PHI ZETA

The Honor Society of Veterinary Medicine
Epsilon Chapter

November 7, 20//

Research Emphasis Day

AUBURN UNIVERSITY
COLLEGE OF VETERINARY MEDICINE

PHI ZETA
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welcomes you to our

PHI ZETA RESEARCH EMPHASIS DAY
November 9, 2011

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 EMPHASIS DAY

November 9, 2011 – 120 Greene Hall (Platform)
Joy Goodwin Center (Posters & Keynote Lecture)

Graduate Student Platform Presentations

8:15 Payal Agarwal

8:30 Amy Back

8:45 Elizabeth Barrett

9:00 Allison Bradbury

9:15 M. Wesley Campbell

9:30 Stacy Soulsby

9:45 Meghan Davolt

10:00 Victoria Jones

10:15 Xiulei Mo

10:30 Benjamin Newcomer

10:45 Anil Poudel

11:00-1:00 Poster Presentations-Joy Goodwin Center
(Poster Session Presenters are present 11:00 – 12:00)

Graduate Student Platform Presentations (continued)

1:00 John Schumacher (Faculty)

1:15 Maninder Sandey

1:30 Evan Sones

1:45 Heather Davis

2:00 Kamoltip Thungrat

2:15 Meghan Umstead

Veterinary Student Platform Presentation

2:30 Jeremy Foote

Post-graduate / Faculty Platform Presentations

2:45 Lenore Bacek

3:00 Manuel Chamorro

3:15 Peter Christopherson

3:30 John Schumacher

3:45 Snack Break – Joy Goodwin Center

4:00 Keynote Lecture – Overton Auditorium – Dr. Elaine Ostrander

How to Build a Dog: Genetics of Complex Traits

Elaine Ostrander, Ph.D.

Dr. Elaine Ostrander is Chief of the Cancer Genetics Branch at the National Human Genome Research Institute of NIH. She also heads the Section of Comparative Genetics. Dr. Ostrander received her Ph.D. from the Oregon Health Sciences University, and did her postdoc training at Harvard. She then went to UC Berkeley and the Lawrence Berkeley National Labs, where she began the canine genome project, initiating work on the canine meiotic linkage maps. She was then at the Fred Hutchinson Cancer Research Center and University of Washington for 12 years, rising to the rank of Member in the Human Biology and Clinical Research Divisions, and Head of the Genetics Program.

Her lab at NIH works in both human and canine genetics and focuses on the genetics of complex disease traits including diseases such as cancer, and morphologic features such as body size, leg length and skull shape. She is the winner of many awards, including the 1995 American Cancer Society Junior Faculty Award, 2000 Burroughs Wellcome Innovation Award, 1999 American Kennel Club Canine Health Foundation President's Award, and the 2005 Canine Health Foundation Asa Mays Award for Excellence in Canine Health Research. She has published over 250 papers and articles, and is currently leading a team of postdocs and students on projects aimed at the mapping of several canine disease genes, finding genes regulating canine morphology, and understanding the population dynamics of modern domestic breeds.

6:30 Phi Zeta Banquet at the AU Hotel & Dixon Conference Center

PLEASE JOIN US FOR THE INDUCTION AND AWARDS BANQUET

Everybody is invited! Tickets $40/person - Reserve ticket by November 4, 2011 with Dr. Missy Josephson (josepem@auburn.edu, 334-844-5423). Deposit check for ticket with Dr. Josephson, 109 Greene Hall, or at the banquet in the **Auburn University Hotel & Dixon Conference Center**.

6:30 **COCKTAILS at cash bar**

7:00 **DINNER**

7:30 **INDUCTION of new Phi Zeta Members**

AWARDS CEREMONY to honor winners of the Platform and Poster Competitions

POSTERS

Veterinary Students

Shelley Ash	Use of TEG ± Cytochalasin D to Evaluate the Role of Hyperfibrinogenemia as a Cause for Hypercoagulability in Sick Animals
LD Cusimano	Evaluation of coagulation factor VIII and von Willebrand factor genes in a colt with presumptive hemophilia A
G.A. Hamlin	The effects of enhanced hypertrophy, reduced oxygen supply, and heat load on breast meat yield and quality
Spencer M. Hubbard	Gene Therapy for Tay-Sachs Disease in Jacob Sheep
Michelle D. Karagas	Dystrophin-Deficient Myopathy in the Springer Spaniel
Ashley Ladegast	Expression of the Putative Tumor Suppressor FOXP3 Gene in Canine Mammary Tumor Cells
Mandy Norris	Live and inactivated filamentous phage for immuno-contraception of wild and feral animals
Daniel Jandrlich	An analysis of feline infertility, as a result of ovarian or uterine pathology
Daniela Pennington	Development of an Adenovirus-Vectored Recombinant Vaccine for Infectious Bronchitis Virus
Devin T. Perry	Daidzein Acts Directly in Testicular Leydig cells to Disrupt Androgen Biosynthesis
Tiffany A Peterson	Comparison of gene expression of TNF-α and IL-1β in brain tissue of normal and GM2 gangliosidosis affected cats
Andrew C. Pfaehler	The soy isoflavone daidzein regulates Leydig cell division in the neonatal rat testis

Tim Twehues	Articular Cartilage Roughness Differs Between Femoropatellar and Femorotibial Joints in the Horse
Marike Visser	Fluoroquinolone-induced Efflux pump Expression in FQR and FQ-S canine and feline E. coli pathogens expressing MDR
E. Whitsett	Propagation of bovine viral diarrhea virus in cells from heterologous species phylogenetically related to cattle

Graduate Students

Michaela J. Beasley	The Pharmacokinetics of single dose extended release Keppra® with and without food in healthy adult dogs
James W. Gillespie	Phage Fusion Peptides Enhance the Efficacy of Liposomal Doxorubicin
Rucha S. Gurjar	Infectious Bronchitis Virus-Specific Effector and Memory T Cell Responses in White Leghorns
Hui Huang	Pharmacological Study of the Transmembrane Domain 6 of Human Melanocortin-4 Receptor
Farruk M. L. Kabir	Sequence determination of canine p16/INK4A alternative exon 1α and differential expression of INK4 tumor suppressors in canine mammary tumors
Victoria J. Jones	Long-Term Phenotypic Correction of Feline Lysosomal Storage Disease by Intracranial AAV Gene Therapy
Jameson Sofge	Comparison of Cyclosporine Preparations based on Therapeutic Drug Monitoring
H. L. Walz	Evaluation of CD25, FOXP3, and CCL5 gene expression in placentomes of pregnant cattle inoculated with bovine viral diarrhea virus

Undergraduate Students

Lauren Eads	Mucosal Vaccine Delivery to Generate Specific Immunity to Infectious Bronchitis Virus
Jeffrey Haney	Towards Development of an IL2R Targeted Therapy for Canine Lymphoma
Nichole Orr	Mucosal and Systemic Immune Responses to Infectious Bronchitis Virus (IBV) after Ocular Vaccination

Post-graduate / Faculty

Deepa Bedi	Nanophage-Mediated Targeted Delivery of siRNA into Breast Cancer Cells
MA Edmondson	Pharmacokinetics of Tramadol and Its Major Metabolites in Alpacas Following Intravenous and Oral Administration
M. Daniel Givens	Evaluation of a modified-live vaccine to protect developing fetuses from stringent natural challenges with bovine viral diarrhea virus and bovine herpesvirus 1
M. Daniel Givens	A rare case of persistent testicular infection with bovine viral diarrhea virus causes consistent shedding of infectious virus in semen
Xiaoqiang Liu	Mechanisms Accounting for Fluoroquinolone Multidrug-resistance *Escherichia coli* Isolated from Dogs and Cats
Ann Marie O'Neill	Enhanced Transduction of Fiber Modified Ad in Canine Lymphoma Cell Lines
K. Parameshwaran	Protective effects of a new antioxidant, PMX-500F, against rotenone induced neuromotor decline, ROS generation and cellular stress in mouse brain

Thomas Passler	Evaluating Transmission of Bovine Viral Diarrhea Virus to Cattle by Exposure to Carcasses of Persistently Infected White-tailed deer (*Odocoileus virginianus*)
Stuart B. Price	Reduction of *Salmonella* Newport Shedding and Disease in Experimentally Infected Calves
Debra Taylor	Management of Acute or Chronic Equine Laminitis with Selected Hoof Care Principles, Dietary Management, and Controlled Exercise
Lixia Wei	Development of Phage Ligands for targeting Non-Small Cell Lung Cancer
H. L. Walz	Evaluation of CD25, FOXP3, and CCL5 gene expression in placentomes of pregnant cattle inoculated with bovine viral diarrhea virus
Regina R. Williams	DNA Sequencing of Labradoodle Duchenne Muscular Dystrophy

Veterinary Student Platform Presentation

Canine PBMC NSG mice: A model of canine specific immunity to allogeneic canine mammary tumor-DC fusion vaccines

Jeremy Foote Ph.D.[1], Patricia Deinnocentes[1], Farruk M.L. Kabir, Bruce Smith Ph.D., VMD[1] and Richard Bird Ph.D.[1]

[1]Department of Pathobiology, College of Veterinary Medicine, Auburn University, AL

Introduction. Invasive breast cancer is the most frequently occurring malignancy in women and canine populations. Both incidence and prevalence of breast cancer are increasing, making development of new strategies for breast cancer treatment, in particular those designed to treat individuals suffering from early stage disease. Recently our lab has developed a cellular vaccine fusion incorporating patient dendritic cells with an immunogenic cell line of canine mammary tumors (CMT28), which has been shown in preliminary trials to generate both humoral and cell mediated tumor specific immunity. However, our ability to assess patient specific immune function in response is limited for client convenience and patient well-being. To evaluate patient immunity to allogeneic DC-CMT28 vaccines we generated a mouse model of canine tumor immune responses**. Our initial emphasis was developing this model using canine peripheral blood mononuclear cells (PBMCs) from healthy donors to determine the feasibility of this project using guide lines established from human PBMC NOD SCID IL2R common gamma chain -/- mouse (PBMC NSG mice) studies.**

Methods. Peripheral blood was collected from 2 healthy Labrador Retrievers and PBMC's were purified using Ficoll density centrifugation. 10^7 PBMC's were injected by the intraperitoneal route into NSG mice 4- 8 hours after radiation treatment with a 100cGy dose of γ radiation. On 1, 7, 14, 21, and 42 days post reconstitution, engraftment of canine WBCs was determined by flow cytometry using antibodies specific to helper T cells (CD45+ CD4+), cytotoxic lymphocytes (CD45+ CD8+), regulatory T cells (CD45+ CD4+ Foxp-3+), naïve (CD45+ IgM+) or class switched B cells (CD45+ IgG+) and IgM (CD45 low IgM intracellular+) or IgG (CD45 low IgG intracellular+) secreting plasma cells. Onset of xenogenic graft versus host disease (GVHD), which has been documented to occur with transfer of human lymphocytes into NSG mice, was assessed by biweekly measurements of weight where a 15% reduction in body weight, or anemia, and thrombocytopenia determined on the day of FACS analysis is indicative of xenogenic GVHD.

Results. Engraftment of NSG with canine PBMC's resulted in detection of CD45+ lymphocytes in the blood, bone marrow, spleen, and peritoneal cavity. The proportions of lymphocytes in the spleen steadily increased over time to represent 70% of the total splenic cells on 42 days post engraftment. In comparison the bone marrow and peritoneal cavity (PEC) canine lymphocytes represented less than 20% of total cells indicating that the spleen was the primary tissue of engraftment. Evaluation of lymphocyte subsets revealed an early predominance of helper and regulatory T cells which decreased over time in comparison to increasing proportions of CTL's and IgM+ secreting plasma cells, which correlated with clinical symptoms (weight loss, anemia, thrombocytopenia) consistent with the onset of GVHD 42 days post engraftment.

Conclusions. NSG mice successfully engraft with canine lymphocytes from healthy donors. The inverse relationship between decreasing proportions of helper and regulatory T cells in comparison CTLs and plasma cells may suggest a potential role in the development of humoral and cell-mediated immunity to mouse tissue auto-antigens signifying an onset of GVHD 42 days post engraftment. These findings suggest that NSG mice have the potential to be a model with which we can model patient specific immunity to allogeneic DC-CMT28 vaccines. Future vaccination studies will be conducted early post reconstitution to precede the activation of canine lymphocytes by mouse auto-antigens and the development of GVHD.

Acknowledgment: NIH, CVM Interdepartmental & Animal Health Research Grants, Allison Church Bird for expert flow cytometry analysis and cell sorting.

Graduate Student Platform Presentations

Tumor Suppressor Gene p16/INK4A Dependent Regulation of Cell Cycle Exit in a Spontaneous Canine Model of Breast Cancer

Payal Agarwal[1], Patricia DeInnocentes[1], Maninder Sandey[1,2], R. Curtis Bird[1]

[1]Department of Pathobiology, College of Veterinary Medicine, Auburn University, AL
[2]Scott Ritchey Research Center, College of Veterinary Medicine, Auburn University, AL

Introduction. Cancer is the result of accumulating genetic/epigenetic changes/mutations in proto-oncogenes and tumor suppressor genes causing loss of control of the cell cycle. Tumor suppressor genes encode proteins that suppress cell growth and most frequently result in exit from the cell cycle. One of the most important tumor suppressor genes, p16/INK4A/CDKN2A, arrests cell cycle in early G1 phase and inhibits binding of CDK4/6 with cyclin D1, which leaves the Rb tumor suppressor protein unphosphorylated and S phase transcription factor E2F bound and inactive. Accumulation of p16 in senescent cells and its inhibitory role in CDK4/CDK6/cyclinD1 complex regulation explains how overexpression of p16 can lead to arrest in G1 phase. We hypothesize that p16 has a role in exit from the cell cycle that becomes defective in cancer cells. We are developing an in-vitro model to elucidate the role of p16 in differentiating and in quiescent cells.

Methods. Well characterized canine mammary cancer cell lines (NCF, CMT28, CMT27, and CMT12) and derived p16-transfected clones (CMT27A, CMT27H, CMT28A, and CMT28F) have been used to investigate the expression of p16 after serum starving cells into quiescence and then refeeding to induce cell cycle re-entry. Cell cycle arrest and synchronous cell cycle re-entry were confirmed by ^{3}H-thymidine incorporation assay and flow cytometry. Expression of p16 and p27 mRNA in starved and exponential cells was compared by semi-quantitative PCR. Expression of p16 protein in starved and exponential cells were compared by western blot reacted with antibodies against p16 to bind homologous canine p16 proteins. To find alternative binding partners of p16, co-immunoprecipitation from non-proliferating cells was performed.

Results. Both NCF and CMT28 express p16 mRNA, while only NCF expresses p16 protein. We have successfully demonstrated cell cycle arrest and synchronous cell cycle re-entry in CMT12, CMT27, CMT28, CMT27A, CMT27H, CMT28A, CMT28F, and normal canine fibroblasts. Cell cycle arrest and synchronous cell cycle re-entry was further confirmed by ^{3}H-thymidine incorporation and flow cytometry. Expression of p16 mRNA and protein was enhanced as each cell line entered the quiescent phase. p53-associated and putative tumor suppressor 14-3-3σ protein co-immunoprecipitated only in quiescent CMT27A cells in comparison to exponential cells. Levels of 14-3-3σ mRNA expression also rose along with p16 in quiescent NCF cells.

Conclusion. All cell lines have demonstrated cell cycle arrest in response to serum-starvation. Because quiescence and differentiation are associated with decreased levels of cyclin D1 and/or CDK6 our data demonstrating that p16 is up-regulated during quiescence suggests the presence of alternative binding partners for p16, such as 14-3-3σ, in promoting and maintaining the quiescent and/or differentiated phenotype.

Acknowledgment. Author acknowledges: NIH, CVM Interdepartmental Grant, Farruk M.L. Kabir, and Allison Church Bird for expert flow cytometry analysis.

A Retrospective Analysis of Canine Spinal Cord Neoplasia in 51 Dogs: Evaluation of the Significance of Adjuvant Radiation Therapy and Other Factors on Prognosis

Amy Back[1]; Annette Smith[1]; Stephanie Schleis[1]; William Brawner[1]; Gregory Almond[1]; Erin Akin[2]; Michaela Beasley[2]; Nora Ortinau[2]; Andrew Shores[2]

[1]Auburn University
[2]Mississippi State University

Introduction. Meningiomas and peripheral nerve sheath tumors (PNST) are the most frequently occurring canine spinal tumors. Clinical findings and treatment have been reported sporadically, but few reports document the long-term follow-up.

Methods. The records of 51 dogs with histopathologically confirmed spinal neoplasms from Auburn University were reviewed. Kaplan-Meyer survival analysis generated median survival times (MST) and log-rank tests (significance $p<0.05$) compared gender, tumor locations, histologic types, mitotic index, and treatment modalities.

