rate of sequence change is consistent with immunoselection of these exposed epitopes. These findings will help to identify optimal peptide antigens and B cell epitopes in *Chlamydia* spp., and in serology of infectious agents in general. Such prediction of immunodominant epitopes with high accuracy is also important for designing peptide vaccines for infectious agents such as *Chlamydia*.

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Experimental Infection of Calves with *Salmonella* Enteritidis: Disease Variability and Peripheral Lymph Node Invasion

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Introduction. In 2012, the Centers for Disease Control and Prevention investigated a human outbreak of *Salmonella enterica* serovar Enteritidis (S.E.) linked to ground beef products from a single supplier. This outbreak was unusual because S.E. is most closely associated with poultry, where it has been implicated in several human outbreaks associated with ingestion of contaminated eggs. To explain this unusual route of transmission of S.E. to humans, we hypothesize that S.E. can cause disease in cattle, where it disseminates from the bovine gut and invades peripheral lymph nodes. Unlike the mesenteric lymph nodes, the peripheral lymph nodes are not removed at slaughter following carcass processing and thus are a potential source of ground beef contamination from infected cattle.

Methods. To test this hypothesis, three pairs of 5-7 week-old calves were challenged orally with between 5.0 x 10^9 and 1.3 x 10^{10} CFUs of a bovine S.E. isolate. Following inoculation, daily fecal samples were enumerated for S.E. and rectal temperatures were measured twice daily. Blood and four peripheral lymph nodes were cultured post-mortem.

Results. Oral challenge of calves with S.E. produced mixed results. A fever spike from 103.6°F to 106.0°F was noted for all calves for days two or three post inoculation. Although each calf received a high dose of S.E., fecal shedding of the organism was not always high and was variable amongst the calves. Calf T1 shed low amounts of S.E. (10^2 - 10^3 CFU/g feces); calves C1, C3 and C4 shed moderate amounts of S.E. (10^4 - 10^6 CFU/g feces); and calves C2 and C2 shed high amounts of S.E. (10^6 – 10^8 CFU/g feces). Bacteremia was noted for two amongst the three most severely affected calves and S.E. was recovered from the peripheral lymph nodes of the same three calves.

Conclusions. This work shows that S.E. is able to cause disease and invade peripheral lymph nodes in a calf model of infection and therefore is a concern for ground beef contamination. Ongoing research is examining a treatment cocktail of seven bacteriophages to reduce S.E. disease and prevent peripheral lymph node contamination. The presence of S.E. in the peripheral lymph nodes of the three most severely affected calves and its presence in the blood of two of these three suggests that S.E. produces a systemic disease in calves. Considering that the S.E. used in these studies was originally isolated from a bovine source, future experimental calf infections using a different S.E. isolate are necessary to determine whether host source of S.E. affects disease outcome.

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Functions of DPLIY motif and helix 8 of Human Melanocortin-3 Receptor

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Introduction. The melanocortin-3 receptor (MC3R) is a member of family A G protein-coupled receptors (GPCRs). The MC3R remains the most enigmatic of the melanocortin receptors with regard to its physiological functions, especially the role in energy homeostasis. The N/DPxxY motif and the eighth helix (helix 8) in the carboxyl terminus of GPCRs have been identified to be important for receptor expression, ligand binding, signal transduction and internalization. To gain a better understanding of the structure-function relationship of MC3R, we have performed systematic study of all the residues of these two parts.

Methods. We identified the function of each residue in the DPLIY and helix 8 of MC3R using alanine-scanning mutagenesis. We studied the cell-surface expression, ligand binding and signaling properties of these 20 residues. ERK1/2 signaling pathway was also studied.

Results. We showed that although all mutants were expressed normally on cell surface, eleven residues were important for ligand binding and one was indispensable for downstream cAMP generation. F347A showed constitutive activity in cAMP signaling while all the other mutants had normal basal activities. All of 9 mutants we selected showed normal basal ERK1/2 phosphorylation levels. The pERK1/2 levels of six binding- or signaling-defective mutants were enhanced upon 1 μM NDP-MSH stimulation.

Conclusions. The DPLIY motif and Helix 8 were important for MC3R activation and signal transduction. The unbalanced cAMP and pERK1/2 signaling pathways suggested the existence of biased signaling in MC3R mutants. Our data led to a better understanding of the structure-function relationship of MC3R.

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Post-graduate/Faculty Platform Presentations

Integrins: Co-conspirator of CAR mediated Adenovirus 5 Transduction in Cells

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Introduction. High efficiency and broad host range transduction, easy genome manipulation, non-integration in the host genome, and well characterized molecular biology have made adenoviral vectors the most commonly employed vector in cancer gene therapies. Adenovirus type 5 (Ad5) infects cells by binding to the coxsackie-adenovirus receptor (CAR) followed by internalization mediated by binding of RGD motifs to integrins ($\alpha_v\beta_3$, $\alpha_v\beta_5$) on the cell surface. Low levels of Ad5 infection of hematopoietic cells in human and mouse have been linked with CAR deficiency as a limiting factor. We have previously reported that a low level of integrins on canine lymphoma cells is a potential obstacle to Ad5 infection. In the current report we have evaluated CAR and integrin levels at the molecular level using qRT-PCR in a broader range of canine tumor cells including lymphoma, mast cells, melanocytes, breast tumor, macrophage and normal canine fibroblasts, PBMCs and HEK293 cells as controls.

Methods. Lymphoma cells; 1771 and OSW, mast cells; BR and MPT1 were cultured in total RPMI media. Canine mammary tumor (CMT28), canine melanocytes (CML) 10 and 7, NCF, DH82, and HEK293 cells were cultured in total DMEM media. Cells were incubated with the Ad5G/L virus vector (expressing green fluorescent protein and luciferase) for 1 hour at 3 different multiplicities of infection (MOI) (10, 100, and 1000 virus particles/cell), and were cultured for an additional 47 hours before analyzing Ad5 infection using flow cytometry. mRNA levels of CAR, alphaV, beta3, and beta5 integrins were compared in all cell lines by relative quantification using qRT-PCR. All mRNA levels were normalized to beta-actin.

Results. Ad5G/L infection in all the cell lines demonstrates that Ad5G/L vector can successfully infects CMT28, DH82, HEK293, CMl10, CML7, and NCF lines. Successful Ad5G/L infection was evident by green fluorescence in cells 48 hrs. post-infection at all 3 MOIs in NCF, CMT28, CML7, CML10, DH82, BR and HEK293There was limited or no Ad5G/L infections in 1771, OSW, PBMCs, and MPT1 mast cells. qRT-PCR results show low level of integrins in MPT1 cells in comparison to CMT28, DH82, and BR cells. Although CAR mRNA levels were comparatively higher in MPT1 cells.

Conclusions. Ad5G/L is a replication incompetent virus vector whose ability to infect cells is mediated by CAR and integrins. Ad5G/L after infecting cells will express both GFP and luciferase. After measuring green fluorescence using fluorescence microscopy and flow cytometry, it was evident that all cells except lymphoma and MPT1 cells (hematopoietic origin) were infected by Ad5G/L. BR cells are an exception that can be attributed to de-differentiation of these cells owing to their long history of cell culture. According to our hypothesis, integrins play a vital role in Ad5 transduction in cells, in addition to CAR. Lack of integrins and not CAR may be the primary reason for the inability of Ad5G/L to infect hematopoietic cells. Our qRT-PCR results agree with our hypothesis, as MPT1 (non-infected by Ad5G/L) cell's levels of alphaV, beta3, and beta5 integrin mRNA were significantly lower than CMT28, DH82, and BR cells (infected by Ad5G/L), although levels of CAR were equal or greater than to those in infectable cells. The levels of mRNA in the lymphoma cell lines and PBMCs are now being examined to determine if they support this hypothesis. Ultimately, these results explain the failure of adenoviral vectors to transduce cells of hematopoietic origin and indicate the need to generate alternate target tropisms for adenovirus 5.

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Determining the influence of obesity on T regulatory lymphocytes in dogs

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Introduction. Obesity is a pro-inflammatory condition that commonly affects dogs. Increased circulating leptin has been associated with obesity in dogs, and human and murine studies have found correlations between leptin concentrations and natural T regulatory lymphocytes (Tregs). Tregs are key players in immune regulation, and suppression of Tregs can result in pro-inflammatory conditions such as allergic, respiratory, and autoimmune diseases. We hypothesized that peripheral Treg percentages would be decreased in obese dogs compared to non-obese dogs and this would correlate with an increase in leptin in obese dogs.

Methods. Twenty healthy medium to large breed client-owned dogs; ten dogs of normal body condition (N BCS) and ten overweight/obese dogs (OW BCS) were included. At a single time point, weight, age, sex, body condition score (BCS), and morphometric measurements were obtained, and blood was collected for flow cytometry and leptin analysis. Body condition score was based on the Purina scale (1-9). Flow cytometry was performed following a previously validated assay to establish %CD24+CD5+foxP3+ Tregs. Circulating leptin concentrations were determined via an enzyme-linked immunosorbent assay previously validated in canines.

Results. Percentage of Tregs was significantly lower in the OW BCS group compared to the N BCS group ($p=0.003$), and leptin concentrations were significantly higher in the OW BCS group compared to the N BCS group ($p=0.012$). A weak positive correlation was found between leptin and Treg % in the OW BCS group ($r=0.607$; $p=0.0478$) while no correlation was evident in the N BCS group. The OW BCS group had significantly higher BCS ($p=<0.001$) and morphometric measurements ($p=<0.001$). In the OW BCS group, there was a strong positive correlation between leptin concentrations and BCS ($r=0.851$; $p=0.0009$). The OW BCS group was significantly older than the N BCS group ($p=0.003$); however, there was no significant correlation between age and any other variables. While the BCS of female dogs was significantly higher ($p=0.031$), there was no effect of sex on Treg % or leptin.

Conclusions. Canine obesity results in a decreased peripheral T regulatory lymphocyte population as compared to a group of normal body conditioned canines. This could theoretically predispose these individuals to a pro-inflammatory state. While a weak positive correlation between Treg% and leptin was identified in the OW BCS group, studies in other species have indicated a negative correlation. Further study will be needed to investigate this correlation in canines.

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Real Time Detection of Bovine Viral Diarrhea Virus Using Detection Dogs

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Introduction. Currently, the detection of pathogens relies on collection of samples from infected animals or contaminated environments, transporting samples to a laboratory, and subsequent laboratory testing, which significantly delays response times and containment efforts. Canids have the potential to be a mobile real-time olfactory sensing technology to detect the presence of pathogens affecting people, animals, and plants. Previous studies demonstrated that disease-specific volatile organic compounds are released from diseased tissues. These volatile organic compounds have unique odor signatures that could be detected by the dog's highly sensitive olfactory system. The purpose of this proof-of-concept study is to evaluate the ability of dogs to detect a pathogen in real time.