Results. Overall MST for all spinal neoplasms (meningiomas, PNST, and lymphoma) was 506 days. Mean time to presentation was 65 days. The most common presenting complaint was conscious proprioceptive deficits (46%). Twenty percent of owners elected not to treat (MST 3 days) or to euthanize immediately after diagnosis. Treatment groups included surgery alone (MST 420 days), surgery and radiation therapy (RT) (MST 640 days), and surgery, RT, and chemotherapy (MST 592 days) with no significant difference between groups ($p = 0.272$). Mitotic index (MI) predicted MST of dogs with meningiomas: MI ≤ 1 890 days vs. MI > 1 160 days, $p = 0.024$. Location influenced MST of dogs with PNST: cervical 596 days vs. thoracolumbar 59 days, $p = 0.028$. Total RT dose was not significant ($p=0.472$) for survival: RT total dose ≥ 40 Gy MST 590 days, < 40 Gy MST 247 days. Gender did not influence survival ($p = 0.2$).

Conclusions. The MST generated from this study can be used as prognostic information for canine patients with spinal tumors, perhaps encouraging treatment. Larger case numbers would strengthen the results.

Acknowledgments. I would like to thank all of the authors for their help with case analysis, case contribution, and assistance with statistics.

Effect of gastric distension with fluid on intra-abdominal pressures in horses

Elizabeth J. Barrett[1], Amelia S. Munsterman[1], and R. Reid Hanson[1]

[1]Department of Clinical Sciences, College of Veterinary Medicine, Auburn University, AL

Introduction. Abdominal hypertension (increased pressure in the abdomen) is a recognized condition in the horse that is suspected to result in reduced blood flow, or ischemia, due to obstruction of venous outflow by the abnormal pressure. This ischemia may lead to gastrointestinal ileus (reduced motility), multi-organ failure, and death. The systemic effects of increased abdominal pressure have been described in humans as "abdominal compartment syndrome, and, if unrecognized and left untreated, results in uniform mortality. Previously performed studies have evaluated the normal abdominal pressures in the standing sedated horse and ongoing research is evaluating the increased abdominal pressure seen in horses with various gastrointestinal disorders leading to colic (large colon impaction, small intestinal disease, peritonitis, etc) both prior to and after exploratory celiotomy (abdominal surgery). Routine procedures performed on horses presenting for signs of colic or having undergone surgery for resolution of signs of colic include passing a nasogastric tube and placing water, electrolyte solution of mineral oil into the stomach. The effect of this procedure on abdominal pressure in this species has not been studied. We hypothesize that instilling fluid into the stomach of normal adult horses will increase their resting abdominal pressure.

Methods. Ten horses were evaluated. Abdominal pressures were measured using a manometer and a standardized right flank approach and values were recorded for end inspiration and end expiration. Abdominal pressures were measured at rest, and after a nasogastric tube was passed and 0, 5, 10, 15 and 20L of water were placed in the stomach. Concurrent systemic blood pressure measurements were taken at the beginning and completion of the study. Statistical comparisons were made using a paired t-test, with significance set at $P<0.05$.

Results. The mean baseline intra-abdominal pressure was -4.5 +/- 3.0 cm H_2O in these horses, which was consistent with previous reports. The intra-abdominal pressures were not altered by placement of the nasogastric tube, but were significantly higher for gastric instillation volumes of 10 L (mean -0.7 +/- 3.1 cm H_2O, $P= 0.02$), 15 L (mean 1.2 +/- 3.4 cm H_2O, $P=0.0009$) and 20 L (mean 5.4 +/- 4.04 cm H_2O, $P=0.00004$) when compared to baseline. Abdominal perfusion pressures, determined from the mean arterial pressures minus the mean intra-abdominal pressures, decreased as the gastric instillation volumes increased, but were not significantly different for any comparison made ($P>0.05$).

Conclusions. Intra-abdominal pressures are significantly elevated when greater than 10 L of water is infused into the stomach. These results indicate the need for further research into clinical cases to determine the significance of gastric pressures in the development of intra-abdominal hypertension.

Acknowledgments. Authors would like to thank the Birmingham Racing Commission for funding.

Role of microglia cells in the pathogenesis of feline GM2 gangliosidosis

Allison M. Bradbury[1,2], Aime K. Johnson[3], Victoria J. Jones[1,2], Ashley N. Randle[1], Jacob A. Johnson[3], Nancy R. Cox[1,4], Miguel Sena-Esteves[5] and Douglas R. Martin[1,2]

[1]Scott-Ritchey Research Center, Departments of [2]APP, [3]Clinical Sciences and [4]Pathobiology, College of Veterinary Medicine, Auburn University, AL
[5]Gene Therapy Center, University of Massachusetts Medical Center, Worcester, MA

Introduction. Microglia cells are the primary resident immune cells of the CNS. Non-activated, resting microglia persist with a ramified morphology, consisting of a small, round cell body with long, branching processes used to survey for bacteria, viruses, cell debris, or degenerating neurons. Once activated, microglia assume an ameboid morphology capable of phagocytosis, express MHCII to present antigens and activate T-cells, and secrete pro-inflammatory molecules. A well-established feline model of GM2 gangliosidosis (GM2) provides a means to further define the role of microglia in neurodegeneration. Additionally, therapeutic intervention with an AAV vector encoding the deficient enzyme, hexosaminidase A, (AAV-Hex), has proven to significantly delay disease onset, and thus should curtail the activation of microglia.

Methods. For immunohistochemistry, 8µm paraffin sections were blocked for 1hr with 5% normal horse serum, treated for 1hr with mouse anti-feline MHCII (1:4), rabbit anti-human/rat/mouse Iba1 (1:100), or mouse anti-feline GM2 (1:500), followed by biotin-labeled horse anti-rabbit/mouse IgG for 1hr, ABC Reagent for 30 min, and visualized with DAB substrate. For double labeling, sections were blocked for 1hr with 5% normal donkey serum and treated for 1hr with MHCII or GM2 antibodies followed by DyLight 488-conjugated donkey anti-mouse IgG (1:250) for 30 min, and Iba1 or GFAP antibodies followed by DyLight 594-conjugated donkey anti-rabbit IgG (1:250) for 30 min and mounted with Vectashield with DAPI.

Results. GM2 cats exhibited an overwhelming population of activated microglia characterized by ameboid morphology and expression of MHCII and Iba1 (a marker of both resting and activated microglia). In comparison, a normal, age-matched control displayed non-activated microglia of the ramified nature and lacked positive staining for MHCII. These results were consistent throughout the cerebrum, cerebellum, and spinal cord. Double labeling unambiguously demonstrated co-expression of Iba1 and MHCII in cells of ameboid morphology, confirming the presence of abundant activated microglia in the GM2 but not normal cat brain.
Three GM2 cats treated with thalamus and deep cerebellar nuclei (DCN) injections of AAV-Hex and euthanized 16 weeks post-treatment, with negligible indication of disease, displayed histological features of microglia indistinguishable from normal cats. One cat treated for long-term evaluation (>1yr), with pronounced symptoms of disease, revealed activated microglia typical of late disease stages. Additionally, in one cat treated in the thalamus but not the DCN, resting microglia predominated in the cerebrum, while activated microglia were readily apparent in the cerebellum.

Conclusions. Excess ganglioside in the neurons of GM2 cats leads to activation of microglia, characterized by ameboid morphology and expression of MHCII. However, AAV mediated gene delivery delays disease onset and activation of microglia. Chronically activated microglia apparent during late stages of disease likely contribute to inflammation, cytotoxicity, and accelerate disease progression.

Acknowledgments. This research has been supported by NIH grant U01NS064096; Scott-Ritchey Research Center; APP; NTSAD; Jewish Community Endowment Fund.

Chronic lymphocytic leukemia in the cat: 19 cases (2000 – 2010)

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Introduction. There is little information regarding the presentation, biologic behavior, treatment, and prognosis in cats with chronic lymphocytic leukemia (CLL), and further investigation is needed to characterize this disease in cats. The goal of this study was to describe the clinical presentation, response to treatment, and prognosis of feline CLL.

Methods. A multi-institutional retrospective study of cats diagnosed with CLL was performed. CLL was defined as the presence of a mature lymphocytosis (≥9,000 lymphocytes/µl) and confirmation of an immunophenotypically monomorphic or clonal lymphoid population. Each patient was required to also have at least 1 of the 2 following criteria: (1) concurrent cytopenia of at least 1 cell line, and/or (2) >15% mature lymphocytes in bone marrow. Data on signalment, history, clinical signs, clinicopathologic features, and response to treatment were reviewed.

Results. Median age of the cats at initial presentation was 13 years (range, 5-20 years). The most common presenting complaint was chronic weight loss, which was present in 8/19 (42%) cats. Sixteen of 19 (84%) cats were treated with chlorambucil and prednisolone; 4 of these cats also received vincristine. Two (10%) cats were treated with multi-agent injectable chemotherapy (L-CHOP). Eighty-nine percent of cats evaluable for response achieved a complete (9 cats) or partial (7 cats) remission. Median overall remission was 15.4 months (range, 1.3 – 27.6 months). The median overall survival in the 18 cats with follow-up data was 17.7 months (range, 0.9 – 27.6 months).

Conclusions. Results of this study suggest that CLL affects older aged cats and responds favorably to treatment with oral chlorambucil and prednisolone.

Uterine Bacterial Isolates of the Mare: a Regional Retrospective Study (2003-2008)

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[1]Department of Anatomy, Physiology, and Pharmacology, College of Veterinary Medicine, Auburn University, AL

Introduction. The aim of this study was to describe the bacteria isolated from the reproductive tract of the mare and their resistance to antimicrobials commonly used for treatment of reproductive tract infections. Authors hypothesized that the population characteristics and resistance pattern would be similar to previously published data in other geographic locations.

Methods. Culture and susceptibility data were acquired from uterine swab, lavage, and biopsy 30 samples (n=8,296) collected in central Florida, between January 2003 through December 2008. Uterine samples were collected for culture and cytology from a population of mares presented either for routine breeding exams or reproductive disorders. Organisms were identified, resistance proportions to anti-microbials were determined, and inflammation was scored (n=880; 33%).

Results. Of the 8,296 samples, at least 95% were collected by uterine swabs. Potentially pathogenic organisms were cultured in 31% (2,576) with *Escherichia coli* (729 cases, 29%) and β-hemolytic *Streptococcus equi* spp. *zooepidemicus* (733 cases, 28%) being the most common. Other organisms considered important potential pathogens were gram negative *Enterobacteriaceae* spp. (10%), *Pseudomonas aeruginosa* (6%), and *Klebsiella pneumoniae* (3%). Among years, *E. coli* was most resistant to trimethoprim-sulfa and ampicillin and least to amikacin and enrofloxacin ($p<0.05$). For *S. zooepidemicus*, resistance was most to oxytetracycline and enrofloxacin and least to ceftiofur and ticarcillin with or without clavulanic acid ($p<0.05$). Inflammatory response was significantly greater for *S. zooepidemicus*.

Conclusions. *E. coli* and *S. zooepidemicus* remain the most 46 common pathogens associated with endometritis, with *S. zooepidemicus* being more likely to initiate an inflammatory response. Current drug recommendations for equine endometritis continue to be practical choices based on this population of mares.

Acknowledgments. There are no financial acknowledgements to be made.

Establishment, Validation and Application of Multispectral Imaging Technology to Studies of Bovine Reproductive Tract Development

Meghan L. Davolt[1], Anne A. Wiley[2], Robyn R. Wilborn[1], Steven E. Ellis[3], Frank F. Bartol[2]

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Introduction. Development of the bovine uterus begins prenatally and is completed postnatally with formation of endometrial glands. Disruption of this process during neonatal life can reduce adult uterine capacity to support pregnancy. However, little is known about mechanisms regulating bovine endometrial development. With a goal of defining molecular and cellular events associated with bovine endometrial histogenesis and cytodifferentiation from birth (postnatal day = PND 0), objectives here were to establish methods for immunohistochemical (IHC) localization of multiple antigens using multispectral imaging (MSI) in order to evaluate patterns of gene expression and cell proliferation in neonatal bovine endometrium. Specific aims were to employ IHC and MSI to acquire qualitative and quantitative data relative to temporospatial expression and labeling patterns for progesterone receptor (PR-A, -B forms), estrogen receptor (ESR1) and two markers of cell proliferation, Ki-67 and bromodeoxyuridine (BrdU).

Methods. For purposes of technical validation, uterine tissue was obtained from a Holstein heifer treated with BrdU (5mg/kg BW/day, i.v.) for five days prior to euthanasia on PND 40. Tissue was fixed in 4% paraformaldehyde, embedded in Paraplast-plus®, sectioned (6µm) and subjected to antigen retrieval in citrate buffer (pH 6). An indirect, non-amplified, simultaneous multi-label IHC method was employed using primary antibodies chosen based upon target specificity, host species and immunoglobulin subtype. Matched, AlexaFluor®-labeled secondary antibodies (Invitrogen Corporation; Carlsbad, CA) were used to produce target-specific fluorescent signals. The Nuance FX MSI system (Caliper Life Sciences; Hopkinton, MA) was used to capture quantifiable image data at specific wavelengths from as many as four unique fluorescent signals simultaneously. Fluorescent signals were analyzed using CellProfiler™ (www.cellprofiler.com). Spectrally unmixed target signal intensities were compared between cell compartments on a within tissue basis after extraction of compartment-specific regions of interest from single- and multi-labeled tissue sections.

Results. Images generated using multi-label IHC and MSI were equivalent, qualitatively and quantitatively, to those obtained using single labeling procedures. Spectrally unmixed images generated from multi-labeled tissues using MSI produced quantifiable data from multiple fluorescent signals. Procedures permitted quantification and comparison of fluorescent signal intensities indicative of ESR1, PR, Ki-67 and BrDU between cell compartments on a within tissue basis from a single preparation.

Conclusions. Establishment and validation of multi-label IHC and MSI procedures for qualitative and quantitative analysis of multiple fluorescent signals in single tissue sections sets the stage for studies designed to evaluate patterns of neonatal bovine endometrial development at cellular and molecular levels simultaneously.

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Safety and Efficacy of Low-dose Intracranial Gene Therapy in a Feline Lysosomal Storage Disease

Victoria J. Jones[1,2], Aime K. Johnson[3], Allison M. Bradbury[1,2], Ashley N. Randle[1,2], Jacob A. Johnson[3], Stanley G. Leroy[5], Nancy R. Cox[1,4], Miguel Sena-Esteves[5] and Douglas R. Martin[1,2]

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Introduction. The GM2 gangliosidoses (Tay-Sachs and Sandhoff disease) are autosomal recessive lysosomal storage diseases caused by deficiencies of lysosomal β-hexosaminidase A (HexA). HexA deficiencies cause accumulation of the glycolipid, GM2 ganglioside, in the central nervous system (CNS), resulting in relentless neurodegeneration and premature death. Following successful results in the GM2 mouse model, gene therapy trials are underway in the GM2 feline model (HexA β subunit deficient) to evaluate adeno-associated virus (AAV)-mediated gene delivery of functional HexA. Since human clinical trials generally begin with a vector dose per kilogram much lower than the effective dose in animal models, it is important to test the feline model with a similar low dose therapy.

Methods and experimental design. In the current study, GM2 cats (n=6) were treated at 1-month old with bilateral injections of an AAV vector formulation encoding feline HexA, to the thalamus and deep cerebellar nuclei (DCN) at 1/10 of the full dose (i.e., 4.4e11 genome copies total). Clinical therapeutic outcome was evaluated using MRI as well as a clinical rating scale reflecting the neurodegenerative course of GM2 cats. Brain and spinal cord were collected at the humane endpoint and cut into consecutive coronal blocks to analyze HexA and AAV vector distribution throughout the CNS.

Results. In treated cats, HexA activity was distributed throughout the brain at 0.2- to 77-fold normal levels, and was highest at injection sites. HexA activity in the spinal cord was 0- to 3.4-fold normal, with highest levels generally in the lumbar region. AAV vector was detected in all brain and spinal cord sections, reaching 58 and 0.7 genome copies/ng genomic DNA, respectively. Of the 6 low-dose GM2 treated cats, 1 is currently 16.8 months old, 4 reached humane endpoint between 13.0- 15.3 months old, and 1 was euthanized at 7.5 months due to severe patellar luxation. By comparison, 3 full dose GM2 treated cats are currently 20.2, 18.3, and 17.0 months old (untreated humane endpoint, 4.5 ± 0.5 months).

Conclusion. This translational study demonstrates that low-dose AAV gene therapy in GM2 cats shows no behavioral evidence of vector toxicity, and serum antibody titers to the AAV vector are low (between 1:8192-1:32) relative to those animals treated with the full dose. Ongoing studies suggest the efficacy of low-dose thalamus and DCN directed gene therapy is limited compared to full-dose therapy with respect to HexA and vector distribution, and phenotypic improvement.

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Functions of transmembrane domain 3 of human melanocortin-4 receptor in ligand binding and activation

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Introduction. The melanocortin-4 receptor (MC4R) is a member of family A GPCRs with seven transmembrane domains that plays an essential role in controlling food intake and energy expenditure. More than 150 distinct *MC4R* mutations have been identified from patient cohorts of different ethnic origins thus far, and recognized as the most common cause of monogenic form of human obesity. Ligands targeting the MC4R have significant therapeutic potential in obesity treatment. Transmembrane domain 3 (TM3) of MC4R has been shown to be critical for ligand binding and receptor activation; however, the structure-function relationship of TM3 has not been fully elucidated. Herein, we systematically analyzed the functions of all 26 residues in TM3.