Methods. Two dogs were trained to detect bovine viral diarrhea virus (BVDV) propagated in cell culture on an eight-arm scent wheel apparatus. The handler was blinded and the dogs were operated off-lead to reduce handler influence. The dogs were presented cytopathic and non-cytopathic BVDV samples containing 5×10^4 to 5×10^5 $CCID_{50}$ (cell culture infective doses, 50% endpoint) per 0.5 milliliter of media. Distractor samples were also utilized and were identical to BVDV samples except they did not contain BVDV. In addition, bovine herpes virus and bovine parainfluenza 3 propagated identically to BVDV were also used as distractors. Approximately 89% of the total number of samples for each dog were blank, only containing distractors and no BVDV virus.

Results. The dogs correctly identified the BVDV samples with a sensitivity of 96.8% (n = 32) for Dog 1 and 82.6% (n = 52) for Dog 2. The dogs correctly identified the distractor samples as non-target samples with a specificity of 99.3% (n = 289) for Dog 1 and 98.4% (n = 453) for Dog 2.

Conclusions. The results of this proof-of-concept study indicate that pathogen-detection-dogs offer the potential for real time detection of pathogens. Dog 1 had a high rate of sensitivity for correctly identifying the positive virus samples while Dog 2 had a moderate detection rate. Both dogs demonstrated a high level of specificity. This capability could identify pathogens during natural disease outbreaks or deter acts of bioterrorism/agroterrorism. Future efforts will evaluate multiple variations of the virus (e.g. cytopathic, non-cytopathic, dried virus, killed virus), whether or not dogs can find the virus in an operational setting, and what type of canine performance characteristics are needed to optimize pathogen detection.

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Undergraduate Poster Presentations

Validation of Canine TRAIL in Canine Tumor Cells

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Introduction. Cancer in dogs shares several important characteristics with human cancers including histological appearance, biological behavior, tumor genetics, inter-individual and intra-tumoral heterogeneity and metastasis to relevant distant sites. Each year, approximately 4 millions new cancer cases are diagnosed in the dog population. Thus, we not only need to design better therapeutics to treat canine cancers, but this vast pool of cancer patients can also be used to better understand the biology of cancer and to evaluate new drugs and approached to treat cancer, be it canine or human. Gene therapy is a promising therapeutic approach that offers several advantages over traditional cancer therapies including selective targeting of cancer cells and minimal other side effects. Tumor necrosis factor-related apoptosis-inducing ligand (TRAIL) is a type II transmembrane protein, which interacts with its receptors (DR4 and DR5) and induces apoptosis in a variety of tumor cells. Several studies have also shown that TRAIL does not induce apoptosis in normal cells, thus highlighting its potential as a cancer therapeutic. The goal of this study was to clone, express and determine apoptotic inducing properties of canine TRAIL in a wide variety of canine tumor cells.

Method. To elucidate biological properties of canine TRAIL, its extracellular domain was synthesized *in-vitro*. The extracellular domain of canine TRAIL was also tagged at its N-terminus with a signal peptide from the canine immunoglobulin (Ig) kappa-chain, for its efficient secretion. This construct was cloned into a mammalian expression plasmid containing the cytomegalovirus (CMV) promoter for high-level constitutive expression. A reverse orientation clone was used as a negative control. The recombinant plasmids were transfected into HEK-293 cells. Cell lysates and supernatants were isolated, resolved on SDS-PAGE gel, blotted and probed with Anti-Human TRAIL antibody. The recombinant plasmids were also transfected into CMT28 (canine mammary tumor), CML10 (canine melanoma) and NCF cells (Normal canine fibroblasts). After 72hrs, transfected cells were assayed with a cell proliferation kit to determine growth inhibitory effects of canine TRAIL.

Results. The extracellular domain of the canine TRAIL was successfully cloned into pDC311 plasmid. The orientation of recombinant plasmids was also confirmed by sequencing, restriction enzyme digestion and agarose gel electrophoresis. Western blot analysis confirmed that canine TRAIL was expressed both in cell lysate and also actively secreted into supernatant. We then transfected various normal and cancerous cell types and performed proliferation assays to determine whether the cells were undergoing apoptosis. Although transfection efficiencies were low, both canine mammary adenocarcinoma (CMT 28) and melanoma (CML 10) cells showed some cell death while normal canine fibroblasts (NCF), which were used as a control, did not. Additional canine and human tumors such as osteosarcoma will be tested in the future. **Conclusions:** Based on the cells that we have tested so far, we have been successful in validating that the canine TRAIL molecule inhibits growth of canine cancer cells. However, due to low transfection efficiency, we have cloned extracellular domain of canine TRAIL into an adenoviral plasmid (pAdenoX adenoviral system). This replication deficient vector expressing canine TRAIL will be further used to characterize its biological properties.

Veterinary Student Poster Presentations

The Effect of N-butylscopolammonium Bromide (Buscopan®) on Equine Intra-abdominal and Abdominal Perfusion Pressures

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Introduction. Compliance of the abdominal walls and the volume of abdominal contents directly influences intra-abdominal pressure (IAP). Elevated IAP, defined as the pressure contained within the abdominal cavity, can result in intra-abdominal hypertension (IAH), which may progress to abdominal compartment syndrome (ACS). Abdominal perfusion pressure (APP), the difference between mean arterial pressure (MAP) and IAP, is a useful measure of visceral perfusion and a valuable prognostic indicator for critically ill patients. A lack of knowledge exists regarding treatment for IAH or ACS in equine patients. This study attempted to narrow this gap by evaluating the effect of n-butylscopolammonium bromide (Buscopan) on IAP and APP. Buscopan, an anticholinergic drug, is primarily utilized in equine medicine to decrease intestinal spasm through inhibition of smooth muscle contraction. We hypothesized that administration of Buscopan at 0.3mg/kg could increase IAP and conversely decrease APP, due to this transient ileus.

Methods. Eight horses were utilized in a dual cross-over design. A solid state pressure transducer (Codman & Shurtleff, Inc) was inserted into the peritoneal space, and an arterial catheter was placed in the transverse facial artery to directly measure the IAP and the MAP respectively. After a 30 minute equilibration period, horses were administered Buscopan or an equivalent volume of saline. During the next 120 minutes, IAP and MAP were recorded at 1 minute intervals; heart rate (HR) and respiratory rate (RR) were noted every 5 minutes.

Results. The data obtained for IAP, APP, RR, HR and MAP was analyzed using a mixed model for repeated measures analysis of variance, and Dunnett's test for multiple comparisons to compare each time period to the baseline values. Analysis indicated no within group significant difference from baseline for the measurements obtained for IAP, APP, MAP, or RR. There was no significant change in heart rate ($P > 0.05$) from baseline in the saline control group. In the group administered Buscopan, HR was significantly increased significantly from minute 5 to minute 45 ($P < 0.003$), and remained above 48 bpm for an average of 60 minutes (SD ± 16.9 mins).

Conclusions. Administration of Buscopan did not affect the measured values for IAP, and caused no statistical differences in APP, MAP, and RR as compared to baseline and the saline control. In the Buscopan treated group, a significant increase in HR from baseline values was observed, which is longer than the 30 minutes reported by the manufacturer for this side effect. Based on these findings, it would be prudent to use caution when assessing HR in horses administered Buscopan for longer than previously recommended.

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Validation and Genetic Profiling of Canine Mammary Tumors

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Canine mammary tumors (CMTs) are widely prevalent—the frequency of neoplasia in the breast tissue of dogs is the highest of domestic species and three times the prevalence of breast cancer in women. Of tumors found in unsprayed female dogs, 50% are CMTs which makes it the most common tumor type in the unsprayed female dog. CMTs are diagnosed via palpation, meaning they are already large enough to be felt upon examination and they often carry a grim prognosis for the effected patient.

This study used QrtPCR with conserved primers for six genes associated with canine mammary tumors and breast cancer and examined them in seven established CMT cells lines. By validating that these genes are present—and the expression pattern there in—a genetic profile of theses tumors can be ascertained. The primers were then used in freshly obtained tumors to create a profile and provide insight into the phenotypes of those tumors based on the expression patterns.

By creating a gene expression profile for CMTs allied to neoplastic transformation, tumors can be better phenotyped so veterinarians can know the most effective courses of treatment and associated prognoses. Identifying the gene expression patterns associated with CMTs allows for a better understanding of malignant behavior of CMTs; this not only has huge implications in management of disease but also in the development of treatments to better combat this highly aggressive form of cancer. Also, this study opens the possibility of further comparison of canine and human breast cancer geno- and phenotypes, by looking at common mutations associated with human breast cancer as well as in CMT. Validation of canine disease as a model system for breast cancer in women will allow development and testing of new treatments for the benefit of both canine and human patients.

Corynebacterium pseudotuberculosis seroprevalence in horses in Alabama

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Introduction. *Corynebacterium pseudotuberculosis* infection in horses, also called pigeon fever, has not been reported in the equine population of Alabama. This study is investigating the seroprevalance of *C. pseudotuberculosis* to determine the significance of detectable titers, which may result from cross-reaction with the ruminant strain of *C. pseudotuberculosis*; residual titers from previous exposure; or false positive reaction due to soil *Corynebacterium* species.

Methods. We intend to obtain samples from 450 horses, with 5-15 samples from each of the 50 Alabama counties. Sixty-one veterinarians practicing in the state were contacted to collect blood from client horses for SHI (synergistic hemolysis inhibition) titer testing. Each owner was asked to complete a comprehensive questionnaire detailing history of previous disease, exposure to ruminants, and travel history.

Results. 217 horses (29 counties) are currently represented. Of these, 95/217 (43.8%) had titers <1:8 (negative), 38/217 (17.5%) at 1:8, 44/217 (20.3%) at 1:16, 28/217 (12.9%) at 1:32, 6/217 (2.8%) at 1:64, 4/217 (1.8%) at 1:128, 1/217 (0.4%) at 1:256 and 1/217 (0.4%) at 1:512. No correlation between exposure to small ruminants and a higher antibody titer was observed. Similarly, no significant correlation between traveling to or having lived in an endemic state and having a higher antibody titer was noted.

Conclusions. With 44% of our samples collected and analyzed, positive titers that would indicate active infection to *C. pseudotuberculosis* are rare in Alabama. However, the incidence of positive titers in a negative population questions the accuracy of the SHI antibody titer test.

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Establishing reference intervals in dogs using the Multiplate® platelet function analyzer

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Introduction. Platelet aggregation is an important element of platelet function and is not routinely evaluated in veterinary species. Multiplate® platelet function analyzer is an impedance analyzer that measures platelet aggregation in a small sample of whole blood. The goal of this study was to establish reference intervals for healthy dogs and to investigate factors that influence measurement of platelet aggregation with this instrument.