Methods. Alanine scanning mutagenesis was used to study the contribution of each residue in TM3, and saturation mutagenesis was used to investigate the effect of side chain of leucine 140 (L140) on ligand binding and signaling. All MC4R mutants were generated by QuikChange™ Site-Directed Mutagenesis protocol. Human Embryonic Kidney (HEK) 293T cells were transiently transfected with wild type or mutant MC4Rs. A superpotent analog [Nle^4, D-Phe^7]-α-melanocyte stimulating hormone (NDP-α-MSH) was used to characterize the ligand binding and signaling properties of these mutants by radioligand binding assay and cyclic AMP (cAMP) radioimmunoassay.

Results. The results of alanine scanning mutagenesis demonstrated that, among the 26 mutants, two (D126A and L140A) had significantly decreased NDP-α-MSH binding and cAMP production, suggesting D126 and L140 were important for both ligand binding and signaling. Two mutants, V128 and I143, had impaired cAMP production but normal ligand binding, suggesting that they were defective in signaling. Interestingly, despite impaired ligand binding and signaling, the basal activity of L140A was higher than that of WT (>8-fold, $p<0.001$), indicating that L140A was constitutively active. To further characterize the function of L140, we mutated leucine to 17 other amino acids. Our data showed that, among the 17 mutants, 9 mutants (L140K, L140E, L140V, L140S, L140P, L140T, L140Q, L140N and L140C) had significantly increased basal cAMP activity (at least 6-fold increase, $p<0.05$), 7 mutants (L140E, L140V, L140S, L140T, L140Q, L140N and L140C) had decreased ligand binding; and 5 mutants (L140D, L140G, L140I, L140M and L140R) had impaired ligand binding and signaling.

Conclusions. Our systematic studies demonstrated that TM3 is essential for MC4R ligand binding and activation. L140 is critical for ligand binding and plays a key role in locking the MC4R in the inactive conformation.

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Characterization of bovine viral diarrhea virus (BVDV) isolates obtained from persistently infected calves resistant to a novel antiviral compound

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Introduction. Treatment with a novel antiviral compound (DB772) effectively inhibited bovine viral diarrhea virus (BVDV) in persistently infected calves but was associated with rapid development of resistance. The objective of this research was to characterize isolates resistant to DB772 isolated from persistently infected (PI) calves treated with the compound.

Methods. Viral isolates were obtained from four Angus-cross beef calves (A,B,C,D) persistently infected with BVDV type 1 or 2 and treated with DB772 intravenously three times a day for six days. Virus obtained from each calf on Day 0 before treatment initiation was shown to be susceptible to DB772 in vitro. Virus obtained during the treatment period (Day 7 for Calf C and Day 3 for Calf D) or following treatment (Day 70 for Calf A and Day 56 for Calf B) shown to be resistant to DB772 in vitro were selected for further study. All viruses were purified by selecting clones created by serial dilutions of virus isolates from passaged white blood cell samples in the absence (susceptible samples) or presence (resistant samples) of 4 μM DB772.
Full genome sequencing was obtained on all pre-treatment and resistant isolates. Additionally, the growth kinetics of each isolate were studied by infecting MDBK cells in 96 well plates at a multiplicity of infection of 3 and incubated in the presence and absence of 4 μM DB772. After incubation for the appropriate time period (1,3,5,7,9,11,13,23,48 or 72 hours) plates were frozen to stop viral replication. Viral titrations were performed using the immunoperoxidase staining assay.

Results. Full genome sequencing revealed an asparagine to aspartic acid mutation in three isolates (B,C,D) at residue 264 (N264D) of the NS5b protein. In addition, the resistant isolate from Calf C demonstrated an isoleucine to methionine change at residue 261 (I261M) and a proline to alanine mutation at residue 262 (P262A) was present in the resistant isolate from Calf B. The resistant isolate from Calf A replaced a tyrosine at residue 289 with histidine (Y289H). All mutations were found in the NS5b protein. The resistant isolate from Calf D containing the single mutation appeared less fit than the susceptible isolate obtained pre-treatment while the remaining resistant isolates replicated at rates similar to isolates sensitive to DB772.

Conclusions. Resistance to DB772 is associated with single or multiple mutations in the NS5b protein or the RNA-dependant RNA polymerase indicating that the mechanism of action of DB772 is through inhibition of RNA replication. Administration of DB772 holds potential as part of a multi-modal approach to pestivirus treatment and control.

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Chlamydia pecorum infection of dairy cows associates with significantly reduced fertility and milk production

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Introduction: Acute infections with species of intracellular *Chlamydia* bacteria have been associated with numerous distinct clinical disease entities in cattle, most prominently abortion and fertility disorders, sporadic encephalomyelitis, kerato-conjunctivitis, pneumonia, enteritis, and polyarthritis. *Chlamydia* (*C.*) *pecorum* has also been routinely detected in cattle. However, the health impact of these ubiquitous low-level subclinical infections remains poorly characterized. In this study, we analyzed the effect of natural *C. pecorum* infection on fertility and milk production in dairy cows.

Methods: From a 3,700-head dairy farm, 451 cows after delivery of the first calf were randomly selected for the investigation. From each cow, cervical cytobrush and plasma samples were collected at first breeding. Cows were estrus-synchronized with dual injection of dinoprost tromethamine, and bred by artificial insemination 2 days later on day 65 ± 3.5 days post partum. Endpoint for determination of fertility was pregnancy 5 weeks post artificial insemination (AI). Additional plasma was collected at pregnancy examination. Cervical samples were obtained immediately after AI by insemination guns modified for cytobrush collection and analyzed by *Chlamydia* 23S rDNA real-time PCR. Luteinizing hormone in plasma was analyzed by radioimmunoassay. IgM plasma antibodies against *C. pecorum* were determined by chemiluminescent ELISA using a *C. pecorum* elementary body lysate as antigen.

Results: Of the 451 cows, 216 (47.9%) were positive in the *Chlamydia* PCR. The detected chlamydial species was in all cases *C. pecorum*. Of the *Chlamydia* PCR-positive cows, 84 (38.9%) became pregnant in the first insemination, and 132 (61.1%) remained open. In contrast, of the *Chlamydia*-negative cows, 112 (48.08%) became pregnant and 123 (52.3%) remained open (p=0.03). Mean plasma luteinizing hormone (LH) concentration at AI was significantly lower in *Chlamydia*-positive cows (109 ng/L) than in *Chlamydia*-negative cows (137 ng/L; p=0.006). Cluster analysis based on liver health index (plasma albumin, cholesterol) and *Chlamydia* immune trend index (day 100/65 anti *C. pecorum* IgM) separated the 451 cows into two clusters. Cows in the cluster with significantly higher liver health and *Chlamydia* immune trend showed 10.9% increased milk production as compared to the cluster with lower liver health index and immune trend (p=0.0002).

Conclusions: These data confirm the negative influence of asymptomatic *C. pecorum* infection on fertility and milk production in dairy cows. The dysregulation of the neuroendocrine axis evidenced by reduced baseline LH is exclusively influenced by the presence of chlamydiae in the genital tract. In contrast, the influence on milk production appears to depend on the immune response to chlamydial infection, evidenced by day 100/65 anti *C. pecorum* IgM, and its effect on circulating inflammatory mediators on liver health, evidenced by the markers for liver health, plasma albumin and cholesterol.

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Absolute quantification of canine *mda-7* mRNA and its splice variants

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Introduction. Human melanoma differentiation associated gene-7 (*mda-7*/IL-24) is one of the few genes that have the potential to be used as an effective anticancer treatment due to its growth inhibitory effect on a wide range of tumor cells. Ectopic expression (high levels) of human MDA-7 protein in cancer cells not only inhibits their growth, but also causes tumor-specific cell killing by apoptosis induction. However, these antitumor effects are not observed at physiological levels of *mda-7*. Physiologically, human *mda-7* is expressed in unstimulated monocytes, skin keratinocytes and melanocytes. Its expression can also be induced in monocytes and T cell with lipopolysaccharides (LPS), phytohaemagglutinin (PHA) and concanavalin A (ConA) treatments. However, *mda-7* is not expressed in any cancer cells with the exception of few colon cancers. We have previously identified, cloned and sequenced the canine ortholog of human *mda-7* gene and showed that the pre-mRNA transcribed from this locus is alternatively spliced to yield five different splice variants. In order to better understand mda-7 function in the dog, we sought to quantify the expression of canine *mda-7* and its splice variants in a variety of cell types.

Methods. Five splice variants were amplified by PCR and cloned into pCDNA3.1+/Hygro and pGEMT easy vectors. These recombinant plasmids were serially diluted to obtain copy numbers ranging from 3,000,000 to 30, and these dilutions were used as template to generate a standard curve by TaqMan PCR. TaqMan® primers and probes were designed to amplify and differentiate among various splice variants. Total RNA was isolated from normal canine epidermal keratinocytes (NCEKs), canine PBMCs, PHA, conA, LPS stimulated canine PMBCs and NECKs and various other dog tissues. One microgram of total RNA was reverse transcribed using a qScript cDNA synthesis kit (Quanta Biosciences). The copy number of splice variants was quantified using TaqMan Fast Universal PCR Master Mix (Applied Biosystem) under optimized PCR conditions.

Results. Canine mda-7 was not expressed in unstimulated canine PBMCs, PHA, ConA or anti-CD3 stimulated canine PBMCs, thymus, lymphnode and spleen. However, canine *mda*-7 was expressed at very high levels in LPS stimulated canine PBMCs. Cultured primary canine keratinocytes also express canine *mda-7* at very high levels, and this expression increased significantly with LPS stimulation. Cancer cell lines derived from various dog tumors types and canine tumor samples including hepatic and splenic hemangiosarcomas were also evaluated for *mda-7* expression. None of the primary tumor samples expressed canine *mda-7.* Five of the six cell lines, namely CMT-28, CMT-27, CML-10, OSW and 17-71, do not express canine *mda-7.* However, CMT-12 expressed *mda*-7 at very high levels. Canine *mda*-7sv1 was the predominant splice variant expressed, sv2 and sv5 were expressed at an intermediate level and sv3 and sv4 were expressed at the lowest levels. Relative expression of the splice variants was similar in both unstimulated NCEKs and LPS stimulated canine PBMCs. Stimulation of NCEKs with LPS caused a two-fold increase in the copy number of canine *mda*-7sv1 and sv2 with no effects on the expression levels of the other splice variants ($p<0.0001$).

Conclusions. Canine *mda-7* is constitutively expressed in cultured normal canine epidermal keratinocytes and LPS stimulation results in increased expression levels of canine mda-7. Expression of canine *mda-7* cannot be detected in unstimulated canine PBMCs. However, expression can be induced in canine PBMCs with LPS stimulation. We also showed that canine mda-7sv1 and sv2 are the predominant splice variants expressed in NCEKs.

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Survival of Dogs Treated with Radiation Therapy for Intranasal Squamous Cell Carcinoma

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Introduction. Carcinomas encompass about two-thirds of all canine intranasal tumors. Squamous cell carcinomas (SCC) comprise a small portion of these cases and little survival data has been presented for this tumor type separate from other intranasal tumors.

Methods. The records of 10 dogs with intranasal SCC from 1997-2007 were reviewed. Histopathologic diagnosis, date of diagnosis, radiation protocol and date of death or last follow-up were collected. Kaplan-Meyer survival analysis was used to generate median survival times (MST) and log-rank tests used to compare radiation protocols, metastatic involvement and tumor stage (significance $p<0.05$).

Results. Definitive radiation therapy consisted of 11 fractions of 4.5 Gy given on Monday, Wednesday, and Friday (MWF RT). The overall MST was 137 days. There was no significant difference in survival between the following: MWF RT (MST 233 days) vs. palliative radiation therapy (MST 115 days) ($p=0.433$); patients with (MST 115 days) and without (MST 137 days) lymph node metastasis at the time of diagnosis ($p=0.486$); tumor stage 4 (MST 384 days) vs. all other stages combined (MST 115 days) ($p=0.201$).

Conclusions. The MST generated from this study can be used when counseling clients about canine patients treated for intranasal SCC. Overall survival for nasal SCC appears shorter than reported for nasal adenocarcinoma. Despite numerical differences, there was no statistical difference in survival when treated with MWF RT vs. palliative RT, or with stage 4 disease. Lymph node metastasis at the time of diagnosis was not a negative prognostic factor. A larger case number would strengthen these results.

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Ultrasonographic evaluation of adrenal gland size compared to body weight and aortic diameter in normal dogs

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Introduction. Hyperadrenocorticism (Cushing's disease) and hypoadrenocorticism (Addison's disease) are two endocrinopathies in dogs. Ultrasonographic evaluation of adrenal gland size and shape aids in reinforcing a diagnosis of these endocrine diseases and in differentiating between pituitary-dependent and adrenal-dependent hyperadrenocorticism. A wide range of values have been reported in for the normal ultrasonographic dimensions of length, width, and thickness of the adrenal glands in healthy dogs. However, it is unclear whether a correlation between ultrasonographically obtained adrenal gland measurements and body weight is present. If an association exists, more precise reference intervals for adrenal gland dimensions in different-sized dogs may be established, allowing for greater confidence in determining if adrenal gland size is abnormal. Additionally, the aorta provides a structure with which to compare adrenal gland measurements. Previous studies have found that ratios of organ measurements to aortic diameter provided more repeatable and reliable measurements for quantifying the size of organs among individuals. Thus, a potential exists to establish a range of normal adrenal gland-to-aorta ratios via ultrasound for dogs of different weights. The purposes of this study were: 1. To examine adrenal gland dimensions as a function of body weight in normal dogs in three weight categories 2. To assess the utility of a ratio of adrenal gland measurements to aortic diameter in an effort to establish more reliable reference intervals for adrenal size.

Methods. The measurements of length (sagittal plane), width (transverse plane), and thickness (sagittal and transverse plane) of both adrenal glands and the diameter of the aorta (sagittal plane) were obtained by ultrasonography in forty-five dogs determined to be healthy on the basis of history, physical examination, complete blood count, serum biochemistry panel, and urinalysis. The dogs were divided by body weight into three groups (< 10 kg, 10-30 kg, and > 30 kg), with 15 dogs in each group to represent small, medium, and large breed sizes. Each adrenal gland measurement was evaluated for repeatability and was compared to the dog's body weight as well as to aortic diameter using a mixed model ANOVA and linear regression analysis.

Results. Preliminary results suggest adrenal gland length is < 2.36 cm in dogs < 10 kg, < 3.06 cm in dogs 10-30 kg, and < 3.67 cm in dogs > 30 kg. Adrenal gland width and thickness are < 0.55 cm in dogs < 10 kg, < 0.67 cm in dogs 10-30 kg, and < 0.79 cm in dogs > 30 kg regardless of the imaging plane.

Conclusions. A correlation between adrenal gland size and body weight in normal dogs is present. More precise reference intervals can be created for adrenal gland size by categorizing dogs as small, medium, or large breed. The aortic diameter can be used to generate a ratio of adrenal gland measurements to aortic diameter.

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Relationship of Virulence and Resistance in Pathogenic Canine and Feline *Escherichia coli*

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Introduction. *E. coli* is the principle cause of urinary tract infections in both human and veterinary professional medicine. A variety of virulence factors (VFs) are necessary for uropathogenesis; variability in these factors results in the extent and impact of infection. Historically, isolates with VFs that facilitate infection frequently do not carry genes associated with antimicrobial resistance. However, recently, among the more virulent strains of *E. coli* to emerge is ST131, isolate strain associated with multidrug resistance. Our laboratory has demonstrated that fluoroquinolone (FQ) use is associated with emergence of multidrug resistance in clinical canine and feline *E. coli* uropathogens. The purpose of this study was to assess the diversity and distribution of the major VFs, including phylotyping and identification of ST131 in canine and feline uropathogenic *E. coli*, and to describe the association of virulence and resistance genes in this sample population.

Methods. A total 232 isolates were randomly selected from a larger population of 1513 canine and feline *E. coli* uropathogens. Susceptibility to 15 antimicrobial agents (6 drug classes) was determined by broth microdilution. Resistance phenotypes were determined, and each isolate was classified as to type of resistance: none (NDR), single (SDR) and multi (MDR; more than two classes). Isolates were then subjected to multiplex PCR for characterization of phylotype (A, B1, B2 and D, with B2 followed by D being most pathogenic) and the presence of 7 specific virulence genes that define *E. coli* as extraintestinal infections (ExPEC), which includes the subset of uropathogens (UPEC). Characterization of ST131 *E. coli* strains was based on Achtman MLST allele SNPs. Among resistance types (NDR, SDR, MDR), proportional comparisons were made with regard to phylotype and virulence profiles, stratified by severity of clinical signs associated with the infections.