Methods. Study included 33 dogs of various breeds, ages and weights. Only clinically healthy dogs were included in the study and were deemed healthy by having values within normal reference intervals for a complete blood cell count (CBC), chemistry panel, standard coagulation tests (PT, aPTT, fibrinogen) and thromboelastography (TEG). Blood samples were collected via jugular venipuncture then transferred to tubes containing the appropriate anticoagulant. Citrated whole blood was used for the Multiplate®, and samples were evaluated at 30 minutes after blood collection. Limited samples were also evaluated at 60 minutes. Adenosine-diphosphate (ADP) and collagen were used to activate platelet aggregation; 0.9% sodium chloride was a negative control. Area under the curve (AUC) and Aggregation units (AU) were measured in duplicate in each test cell.

Results. Overall, ADP proved to be a reliable agonist whereas collagen produced inconsistent results. Calcium chloride was added in 20 samples and resulted in a mean ADP AUC value of 82.4 U (56-120) and mean aggregation of 101.9 AU (62.6-131.3) at 30 minutes after the blood draw. Average values tended to be lower at 60 minutes but were not significantly different. Aggregation using ADP as an agonist without recalcification was evaluated in 13 dogs and yielded significantly lower platelet aggregation at 30 minutes. Adjustment of sample pH to ~7.0 through addition of lactic acid resulted in significantly lower ADP AUC and aggregation values.

Conclusions. The findings indicate it is important to establish a consistent protocol when operating the Multiplate® as many factors can influence the results.

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Validation of Equine Distal Metacarpal III Cartilage Thickness through Multiple Imaging Modalities

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Introduction. Equine osteoarthritis (OA) is a degenerative condition commonly affecting the articular cartilage (AC) of high motion joints due to impact trauma. AC is a complex network of hydrophilic protein aggrecans and type II collagen fibers which, create a low friction-gliding surface that allows for joint movement and load bearing. Early detection of OA is of great interest to many orthopedic surgeons; therefore, creating an accurate equine joint model is essential not only for equine orthopedic studies, but also human studies due to the extreme similarities in cartilage anatomical properties. The purpose of this study was to compare cartilage thickness measurements of distal metacarpal III (dMCIII) taken from 7 Tesla magnetic resonance imaging (7T-MRI) and computed tomography (CT) images to histological measurements and needle probe thickness testing.
Methods. Three equine cadaver limbs with no history of degenerative joint disease were transected below the carpus prior to imaging. 7T-MRI, CT, and CTA imaging studies were performed on each limb. Using the medical engineering software, Mimics®, three-dimensional models of the AC on dMCIII were generated for thickness measurement purposes. AC sections were obtained from the medial paracondylar groove of distal MCIII by disarticulation of the fetlock joint. Samples were processed, stained with an H & E, Trichrome, or Safranin O stain, and measurements of AC thickness were taken perpendicular to the joint surface to the tidemark; as well as, to the beginning of the subchondral bone. Needle probe thickness data acquired from prior studies done by this laboratory was used for comparison.
Results. Needle probe data found no statistical difference in AC thickness between horses across the surface of the dMCIII condyle ($P>0.05$). Average AC thickness measurements taken from Mimics® 3D models, found AC thickness to be 0.42mm ± 0.068 and 0.38mm ± 0.11 for left and right forelimbs respectively, with no statistical difference between the left and right forelimbs ($p>0.05$). Average cartilage thickness was found to be 0.5mm ±0.05mm with histological measurements. Histological measurements showed statistical difference when comparing both horse one and horse two to horse three ($p<0.0001$) and no statistical difference was shown when comparing horse one to horse two ($p=0.2643$). A statistical difference is seen when comparing the average MRI and histology AC thickness measurements to the measurements acquired with the needle probe testing ($p=0.0108$ and 0.0339 respectively). No statistical difference is observed when comparing MRI and histology AC thickness measurements ($p=0.7579$). An ordinary one-way ANOVA with a Tukey's multiple comparisons test was used to make these comparison.
Conclusions. It can be concluded from this study that 7T-MRI using the T2-weight FLASH pulse sequence can accurately evaluate equine articular cartilage of distal metacarpal III. These measurements mirror those taken from histology slides to the tidemark. When comparing needle point testing and histological measurements there is a difference observed, which can be hypothesized as a lack of sensitivity of the needle test in differentiating the calcified from uncalcified cartilage further testing needs to be done to validate what the needle probe testing is measuring.
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Pharmacokinetics of altrenogest (Regu-Mate®) in lactating mares and suckling foals: Potential impacts on foal performance and fertility

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Introduction. Altrenogest (Regu-Mate ®) is labeled for use in healthy mares as an estrus suppression method, but is also commonly used in lactating broodmares for the purposes of reproductive management. To date, no research has investigated the pharmacokinetics of altrenogest in the lactating mare, and whether or not altrenogest may be bioavailable to the foal through the dam's milk. Data in cattle and humans confirms that progesterone and related compounds are secreted in milk, yet to date no research has investigated this possibility in horses. Current research from our lab demonstrates that uterine development is occurring in the neonatal filly and confirms the presence of progesterone receptor in developing uterine tissue. In this study, our objectives are to determine the concentration of altrenogest in 1) the blood of the treated mares to confirm adequate dosing and absorption of the drug in all mares, 2) the milk of the mare in order to develop a pharmacokinetic profile for lactating mares, and 3) the blood of the suckling foal to confirm transfer of this steroid hormone to the foal via consumption of milk from the treated mare.

Methods. A total of ten mare/foal pairs from the Equine Reproduction Center teaching herd will be sampled. The sampling protocol described below will begin when each foal reaches 6 weeks of age to correspond with the peak lactation period of the mare. Altrenogest dosing and sample collection will occur over the course of four days and each mare/foal pair will be subjected to only one four-day sampling period. For blood and milk collection, 6 mL of blood will be collected from the jugular catheters of both mares and foals at each time point described. At the same time points, five mL of mammary secretions (milk) will be harvested from the mare. The initial blood and milk samples will be taken at time zero (T = 0) prior to the first administration of altrenogest (altrenogest is given daily). Thereafter, blood (for serum) and milk samples will be collected at the following timepoints: 30, 60, 90 minutes, 2, 4, 6, 12, 18, and 24 hours after the administration of the initial altrenogest dose. Blood and milk will then be collected at 6, 12, 18 and 24 hours after each subsequent dose of altrenogest for a total sampling time of 72 hours.

Results. Results will be obtained by analysis of previously obtained milk and serum samples, as described above. Altrenogest will be quantitated in serum and milk by the Auburn University Veterinary Teaching Hospital Clinical Pharmacology Laboratory using liquid chromatography- tandem mass spectrometry (LC-MS/MS) methods and modified as necessary for validation in equine serum and milk. Serum (dam and foal) and milk altrenogest concentrations versus time data will be subjected to non-compartmental pharmacokinetic analysis. Pertinent parameters to be described in both serum and milk will include (but not be limited to): maximum and minimum drug concentrations (Cmax, Cmin) and time to each (Tmax, Tmin), area under the curve (AUC), mean residence time (MRT), and disappearance rate constants (kd) and half-life (t ½).

Conclusions. For this study, analysis of the collected data has not yet occurred. Conclusions concerning this study will be drawn based on whether or not detectable concentrations of altrenogest are present in the dam's milk and in the foals serum.

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The Herbicide, Atrazine, Mimics Restraint Stress in Adrenal Morphological Changes but Potentiates Stimulation of Aldosterone Synthesis

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Introduction. Atrazine (2-chloro-4-ethylamino-6-isopropylamino-s-triazine; ATR) is one of the most commonly used broadleaf herbicides. ATR has been shown to inhibit both pulsatile luteinizing hormone (LH) release and preovulatory LH surge. Recent evidence suggests that some of these effects are mediated by ATR's effects on the hypothalamic-pituitary-adrenal (HPA) axis. ATR treatment leads to an increase in adrenocorticotropic hormone (ACTH) and, subsequently, corticosterone (CORT). We have shown that adrenalectomy will abolish ATR's inhibition of LH pulsatile release.

Methods. To determine how ATR activates the adrenal gland, adult ovariectomized Sprague Dawley rats were gavaged daily with vehicle, ATR (6.5, 50 or 100 mg/kg) or restrained for 30 min for 4 consecutive days. One hour after the final treatment, trunk blood was taken along with adrenal glands. The right adrenal was fixed for histology and the left was fast frozen for RNA quantification. A second cohort of animals was similarly treated but on the final day of treatment animals were treated with dexamethasone to inhibit endogenous ACTH release and then treated with either angiotensin II (Ang II) or ACTH. Blood samples were taken before and 5, 15, 20 and 25 minutes after treatment and assayed for aldosterone (ALDO) and CORT

Results. ATR treatment led to a reduction in zona glomerulosa thickness and aldosterone synthase immunoreactivity with no change to zona fasciculata or 11β-hydroxylase. There were no differences found between ATR treated animals and stressed animals in adrenal morphology. However, Ang II treatment elicited a higher level of ALDO in ATR treated animals than both control and stressed animals. Interestingly, both ATR and stressed animals displayed reduced ALDO levels compared to vehicle treated animals when stimulated with ACTH. There was no difference in CORT levels between any groups after ACTH stimulation.

Conclusions. In conclusion, ATR-induced adrenal activation and morphological changes mimic those of repeated stress whereby there is a reduction in the adrenal cortical region responsible for aldosterone synthesis, however, when stimulated by Ang II but not ACTH, the remaining cells capable of producing ALDO in the adrenal of ATR treated animals, produces several fold higher level of hormone than in control and stressed animals. These findings have profound ramifications on our understanding of how a ubiquitous herbicide commonly found in water can alter adrenal response to stimulation.

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Effects of intracranial gene therapy on peripheral pathology in cats with inherited neurologic disease

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Introduction. GM_1 gangliosidosis is a storage disease that results from a deficiency in the β-galactosidase (βgal) enzyme and subsequent GM_1 ganglioside accumulation in lysosomes. Clinical signs are primarily neurologic, with affected children often dying before 5 years of age. To treat cats with GM1 gangliosidosis in preclinical studies, feline βgal was delivered via adeno-associated virus (AAV) vectors using either of 2 capsid proteins, AAV1 or AAVrh8. Vector was injected into the thalamus and deep cerebellar nuclei at ~2 months of age, before the onset of symptoms. Cats were followed for either 16-weeks post-treatment or to the humane endpoint (~8 months of age in untreated GM1 cats). In a previous study, βgal was normalized throughout the brain and spinal cord in treated cats, which lived >4 times longer than untreated cats, often with few or no clinical signs. However, peripheral disease was suspected in a subset of treated animals.

Methods. The current study analyzed βgal distribution to the following peripheral tissues: liver, heart, skeletal muscle, small intestine, kidney, spleen, adrenal gland.
4-Methylumbelliferone (4MU) Enzyme Assay *(McCurdy et al, 2014)*-Tissue homogenates were incubated at 37°C with 4MU fluorogenic substrates for βgal, hexosaminidase (A and total), and α-mannosidase. Protein concentration was determined by the method of Lowry, and specific activity was expressed as nmol 4MU/mg protein/hour.
Echinocytosis**-**Blood smears were made from 6-8 month old cats. Percent of echinocytes was calculated by manual counting of 40X microscopic fields (n=3).

Results. βgal activity, <5% of normal in untreated GM1 tissues, was restored to ~25% in liver of all treated cats and to 30% in the heart of AAV1-treated cats at humane endpoint. In general, only 10% of normal levels of lysosomal enzyme activity is considered sufficient to prevent clinical disease. Secondary lysosomal enzymes (such as α-mannosidase) were elevated in some peripheral tissues of untreated GM1 cats, with variable normalization after treatment. Echinocytosis in untreated GM1 cats also was variably normalized after treatment.

Conclusions. The enzyme assay results demonstrate that intracranial gene therapy corrects pathology in some peripheral organs of GM1 cats, though more global distribution to the periphery may be desirable. Echinocytosis data, an easily accessible and potentially useful biomarker, reflected minimal correction of the disease phenotype in most peripheral organs.

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Evaluation of Vetericyn Plus™ Pinkeye Spray as a Healing Aid for Infectious Bovine Keratoconjunctivitis caused by *Moraxella bovis.*

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Introduction. Infectious Bovine Keratoconjunctivitis, commonly called "pink eye", is painful condition affecting beef and dairy cattle worldwide. Although, it is not a fatal disease, it has a tremendous negative economic impact on the cattle industry including significant losses in body weight and milk production. It has been estimated that annual losses associated with only decreased gain from infected cattle exceeds 150 million dollars. Currently, antibiotics are the treatment of choice. However, new legislation is making it necessary to reduce and or eliminate the use of antibiotics in food producing animals. Hence, Vetericyn Plus™ Pinkeye Spray may be an effective alternative to antibiotics that aids in healing. Therefore, we hypothesized that Vetericyn Plus™ Pinkeye Spray will significantly aid in corneal healing following experimentally induced Infectious Bovine Keratoconjunctivitis due to *Moraxella bovis* and will not result in any detectable tissue residues following ocular administration.

Methods. 30 dairy bull calves were randomly assigned to 3 groups for a single eye randomized controlled challenge. Groups 1 and 2 were inoculated with the infectious agent *Moraxella bovis* after a standardized .6mm corneal lesion was induced using n-heptanol. Corneal irritation and/or lesions are required for the organism to cause an active infection in the eye. We used group 3 as an additional control group, in which the calves received an inoculation of *Moraxella bovis* without a corneal lesion. Group 1 was the experimental treatment group, and Vetericyn Plus™ Pinkeye Spray was administered twice a day. Group 2 was the inoculated control group and 0.9% Saline was administered twice a day for 10 days. Each animal was given a pain score twice a day (based on blepharospasm, discharge and tearing) and photographs were taken to allow the digital measurement of the change in size of the lesion. Starting on day 11, each animal in Group 1 was evaluated for detectable tissue residues in the serum, plasma, milk, liver, fat, muscle and urine. Liver, muscle and fat biopsies were only performed on days 11 and 17.

Results. At this point, only the pain scores of the first 6/10 treatment and saline groups were able to be analyzed. Between Days 1 and 2, the Vetericyn group decreased in pain score by 2.67/4.0, or 67%. The Saline group decreased by 9%, or 0.33/4.0 in pain score. After the 10 day study, pain scores for the Vetericyn group decreased by 58%, or 2.33/4.0 whereas the Saline group decreased by 43%, or 1.66/4.0.

Conclusions. Based on the data collected to this point, the Vetericyn group was able to exhibit a lower pain score faster than the Saline group. In the end, the Vetericyn group had a pain score that had decreased by 58% compared to the Saline group that decreased by only 43%. Those numbers allows us to preemptively say that Vetericyn Plus™ Pinkeye Spray will serve as a non-antibiotic healing aid for Infectious Bovine Keratoconjunctivitis, helping to not only decrease pain due to the *Moraxella bovis* infection, but do so faster as well. Once the full study has been completed, we will be able to evaluate the change in the size of the corneal lesion, as well as the tissue residues. After all data has been collected and analyzed, it is expected that the conclusions remain the same.

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Plant-based Omega-3 stearidonic acid (SDA) enhances anti-proliferative activity of docetaxel in human prostate cancer cells

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Introduction. Our unpublished data show that stearidonic acid (SDA), a plant-based omega-3 fatty acid, enhances doxorubicin cytotoxicity in human prostate cancer cells (PCa) but has no measurable effect on normal prostate epithelial cells. Similarly, others showed that the fished-based omega-3 fatty acid DHA enhances docetaxel's cytotoxicity in PCa. These observations suggest that PCa therapy development using omega-3 fatty acids and chemotherapeutic drugs in combination is an attractive possibility. We intended to quantify the effects of docetaxel combined with SDA on nuclear factor kappa-light-chain-enhancer of activated B cells (NF-κB) in LNCaP and the proliferation/viability effects in PC3 cells. We also aimed to qualitatively assess the treatment's effect on androgen receptor (AR) and peroxisome proliferator-activated receptor gamma (PPARγ) expression in LNCaP and PC3 cells, respectively.

Methods. We used the MTT assay to determine PC3 cell viability and a luciferase reporter assay to quantify the combined drug/SDA effects in LNCaP cells transduced with a lentiviral vector carrying the NF-κB response element sequence upstream of the luciferase coding sequence. We assessed AR and PPARγ expression qualitatively by immunocytochemistry and quantitatively by immunoblot analysis.

Results. The cytotoxic effect of docetaxel is enhanced by SDA when the two are administered to PC3 cancer cells. The combination of docetaxel and SDA at a constant 1:1 ratio increased cancer cell death compared to each drug alone. SDA suppressed TNFα-induced NF-κB activity and down regulated AR-induced expression in androgen dependent LNCap cells. PPARγ expression was enhanced by DOC but not SDA suggesting that SDA does not use the PPARγ-pathway to suppress NF-κB. In summary, our results suggest that SDA may be an effective addition to docetaxel in treating PCa.

Conclusions. We showed that SDA enhances DOC induced cell death in PC3 cells. Our data suggest the anti-proliferative activity of SDA is mediated at least in part by the down regulation of NF-κB and AR in LNCaP cells.

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Effects of Vaccination Age on IBV-Specific Antibody Production and Avidity in Chickens

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Introduction. Infectious bronchitis virus (IBV) is an economically important pathogen of chickens worldwide. Although IBV vaccination has been instrumental in decreasing economic losses, infections have persisted in chicken flocks. IBV's persistence may be facilitated by vaccination of immunologically immature chicks. Therefore, we hypothesized that age of vaccination is one of the contributing factors of IBV persistence by inducing lower, less-protective IBV-specific immune responses.

Methods. IBV-specific IgG and IgA antibody levels were measured in tears and plasma using an IBV-specific ELISA as previously described (Orr-Burks et al., 2014). Tears and plasma were collected 14 days after vaccination. The indirect IBV-specific ELISA was also used to measure avidity indices using the method of Jones et al. (1987). The avidity index was defined as the concentration of KSCN inhibiting 50% of the antibody reactivity on the ELISA. The avidity index was measured by incubation with increasing molarities of potassium thiocyanate (KSCN) for 30 minutes. The 50% inhibition point was defined using 3rd order polynomial regression models.

Results. Our research demonstrated that vaccination of chickens at 3-4 weeks of age produced a significantly higher IgG antibody response, but not IgA antibody responses, than those vaccinated at 1-7 days of age. To determine if age of vaccination affects not only the quantity but also the quality of antibodies, the relative antibody avidities in plasma and tears of 1 day and 4 week old chickens was measured. Avidity indices demonstrated a concentration-independent inhibition by KSCN of plasma antibodies and limited concentration dependence of tear antibodies. A significantly higher avidity index of plasma and tears IgG antibodies was observed in 4 weeks old IBV-vaccinated chickens when compared with 1 day old chickens, while no significant difference in avidity indices of plasma or tears for IgA antibodies was observed.

Conclusions. Our finding expanded current understanding of age of vaccination on induction of IBV-specific antibodies and confirmed the immaturity of the immune system at 1 day of age as hypothesized. We demonstrated that the IBV vaccine induced lower IgG antibody responses to IBV at 1 day of age compared to 4 week old chickens and these antibodies also expressed significantly lower avidity indices. The observation that this deficiency at day 1 of age is not observed for IgA antibodies, argues that these antibody isotypes are differentially regulated and/or differentially influenced by route of vaccination at different ages.

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Characterization of the Ocular and Neuromuscular Pharmacodynamic Effects of Rocuronium in Raptors

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Introduction. Raptors are frequently admitted to rehabilitation centers with ocular conditions that require surgery, such as cataracts. These conditions can prevent an otherwise healthy bird from being rehabilitated and released. In order to facilitate intraocular surgery, the iris must be dilated. However, mydriasis is difficult in avian species as birds can voluntarily control the skeletal muscle in the iris. A drug capable of causing paralysis of voluntary muscle, such as a neuromuscular blocking agent (NMBA), may provide adequate dilation to facilitate eye surgery in raptors. The NMBA we are investigating is rocuronium. The main objective of this research was to intravenously administer rocuronium to red-tailed hawks *(Buteo jamaicensis)* and evaluate the ocular and neuromuscular pharmacodynamics. The hypothesis was rocuronium will cause significant dilation of the pupil.

Methods. Seven red-tailed hawks from the Southeastern Raptor Center (SERC) were included in this study. All birds were induced with the gas anesthetic isoflurane (5%) by mask until general anesthesia was achieved and then intubated and maintained on a positive pressure ventilator with isoflurane at 2-3%. Vitals monitored included heart rate, ECG rhythm, pulse rate, temperature, expired CO2, and direct arterial blood pressure. Rocuronium was administered at a dose of 1.0mg/kg given once via a 22 Ga intravenous catheter placed in the wing vein. Ocular pharmacodynamic effects were assessed by measuring pupil diameter with calipers each minute for the first 10 minutes after treatment and then every five minutes for the next 50 minutes. Peripheral pharmacodynamic effects were evaluated utilizing a peripheral nerve stimulator placed over the ischiatic nerve in order to conduct a train of four (TOF) ratio to monitor the depth of paralysis. Four equal twitches (TOF=1) were confirmed prior to administration of the paralytic and then stimulation was applied every minute for 60 minutes.