Results. Of the 232 *E. coli* uropathogens, the far majority were from dogs (80.6%) compared to cats (19.4%). The proportion of each resistance type was: NDR (n=27; 17.5%), SDR (n=96; 56.4%) and MDR (n=109; 26.12%); for MDR isolates, resistance included beta-lactams and FQ. The proportion of phylotypes expressed overall were A (15%), B1 (25%), B2 (41%), and D (19%). MDR isolates exhibited significant shifts ($p<0.05$) in phylogenetic distribution (type B1> A> D>B2; 25.6% expressing D or B2) and virulence profiles, compared to NDR isolates (B2=D>B1>A; 71.1% expressing D or B2). The percent of B2 and D isolates differed ($p<0.0001$) with severity of disease at 57, 52, 77, 67 and 80% for no clinical signs, mild, moderate, severe and life-threatening disease, respectively. Of the 232 isolates, 9 isolates (3.8%; all MDR) were characterized as ST131 (8.3% of MDR isolates).

Conclusions. Virulence profiles of clinical canine and feline *E. coli* uropathogens differ significantly with severity of infections and resistance patterns. ST131 occurs at a frequency similar to that in humans. These results may have implications with regards to the spread and persistence of resistance and virulence genes in bacterial populations and to the prudent use of antimicrobial agents.

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Accuracy & Precision of Compounded Cyclosporine Capsules & Solution

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Introduction. Drug products compounded by pharmacists are intended for situations in which a commercially available product is not available to meet the need of the patient. However, compounded products frequently are prescribed because they are cheaper; this is particularly true for cyclosporine (CsA). FDA approved products such as Atopica® are subject to extensive premarket assessment of product strength, purity and potency, and stability. In contrast, compounded drugs undergo no pre-dispensing regulatory assessment; indeed, protocols used by compounded pharmacists are not standardized leading to potential marked variability among preparations. This study described the variability of CsA preparation accuracy and precision when compounded as either a capsule or a solution at a low or high concentration. We hypothesize that $\geq$ 25% of compounded CsA products will fail to be between 90% and 110% of the labeled concentration of active ingredient.

Methods. Compounded CsA was acquired from 5 compounding pharmacies. A prescription was filled for 10 mg and 300 mg capsules, and 50 mg/ml and 150 mg/ml solution at 3 different times, at 14 to 45 days apart. Physical characteristics (color, clarity, pH, particle size, etc.) of solutions were recorded. Accuracy (actual compared to predicted) was based on CsA content (strength) in each preparation. Precision (variability measured as coefficient of variation [CV%] surrounding product strength) was based on replications (n=3) from each pharmacy (n=5) for each preparation. CsA was analyzed by HPLC (High Performance Liquid Chromatography) in 3 separate aliquots for each sample. FDA approved CsA (Atopica® and a human USP modified generic) served as positive controls. The proportion of preparations for which CsA strength was not within 90 to 110% % of the labeled strength were determined.

Results. Drug recovery from the FDA approved preparations was 98$\pm$1.5% for capsules and 84% for solutions; CV for % recovery was 1 to 2.7%. For compounded preparations, accuracy (actual mean $\pm$ sd (mg) and % of predicted) among all pharmacies for the capsules were as follows: 290$\pm$17 (300; 97%) and 9.8$\pm$0.7 (10 mg; 96%); and for solutions: 41$\pm$ 2.5 (50 mg/ml; 84%) and 119$\pm$ 11 mg/ml (150 mg/ml; 79%). One pharmacy particularly performed poorly with strength = 31.4$\pm$ 12.5% less than labeled. Precision (CV%) for within pharmacies ranged from 5.5 to 24% (higher for solutions) and between 5 pharmacies was as follows: for capsules, 3.96% to 10.91% (300 mg), and 1.58% to 12.24% (10 mg); and for the solutions 5.98% to 18.86% (150 mg/ml) and 2.14% to 8.69% (50 mg/ml). Physical characteristics differed markedly among pharmacies but similar within pharmacies.

Conclusions. Compounded capsules appear to be accurate and precise but >60% of the oral solutions did not contain 90% to 110% of the labeled CsA concentration. Selected pharmacies appear to be more inaccurate. The degree of precision suggests that standardizing CsA preparation protocols is warranted.

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Post-graduate/Faculty Platform Presentations

Determination of the *in vitro* effects of two forms of hydroxyethyl starch solutions on thromboelastography and coagulation parameters in healthy dogs

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Introduction. Synthetic colloid dosages are typically limited to 20 ml/kg/day due to their adverse effects on coagulation. Newer low molecular weight hydroxyethyl starch solutions, such as Voluven®, have been developed to avoid this issue. The goals of this study were to determine if Voluven® was less detrimental to coagulation when compared to Hetastarch as well as to determine what dilutional volume of these two solutions was needed to observe changes in coagulation parameters *in vitro*.

Methods. Whole blood samples were obtained from 25 healthy canine volunteers and diluted with Hetastarch and Voluven® in test tubes to dilutions of 10:2 (~ 18 ml/kg dose), 10:4 (~ 36 ml/kg dose) and 10:6 (~ 54 ml/kg dose) as well as with 0.9% NaCl at a dilution of 10:6. A coagulation profile and thromboelastogram were performed on each sample at baseline and with each dilution.

Results. A significant difference was seen at the 10:6 dilution between Hetastarch and Voluven® with the following thromboelastography variables: maximal amplitude, α (angle), and K (clot formation time). 0.9% NaCl was also significantly different from both Hetastarch and Voluven® at the 10:6 dilution with respect to the same TEG variables. There was no significant difference in prothrombin time or activated partial thromboplastin time when Hetastarch and Voluven were compared at different dilutions.

Conclusions. As detected by thromboelastography, Voluven® appears to cause less of an effect on coagulation at higher dilutions than Hetastarch *in vitro*. A dilutional coagulopathy does not appear to be the cause of this effect. Thromboelastography may be a more sensitive monitoring tool for the presence of coagulopathy secondary to hydroxyethyl starch administration than the standard coagulation profile parameters.

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Comparison of BVDV-specific antibody titers and decay between calves fed maternal colostrum or colostrum replacer at birth

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Introduction. Providing high concentrations of specific antibodies through colostrum is a key management strategy to reduce clinical disease caused by bovine viral diarrhea virus (BVDV) in young calves. The objective of this study was to compare the quantity and persistence of BVDV-specific neutralizing antibodies in serum of calves fed maternal colostrum (MC) or a commercial colostrum replacer (CR) at birth.

Methods. Forty newborn male Holstein calves were removed from their dams at birth and assigned to 1 of 2 treatment groups. **Group CR** (n=20) received 2 packets of Land O'Lakes-Bovine IgG® colostrum replacer (100 g of IgG per packet). **Group MC** (n=20) received 3.8 Lt. of high quality fresh or frozen maternal colostrum (> 50 g of IgG per liter). All calves were moved to an isolated BVDV negative facility to prevent inadvertent infection with BVDV. A pre-colostral serum sample was collected from each calf for BVDV virus isolation, detection of BVDV-neutralizing antibodies, and measurement of IgG concentrations. A second measurement of total IgG concentration in serum was performed at 48 hours of life. Blood samples for detection of BVDV neutralizing antibodies were collected after maternal colostrum or colostrum replacer feeding at 48 hours, 7 days of life, and monthly until the calves became seronegative (antibody titer < 1:2). Total quantities and decay of BVDV neutralizing antibodies and total IgG concentration at 48 hours of life was compared between treatment groups by repeated measures ANOVA.

Results. Calves in the MC group had higher levels of serum IgG, but lower levels of BVDV1 and BVDV2 antibody titers at 48 hours of life compared to calves in the CR group. Although BVDV1-specific antibody titers remained higher in CR calves as compared with MC calves during the first 5 months, statistically significant differences were detected only at 48 hours and 3 months ($P < 0.05$). Compared to MC calves, BVDV2-specific antibody titers remained significantly higher in CR calves during the first 4 months ($P < 0.05$). The mean time to become seronegative to BVDV1 and BVDV2 was 7 and 6 months respectively for all the calves and was not significantly different between groups ($P > 0.05$).

Conclusions. Calves in the CR group had higher BVDV-specific serum antibody levels at 48 hours and during the first months of life compared with calves in MC group; however, calves in both groups became seronegative to BVDV at the same time. Persistence of BVDV-specific antibodies was similar in CR and MC groups. Colostrum replacers provide adequate BVDV-specific antibodies protecting calves against acute BVDV infection; however higher total serum IgG concentrations at 48 hours are reached when maternal colostrum is used, thus reducing failure of passive transfer.

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A Novel Missense Mutation associated with Hemophilia A in two Boxers.

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Introduction. Hemophilia A is an inherited, X-linked coagulation disorder that is caused by a deficiency in coagulation factor VIII. Over 100 mutations in the gene encoding factor VIII have been documented in people, but only intron inversions have been documented in dogs. An eleven-week-old male Boxer (Boxer 1) from a litter of twelve puppies (5 males, 7 females) developed multiple subcutaneous hematomas and lameness. Two male siblings died after tail docking, one male sibling had no clinical signs and one male sibling was euthanized after developing large ulcerated hematomas over the shoulder. The females in the litter had no clinical signs. The puppy was documented to have <1% factor VIII activity and was diagnosed with hemophilia A. Subsequently, a second male Boxer (Boxer 2) with similar clinical signs and from the same region of the country was also diagnosed with Hemophilia A. The purpose of this study was to identify the causative mutation(s) in both dogs.

Methods. Genomic DNA was isolated from EDTA blood samples from Boxer 1, the dam, three female siblings, and one asymptomatic male sibling. Similar samples were prepared from Boxer 2 and its dam and sire. Primers were designed in non-coding regions to amplify the 26 exons of the factor VIII gene via PCR. Target bands were excised, and DNA was isolated and sequenced directly.

Results. In both Boxers, DNA sequencing revealed a single nucleotide change, C to G, at nucleotide position 1487 (1487C>G) in Exon 10. The change is predicted to result in the substitution of an arginine for a proline at amino acid 477 (P477R) in the A2 domain of Factor VIII. The dam and 3 female siblings of Boxer 1 were heterozygous for the mutation while the asymptomatic male sibling was clear of the mutation. The dam of Boxer 2 was a carrier for the mutation, and the sire was clear.

Conclusions. This is the first published documentation of a missense or any single nucleotide mutation associated with hemophilia A in dogs and the first reported case of Hemophilia A in a Boxer. Based on pedigree evaluation, the dam of Boxer 1 was the great, great grandmother of Boxer 2. The same mutation has been documented in people, also resulting in <1% factor VIII activity.

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Function of the *ramus communicans* of the medial and lateral palmar nerves of the horse

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Introduction: Reasons for performing study: The role of the communicating branch between the medial and lateral palmar nerves of the thoracic limbs of horses (*i.e.*, the *ramus communicans*) in conveying sensory impulses proximally should be determined to avoid errors in interpreting diagnostic anaesthesia of the palmar nerves.
Hypothesis: The sensory nerve fibers in the *ramus communicans* of the thoracic limb of horses travel proximally from the lateral palmar nerve to ramify with the medial palmar nerve, but not *vice versa.* The objective is to determine the direction of sensory impulses through the *ramus communicans* between the lateral and medial palmar nerves.

Methods: Pain in a thoracic foot was created with set-screw pressure applied to either the medial or lateral aspect of the sole. The palmar nerve on the side of the sole in which pain was created was anaesthetised proximal to the *ramus communicans* with local anaesthetic solution. Lameness was evaluated objectively using a wireless, inertial, sensor-based, motion analysis system (Lameness Locator®). Lameness was also evaluated subjectively using a graded scoring system. Local anaesthetic solution was then administered adjacent to the *ramus communicans* to determine the effect of anaesthesia of the *ramus communicans* on residual lameness.

Results: When pain originated from the medial or the lateral aspect of the sole, anaesthesia of the ipsilateral palmar nerve proximal to the *ramus communicans* did not entirely resolve lameness. Anaesthesia of the *ramus communicans* further attenuated or resolved lameness.

Conclusions: Sensory fibers travel in both directions in the *ramus communicans* to connect the medial and lateral palmar nerves. When administering a low palmar nerve block, both palmar nerves should be anaesthetised distal to the *ramus communicans* to avoid leaving non-desensitised sensory nerve fibers travelling through this neural connection. Alternatively, local anaesthetic solution could also be deposited adjacent to the *ramus communicans* when anaesthetizing the palmar nerves.

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Comparison of four techniques of centesis of the lateral compartment of the femorotibial joint of the horse

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Introduction. *Reasons for performing study*: Our experiences indicate that centesis of the lateral compartment of the femorotibial joint is often unsuccessful.
Hypothesis: We hypothesized that a drug can be administered into the lateral compartment of the femorotibial joint via a diverticulum of this joint that surrounds the medial aspect of the long digital extensor tendon and that this technique is more accurate than described techniques of centesis of this compartment.

Objective. To determine the accuracy of 2 published and 2 unpublished techniques of centesis of the lateral compartment of the femorotibial joint.

Methods. Twenty-four stifles of 12 horses were divided equally into 4 groups, and a radiocontrast medium was injected into the lateral compartment of the femorotibial joint of each group using a hypodermic needle inserted: 1) caudal to the lateral patellar ligament proximal to the tibial plateau 2) caudal to the long digital extensor tendon proximal to the tibial plateau and 3) between the long digital extensor tendon and bone of the extensor groove of the tibia, or 4) directly through the long digital extensor tendon until it contacted bone. Twelve veterinary students who had no experience using any of these techniques performed the injections. Accuracy of each technique was determined by examining radiographs obtained after injecting the contrast medium.

Results. The incidence of success for the technique that accessed the lateral compartment by inserting a needle through the tendon was 100%. Successful centesis of the lateral compartment using other techniques was far less.

Conclusions. The lateral compartment of the femorotibial joint can be accessed accurately by inserting a needle through the long digital extensor tendon as it lies within the extensor groove. Other techniques may not be as accurate for clinicians inexperienced in centesis of the lateral compartment of the femorotibial joint.

Veterinary Student Poster Presentations

Use of TEG ± Cytochalasin D to Evaluate the Role of Hyperfibrinogenemia as a Cause for Hypercoagulability in Sick Animals

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Introduction. Thrombelastography (TEG) allows a global view of coagulation by combining the effects of plasma coagulation proteins and blood cells into one tracing called a thrombelastogram. The measured values, R, K, α-Angle, and MA, correlate with the time to initial fibrin formation, rate of clot formation and clot strength. Fibrinogen concentration, platelet number and platelet function contribute substantially to maximum clot strength. Fibrinogen is a positive acute phase protein produced in high concentration during inflammation. TEG shows that many sick patients are hypercoagulable but fibrinogen's contribution has not been evaluated in veterinary patients. Cytochalasin D, a fungal toxin that inhibits platelet actin polymerization, and produces a thrombelastogram showing only the plasma protein contribution to coagulation. Evaluation of the relationship between fibrinogen concentration and clot strength will determine if hypercoagulability in sick animals is entirely a result of hyperfibrinogenemia.

Methods. Blood was collected from 33 healthy dogs and 10 sick dogs. CBC and coagulation panels (PT, APTT, Fibrinogen, AT, D-dimer, and FDP) were performed along with TEG. For each sample, one TEG reaction contained Cytochalasin D to eliminate the platelet contribution to the thrombelastogram. Standard TEG was performed for the other reaction, with an equal volume of saline as a control. Results obtained from healthy and sick dogs were compared and the association between fibrinogen concentration and clot strength was assessed.

Results. Clot strength and fibrinogen concentration were significantly greater in sick dogs than in healthy dogs. In sick dogs clot strength was directly correlated with fibrinogen concentration.

Conclusion. Hyperfibrinogenemia contributes substantially to hypercoagulability in sick dogs. This information may help to guide anti-thrombotic therapy in animals with hypercoagulability.

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Evaluation of coagulation factor VIII and von Willebrand factor genes in a colt with presumptive hemophilia A

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Introduction. Hemophilia A, the most common inherited coagulopathy in horses, is an X-linked disorder caused by a functional or quantitative deficiency in coagulation factor VIII (FVIII). Equine patients with this disorder have a severe bleeding diathesis which often leads to death. The coding regions of the FVIII and von Willebrand factor (VWF) genes have not been evaluated in horses with hemophilia A to identify causative mutations associated with decreased FVIII in these patients. The purpose of this study was to evaluate the coding regions of the FVIII gene and the VWF FVIII binding site in a hemophilia A colt and its dam in an effort to identify a causative mutation.

Methods. Genomic DNA was isolated from a Tennessee Walking Horse colt with hemophilia A (15% FVIII activity), its dam, and seven control horses. DNA segments were amplified using PCR and primer sets designed in the non-coding regions flanking the 26 exons of the FVIII gene and exons 17 through 28 for the VWF gene. PCR products were separated on agarose gels using electrophoresis, and products were extracted and submitted for sequencing. Foal and mare FVIII and VWF sequences were compared to normal equine sequence and the equine genome available on GenBank.