Results. Examination of the ocular pharmacodynamics effects reveal pupil size was significantly different from baseline ($p<0.05$) at all time periods out to 60 minutes. The average base pupil size in all birds was measured to be (3.7mm +/- 0.48)SD. The maximum pupil size occurred two minutes after rocuronium administration (6.1mm +/- 1.07), resulting in a 65.5% increase in size. On average, pupil size increased by 2.4mm in each bird. The average duration of increased pupil size was 51 minutes. The average time until pupil size began to decrease from maximum pupil size was 20 minutes. In review of the peripheral pharmacodynamics effects, compared to baseline, a significant effect ($p<0.05$) was observed at one minute and remained significant through 25 minutes. The average time to return of peripheral response was 29.4 minutes. The average time to reach TOF base was 37 minutes with all birds returning to baseline by 48 minutes.

Conclusions. These results support the hypothesis that rocuronium bromide provides adequate pupil dilation in red tailed hawks to enable ocular surgery. No noticeable adverse effects were observed in any of the birds during the study. Pharmacodynamic effects are consistent in both pupil size and TOF across all birds. Finally, a rapid time to maximum pupil dilation observed in all birds is ideal for many surgical procedures.

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Impact of Cyclophosphamide and Doxorubicin on Antimicrobial Minimum Inhibitory Concentration and Resistance in Escherichia coli

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Introduction. Efflux pumps are documented to be a significant cause of multidrug resistance in gram negative bacteria. Efflux pumps are constitutively expressed, have a broad substrate specificity and aid in removing noxious stimuli that would hinder bacterial survival. Previous studies have illustrated that fluoroquinolones cause Escherichia coli (E. coli) to increase efflux pump expression, leading to increased MIC and decreased susceptibility. Anticancer chemotherapy drugs that target eukaryotic DNA-such as doxorubicin (DXO), a topoisomerase inhibitor, and cyclophosphamide (CPD), a DNA alkylator may be perceived by bacteria as toxins, resulting in the up-regulation of efflux pumps. E. coli is a commensal organism that becomes opportunistic in immunocompromised patients. Therefore, an increase in efflux pump expression due to anticancer chemotherapy exposure aids in contribution of antimicrobial resistance in E. coli, contributing to therapeutic failure.

Methods. To determine the impact of two anticancer drugs doxorubicin and cyclophosphamide on E. coli efflux pump activity, E. coli isolates were exposed to chemotherapeutic agents in the presence and absence of a non-specific efflux pump inhibitor Phenyl-Arginine-Beta-Naphthylamide (PAβN). To determine the impact of antimicrobial resistance after exposure to doxorubicin, E. coli isolates underwent susceptibility testing to determine MIC against 18 antimicrobials on 96-well custom antimicrobial plates.

Results. The addition of the efflux pump inhibitor dramatically decreased the MIC for E. coli isolates in the presence of DXO. However, with the addition of the efflux pump inhibitor no increased susceptibility was noted in the presence of CPD. Antimicrobial susceptibility testing for E. coli isolates after exposure to doxorubicin did not illustrate an overall increased resistance, however doxycycline did illustrate an increased MIC.

Conclusions. The difference of E. coli isolates exposed to DXO in the presence and absence of PAβN is a five-fold MIC decrease illustrating increased efflux pump activity suggesting increased efflux pump expression. However, no notable MIC difference was observed when E. coli isolates were exposed to CPD in the presence or absence of PAβN. Increased antimicrobial resistance after exposure to doxorubicin did not reveal an increased MIC for E. coli isolates with the exception of doxycycline, suggesting other protective bacterial survival mechanisms were utilized in E. coli isolates besides efflux pumps.

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Investigation of alternate sources for the isolation of endothelial progenitor cells from horses

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Introduction. Endothelial progenitor cells (EPCs) are rare progenitor cells that originate in the bone marrow and circulate in peripheral blood. EPCs have the unique ability to undergo vasculogenesis, making EPCs a promising source for therapeutic management of ischemic diseases. The purpose of this study was to establish alternative methods in order to increase the success rate of isolating endothelial progenitor cells from horses.

Methods. Endothelial colony forming cells (ECFCs), a subtype of endothelial progenitor cells, were isolated from peripheral blood from the jugular and cephalic veins using two protocols. In the whole blood method, blood was added directly to 225 cm^2 flasks containing endothelial growth media. A density gradient centrifugation method was also used, and the cells were harvested from the buffy coat and allowed to adhere in 75 cm^2 flasks. Colony numbers and emergence times were recorded, and the cells were characterized by functional assays of vascular tubule formation on extracellular matrix and acetylated low density lipoprotein (DiI-Ac-LDL) uptake.

Results. Seven out of eight sampled horses produced colonies at day 6.6 ± 0.8 and formed 3.3 ± 1.3 colonies/mL using the whole blood method, and 6.9 ± 5.5 colonies/mL using the density gradient centrifugation method. The success rate of isolating cells was 75% from the cephalic vein and 25% from the jugular vein. Utilization of both sites in the same horse had a success rate of 87.5%. All colonies demonstrated the typical single-layer cobblestone morphology and rapid proliferation. The isolated cells demonstrated ability to uptake LDL and to form vascular tubules on the extracellular matrix material.

Conclusions. EPCs can be isolated more reliably from the smaller diameter cephalic vein than from the jugular vein, potentially due to the affinity EPCs have for the vessel wall. This holds great promise for the future use of EPCs in autologous transplants for the treatment of equine disease.

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Microgliosis in Tay-Sachs Jacob Sheep

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Introduction. Tay-Sachs disease (GM2 gangliosidosis), a neurodegenerative lysosomal storage disease, has been recently discovered in Jacob sheep. With this finding, Jacob sheep have been utilized in research to learn more about the disease process along with finding suitable treatment methods. An adeno-associated viral vector treatment has been introduced to increase levels of the dysfunctional enzyme (hexosaminidase A) to promote breakdown of accumulated GM2 ganglioside. An aspect of the disease that requires better understanding is the inflammatory and neurodegenerative process that arise from the accumulation of GM2 ganglioside. The resident immune cells, microglia, may play an important role in neurodegeneration.

Methods. In order to gain a better knowledge of this process, microglia in a normal (control), affected/untreated, and affected/treated sheep were analyzed. Sections were obtained from the cerebral cortex, diencephalon, cerebellum and brainstem. Immunohistochemistry (IHC) was performed using Iba1 and MHC class II antibodies to look at overall microglia activation and microgliosis in sections for each group of Jacob sheep. Microscopic images were taken of differentiated areas within each section which were then used to obtain quantitative data (ImageJ software). Microglia cell width, process length and overall antibody stain density were measured.

Results. Qualitatively, microglia in affected sheep had larger cell bodies, shorter process lengths and greater numbers of activated cells. Microgliosis in affected/treated sheep did not appear as severe as affected/untreated sheep. The average microglia cell body diameter was longest in affected/untreated sheep, and affected/treated were longer compared to normal (23.6, 19.2, and 13.8 microns respectively; $P < 0.01$). Affected/untreated sheep had shorter microglia process lengths compared to both affected/treated and normal, while affected/treated and normal were not significantly different (10.1, 15.7, and 14.9 microns respectively; $P<0.01$). Iba1 staining was the densest in affected/untreated animals, and was denser in affected/treated compared to normal (6.0, 3.6, and 1.9 % area respectively, $P< 0.01$). Stain density in MHC class II IHC was greater in both affected/untreated and affected/treated compared to normal (1.26, 1.20, and 0.3 % area respectively, $P<0.01$).

Conclusions. Both the cerebrum and thalamus had more activated microglia and microgliosis than the cerebellum in affected Jacob sheep. Affected/treated sheep showed partial normalization of microgliosis, with levels of microglial activation intermediate between untreated/affected and normal animals.

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Evaluation of the Ability of Adenovirus Vectors to Infect Canine Mast Cell Tumors

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Introduction. Gene therapy is a promising new approach to cancer treatment. One method involves direct injection of vector viruses into a cancerous tumor to kill the local tumor cells, and subsequently, through the bystander effect, kill other tumor cells elsewhere in the body. This is based on the suggestion that tumor cells communicate well with other tumor cells and will minimally affect healthy cells. This project addresses whether adenovirus vectors can potentially be effective in treating canine mast cell tumors: a tumor that is typically accessible for direct injection.

Methods. Adenovirus type 5 (Ad5), encoding purine nucleoside phosphorylase (PNP), a pro-drug convertase enzyme, was used. The cells that express PNP then convert a subsequently injected pro-drug to its active form, killing the infected cells. Current research indicates that lymphocytes and related cells cannot readily internalize Ad5, because they lack two specific types of receptors. These receptors, known as coxsackie adenovirus receptor (CAR) and αVβ3/αVβ5 integrins, interact with specific amino acid sequences on the adenovirus to allow internalization of the virus into the cell. Our hypothesis is that mast cells, being related to lymphocytes, may also be resistant to Ad5 infection. In order to test this hypothesis, mast cell tumor cell lines (BR, MPT1) and control tumor lines (CMT28, DH82) were evaluated for CAR and αVβ3/αVβ5 integrin expression by quantitative RT-PCR. Additionally the efficacy of virus infection was evaluated with an Ad5 virus expressing a marker gene, green fluorescent protein (GFP). Fluorescence microscopy was used to identify virally infected cells. Flow cytometry measured both the number of cells expressing GFP and the intensity per cell. These data were used to estimate the efficiency of using an Ad5 based vector to infect and subsequently kill canine mast cell tumors.

Results. At 48 hours post-infection and at a multiplicity of infection of 1,000, CMT28 had the highest percentage of cells fluorescing with GFP (83.3%), followed by BR and DH82 (66.6% and 65.8%, respectively). MPT1 had the lowest percentage with 6%. The qPCR results showed that when normalized to the cell line expressing the least amount of CAR (CMT28) the magnitude of difference between cell lines was up to 1.95 fold difference (with BR expressing the most). The relative gene expression of the integrins was normalized to the lowest expressing cell line (MPT1) with fold differences variable and as high as 184.7 fold difference (β5 expression in DH82).

Conclusions. According to the flow cytometry results Ad5 infection was most efficient in CMT28 (a canine mammary tumor cell line) and was possible though less efficient in DH82 (canine macrophage like cells) and BR (canine mast cells). MPT1 is the most accurate cell line for in vivo mast cells but Ad5 infection was inefficient. According to qPCR, DH82 expressed the highest amount of integrins, while MPT1 expressed the least. BR expressed the most CAR with CMT28 expressing the least. This suggests that integrins play an essential role in internalizing the adenovirus.