Results. Evaluation of the FVIII coding region sequences for the colt and mare did not reveal any significant changes when compared to normal equine sequence and published equine GenBank sequence. No significant changes were noted in the exons encoding the FVIII binding site in the VWF gene in either horse.

Conclusions. The reason for the colt's reduced FVIII activity was not identified in the evaluated areas of the FVIII and VWF genes. Genes encoding proteins involved in intracellular transport of coagulation factors and non-coding regions of the FVIII gene, including the promoter, are possible locations for a causative mutation in the colt and warrant further examination.

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The effects of enhanced hypertrophy, reduced oxygen supply, and heat load on breast meat yield and quality

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Introduction. Skeletal muscle development proceeds from early embryogenesis through marketing age in broiler chickens. Although myofiber formation is essentially complete at hatching, myofiber hypertrophy often occurs post hatch through the assimilation of satellite cell nuclei into the myofibers. As the diameter of the myofiber increases, capillary density peripheral to the myofiber is marginalized, thus limiting oxygen supply and its subsequent diffusion into the myofiber, causing micro-ischemia. The superficial and deep pectoralis muscles constitute 25% of the total body weight in a market age bird, thus compromise of those muscle groups can have a profound economic impact on broiler production. We hypothesized that marginal capillary support relative to the hypertrophic myofibers increases the incidence of micro-ischemia, and therefore myofiber damage, especially in contemporary high-yield broilers under stressful conditions such as high environmental temperature.

Methods. We evaluated the following parameters in four different broiler strains at 36 and 49 days of age when reared under thermoneutral (20-25C) and hot (30-35C) environmental conditions: Capillary and vessel density, myofiber density and diameter, and amount of edema. Myofiber density and edema were histomorphometrically evaluated using Image J.

Results. Least squares means analysis revealed significant differences in myofiber diameter, muscle cross sectional area, myofiber density, vascular density including total number of vessels (capillaries and larger vessels), and number of capillaries per fiber ($p \geq 0.0001$) among the different age groups. In addition, myofiber diameter ($P \geq 0.001$), number of fibers ($P \geq 0.0001$) and number of capillaries ($P \geq 0.05$) per fiber differed between birds reared in thermoneutral versus hot conditions. Significant strain specific differences were not observed.

Conclusions. It has been hypothesized that heat stressed broilers cannot synthesize protein as efficiently as birds raised in thermoneutral conditions and therefore, muscle growth by hypertrophy is limited. The present study demonstrated that broilers in thermoneutral environments had an average fiber diameter of 597 pixels, while those in heat stressed environments had an average diameter of only 547 pixels ($p \geq 0.001$). As a result of smaller myofibers, the number of fibers per field of view increased 17% in heat stressed broilers as compared to non-heat stressed birds($p \geq 0.0001$). Heat stressed broilers also had roughly the same number of capillaries per field, giving them a lower capillary to fiber ratio ($p \geq 0.05$) than the non-heat stressed broilers. Therefore, reduced vascular support per fiber in broilers raised in hot environments may predispose the birds to micro-ischemia and muscle damage.

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Gene Therapy for Tay-Sachs Disease in Jacob Sheep

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Introduction. Tay-Sachs Disease (TSD) is a fatal lysosomal storage disease in humans caused by build-up of GM2 ganglioside in lysosomes of the CNS. Ordinarily, GM2 ganglioside is catabolized by β-hexosaminidase A (HexA), a heterodimer composed of alpha and beta subunits, encoded by *HEXA* and *HEXB* genes. In TSD, *HEXA* is mutated causing malformation of the alpha subunit, resulting in nonfunctional enzyme. Excess GM2 ganglioside storage leads to progressive neurodegeneration and death, usually by five years of age. Adeno-associated virus (AAV) vectors encoding wild type *HEXA* have been successful in transducing cells and producing functional HexA alpha subunit both *in vitro* and *in vivo*. Though lack of authentic animal models has hindered therapy development for TSD, the recent discovery of TSD in Jacob sheep has provided invaluable opportunities to perform translational research in animals whose brain size and complexity are similar to that of human infants.

Methods. In this study one affected Jacob sheep was treated with AAV vector encoding *HEXA*, and another was treated with AAV vectors encoding both *HEXA* and *HEXB*. Injections were performed intracranially into the thalamus and lateral ventricle.

Results. Untreated TSD sheep lived for an average of 7 months, while *HEXA* and *HEXA+HEXB* treated sheep lived to 14.8 and 14.0 months, respectively. In untreated brains, HexA activity was 7.0% of normal controls. In *HEXA* and *HEXA+HEXB* treated sheep brains, HexA activity was detected throughout the cerebrum and cerebellum reaching up to 2.1 fold normal and 155.8 fold normal, respectively.

Conclusions. These results demonstrate the effectiveness of AAV gene therapy in transducing cells and producing functional enzyme throughout the brain. The supranormal enzymatic activity levels seen in *HEXA+HEXB* treated sheep brain suggest that optimal production of HexA is achieved by co-expression of both subunits simultaneously. These results will be used in the design and implementation of human clinical trials for TSD.

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Dystrophin-Deficient Myopathy in the Springer Spaniel

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Introduction. Duchenne muscular dystrophy (DMD) is the most common form of muscular dystrophy occurring in humans, and is caused by mutations in the dystrophin gene. Canine Duchenne-like muscular dystrophy is an excellent model of human DMD genetically, phenotypically, and in response to therapy. Many mutations of the canine dystrophin gene have been identified and sequenced in a number of breeds. The dystrophin gene, found on the X chromosome, is large, spanning 2.4 million bases. The mRNA transcript contains 13,887 bases, and is translated into the protein dystrophin. This protein plays a crucial role in the structural integrity of muscle and muscle tissue maintenance. The absence of dystrophin causes muscle necrosis, fibrosis, and progressive muscle wasting. The objective of this study was to identify the location of the error responsible for dystrophin-deficiency in the English springer spaniel. Therefore, the entire coding region as well as the 5' and 3' untranslated region were evaluated for possible mutations.

Methods. The majority of the dystrophin mRNA from an affected English springer spaniel was PCR amplified, from skeletal muscle, in overlapping segments of approximately 2000 bases. In some instances, smaller amplicons were used to confirm or better amplify regions of the mRNA. The 5' and 3' untranslated regions were analyzed by rapid amplification of cDNA ends (RACE). For the 5' untranslated region, mRNA was reverse transcribed and amplified using the FirstChoice ® RLM-RACE Kit, which is designed to select for cDNA from only full length, capped mRNA. The 5' RLM-RACE PCR product was also cloned into a plasmid vector using the Promega pGEM ®-T Easy Vector Systems. Bacteria carrying appropriate clones were identified by restriction digestion and electrophoresis. A classic RACE protocol was used for the 3' untranslated region. PCR products, cloned 5' RACE products and bulk 5' and 3' RACE products were sequenced. All sequenced results were compared to the published normal canine dystrophin gene sequence.

Results. Many differences between the English springer spaniel and published canine cDNA sequence were identified. The majority of changes were single base nucleotide polymorphisms, with no effect on the amino acid sequence or were conservative changes with only a small effect. The 5' and 3' untranslated region did not contain any significant base changes that would cause the gene to be expressed improperly. Three non-conservative amino acid changes were found at positions 7992, 10607, and 10702 within the coding region of the sequence. One of these mutations is most likely the candidate for the causative mutation for DMD in this breed.

Conclusions. Currently, work is being done to verify or duplicate some small portions of the sequence. However, these missense point mutations must be analyzed further to assess which is responsible for modifying the dystrophin protein causing dystrophin-deficiency in the springer spaniel.

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Expression of the Putative Tumor Suppressor FOXP3 Gene in Canine Mammary Tumor Cells

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Introduction. FOXP3 is a transcription factor originally found in regulatory T lymphocytes, as well as epithelial cells of many tissues including mammary epithelium. Research has shown FOXP3 plays a key role in up-regulating tumor suppressor genes and down-regulating tumor oncogenes. FOXP3 mutations or deletions have also been shown to contribute to mammary tumor growth in human mammary tumor cell lines and to be highly expressed in cells derived from malignant tissues.

Methods. We investigated canine mammary tumor (CMT) cell lines with known defects in oncogene and tumor suppressor gene expression. 6 CMT cell lines included CMT9, CMT12, CMT27, CMT28, CMT47, CMT119 derived from different dog breeds and normal canine fibroblasts (NCF) grown in culture and total RNA was isolated. CMT cells were evaluated for presence of FOXP3 mRNA following RNA extraction and semi-quantitative reverse transcription polymerase chain reaction (PCR). Primers for FOXP3 amplicons were designed from the canine genome using Vector NTI software. FOXP3 amplicons were evaluated by agarose gel electrophoresis and their identity confirmed by DNA sequencing. Additionally, fluorescence activated flow cytometry was used to detect the presence and amount of the nuclear transcription factor FOXP3 protein in each of the CMT cell lines.

Results. FOXP3 was detected by rt PCR and flow cytometry in CMT cell lines. Normal thoracic canine fibroblasts (NCFs) were also evaluated for the presence of FOXP3 expression using both RNA extraction/rt-PCR and flow cytometry as non-neoplastic cell controls. The expression of FOXP3 in canine mammary cancers provided indications of defects in an important and potent transcription factor known for its effects on the immune system cell subsets and several cancer types. Comparing results from rt-PCR and flow cytometry, there is conflicting data where some cell lines express RNA yet fail to express the protein. The cell lines that are in the low FOXP3 category (CMT 9, 119 and 47) were not expressing the protein in abundance despite the presence of the mRNA.

Conclusions. One possible explanation for this phenomenon could be that the cells still maintain the necessary machinery to produce the FOXP3 RNA, yet a mutation in the gene could cause failure of mRNA translation into FOXP3 protein or a mutated protein is formed which is unstable. Failure of FOXP3 protein expression could lead to less tumor suppression thus allowing tumor growth. Other possible explanations for CMT 9, 119, and 47 expressing little FOXP3 protein could be a malfunction in cell cycle pathways. It has been shown that p53 function is critical for FOXP3 activation. If there was a defect in p53 expression this could lead to failure of FOXP3 activation and subsequent tumor suppression. Other CMT cell lines expressed abundant FOXP3 mRNA and protein (CMT 12, 27, and 28) and were surprising given FOXP3's role in tumor suppression. If such large amounts of FOXP3 protein were expressed tumor growth should be suppressed as well. A possible explanation for this phenomenon could be that aberrant cell growth is occurring due to mutation in FOXP3, and in response, abundant levels of FOXP3 were produced in response. Increased FOXP3 expression in human breast cancer has been associated with poor prognosis survival and these canine cell lines, expressing FOXP3 at intermediate to abundant levels, were obtained from terminal cases. Evaluation of FOXP3 expression defects may provide an important new gene prognostic indicator or therapy target for the treatment of canine mammary cancer.

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Live and inactivated filamentous phage for immunocontraception of wild and feral animals

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Introduction. Overpopulation of certain animal species remains a serious economic, health, and welfare concern and is a worldwide problem. Inexpensive humane methods to reduce the number of animals born are urgently needed to provide practical cost-effective safe alternatives to current animal control methods. Our focus is on development of contraceptive vaccines for wild and feral animals using filamentous phage as a delivery vector for immunogenic peptides. The use of phage as a carrier for immunogenic peptides provides significant benefits such as high immunogenicity, low production cost, and high stability of phage preparations. However, introduction of live recombinant phage into environment for control of free-roaming animals might represent a potential ecological problem. To address this issue, we accomplished comparative studies of antigenic properties of live and inactivated (not viable) phage, which, if released, will degrade as any other protein, thereby not contaminating the environment.

Methods. In our experiment, we used a phage clone that was previously selected from a landscape phage display library and shown to stimulate production of anti-sperm antibodies with contraceptive properties. Several methods of phage inactivation were tested, including drying, freezing, heating, and UV irradiation.

Results. Under studied conditions, only heating at 76ºC for 3 hrs and UV irradiation resulted in complete phage inactivation. Phage samples treated by heat and UV were characterized using spectrophotometry and electron microscopy. To test phage antigenicity, live and inactivated phage preparations were injected into mice and antibody responses assayed by ELISA. It was found that phage killed by heat causes little to no immune responses probably due to destruction of phage particles. UV inactivated phage stimulated production of IgG serum antibodies at the levels comparable to live phage.

Conclusions. If proper phage inactivation methods are used, peptides displayed on the capsid of filamentous bacteriophage remain antigenic. Vaccines based on filamentous phage inactivated by UV irradiation might represent safe alternatives to vaccines composed of infective phage particles, allowing their use in situations where release of the vaccine components into the environment is problematic to control.

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An analysis of feline infertility, as a result of ovarian or uterine pathology

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Introduction. Feline breeding colonies are important to the feline industry by either preserving traits desirable for a particular breed, or in research settings by maintaining genetic traits necessary for medical treatments. Work in the domestic feline population can also be extrapolated to the endangered wild feline populations. As breeding females' age, their reproductive efficiency declines. The objective of this study is to determine the most common causes of infertility in breeding females that are no longer producing litters of kittens.

Methods. 24 queens within the gangliosidosis feline breeding colony housed at the Scott Ritchey Research Center were selected for inclusion into the study. Cats were entered into the study if they had a history of infertility for more than one year duration, reduced litter size or survival of kittens following at least two pregnancies, or were diagnosed with pathology leading to infertility. For comparison, 2 normal cats and 1 juvenile cat were also evaluated. The breeding records of each queen were evaluated for age at last litter, number of litters produced, average litter size, and previous fertility issues. The uterus and ovaries of each queen were evaluated for gross pathology and histopathology. Three tissue samples were taken from four regions of each reproductive tract collected and are as follows: left ovary, right ovary, left uterine horn, and right uterine horn. The tissue samples were processed by means of dehydration using a series of ethanol dilutions [70%, 80%, 95%, and 100% respectively], followed by placement of the dehydrated samples in paraffin wax. Once the paraffin blocks containing the tissue samples solidified, 6 micrometer (µm) sections were cut using a rotary microtome. The tissue sections were placed in a warm water bath (43°F), and adhered to a microscope slide. The slides were stained with hematoxylin and eosin (H&E stain) and evaluated for abnormal pathology.

Results/Discussion. In 6 out of 24 (25%) cases, atrophy was observed in the uteri and ovaries characterized by deficient numbers of endometrial glands and ovarian follicles, respectively. Evidence of pyometria, cystic endometrial hyperplasia (CEH), and endometritis were found in 18 of 24 (75%) queens. Seventeen of the 18 (94%) queens that were diagnosed with one (16%) or a combination (58%) of the pathologies listed, started showing signs of infertility by two years, eight months of age. Prior to the onset of infertility, the queens were exposed to an average of 4 male cats, and produced an average of 2 litters. This data suggests that mature, continuously breeding, female cats may show signs of infertility by 3 years of age as a result of CEH, endometritis, pyometria and/or ovarian cysts.

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Development of an Adenovirus-Vectored Recombinant Vaccine for Infectious Bronchitis Virus

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Introduction. Infectious bronchitis virus (IBV) causes significant economic losses to the poultry industry in the form of decreases in egg production and weight gain, and increased condemnations due to airsacculitis. Previous work has shown that Ark-type IBV vaccine strains undergo intraspatial variation in the host which results in selection of new predominant IBV subpopulations with non-synonymous changes in the S1 subunit of IBV's spike protein (Gallardo, R.A., V.L. van Santen, & H. Toro 2010). Due to the importance of the spike protein for host cell attachment and phenotypic variability among IBV strains, it is conceivable that a recombinant vaccine encoding the spike protein of host-selected IBV populations would confer more efficient protection than vaccines developed by serial passaging in embryonated eggs. Replication deficient adenovirus (Ad) vectoring the IBV S1 gene (AdIBV) of two distinct subpopulations were constructed as described (Toro et al 2007) and tested in chickens.

Methods. Chickens were vaccinated *in ovo* on day 18 of embryonation with one of two AdIBV constructs (~10^5-10^7 ifu/bird) and challenged via mucosal routes at 22 days post-hatch using a virulent Ark IBV strain. A positive control group was vaccinated intramuscularly at 1 day of age with a commercially available attenuated ArkDPI. On Day 20, pre-challenge sera and, on Day 27, post-challenge sera were obtained from all birds for virus neutralization assays (VNT) as accepted (Gelb & Jackwood, 2008). On Day 27 (5 days post-challenge) clinical signs were evaluated followed by euthanasia. Lacrimal fluid samples were obtained to determine viral load by qRT-PCR and tracheal samples were collected for histomorphometry using ImageJ (Rasband, 2011).

Results. Vaccine construct designated AdIBVC2 provided slight reduction in clinical signs, reduction of the viral load in lachrymal fluids, and increased virus neutralization titers as compared to negative controls. The histopathological findings were consistent with the clinical signs and virus concentration in the upper respiratory tract. AdIBV C4 provided neither protection nor reduction of the viral load.

Conclusions. AdIBVC2 delivered at a dose of 10^7 ifu/bird moderately decreases severity of IBV clinical signs when compared to the unvaccinated chickens. Future studies may focus on evaluating protection induced by vaccination with higher doses of the AdC2 recombinant vaccine.