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Effects of bisphenol A (BPA) and diethyl (hexyl) phthalate (DEHP) on epididymal development in the rat

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Introduction. There has been increasing public concern that exposures of the population to chemicals present in food, air and water may cause adverse effects on reproductive health. BPA and DEHP are found in plastics and several other consumer products. The Center for Disease Control and Prevention estimates daily phthalate exposure levels at 30 µg/kg body weight, and over 90% of the population has detectable levels of the industrial chemical BPA in their body (0.2-20 ng/ml of unconjugated BPA in serum). DEHP is considered a prototype anti-androgen, whereas BPA is a classical estrogenic compound. Therefore, exposure to hormonally active environmental agents during the perinatal period can have adverse effects on reproductive tract development. Indeed, several reports have demonstrated that exposure of laboratory species to BPA decreased testis size and gene expression of androgen receptors (AR) in the gonads. Similarly, exposure to phthalates impaired development of the male reproductive tract by inhibiting androgen secretion. Also, the Wnt4/β-catenin-mediated pathway, in concert with the antimüllerian hormone (AMH), supports regression of Müllerian ducts (MD) and it has been suggested that MD remnants, arising from deficits in AMH function, are incorporated into the epididymis. Historically, diethylstilbestrol, a synthetic estrogen, was given to pregnant women to prevent miscarriages, but caused increased rates of cryptorchidism, epididymal cysts and other reproductive abnormalities in adult male offspring. Postnatal development of the epididymis is androgen-dependent and is subject to regulation by estrogen. However, the mechanisms of chemical-induced disruption of epididymal development have not been investigated.

Methods.

- Pregnant Long Evans rats were gavaged with low and high doses of BPA and DEHP in corn oil as a vehicle from gestational day 12 to 21. Male offspring were sacrificed at postnatal day 35.
- The head of the epididymis (caput epididymis) (3 animals/group) were separated from testis at the time of sacrifice and were homogenized and processed for western blot analysis.
- Protein expression levels were analyzed by densitometry and normalized to β-actin.
- Differences between groups were evaluated by one-way ANOVA (Graph Pad, San Diego, California).

Results.

- Developmental exposure to BPA, but not DEHP, increased AR protein expression. However, exposures to both BPA and DEHP significantly increased ($P<0.05$) Wnt4 protein and β-catenin protein expression at 35 days of age.

Conclusions.

- BPA and/or DEHP increased AR, Wnt4 and β-catenin protein expression in the epididymis during development
- Changes in protein gene expression may impair signaling pathways that regulate development of the reproductive tract, including the epididymis.
- Altered developmental patterns in the epididymis may be a contributing factor to the increase in obstructive azoospermia in the population.

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Graduate Student Poster Presentations

Subconjunctival Space Temperatures in Horses

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Introduction. An ideal eye drug delivery technique should be minimally invasive with a drug depot that delivers a steady drug concentration over an extended period of time. Thermosensitive hydrogels injected into the subconjunctival space (SCS) show promise as a sustained delivery system targeting the cornea and anterior chamber in horses. The aim of this study was to describe the subconjunctival space temperature in normal horse eyes.

Methods. Temperatures measurements were obtained from five adult horses (10 eyes) within 10 minutes of induction of general anesthesia. SCS temperatures were measured under the dorsal and ventral bulbar conjunctiva using a type T thermocouple and rectal temperatures were measured using an electronic rectal thermometer. Results were analysed for range, mean and standard deviation. A student's paired t–test was used to determine if there was a significant difference between rectal and subconjunctival temperatures, with $P < 0.05$ considered significant.

Results. SCS temperatures ranged from 33.3°C-35.3°C (mean ± SD; 34.29°C ± 0.69°C) in the dorsal, and 33.5°C-35°C (34.33°C ± 0.50°C) in the ventral, bulbar SCS. The mean rectal temperature (37.39°C ± 0.93°C) was within reference ranges. Subconjunctival temperatures were significantly lower than rectal temperatures ($P = 0.0004$).

Conclusions. The temperature range in the bulbar SCS of horses in this study was 33.3°C-35.3°C i.e. 2.5°C-4.1°C below recorded rectal temperatures. These findings were consistent with previously reported findings in human and rabbit eyes, and support use of a thermosensitive hydrogel with a gelation setpoint of 32°C-36°C for subconjunctival injection in horses.

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Diindolylmethane, a naturally occurring compound, induces cytochrome p450 3A4 and multidrug resistance protein 1 gene expression by activating human pregnane X receptor

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Introduction. Activation of human pregnane X receptor (hPXR)-regulated expression of cytochrome p450 3A4 (CYP3A4) and multidrug resistance protein 1 (MDR1) plays an important role in mediating adverse drug interactions. Given the common use of natural products as part of adjunct human health behavior, there is a growing concern about natural products for their potential to induce undesired drug interactions through the activation of hPXR-regulated CYP3A4 and MDR1. Here, we studied whether 3,3'-Diindolylmethane (DIM), a natural health supplement, could induce hPXR-mediated regulation of CYP3A4 and MDR1 in human hepatocytes and intestinal cells.

Methods. PXR transactivation and Rhodamine 123 accumulation assays were performed to determine the function of PXR and MDR1, respectively. Quantitative RT-PCR and western blot analyses were conducted to study gene and protein expression. RNAi experiments were performed for gene knockdown studies.

Results. DIM, at its physiologically relevant concentrations, not only induced hPXR transactivation of CYP3A4 promoter activity but also induced gene expression of CYP3A4 and MDR1. DIM decreased intracellular accumulation of MDR1 substrate rhodamine 123, suggesting that DIM induces the functional expression of MDR1. Pharmacologic inhibition or genetic knockdown of hPXR resulted in attenuation of DIM induced CYP3A4 and MDR1 gene expression, suggesting that DIM induces CYP3A4 and MDR1 in an hPXR-dependent manner.

Conclusions. The results support our conclusion that DIM induces hPXR-regulated CYP3A4 and MDR1 gene expression. The inductive effects of DIM on CYP3A4 and MDR1 expression caution the use of DIM in conjunction with other medications metabolized and transported via CYP3A4 and MDR1, respectively.

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The Ideal Square and Surgeon's Knots Evaluated in Large Gauge Suture

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Introduction. This study investigated optimum knot configuration using large gauge suture (2USP and 3USP polyglactin 910; 2USP polydioxanone). We hypothesized surgeon's knots would: 1) have higher relative knot security (RKS) and knot holding capacity (KHC) than square knots; 2) have higher volume and weight than square knots; 3) require an additional throw to provide a secure knot at the end of a suture line, compared to the start.

Methods. Surgeon's and square knots were tested under linear tension on a Universal testing machine, recording failure mode and KHC. A digital micrometer measured knot size. An ANOVA and Tukey's post hoc test compared strength between number of throws, suture, suture size, and knot type. Significance was set at $p \leq 0.05$.

Results. Comparing knots with the same number of throws, there was no significant difference in KHC (p=0.299) and RKS (p=0.317) between square and surgeon's knots. There was no significant different between volume (p=0.128) and weight (p=0.310) of square and surgeon's knots. Overall, there was no significant difference in KHC (p=0.255) and RKS (p=0.267) between knots at the start and end of a suture line, however, seven throws were required to form a secure knot ending a suture line, compared to six throws at the start.

Conclusions. Comparing knots tested, there was no significant difference between strength and size of square and surgeon's knots with the same number of throws. An additional throw was required to form a secure knot at the end, compared to the start, of a continuous suture line.

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Defect in MAPK Signaling As a Cause for Monogenic Obesity Caused By Inactivating Mutations in the Melanocortin-4 Receptor Gene

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Introduction. The melanocortin-4 receptor (MC4R) is a Family A G protein-coupled receptor that plays an essential role in regulating energy homeostasis, including both energy intake and expenditure. Mutations leading to a reduced MC4R function confer a major gene effect for obesity. More than 170 distinct mutations have been identified in humans. In addition to the conventional Gs-stimulated cAMP pathway, the MC4R also activates MAPKs, especially ERK1/2. We also showed there is biased signaling in the two signaling pathways, with inverse agonists in the Gs-cAMP pathway acting as agonists for the ERK1/2 pathway. In the current study, we sought to determine whether defects in basal or agonist-induced ERK1/2 activation in MC4R mutants might potentially contribute to obesity pathogenesis in patients carrying these mutations.

Methods. The constitutive and ligand-stimulated ERK1/2 activation were measured in wild type and 73 naturally occurring MC4R mutations.

Results. We showed that nineteen mutants had significantly decreased basal pERK1/2 level, and five Class V variants (where no functional defects have been identified previously), C40R, V50M, T112M, A154D and S295P, had impaired ligand-stimulated ERK1/2 activation. We also observed biased signaling in 25 naturally occurring mutations in the Gs-cAMP and ERK1/2 pathways.

Conclusions. Our studies demonstrated for the first time that decreased basal or ligand-stimulated ERK1/2 signaling might contribute to obesity pathogenesis caused by mutations in the MC4R gene.

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Neuroprotective mechanisms of carnitinoid antioxidants in rodent models of mitochondrial dysfunction

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Introduction. The pathological effects of mitochondrial dysfunction result from both oxidative damage and bioenergetic deficiency, and are more severe in cells and tissues with high metabolic energy demands such as neurons, skeletal muscle and cardiac muscle. In this context, our research efforts have focused on a group of proprietary synthetic lipoylcarnitine and butyrylcarnitine derivatives as potential therapies to minimize oxidative damage and maximize mitochondrial energy production in animal and cellular models of mitochondrial disease. Previous results from experiments using lipoylcarnitine methyl ester Iodide (PMX-500FI) and butyrylcarnitine hydrochloride (PMX-550B) showed significant improvement from rotenone-induced complex I inhibition in mice. Taken together, utilizing these mitochondrial dysfunction modelling systems may provide data critically needed for building a strong case for continued development of these unique lipoylcarnitine and butyrylcarnitine compounds.

Methods. We are extending our studies to include two new carnitinoid compounds using two additional animal models: a rotenone-induced rat model and a genetically engineered mouse model of mitochondrial dysfunction. Establishing a useful rat model of mitochondrial dysfunction by administration of rotenone, a mitochondrial complex I (CI) inhibitor, was challenging. In rats, rotenone treatment results in symptoms that resemble human mitochondrial CI diseases and neurological symptoms similar to humans with Parkinson's disease (PD). There is a known mitochondrial involvement in PD, i.e., mitochondrial dysfunction in a discrete area of the brain that leads to the death of these neurons. In addition to the rotenone-treated rats, we are also using a line of transgenic mice that develops a condition similar to human juvenile PD due to an engineered mutation in the Parkin gene that results in deficiency of the Parkin protein. Methodology used includes: neuromotor assessment by Open Field and Rota-rod tests, western blotting to assess modulation of apoptosis and mitophagy pathways, and immunohistological analyses of substantia nigra *pars compacta* to assess loss of dopaminergic neurons and protective effects of PMX compounds.

Results. Experiments are ongoing. Preliminary data show that, in rotenone-treated cultured H19-7/IGF-IR rat primordial hippocampal cells and neuronal cells from rotenone-treated mice, PMX-500FI reduced ROS and increased ATP production, was antiapoptotic, reversed deficits in long term potentiation, and reduced presynaptic release and basal synaptic transmission.

Conclusions. Continued testing of these unique antioxidant compounds in rodent models is warranted, and will provide important insight into the mechanisms of mitochondrial dysfunction-induced neurodegeneration.

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In vitro efficacy of anti-protozoal compounds against *Tritrichomonas foetus*

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Introduction. Bovine trichomoniasis is a sexually transmitted disease caused by *Tritrichomonas foetus*, an obligate parasite of the reproductive tract of cows and surface of the bull's penis and prepuce. Significant economic losses to cattle producers occur annually due to *T. foetus* with annual losses exceeding $650 million. Currently there are no known drugs that are legal for use in food animals that are a treatment for *T. foetus*. Oxibendazole and ponazuril are anti-protozoal drugs that are legal for use in food animals and were found to be efficacious against *T. foetus* by this research team in an *in vitro* limited replication, preliminary study. The overall hypothesis of this study is that oxibendazole or ponazuril combined with a polymer enhancer, forming a topical formulation, can be effective at treating bulls infected with *T. foetus* when applied to the penis and prepuce. The specific aim of this study is to validate this hypothesis by *in vitro* testing of the formulations against cultures of *T. foetus.*

Methods. The efficacy of selected formulations of ponazuril (PO) and oxibendazole (OX) with and without a polymer enhancer (PE) to kill *T. foetus* organisms using an *in vitro* culture will be tested. Five replication of each treatment were completed to investigate variations in efficacy and to evaluate the survivability of pseudocysts. The efficacy of the formulations were compared to a control of *T. foetus* with no treatments. The *T. foetus* isolate of bovine origin was cultured in Diamond's Media (DM) at 37°C. Following culture of the organism, $3.5x10^5$ of *T. foetus* organisms were washed and resuspended in 1 mL of sterile phosphate buffered saline (PBS) and then inoculated into tubes containing 10 mL of DM. At time 0, one of the following treatments were added to individual culture tubes as follows: 1) 0.5 mL DM (control); 2) 0.5 mL PE; 3) 75 mg PO (0.5 mL); 4) 50 mg OX (0.5 mL); 5) 37.5 mg PO (0.25 mL) + 0.25 mL PE; or 6) 25 mg OX (0.25 mL) + 0.25 mL PE. Each treatment was applied to individual culture tubes in 5 replicates. The tubes were vortexed and a 20 μL sample removed every 2 hours for a total of 12 hours. From each sample, the surviving organisms were counted utilizing disposable Neubauer hemocytometers. At 12 hours post-treatment, each formulation was recultured in fresh DM, passaged daily for 1 day and evaluated microscopically until the presence of *T. foetus* organisms in culture were noted.

Results. During the 24-hour drug exposure, a significant reduction of viable T. foetus organisms was detected in all treatment groups. In the untreated control tubes the number of viable organism reached a plateau at 4 hours and contained an expected number of viable organisms until the end of the observation period. Following replacement of culture media at 24 hours, a reemergence of viable protozoa was noted in all treatment groups, suggesting that drug exposure resulted in pseudocyst formation rather than destruction of the protozoa.

Conclusions. While the drug formulations did cause significant decrease in number of *T. foetus* organisms a complete kill of the protozoa was not appreciated. Formulation of the drug with the polymer enhance may have caused the drug to be tightly bound and unable to reach concentrations sufficient to kill the protozoa.

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Evaluation of Mitoxantrone in CHOP-like Chemotherapy for Canine Lymphoma

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Introduction. Standard of care for canine lymphoma involves treatment with cyclophosphamide, doxorubicin, vincristine, and prednisone (CHOP). The addition of doxorubicin to treatment protocols has been shown to improve remission and survival times, but may cause cardiotoxicity with cumulative dosing, particularly in patients with underlying cardiac disease. When doxorubicin cannot be safely administered, mitoxantrone is often substituted (Mx-COP); however the efficacy of mitoxantrone in first-line combination chemotherapy for lymphoma has not yet been evaluated.

Methods. Records at twelve institutions were queried for dogs with newly diagnosed high-grade multicentric lymphoma treated with Mtx-COP. Demographic data, stage, and immunophenotype were recorded, along with remission duration and survival time. Results were compared to contemporaneous institutional data.

Results. Forty-four patients met inclusion criteria. Median remission duration was 184 days (95% CI 132-236 days). Median survival time was 234 days (95% CI 167-301 days). Eleven cases were censored due to loss of follow-up, still being alive, or death from non-lymphoma causes. Contemporaneous institutional data for 53 dogs treated with CHOP showed disease free interval and median survival times of 219 days and 344 days respectively. There was no statistically significant difference between disease free interval and median survival times for these two groups.

Conclusions. Remission and survival times were similar between the two protocols, although there was a trend toward better outcome in the CHOP group. Males were overrepresented in the Mtx-COP group and this may have contributed to differences in outcome. Additional investigation is needed to further evaluate whether the substitution of mitoxantrone for doxorubicin significantly shortens survival time in dogs treated with multidrug chemotherapy, but this study supports the use of mitoxantrone in cases where doxorubicin carries unacceptable risk.

Acknowledgments. Case contributions were from the authors listed.

Modified Equine Teno-Fix Tenorrhaphy Repair

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Introduction. Traumatic lacerations of equine flexor tendons can be career-ending and life-threatening, with 18-55% of cases returning to athletic performance. In addition, repairs must be able to withstand strains associated with immediate weight bearing and locomotion, reported up to 3559N at a walk. Current techniques are not strong enough to support these strains. Previous evaluation of the Teno Fix® (TF) device for equine tenorrhaphy revealed that four TF implants were similar in strength to the current clinical standard, the three-loop pulley (3LP), at a 2mm gap. However, the TF was weaker in maximum load at failure. A different implant configuration using the TF device may create a stronger repair. The objective of this study was to compare the strength and failure characteristics of the TF to the 3LP *ex vivo* and evaluate the effect of implant pattern and number of TF implants on maximum strength and gap formation. It was hypothesized that a modified TF repair distributing strain at a staggered distance will increase the ultimate strength of repair and make it stronger than the 3LP. It will also allow for testing an additional implant (total of 5), which will increase overall repair strength.

Methods. Twenty superficial digital flexor tendon pairs were randomized and repaired with a 3LP or one of two TF patterns; four staggered TF implants (4TF) or five staggered TF implants (5TF). Ultimate load to failure and 2mm gap (newtons) mode and gap at failure (mm) were obtained using a materials testing system and high speed camera. Statistical analysis was performed using one-way analysis of variance (ANOVA). Post-hoc testing was performed using Tukey-Kramer HSD. Significance for all analyses was set at $p \leq 0.05$.

Results. The 3LP failed at a greater load than both the 4TF and 5TF ($p<0.001$; $p<0.001$). Load to a 2mm gap was significantly higher for the 3LP than both TF repairs ($p<0.001$; $p<0.001$). Mode of failure was suture pull-out or breakage for the 3LP, and anchor pull-out or failure for the TF. Gap at failure was significantly larger for the 3LP than both TF repairs ($p<0.001$; $p<0.001$). Use of a fifth TF implant had no significant impact on the strength of repair ($p=0.37$).

Conclusion. Changing the pattern and number of TF implants had no significant impact on repair strength and the TF was not as strong as the 3LP in load at failure or load to a 2mm gap. Low ultimate strength suggests that the TF repair patterns used in this study may not be ideal for equine flexor tendon tenorrhaphy.

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Towards Multilevel Targeting of Adenoviral Vectors to Malignant Cells of Lymphocyte Origin

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Recombinant Adenoviral vectors are currently the most common vector for efficient *in vivo* transgene delivery in cancer gene therapy approaches. Internalization of adenovirus into target cells demands initial binding of virus fiber knob to the cell surface coxsackie-adenovirus receptor (CAR) followed by secondary binding with $\alpha_v\beta_{3/5}$ integrins on the cell surface. However, several studies have shown that cell types of lymphocyte origin (B cell, T cell, NK cells etc.) are poorly infected by adenovirus due to the paucity of both CAR and integrins. Hence, retargeted adenovirus must be developed that will bypass both CAR and integrin requirements. Our goal is to explore mechanisms to target lymphomas at three levels, transduction (or infection), transcription and transgene effect. We hypothesize that, transductional targeting of adenovirus by utilizing new targeting ligand and receptor pairs such as Interleukin-2-receptor (IL-2R) and anti-IL-2R antibody will allow us to conduct CAR and integrin independent cell specific targeting of adenovirus to malignant lymphocytes. To accomplish this goal, the sequences of canine, human and murine IL-2 and IL-2R were compared to each other. This data shows that human and canine IL-2 and IL-2R may cross react whereas murine IL-2 has diverged significantly. Based on these results, canine IL-2 and IL-2R were cloned, followed by sub-cloning these constructs, along with the ectodomain of IL-2R (Ecto-IL-2R), into eukaryotic expression vectors. Unique camelid single chain antibodies against IL-2R (sc-IL-2RAb) will be generated by immunizing alpacas with Ecto-IL-2R. The DNA encoding these antibodies will be cloned and inserted into the adenoviral fiber gene to allow knob-scIL-2RAb fusion proteins to be produced. Further, tumor specificity may be developed through the use of promoters that are active in tumors, but not normal cells, driving transgenes that act on transformed cells and spare the non-cancerous cells. If the approach is successful, then we can propose a new way for efficient utilization of recombinant adenovirus as gene therapy vector in the treatment of various cancers like B or T cell Lymphoma, Leukemia or in case of other hematopoietic genetic diseases.

Population Pharmacokinetics and Pharmacodynamics of Itraconazole in Panamanian Golden Frogs (*Atelopus zeteki*)

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Introduction. Panamian Golden Frogs (*Atelopus zeteki*), is a critically endangered amphibian, and has been extinct in the wild since 2009. One of the factors which have decimated both wild populations and zoological collections is the emergence of the chytridiomycete fungus *Batrachochytrium dendrobatidis*. Current recommendations for treatment include submerging the ventral abdomen in a 0.0025-.01% itraconazole solution for 5-10 minutes for 10 consecutive days. A minimum inhibitory concentration (MIC) for itraconazole has been reported to be < 1.56µg/ml, but no pharmacokinetic data is available regarding efficacy the recommended doses or whether the drug is distributed systemically. The purpose of this study was to determine the population pharmacokinetic parameters of itraconazole, establish whether the published MIC is reached and the distribution of itraconazole within the frog.