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Daidzein Acts Directly in Testicular Leydig cells to Disrupt Androgen Biosynthesis

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Introduction. Endocrine disruptors (EDs) are chemicals in the environment that mimic and/or antagonize the function of endogenous hormones. Soybeans are the primary ingredient in soy infant formulas. Interestingly, it has been determined that soybeans contain high concentrations of the chemicals genistin and daidzin, which are capable of acting as phytoestrogens (dietary estrogens). Genistin and daidzin are hydrolyzed by gut microflora to their biological active forms genistein and daidzein with the capacity to utilize a number of mechanisms, including binding of estrogen receptors, affecting Leydig cells. Testicular Leydig cells produce testosterone (T), the androgen that supports male reproductive activity and maintain the male phenotype. The literature has only little information on the effects of daidzein, giving the impression that biological effects associated with consumption of soy-based diets are related only to genistein action.

Methods. In the present study, Leydig cells were isolated from 35 day-old Long Evans male rats and incubated in DMEM/F-12 culture media containing luteinizing hormone at 10 ng/mL with or without daidzein (0, 100 nM) for 18 h. At the end of treatment, Leydig cells were harvested to normalize their numbers. One group of cells was then incubated further in microcentrifuge tubes for 3 h. The amounts T production was measured in spent media by radioimmunoassay. The remaining group of cells was lysed and Steroidogenic Acute Regulatory Protein (StAR) and steroidogenic enzyme protein expression were assessed using Western blot analysis.

Results. The results indicated that T production by daidzein-treated Leydig cells was decreased (27 ± 2.5 ng/ml • 3 h) compared to control, untreated cells (44 ± 7.5)($P<0.05$). Furthermore, analysis of the androgen biosynthetic pathway by Western blot analysis showed that levels of the StAR were increased after treatment with daidzein in comparison to control. There were no significant differences in steroidogenic enzyme protein expression between the control and treatment groups ($P > 0.05$).

Conclusions. Increased StAR protein levels in Leydig cells have been attributed to decreased rates of phosphorylation, which disrupts cholesterol mobilization and availability for androgen biosynthesis. The results demonstrate clearly that daidzen has the capacity to regulate androgen secretion. Because genistein also exerts similar effects, it is likely that daidzein and genistein act in a dose additive manner to effect Leydig cells and perhaps other tissues. Altogether, these findings have implication for the use of soy-based infant formulas because neonates are especially sensitive to changes in hormonal activity during development.

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Comparison of gene expression of TNF-α and IL-1β in brain tissue of normal and GM2 gangliosidosis affected cats

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Introduction. GM2 gangliosidosis is a lipid storage disease in which deficiency of a hydrolytic enzyme leads to accumulation of GM2 ganglioside in lysosomes, resulting in degeneration of neuronal tissue and subsequent inflammatory responses. Cats affected with GM2 gangliosidosis provide a model to study the effectiveness of gene therapy to deliver the insufficient enzyme and impede disease progression. The marked inflammatory response associated with neurodegeneration, namely the production of inflammatory cytokines such as IL-1β and TNF-α, offers a way to track disease progression or correction. TNF-α and IL-1β are produced by microglial cells, T-cells, astrocytes, and macrophages and are known to activate vascular endothelium

Methods. 3 cats affected with GM2 gangliosidosis were treated by intracranial injections of an AAV vector encoding feline hexosaminidase (AAV-fHex), the lysosmal enzyme of which they are deficient. 16 weeks post treatment the treated cats and age-matched normal and affected untreated controls were sacrificed. Brain tissue was sectioned and frozen. mRNA was isolated from frozen feline brain sections, treated with DNAse, and converted to cDNA for quantitative PCR. Comparison of housekeeping genes to be used for normalization established RPL-17, a gene encoding a ribosomal protein, to be the most consistent in brain tissue regardless of disease status and age. Expression levels of IL-1β and TNF-α were compared between normal, affected, and AAV-treated cats throughout the brain.

Results and Conclusions. Normal cats displayed higher levels of IL-1β and lower levels of TNF-alpha compared to affected cats. Affected cats that were treated with AAV-fHex appeared more similar in cytokine expression levels to normal cats than those affected but untreated. The higher levels of TNF-alpha in affected untreated cats is likely a result of the widespread inflammatory response noted with accumulation of GM2 ganglioside, while the decreased levels in treated cats suggest that therapeutic intervention may alleviate disease progression.

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The soy isoflavone daidzein regulates Leydig cell division in the neonatal rat testis

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Introduction. Soybeans have become an increasing source of dietary protein around the world, especially as a constituent of infant formulas. However, soy beans contain approximately equal amounts of the isoflavones genistein and daidzein, which are able to interact with estrogen receptors and other transcriptional factors in testicular Leydig cells. The latter produce the male androgen testosterone (T) to support male reproductive activity and the male phenotype. Much research has been done to establish the capacity of genistein to interfere with the endocrine axis, but much less is known about the biological activities of daidzein.

Methods. The current study was designed to determine the effects of daidzein on Leydig cell proliferation, the initial step in the differentiation process of Leydig cells. Therefore, testes were collected from 21 day-old Long Evans male rats for the isolation of Leydig cells using enzymatic digestion and Percoll gradient centrifugation. Subsequently, purified Leydig cell fractions were incubated in DMEM/F-12 culture media containing 10 ng/mL luteinizing hormone and 0, 100, and 200 nM daidzein for 18 h. At the end of the treatment period, Leydig cells were incubated further in culture media containing tritium labeled (^{3}[H]) thymidine for 3 h in order to assess proliferative activity. In addition, unlabeled cells were processed to obtain lysates for Western blot analysis to measure cell cycle protein regulators.

Results. The results of ^{3}[H] thymidine incorporation assays showed that daidzein induced proliferative activity in Leydig cells at the 200 nM concentration (0.73 ± 0.05 CPM/10^3 cells) compared to control (0.48 ± 0.08 CPM/10^3 cells; $p < 0.05$, $n = 4$), which was related to increased expression of the proliferating cell nuclear antigen (PCNA). Daidzein-induced mitotic activity was also associated with activation of the protein kinases AKT and extracellular regulated kinase (ERK) as determined by increased phosphorylation status (AKT^{Ser478}; ERK $^{Thr202/Tyr204}$ or p-p42/44 MAPK).

Conclusions. The neonatal period is a critical window of sensitivity of developing Leydig cells to the mitogenic effects of daidzein. Because the final numbers of Leydig cells in the adult testes depend on mitotic activity in the prepubertal period, exposures of male neonates to soy-based diets has implication for the endocrine function of the adult testis.

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Articular Cartilage Roughness Differs Between Femoropatellar and Femorotibial Joints in the Horse

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Introduction. The integrity and durability of a joint is influenced by the roughness of the articular cartilage. The physical nature of articular cartilage allows for it to deform and adapt to the force being placed upon the cartilage. Each joint has different forces applied upon it, which may have an effect on the surface roughness of the articular cartilage.

Methods. Articular cartilage roughness was tested on four equine stifles using a stylus surface profilometer. The medial trochlear ridge, patella, medial condyle, and lateral condyle of the femur within the joint were tested to represent a different type or amount of force being applied to the articular cartilage. The scan was taken at the apex of the cartilage

Results. The average micrometer roughness was measured for each joint location. The average surface roughness of the femoropatellar joint was 0.71 micrometers, whereas the average surface roughness of the femorotibial joint was 0.79 micrometers. The medial and lateral condyles within the femorotibial joint displayed a surface roughness of 1.13 and 0.44 micrometers respectively. The average surface roughness of the medial trochlear ridge was 0.68 micrometers, and the roughness of the patella was 0.73 micrometers.

Conclusions. This project measured roughness of the joints in the equine stifle. The femoropatellar joint has a different roughness compared to the femorotibial joint. The lateral condyles are slightly smoother when compared to the medial condyle of the femorotibial joint. These differences may be due to the type of joint, wear, and self-adaptations performed by the articular cartilage. This information can lead to the development of new products to aid veterinarians in the repair and the preservation of joint integrity. If this biological architecture could be applied to industrial bearings or an artificial joint, the integrity and durability of such products would be greatly increased.

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Fluoroquinolone-induced Efflux pump Expression in FQR and FQ-S canine and feline E. coli pathogens expressing MDR

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Introduction. Efflux pump expression is among the mechanisms by which *Escherichia coli* develops clinical drug resistance. Resistance to fluoroquinolone (FQ) has become increasingly problematic since the approval of the 2nd generation drug, enrofloxacin (ENR) in 1989. Frustratingly, resistance to FQ generally is not limited to FQs, but crosses multiple drug classes, causing multidrug resistance (MDR; resistance to 2 or more drug classes). Our lab has demonstrated that efflux pump expression is necessary for canine or feline *E. coli* isolates to express high-level multidrug resistance. Newer generation FQs such as Pradofloxacin (recently approved for veterinary use in the UK) may be associated with less resistance. Up to 5 major efflux pump families are expressed by clinical *E. coli* isolates. However, the impact of the various FQ to induce pump expression is not well documented, particularly in canine or feline isolates. This study compared the impact of earlier versus later generation FQ on efflux pump activities in canine and feline *E. coli* MDR pathogens

Methods. Canine or feline *E. coli* pathogens expressing MDR were studied. Isolates either were (FQ-R; n=15) or were not (FQ-S;n=15) resistant to FQ. Four pumps were studied (AcrB, EmrE, MacB and cmr) after exposure to FQ drugs (n=7): 2nd generation drugs ciprofloxacin (CIP (2nd)), enrofloxacin (*ENR) and orbifloxacin (*ORB); 3rd generation drug, marbofloxacin (*MAR); and the 4th generation drugs, gatifloxacin (GAT), moxifloxacin (MOX) and prafloxacin (*PRA) (*veterinary approved). *E. coli* isolates were exposed to each drug (100 µg/mL) for one hour. Isolates were harvested, RNA collected, and reverse transcriptase was performed. Pump expression relative to a house keeping gene and relative to a control isolate (ATCC 28596) was measured via real-time PCR. ANOVA and PostHoc's statistical analysis was applied to compare efflux pump activity among MDR phenotypes, drugs and pumps.

Results. Relative activity for each pump was compared between ENR-S and ENR-R isolates for each drug. Efflux pump expression differed among resistant types and drugs, with expression greater ($p<0.001$) in FQ-R compared to FQ-S isolates. Among drugs, expression was greatest for ENR compared to MAR, MOX, GAT, and PRA ($p<0.001$). Among pumps, expression of EmrE was greater than acrB, MacB and cmr ($p<0.001$). Interestingly, exposure to MAR increased the expression of EmrE and acrB but not macB and cmr.

Conclusions. Earlier generation drugs induce pump expression more than later generation drugs. The lower relative expression of pump activity induced by 3rd generation FQ may reflect their use within the veterinary field, or molecular differences in drug structure. This lower level of pump expression supports their (judicious) use in lieu of earlier generation FQ.

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Propagation of bovine viral diarrhea virus in cells from heterologous species phylogenetically related to cattle

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Introduction. The objective of this research was to compare the ability of Bovine Viral Diarrhea Virus (BVDV) to infect cells of four heterologous hosts with different levels of phylogenetic relatedness to cattle.

Methods. Primary kidney cells were selected from mammalian families within the order Artiodactyla, including *bovidae*, *cervidae*, *camelidae*, and *suidae*. Additionally, lagomorph (rabbit) kidney cells served as a phylogenetically removed growth environment. Cell lines were determined free from BVDV infection, and the camelid, porcine, and lagomorph cell lines were determined free from contaminating bovine cells. 36 BVDV isolates obtained from persistently infected cattle were used, including subgenotypes 1a, 1b, and 2. Propagation of the 36 isolates was performed with an MOI of 1.0 in triplicate wells of 96-well culture plates resulting in 12,500 cells/well/species. Following incubation for 96 hours, an immunoperoxidase monolayer assay was performed, and evaluated by two blinded reviewers (0= negative; 1= 1-5%; 2= 6-25%; 3 = 26 - 50%; 4 = 51-75%; 5 = 76 - 100% of cells infected). Results were analyzed by logistic regression in SAS 9.2 (SAS Institute Inc., Cary, NC).

Results. BVDV infected four of the five cell types, with the following percentages of wells confirmed positive: 100% bovine, 97% cervid, 75% camelid, 50% porcine, and 0% lagomorph. The probability of infection between species revealed cervid cells were 55 times more likely to become infected than porcine cells and 8 times more likely than camelid cells. Camelid cells were 7 times more likely to become infected than porcine cells. 36% of BVDV isolates infected all four cell types. An odds ratio comparing BVDV-1 to BVDV-2 revealed that BVDV-2 was 3 times more likely to infect heterologous cells than BVDV-1.

Conclusions. BVDV established infection in all cultures in the following order: bovine > cervid > camelid > porcine > lagomorph, which readily follows phylogenetic relatedness to cattle within the order Artiodactyla. Lagomorphs appeared to be too phylogenetically distant from Artiodactyls to be susceptible to BVDV, and this result contrasts previous published reports. These findings contribute to the understanding of BVDV ecology and control in heterologous species, but further research will be necessary.

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Graduate Student Poster Presentations

The Pharmacokinetics of single dose extended release Keppra® with and without food in healthy adult dogs

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Introduction. Levetiracetam appears to be safe and potentially effective anticonvulsant for the control of epilepsy in dogs. However, its efficacy is limited by a short half-life (often less than 2 hours). This half-life necessitates often results in subtherapeutic concentrations despite high doses being administered at 8 hour dosing intervals. An extended release preparation recently has been approved in humans that allows less frequent dosing intervals. The purpose of this study was to evaluate the pharmacokinetics of extended release levetiracetam (Keppra XR®) tablets in normal healthy dogs after a single oral dose with the goal of establishing a dosing regimen for the commercially available tablet that would allow 12 to 24 hour dosing intervals. The effect of food on drug movement was also evaluated.

Methods. Fourteen dogs were studied using a randomized crossover design. All dogs were administered intravenous levetiracetam first, followed by extended release levetiracetam orally (6 with and 6 without food) after a 24-hour washout period, both at the same average dose of 32.67mg/kg. Blood samples were collected for 24 hours after intravenous administration and for 36 hours after oral administration. Animals in the fasted group were withheld food for a minimum of 12 hours prior and 4 hours post administration. Serum levetiracetam concentrations were detected using ARK Diagnostic Levetiracetam Assay®.

Results. Keppra XR® was well tolerated. Data extrapolated from time concentration plots show that without food and with food serum levetiracetam is within therapeutic range by 100 minutes and 200 minutes, respectively, and stays within the range for an average of 19.8 hours (range 15-24.2) and 20.7 hours (range 16.7-28.7), respectively.

Conclusions. The authors concluded that the pharmacokinetics of Keppra XR® supports dosing twice daily at approximately 30 mg/kg in dogs. With appropriate drug monitoring, some dogs may only require once daily dosing.

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Phage Fusion Peptides Enhance the Efficacy of Liposomal Doxorubicin

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Introduction. Liposomal doxorubicin (Doxil/Caelyx) has been used extensively for treatment of various types of cancers. Targeted chemotherapy treatments have shown to be effective in reducing the dose required for killing cancer cells in various types of solid tumors, such as breast cancer and non-small cell lung cancers. Current targeting methodologies use antibodies or proteins that interact with overexpressed receptors on various types of cancer cells. One of the limiting steps to scaling up production of targeted nanomedicines is the requirement for a complex chemical conjugation and subsequent purification step to link the antibody or protein to the surface. We have previously identified a system of spontaneous protein incorporation into liposomal nanomedicines by taking advantage of the intrinsic membrane properties of filamentous phage major coat protein to insert into liposomal nanomedicines such as Doxil. To obtain targeting moieties to various cancers, we employ a phage display library that contains an 8 or 9 amino acid fusion protein at its N-terminus that was biopanned for phage that bind to specifically to cancer cells. These proteins were then isolated and introduced into Doxil. The phage-Doxil preparations were screened *in vitro* in breast cancer cell lines for increased cytotoxicity and apoptosis activations compared to untargeted Doxil.

Methods. Phage major coat protein was inserted into Doxil at a rate of 0.5% v/v protein/lipid concentration by incubation in 15 mM cholate buffer at 37ºC overnight. Cholate was removed by dialysis in decreasing concentrations of cholate. Samples were purified by size exclusion chromatography to remove unencapsulated doxorubicin and unincorporated protein. Phage-Doxil preparations were then concentrated and treated with proteinase K to determine orientation and quantify the percent of protein incorporation. Doxorubicin was freed by lysis of the liposomes with detergent and quantified using UV/Vis spectroscopy. All phage-doxil samples were assayed for size distribution and zeta potential using dynamic light scattering with a Malvern ZetaSizer Nano. Cells were plated at 5,000 cells/well and incubated with various concentrations of phage-Doxil for 24 hours in a 37ºC/5% CO_2 incubator. Cytotoxicity and caspase activation was assayed using a Promega ApoLive-Glo kit according to the manufacturer's recommendations.

Results. Our results show that major coat protein insertion is a pH dependent process and insertion of DMPGTVLP protein reaches close to 100% at a pH of 8.5 in 1X TBS with minimal doxorubicin leakage. We also show that the insertion procedure introduces phage fusion proteins in the correct orientation (N-terminus exposed on the surface). Incorporation of major coat protein appears to produce a slight increase in particle size (100nm to ~130nm) and a slight decrease in zeta potential depending on the charge of the inserted protein. DMPGTVLP-Doxil increases cytotoxicity and induces apoptosis in target MCF-7 breast cancer cells, but not Calu-3 NSCLC cells suggesting that our fusion protein is involved in targeting to specific cells.

Conclusions. We suggest that insertion performed at a pH of 8.5 in 1X TBS produces optimal insertion properties and reduces leakage of entrapped doxorubicin. Studies *in vitro* suggest that DMPGTVLP-Doxil increases the efficacy of Doxil by inducing caspase pathways leading to apoptosis at a lower dosage of doxorubicin. We suggest that the increase in efficacy of Doxil is due to the targeting motif provided by the phage fusion proteins to target select cells.

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Infectious Bronchitis Virus-Specific Effector and Memory T Cell Responses in White Leghorns

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Introduction. Infectious bronchitis virus (IBV) causes huge economic losses in the poultry industry of South-Eastern USA, including Alabama, despite existing vaccination programs. Current vaccines used to protect chicken flocks against IBV include Arkansas (Ark)-type IBV strains. Very little is known why these vaccines fail to control IBV outbreaks. It was shown by Collisson *et al.* that cell-mediated immunity is important in controlling IBV. *We hypothesize, that effector and memory T cell responses induced by an Ark-type IBV vaccine strain induces limited T cell responses, which contributes to vaccine failure.* Thus, in this study, we are measuring the magnitude of T cell responses elicited by an Ark-type IBV vaccine.

Methods. To measure T cells responses induced by an Ark-type IBV vaccine, chickens were ocularly immunized with $3x10^4$ or $3x10^5$ EID_{50} (egg infectious dose of 50%) per bird. To measure lymphocyte accumulation in spleen, Harderian glands (HDGL) and conjunctiva-associated lymphoid tissue (CALT), lymphocytes were counted using a hemocytometer and tryphan blue exclusion. Memory T cells were measured by flow cytometry using commercially available antibodies specific for $CD3^+CD44^+$ T cells (Southern Biotechnology, Birmingham, Al). IBV vaccine induced interferon-ɣ (IFNɣ) responses were measured using Enzyme-linked immuno-spot (ELISPOT) assay and the quantitative reverse transcriptase–polymerase chain reaction (RT-PCR).

Results. The number of lymphocytes in spleen, HDGL and CALT gradually increased between 7-9 days post IBV challenge reflecting the expansion phase of the immune response, and on day 10 rapidly declined reflecting the contraction phase. Thus, the IBV-specific immune response followed the normal pattern of a lag phase, expansion phase, and contraction phase as has been reported for mammals. Flow cytometry of spleen, HDGL and CALT lymphocytes post IBV vaccination demonstrated significant increase in $CD44^+$ memory/effector T cells between 9-11 days after vaccination. These cells declined after day 11. The primary IFN-ɣ response by the HDGL to ocular IBV challenge showed high IFN-ɣ levels during 3 stages (day 2, days 4-6 and days 10-11 of the immune response) as measured by quantitative RT-PCR, while CALT and spleen lymphocytes showed increases on day 6 and 11, presumably reflecting the inductive and effector phases of the immune response, while HDGL also displayed a robust innate response. *In vitro* stimulation of immune lymphocytes with IBV showed a high and rapid IFN-ɣ memory response in spleen but not in HDGL and CALT lymphocytes, indicating that memory responses may be limited to the spleen.

Conclusions. Ark-type IBV vaccination showed the standard kinetics in both mucosal and systemic lymphoid tissues reported for mammalian immune responses. A strong effector response was observed in mucosal tissues after ocular immunization based on IFN-ɣ production and the increase of memory/effector T cell numbers. However, *in vitro* IBV recall responses were restricted to the systemic immune compartment. Future experiments will address the importance of these T cell responses for the control of IBV.

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Pharmacological Study of the Transmembrane Domain 6 of Human Melanocortin-4 Receptor

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Introduction. The melanocortin-4 receptor (MC4R) is a G protein-coupled receptor (GPCR) primarily expressed in the central nervous system. It is a critical regulator of energy homeostasis, regulating both food intake and energy expenditure. Mutations of *MC4R* gene have been identified as the most common cause of monogenic obesity. The MC4R has emerged as a premier target for obesity treatment. To gain a better understanding of the structure-function relationship of the MC4R, we sought to determine the function of each residue in transmembrane domain 6 (TM6) of MC4R using alanine scanning mutagenesis.

Methods. MC4R mutants were generated by QuikChange site-directed mutagenesis kit. HEK293T cells were transiently transfected with WT or the mutant MC4Rs. Ligand binding assays were performed on intact cells using ^{125}I-NDP-MSH with or without different concentrations of unlabeled α-MSH. Cyclic AMP levels were measured by radioimmunoassay. The cell surface expression of these mutants was observed by confocal microscope. The levels of ERK phosphorylation were measured by western blotting.

Results. Of the 31 mutants, three mutants (H264A, L265A and Y268A) were severely defective in ligand binding, and eight mutants (G243A, T246A, L250A, L247A, I251A, W258A, P260A and F261A) displayed significant signaling impairments. H264A had no detectable binding and showed no response to α-MSH. The cell surface expression levels of H264A as well as L265A were not different from that of the WT MC4R while the expression level of P260A was decreased. Seven mutants (M241A, A244G, L250A, A259G, I266A, F267A and I269A) were constitutively active and their basal activity could be inhibited by two inverse agonists of the MC4R, Ipsen 5i and ML00253764 with maximal inhibition varied from 40% to 88%. These mutants that were constitutively active in the cyclic AMP pathway were also constitutively active in the MAPK pathway with enhanced ERK phosphorylation.

Conclusions. These data provided detailed information regarding the structure-function of the TM6 of MC4R in receptor function. We identified residues that are important for cell surface expression, ligand binding, and signaling. We also showed that MC4R mutants that constitutively activated the cyclic AMP pathway also constitutively activated MAPK pathway.

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Sequence determination of canine p16/INK4A alternative exon 1α and differential expression of INK4 tumor suppressors in canine mammary tumors

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Introduction. The cyclin-dependent kinase inhibitors (CKIs) are the cell cycle regulators that act as endogenous tumor suppressors in many mammalian tumors. Two families of CKIs –INK4 (p16, p15, p18, p19) and CIP-KIP (p21, p27, p57), block the G1/S phase transition of the cell cycle. p16/IKN4A is an important tumor suppressor gene defects in which have been associated with a number of cancers including human and canine breast cancers. p16 is encoded from the INK4A gene locus and then alternatively spliced into two distinct messages called p16 and p14ARF that share two common exons (exon 2 and 3) but different first exons- p16 exon 1α and p14 exon 1β- that harbor their respective native promoters. In dogs, the p16 transcript has not been fully elucidated since there are no full-length mRNAs or express sequence tags available that would completely define this transcript. An extremely GC-rich content has also made it difficult to clone and sequence and it is missing from the published canine genome. The complete sequence information of p16 is important to assess its expression in canine mammary tumors and evaluate molecular mechanisms that depend on its sequence. The objectives of this study were to determine the sequence of exon 1α that distinguishes p16 from p14ARF and the expression of INK4 tumor suppressors in canine mammary tumor (CMT) cell lines.

Methods. Six CMT cell lines (CMT9, CMT12, CMT27, CMT28, CMT47 & CMT119) from different dog breeds and normal canine fibroblasts (NCF) were cultured and total RNA isolated by phenol chloroform extraction. The primers for p16 and other CKIs were designed by Vector NTI. The semi-quantitative (sq) PCR and the PCR for rapid amplification of cDNA ends (RACE) were used to determine the sequence of p16 as well as expression of INK4 CKIs in all cell lines. The NCBI BLAST and Vector NTI sequence alignX tools were employed to identify canine p16 exons.

Results. From sq-PCR data, we found differential expression patterns for the INK4 genes in CMT cell lines. p16, p14ARF and p15 were not expressed in CMT12/27/119 cell lines and the expression of p14ARF was also absent in the CMT47 cell line. Whereas, p18 and p19 were expressed in all CMT cell lines including NCF. The nested RACE PCR amplified p16 mRNA to the 3'-end resulting in an authentic sequence which was confirmed by NCBI BLAST. This extended our previously identified partial p16 sequence with a complete 3'-end. To identify the exon 1α, we amplified a region of p16 mRNA using new primers that were designed from an upstream conserved sequence. The amplicon sequenced from the sq-PCR product indicated a partial sequence of the p16 exon 1α when aligned with other species.

Conclusions. Differential expression of the INK4 genes in our CMT cell lines suggested that defects in these tumor suppressors frequently promote cell transformation in canine breast cancers. The sequencing of the p16 alternative exon 1α might solve critical problems in future studies including, distinguishing the expression of p16 from p14ARF in CMT cells and biopsies, determining the complete sequence of p16 transcript and evaluating molecular mechanisms promoting canine breast cancers by targeting p16.

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Long-Term Phenotypic Correction of Feline Lysosomal Storage Disease by Intracranial AAV Gene Therapy

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Introduction. Deficiency of lysosomal β-galactosidase (βgal) causes storage of GM1 ganglioside, resulting in progressive neurodegeneration and premature death, often by 5 years of age. Adeno-associated virus (AAV) gene therapy has been extraordinarily successful in the GM1 mouse model, resulting in enhanced survival and complete clearance of storage in the brains of GM1 mice. Because the mouse brain is ~1000 times smaller and much less complex than the human brain, it is important to test AAV gene therapy in an animal model whose brain size and complexity more closely resemble humans. The feline GM1 model presents an unparalleled opportunity to evaluate AAV gene therapy in a non-rodent, 'large animal' prior to initiating human clinical trials.

Methods and Experimental Design. In the current study, AAV2/1 or AAV2/rh8 vectors, expressing feline βgal cDNA (3.1-12.0e12 genome copies total), were injected bilaterally into the thalamus and deep cerebellar nuclei (DCN) of 2-month old GM1 cats (disease onset ~3.5 months). In the short-term experimental group (n=7), brain and spinal cord were collected 4-16 weeks post injection, and cut into consecutive coronal blocks to analyze βgal distribution. In the long-term experimental group, clinical therapeutic outcome was evaluated by (1) magnetic resonance imaging and (2) a clinical rating scale reflecting the neurodegenerative course of GM1 cats.

Results. In treated GM1 cats, βgal was distributed throughout the entire anterior-posterior axis of the brain at levels up to 5 times normal, and filipin staining (a surrogate marker of GM1 levels) demonstrated extensive clearance of storage material. βgal activity was evident in all spinal cord sections, reaching 2.5 times normal levels. Currently, AAV-treated GM1 cats in the long term experimental group are 25, 23, and 21 months old, with no evidence of clinical neurological disease (untreated humane endpoint, 7.7±0.8 months).

Conclusions. These translational studies provide strong support for the initiation of AAV-based clinical trials for human GM1 gangliosidosis.

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Comparison of Cyclosporine Preparations based on Therapeutic Drug Monitoring

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Introduction. Cyclosporine (CsA) is a common choice for treatment of immune-mediated disorders in dogs or cats. A variety of preparations are prescribed by veterinarians. FDA approved products include Atopica®, a canine and feline approved microemulsion; Neoral®, a human approved microemulsion, and at least 8 different generic human microemulsions. In addition, veterinarians often prescribe compounded CsA preparations prepared by pharmacists. Therapeutic drug monitoring (TDM) is a tool used by veterinarians to guide dosing regimens of CsA. Samples are collected at 2 and 12 hours after dosing to determine if drug concentrations in the patient blood reach recommended target therapeutic ranges. Although disease and physiologic (species, gender, age) factors contribute to variability in drug concentrations, preparation source is likely to be a major contributor. The purpose of this study is to explore potential reasons for differences in CsA concentrations in animals, with a focus on preparations. We hypothesize that human generic products are characterized by the greatest and Atopica® the lowest variability; and that, compounded products are least and Atopica® most likely to achieve target concentrations.

Methods. Data was retrospectively retrieved from TDM samples received by the Clinical Pharmacology Lab from January 2003 through October 2011. Only those samples for which the specific preparation (eg, Atopica®, Neoral®, generic or compounded) was identified was retrieved. Data of interest included drug preparation, dose, timing of sample (2 hr peak versus 12 hr trough used for comparisons), drug concentrations and patient relevant information including species, breed, gender and disease. Signalment data was summarized descriptively. Mean concentrations adjusted for dose were compared among preparations at 2 and at 12 hrs. Further, the proportion of patients for which a peak target of 800 ng/ml and trough target of 100 ng/ml were compared.

Results. A total number of 180 samples with the specific preparation were identified: Atopica (n=111), Neoral (n=17), generic (n= 22; the number of different generic products could not be identified) and compounded (n=30). The most common indications were autoimmune hemolytic anemia (40%) followed by granulomatous meningoencephalitis (20%), perianal fistulas (18%) immune mediated hemolytic anemia (10%), and inflammatory bowel disease (8%). Mean peak CsA blood concentrations (adjusted for dose) were higher ($p=0.05$) for generic (454.8 ± 316.4) compared to all others (lowest for compounded at 27.6 ± 14.5) and at trough, significantly higher for generic (159.8 ± 184.5) compared to compounded (18.6 ± 9.4). The proportion of patients for which the peak target concentration was achieved was greatest for the generic products (90% at peak and 89% at trough) and least for compounded (0 at peak and 40% at trough).

Conclusions. This study demonstrates marked variability in blood CsA concentrations due, in part, to preparation source. Generic products appear to perform most and compounded least favorably. This study suggests that further research is warranted to establish which preparations are most likely to achieve targeted concentrations.

Evaluation of CD25, FOXP3, and CCL5 gene expression in placentomes of pregnant cattle inoculated with bovine viral diarrhea virus

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Introduction. CD4+CD25+ T regulatory cells have been associated with immunosuppressive activity in the pregnant uterus of humans and some animal species, playing a significant role in maintaining pregnancy by suppressing placental inflammation. Despite intrauterine infection with bovine viral diarrhea virus (BVDV), a significant inflammatory response is often absent in histologic sections of virally infected placentas between gestational days 75 to 150. The objectives of this study were to evaluate if BVDV is recognized in the placenta by the maternal immune system, despite the absence of significant microscopic lesions. Additionally, we wanted to characterize the immune response to bvdv at the maternal-fetal interface and evaluate mechanisms by which BVDV alters normal local immunoregulatory processes at this site.

Methods. Heifers naive to BVDV were synchronized, bred, and upon confirmation of pregnancy were divided into principal (n=11) and control groups (n=11). The principal group was inoculated intranasally with $1.0x10^5$ $TCID_{50}$/ml of the type-2 strain PA131 between gestational days 89 to 110. Randomly selected samples from the uterus, placenta, and fetal tissues were collected between 150-157 days gestation. Total RNA was extracted from placentomes and prepared for real-time reverse transcription PCR to quantify gene expression.

Results. Expression of CD25, FOXP3, and CCL5 in placentomes of principal, control groups, and immunologically active control tissues (mesenteric lymph nodes) was observed. Following normalization of target genes with housekeeping genes, FOXP3 and CCL5 were expressed at higher levels in placentomes of heifers infected with BVDV than control heifers.

Conclusions. Detection of CD25, FOXP3 and CCL5 in bovine placentomes has not been documented previously and provides a baseline for characterization of the immunological profile of the normal placenta at 150 days gestation. These findings suggest there is immune recognition of the virus at the maternal-fetal interface and the virus may alter normal expression of T regulatory cell markers.

Undergraduate Student Poster Presentations

Mucosal Vaccine Delivery to Generate Specific Immunity to Infectious Bronchitis Virus

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Introduction. Infectious Bronchitis Virus (IBV) is a coronavirus that causes acute respiratory illness in chickens. Signs of infection are observed 14 to 48 hours after exposure and normally last about a week; symptoms include dyspnea, thickened air sacs, decreased egg production, misshapen eggs, and a stunt in growth. IBV causes significant losses for the poultry industry. Chicken farmers vaccinate their flocks with live attenuated multivalent IBV vaccines normally through mucosal routes, such as spray, water, or ocular application to remedy the problem. The importance of mucosal immunity to control IBV was demonstrated with chicken lines that were resistant or susceptible to IBV. The resistant lines generated lower levels of IgA in tears and saliva than susceptible lines (Cook *et.al*. Avian Path. 1992). *Our objective is to compare two mucosal vaccination routes for IBV, i.e., oral versus ocular, for their ability to generate mucosal and systemic immunity to IBV. We hypothesize that ocular immunization will provide better mucosal and systemic immunity to IBV than oral vaccination in chickens.*

Methods. Specific pathogen free (SPF) white leghorn chicken eggs (Sunrise Farms, Inc., Catskill, NY) were incubated and hatched. Experiments were performed under Biosafety Level 2 (BSL 2) conditions in compliance with federal and institutional care and use guidelines. The chickens were divided into five groups, which received 0x, 0.5x, 1x, 2x, or 4x vaccine doses in water, respectively. A 1x oral vaccine dose consisted of 1.2×10^6 egg infective dose (EID_{50}) per bird. The ocular vaccine contained 3×10^5 EID_{50} per chicken. Blood and tear samples were collected on 3, 8, and 14 days after vaccination. Feces and saliva were collected 21 days after vaccination. IBV-specific IgG and IgA levels were measured by ELISA. All groups were boosted by the ocular route to measure memory responses. A separate group of chickens were vaccinated with 1x oral vaccine and lymphoid tissues of interest were harvested to measure IBV-specific IgA spot-forming cells (SFC).