Methods. Healthy adult frogs (n=50) ventral abdomens were submerged in a 0.01% itraconazole solution for 10 minutes, rinsed with a saline solution and placed into individual humidified containers. Frogs were sacrificed at 0, 0.5, 1, 2, 4, 6, 8, 12, 24, 36hrs and the ventral skin, liver and heart removed. Each individual sample was weighed, placed in methanol and macerated. The supernatant was isolated and itraconazole was measured via HPLC. Pharmacokinetic parameters were determined via non-compartmental analysis and a population pharmacokinetic model determined via AIC and BIC score. Bias and precision of the final model was determined via bootstrap analysis (Phoenix®, St. Louis, MO).

Results. Detection of itraconazole in the heart was minimal at the 12-24 hour sample, preventing further pharmacokinetic analysis. Detection at the level of the skin was present throughout the 36 hour analysis period. Tmax was 24 hours, Cmax 3.54+/-2.1ng/mg, area under the curve (AUC) 176.6 +/-28.6 hr *ng/ml and mean residence time (MRT) 20.4hrs. Due to the continued presence of itraconazole past the data collection points, the elimination half-life could not be determined for the skin, however the absorption constant (Ka) was 0.044+/-0.009/hr and the volume was 0.006+/- 0.0008. Itraconazole was also detected in the liver, with a Tmax at 15.6 hours, Cmax 9.79+/- 41.7 ng/ml, AUC 177.67 +/= 78.8 and a MRT 18.8+/- 1.33 hr. The Ka was 0.23 +/- 0.146/hr, the volume was 0.06+/-1 0.0062 and the Ke was 5.61E-05 +/- 0.014604/hr.

Conclusions. Due to the presence of itraconazole in the liver as well as the heart, we conclude that itraconazole is absorbed through the ventral skin and systemically distributed. Furthermore, the indicated dose (0.01%) reaches concentrations for *B. dendrobatidis* above the published MIC for at least 24 hours, with the peak skin concentration at 3.54 ng/mg. The mean elimination half-life could not be determined since a large amount of drug remained at both sites after the final data collection point.

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Post-graduate/Faculty Poster Presentations

Effect of prenatal exposure to di (2-ethylhexyl) phthalate (DEHP) and bisphenol A (BPA) on sexual differentiation and steroid hormone secretion capacity in male rats

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Introduction. In recent years, there have been reports of impaired reproductive capacity in laboratory species and wildlife associated with exposure to environmental chemicals. Hormonally active chemicals or endocrine disruptors (EDs) are found in the environment as constituents of several consumer products, e.g., bisphenol A (BPA), phthalates, polychlorinated biphenyls, pesticides, polybrominated diphenyl ethers (PBDEs) and cadmium chloride. There is evidence that prenatal exposure to EDs such as BPA and diethyl (hexyl) phthalate (DEHP) interferes with sexual differentiation in the fetal period and affect reproductive tract development in the postnatal period. It is likely that reproductive anomalies in the adult are directly linked to chemical exposures occurring early in development. The present study investigated the effects of prenatal exposure to BPA and DEHP on expression of transcriptional factors regulating sexual differentiation, e.g., steroidogenic factor 1 (SF-1), Sox9, antimüllerian hormone (AMH) and GATA-4. In addition, we analyzed gene expression in the pituitary gland and measured testicular steroid hormone secretion capacity.

Methods.

- Timed pregnant Long-Evans female rats were gavaged with DEHP at 5 and 50 and BPA at 2.5 and 25 µg/kg body weight (bw) (8 dams/group) from gestational days 12 to 21. Cohorts of male offspring were evaluated at 10 and 90 days of age.
- At sacrifice, blood was collected to obtain serum, and testes and pituitary glands were collected and processed for western blot analysis.
- Protein expression of SF-1, Sox9, AMH, and GATA-4 in the testis was analyzed at day 10 post-partum, whereas LHβ- and FSHβ-subunit protein levels were measured in pituitary glands obtained at 90 days of age.
- Steroidogenic capacity of testes was assessed *ex vivo* at 90 days of age by radioimmunoassay of steroid hormones.

Results:

- Expression of Sox9 and AMH protein were increased ($P<0.05$) in testis of male rats whose dams were exposed to DEHP and BPA compared to control. Expression of GATA-4 was increased in testis of male rats from the higher BPA and lower DEHP dosage groups but was decreased at the higher DEHP dose ($P<0.05$).
- Prenatal exposure to both DEHP and BPA decreased ($P<0.05$) FSHβ- and LHβ-subunit protein levels in the pituitary glands of adult male rats compared to control unexposed animals.
- At 90 days of age, serum testosterone (T) was decreased only in male rats from the lower DEHP dosage group, whereas serum 17β-estradiol concentrations were similar in chemical-exposed and control rats ($P>0.05$). Furthermore, Leydig cell T secretion was decreased ($P<0.05$) by maternal exposure to BPA at 25 µg/kg bw but was increased by exposure to DEHP at 50 µg/kg bw ($P<0.05$).

Conclusions. Data demonstrate that developmental exposures to chemicals alter gene expression affecting sexual differentiation and testicular function in the adult. The present study supports the view that exposure of the population to chemicals in the environment has implications for male reproductive health.

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Vaccinal prevention of reproductive disease due to bovine viral diarrhea virus: a meta-analysis.

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Introduction. Bovine viral diarrhea virus (BVDV) is a common cause of reproductive inefficiency and failure in cattle populations worldwide. The reproductive sequelae of BVDV infection depends largely on the immune status of the dam and the stage of gestation at which infection occurs; possible adverse outcomes of infection include poor conception rates, early embryonic death, abortion, congenital malformations and the creation of persistently infected animals. The aim of this study was to evaluate the efficacy of BVDV vaccination to prevent reproductive disease by performing a quantitative synthesis of previously published studies.

Methods. Relevant articles were found by performing a search of four relevant scientific databases (PubMed, CAB abstracts, Agricola and Web of Science) using the search term "BVDV vaccine" and by examining the reference lists of 10 systematic reviews. Inclusion criteria for the meta-analysis mandated that the studies were controlled, primary studies that included necessary data for use in the meta-analysis, such as the number of pregnancies, abortions or fetal infection events in the treatment and control groups. Risk ratio effect sizes were used in random effects, weighted meta-analyses to assess the impact of BVDV vaccination on three outcomes: pregnancy risk, abortion risk, and risk of fetal infection. Within each outcome, sub-analyses were performed to evaluate the effect of modified live and inactivated or polyvalent and monovalent vaccines, homologous and heterologous or field challenge and vaccination using only bovine studies.

Results. The analysis demonstrated that the probability of fetal infection in vaccinated cattle is approximately one-seventh the risk in unvaccinated cattle exposed during gestation. Use of a polyvalent vaccine may further reduce the risk of fetal infection. The risk of abortion is reduced by more than 40% with vaccination (risk ratio = 0.597). Pregnancy risk was significantly improved in vaccinated animals (risk ratio = 1.05) subjected to field exposure relative to unvaccinated animals and was not adversely affected by vaccination when viral challenge was delayed until animals were gestating.

Conclusions. This meta-analysis provides quantitative support for the benefit of vaccination in the prevention of BVDV-associated reproductive disease. Vaccinal protection of fetal infection has the potential to significantly decrease the number of calves born persistently infected with the virus and thus decrease viral transmission amongst susceptible populations. In regions where virus eradication is not feasible or practical, vaccination represents the most effective biosecurity management tool to decrease the impact of BVDV infections in cow-calf operations.

Impact of passive immunity induced by maternal vaccination on subsequent immunization and disease-sparing in early-weaned beef calves challenged with highly virulent BVDV

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Introduction. Vaccination programs have been developed to limit disease associated with *Bovine viral diarrhea virus* (BVDV) infection. The ultimate goal of BVDV vaccination is to induce immunity that prevents viral replication after infection; however, passively acquired BVDV-specific neutralizing antibodies can impact immunity and vaccine responses in the young calf. The purpose of this study was to examine the impact of passively-derived antibody on vaccine response and mitigation of disease in early weaned beef calves whose passive immune status was documented and when vaccination and challenge were separated by only 5 days.

Methods. Sixty-three crossbred beef calves were utilized in this study. All calves were born to heifers raised and bred in biosecure herds, and whose vaccination histories or absence of vaccination were known. To assess the decay of passively-acquired BVDV-specific neutralizing antibodies, blood (serum) was collected from all calves at birth and monthly until early weaning at calf ages of 2-4 months. Prior to early weaning and vaccination, calves were stratified by serotiter to BVDV 2 from serum samples obtained on calves 30 days prior to study and then randomly assigned to unvaccinated or vaccinated (Inforce 3/Bovi-Shield BVD) groups. Calves were weaned (day -7) shipped (day -6) and vaccinated/unvaccinated (day -5) prior to challenge (day 0) with highly virulent BVDV 2 strain 1373. Virus isolation from whole blood, serum, and nasal swabs, clinical pathology, clinical illness scores, and virus neutralization (BVDV 1, BVDV 2, and BVDV 2 1373) assays were performed on calves following challenge.

Results. Calves vaccinated with Inforce3/Bovi-Shield BVD exhibited significantly lower rectal temperature measurements and lower proportions on calves exhibiting clinical illness. Vaccinated calves had significantly higher white blood cell and differential cell counts and significantly better average daily gains as compared to unvaccinated calves. Vaccinated calves had less frequent isolation of virus from clinical specimens that unvaccinated calves. In contrast to 100% of unvaccinated calves becoming viremic, BVDV was not isolated from any clinical sample at any time point from 47% (15/32 calves) of the calves vaccinated with Inforce3/Bovi-Shield BVD. Also of interest is the lack of nasal swab positive results for calves that had been vaccinated with Inforce3/Bovi-Shield BVD. Vaccinated calves did not develop as great a degree of antibody response to BVDV 2 strain 125c and the challenge strain BVDV 1373 as the unvaccinated group of calves, even though all calves had been challenged with BVDV strain 1373.

Conclusions. Administration of Inforce3/Bovi-Shield BVD to early weaned beef calves provided protection from clinical disease when vaccination and challenge were separated by 5 days. Passively derived BVDV-specific antibodies do not appear to decease efficacy of Inforce3/Bovi-Shield BVD vaccination of early weaned beef calves.

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