Results. The IgG response in tears 14 days after ocular vaccination was significantly higher than in all oral vaccination groups, while all groups were significantly higher than controls. The IgG response in feces was undetectable. The IgG response in the 0.5x and ocular groups was higher on day 21 in saliva and on day 14 in plasma when compared to controls,. The mean IgA response in tears on day 14 in the 2x oral vaccination was somewhat higher than the other groups; all groups were higher than the control. The IBV-specific IgA response in feces did not significantly differ between groups, while the mean IgA response in saliva and plasma displayed a trend of being higher after oral immunization. The ELISPOT assay revealed that Harderian glands contained the highest number of IgA SFC on days 8 and 10 after oral immunization, while the spleen and CALT were very low and the cecal tonsil did not display any IgA SFC.

Conclusion. Ocular immunization for IBV does provide better systemic immunity, but not better mucosal immunity than water-based oral immunization. Ocular immunization induced higher IgG levels in tears and to a lesser extent in plasma than observed in the oral immunization groups, while IgA levels were highest in the 2x oral immunized group. No induction of an IBV-specific immune response was observed in the intestinal tract by either immunization route. The memory response is currently being evaluated.

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Towards Development of an IL2R Targeted Therapy for Canine Lymphoma

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Introduction. Adenoviral (Ad) vectors have been widely used to deliver therapeutic genes to target cells in cancer gene therapy applications. We propose to develop a therapeutic approach for lymphoma that will target lymphoma cells and deliver a protein that will trigger cell death specifically in the cancer cells. Lymphoma cells lack the molecules required for adenovirus to bind and enter the cell. Therefore, in order for any gene therapy application to be successful, identification of cell receptors that will permit uptake of Ad vectors is necessary. Interleukin 2 receptor (IL2R) overexpression has been observed on a number of cancer cells, including those of hematopoietic origin. Following binding by its ligand, the IL2R-IL2 complex is internalized. We wished to assess the feasibility of developing an IL2R targeted therapy incorporating the tumor-specific tumor necrosis factor related apoptosis inducing ligand (TRAIL) for the treatment of canine lymphoma, as the delivery of TRAIL cDNA via Ad vectors has been shown to selectively induce apoptosis in tumor cells but not in normal cells.

Methods. We analyzed cell surface expression of IL2R to confirm that it is overexpressed on lymphoma cells and therefore a potential target for gene therapy; additionally we assessed the ability of TRAIL to selectively induce apoptosis in cancer cells. Expression of CD25, the alpha subunit of IL2R, was analyzed on cells from normal canine lymph nodes, canine lymphoma cell lines and primary cells from canine lymphoma by flow cytometry. To assess the ability of TRAIL to induce apoptosis in canine lymphoma cells, we used the canine lymphoma cell line OSW and compared activity of human recombinant TRAIL in these cells to HEK293 cells, a non-tumor cell line and assessed apoptosis by staining with Annexin V antibody and propidium iodide.

Results. Analysis of CD25 expression demonstrated that IL2R is overexpressed on lymphoma cell lines and primary lymphoma cells in comparison to normal lymphoid cells. We found that the number of apoptotic and dead HEK293 cells, as measured with Annexin-v-FITC and propidium iodide was the same in both untreated and TRAIL-treated HEK293 cells. In the tumor cell line, there was an increase in both apoptotic and dead cells in response to human recombinant TRAIL.

Conclusions. We have demonstrated that IL2R is overexpressed on canine lymphoma cells therefore this receptor may be a potential target for a novel Ad gene therapy application for canine lymphoma. We have also shown that TRAIL is capable of inducing apoptosis in cancer cells, while normal cells remain unaffected making it an ideal candidate gene for incorporating into an IL2R targeted Ad vector.

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Mucosal and Systemic Immune Responses to Infectious Bronchitis Virus (IBV) after Ocular Vaccination

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Introduction. Infectious Bronchitis Virus (IBV) is a highly contagious coronavirus that is prevalent in all countries with extensive poultry industry. IBV outbreaks have a major economic impact by reducing weight gain in broilers, increasing condemnation at processing plants, and sometimes even leading to mortality. In layers IBV infection causes decreased egg production as well as a decrease in egg quality. Furthermore, post-infection egg production generally does not return to pre-infection rates. IBV is normally transmitted by aerosol inhalation or by the ingestion of contaminated feed, water, or feces. Thus, IBV enters the host through mucosal surfaces, which is consistent with the observation by Cook et al. that resistance or susceptibility to IBV in inbred chicken lines was correlated with mucosal protection, i.e., higher IgA levels in tears and saliva. Multiple serotypes of IBV have been identified and their prevalence varies between different geographic regions. In the South-Eastern USA, including Alabama, the Ark-serotype is very prevalent despite extensive vaccination efforts. *We hypothesize that Ark-type IBV vaccines, used in the South-Eastern USA, have an inability to induce strong mucosal immune responses to IBV, thus allowing IBV infection after vaccination*. This research provides a better understanding of the contribution of mucosal versus systemic immunity to control IBV infection.

Methods. Chickens were ocularly vaccinated with 100 µL containing 3 x 10^5 EID_{50} of IBV Ark serotype vaccine strain. Control chickens were kept in a separate area. Tears and plasma was collected prior to vaccination and daily after vaccination. Challenged chickens were re-vaccinated on day 28. Tears were centrifuged at 16,100 g for 10 minutes and the liquid phase was collected and stored at -80° C until tested. Blood samples were collected, kept on ice for 45 minutes, and were centrifuged at 500 g for 30 minutes. Plasma was collected and was frozen at -80° C until tested for antibodies. ELISA protocols measuring IBV-specific IgG and IgA levels were developed. IBV was propagated using Specific Pathogen Free (SPF) White Leghorn eggs from Sunrise Farms (Catskills, NY).Virus was heat-inactivated and purified using a series of ultracentrifugation steps. The IgA ELISPOT was performed as previously reported (van Ginkel et al.,2009)

Results. An IBV-specific ELISA was developed. Plates were coated with 5µg/ml of killed IBV in carbonate buffer, pH 9.4 overnight. Plates are blocked with PBS with 1% BSA, 0.05% Tween 20 and samples are incubated overnight. Biotinylated monoclonal antibodies specific for chicken IgG (0.5 µg/ml) and IgA (1.0 µg/ml are used for detection, followed by Streptavidin conjugated to HRP. The primary plasma IgG and IgA antibody response to IBV gradually increases from control values to peak levels around day 9, after which they gradually decline. The IBV-specific antibody response in tears follows similar kinetics with the exception that the IgG antibody levels peak one day later on day 10. The IBV boost given 4 weeks after the primary vaccination did not translate into a major increase in IBV-specific IgG and IgA antibody in plasma or tears, but antibody levels remained constant for the 2 weeks after the boost. A peak IgA ELISPOT response in the spleen was observed 2 days prior to that in CALT and HDGL. The IgA ELISPOT reflected the observed IgA levels in tears and plasma, in that plasma IgA levels reached significantly higher than in controls 2 days earlier when compared to IgA levels in tears.

Conclusions. Although ocular vaccination with IBV Ark-type vaccine strains induced mucosal immunity as measured by induction of IgA production in mucosal secretions and lymphoid tissues, no elevated memory humoral response was observed after revaccination, although IBV boosters have been reported to increase protection. Whether this indicates that cell-mediated immunity rather than humoral immunity at mucosal surfaces is more important is currently being investigated.

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Post-graduate/Faculty Poster Presentations

Nanophage-Mediated Targeted Delivery of siRNA into Breast Cancer Cells

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Introduction. Although synthetic nucleic acids (NAs), such as siRNA and antisense oligo's demonstrated high efficiency as gene-controlling drugs in numerous experiments *in vitro*, their systemic use is hindered by their instability in physiological liquids, insufficient tissue bioavailability and inability of intracellular accumulation in the site of action. Efficacy of NKs as potential anticancer therapeutics can be increased by their targeted delivery into cancer cells via tumor-specific ligands. Phage display offers a unique approach to identify highly specific and selective ligands that can deliver nanocarriers to the site of disease. In this study, we proved a novel approach for intracellular delivery of siRNAs into breast cancer cells through their encapsulation into nanophage targeted to the tumor cells with preselected intact landscape phage proteins.

Methods. The breast cancer specific phage protein (VEEGGYIAA and DWRGDSMDS) were isolated by size exclusion chromatography, complexed with siRNA in the molar ratio of 80/1 and analysed by UV spectroscopy, agarose gel analysis by gel-green staining and atomic force microscopy. Binding of the phage fusion protein-siRNA complex to their respective antigen was tested by ELISA using 7b1 phage protein (streptavidin binder) complexed with siRNA as a model. Delivery of phage fusion protein-siRNA complex into MCF-7 cells was visualized by immunofluorescence microscopy. Efficeincy of protein knockdown in MCF-7 cells by the complex was assesed by western blot analysis.

Results. In this study, MCF-7 cell targeting phage proteins with structure VEEGGYIAA and DWRGDSMDS complexed with Alexa-fluor 488 siRNA demonstrated its efficient delivery into breast cancer MCF-7 cells and not in normal mammary MCF-10 A cells. Efficient delivery was also accompanied with gene silencing ability as demonstrated by silencing of GAPDH in breast cancer MCF-7 cells and not in normal mammary MCF-10 A cells confirmed by western blot analysis.

Conclusion. Our approach of targeted delivery of siRNA via phage proteins exploits the ability of phage coat protein to interact with siRNA and form a self-assembled complex, which circumvents any chemical modification technique. Moreover, rapid identification of target specific probes by screening with polyvalent phage display provides an opportunity to increase the repertoire of ligands that can demonstrate efficient target specific siRNA delivery and gene-silencing in vivo and offers the potential for development of new anticancer siRNA-based targeted "individualized" nanomedicines.

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Pharmacokinetics of Tramadol and Its Major Metabolites in Alpacas Following Intravenous and Oral Administration

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Introduction. Tramadol is an analgesic with opioid and monamine reuptake inhibition. Tramadol has been used in humans and dogs for postoperative pain management following gynecological and orthopedic surgeries. Because of the apparent efficacy and high therapeutic safety levels, tramadol is an appealing drug for use in alpacas.

Methods. was administered to six alpacas (43-71kg) at a dose of 3.4 - 4.4 mg/kg intravenously (IV) and, after a washout period, 11 mg/kg orally. Serum samples were collected and stored at -80 °C until assayed by HPLC. Pharmacokinetic parameters were calculated.

Results. The mean half-lives ($t_{1/2}$) IV were 0.85 ± 0.463 and 0.520 ± 0.256 hrs orally. The C_{max} IV was 2467 ± 540 and 1202 ± 1319 ng/mL orally. T_{max} occurred at 0.036 ± 0.041 and 0.111 ± 0.068 hrs, respectively. The area under the curve ($AUC_{0-\infty}$) IV was 895 ± 189 and 373 ± 217 ng*hr/mL orally. The volume of distribution ($V_{d[area]}$) IV was 5.50 ± 2.66 and the $V_{d[area]}$/F orally was 26.8 ± 13.6 L/kg. Total body clearance (Cl) IV was 4.62 ± 1.09 hrs; Cl/F for oral administration was 39.5 ± 23 L/hr/kg. The IV mean residence time (MRT) was 0.720 ± 0.264. Oral adsorption (F) was low (5.9-19.1%) at almost 3 times the IV dosage with a large inter-subject variation.

Conclusions. This may be due to protein binding with the rumen contents or enzymatic destruction. A dosage of 5-10 times orally would be needed to achieve the same IV serum level of tramadol. The $t_{1/2}$ of all 3 metabolites was longer than the parent drug; however, O-DMT, N-DMT, Di-DMT metabolites were not detectable in all of the alpacas.

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Evaluation of a modified-live vaccine to protect developing fetuses from stringent natural challenges with bovine viral diarrhea virus and bovine herpesvirus 1

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Introduction. The objective of this research was to determine if two pre-breeding vaccinations of heifers with a multivalent, modified live vaccine (Express® FP5, Boehringer Ingelheim Vetmedica) would provide fetal and abortive protection following prolonged field exposure to animals persistently infected (PI) with bovine viral diarrhea virus (BVDV) and animals acutely infected with bovine herpesvirus 1 (BoHV-1).

Methods. Ten unvaccinated and twenty vaccinated pregnant heifers underwent exposure in a 1.94 hectare pasture to three steers PI with type 1a, 1b or 2 BVDV for 56 days beginning 102 d after the second administration of vaccine. Eighty days after removing PI animals, heifers were commingled for 14 d with three bulls acutely infected with BoHV-1.

Results. Following BVDV challenge, one vaccinated heifer aborted a fetus which was not obtainable for diagnostic evaluation. None of the calves (0/19) from vaccinated heifers were PI with BVDV whereas all fetuses and calves (10/10) from unvaccinated heifers were infected. Bovine herpesvirus 1 was not detected in any fetus, calf or associated placenta in either treatment group. However, four unvaccinated heifers aborted following BoHV-1 challenge. Calves birthed by vaccinated and subsequently exposed heifers exhibited longer gestation lengths, higher birth weights, higher weaning weights, higher average daily gains and greater market value at weaning than calves birthed by unvaccinated, exposed heifers.

Conclusions. Pre-breeding immunization with a modified-live vaccine prior to a stringent viral challenge during pregnancy had a very positive economic impact due to significantly fewer abortions and PI offspring, improved growth of offspring and increased market value of weaned calves.

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A rare case of persistent testicular infection with bovine viral diarrhea virus causes consistent shedding of infectious virus in semen

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Introduction. Recently, a dairy bull in the United States was diagnosed as the second confirmed case of persistent testicular infection with BVDV; thus, the objective of this investigation was to characterize the bull's infection, humoral immune response, and epidemiologic significance.

Methods. Virus isolation, antigen capture ELISA, and various PCR assays to detect BVDV were performed on serum. Virus neutralization assays were performed to detect anti-BVDV antibodies in serum. PCR assays to detect BVDV were performed on whole blood samples. Direct immunoperoxidase staining of cryopreserved semen, closed one-tube, RT-nested PCR, titration with subsequent immunoperoxidase monolayer assay and passage with subsequent virus isolation were performed to detect BVDV in cryopreserved semen. Centrifugal separation with PCR assay of the supernatant and cell pellet were performed to determine if virus was cell associated and/or detectable free in seminal plasma. Sequencing of 248 nucleotides from the 5′ nontranslated region of the viral genome was performed to determine viral subgenotype. Epidemiologic investigation involved sequential virus neutralization assays of serum obtained from bulls and steers before, during, and after contact with the infected bull.

Results. Between 6 and 24 months of age, this bull lacked BVDV in seven sequential serum samples and two peripheral white blood cell samples. The bull was seropositive to type 1, BVDV strains with serologic endpoints of 256, 2048, and 4096 at 8, 19, and 22 months of age, respectively. At 24 and 29 months of age, the bull exhibited serum neutralizing antibody titers of 4096 and 16384, respectively, to the strain isolated from his semen. The bull produced 25 collections of semen from 14 to 22 months of age that consistently contained BVDV as determined by direct immunoperoxidase staining, PCR and virus isolation when semen was shipped to the laboratory in a liquid nitrogen dry shipper. The concentration of infectious virus in semen ranged from < 250 to 6250 $CCID_{50}$/mL with a median of 1250 $CCID_{50}$/mL. Using centrifugal separation and PCR, virus was not detected free in seminal plasma but was readily detected in association with pelleted cells. Sequencing revealed that the persisting virus was a 1a subgenotype of BVDV. Epidemiologic investigation provided evidence that BVDV was not transmitted to directly contacted bulls and steers.

Conclusions. In conclusion, this investigation validates that exposure to a 1a strain of BVDV in the field can result in persistent testicular infection of a seropositive, non-viremic bull causing contamination of semen with readily detectable infectious virus for a duration of at least eight months.

Mechanisms Accounting for Fluoroquinolone Multidrug-resistance *Escherichia coli* Isolated from Dogs and Cats

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Introduction. Fluoroquinolones (FQs) are among the most potent antibacterial drugs against *Escherichia coli*. However, FQs resistance is becoming a serious problem in *E.coli* and it is often associated with multidrug resistance (MDR), as has been found in *E.coli* isolates from dogs and cats. The purpose of this study was to further characterize the relationship between the FQs MICs, the number of mutations in target genes, efflux pump activity, and plasmid-mediated genes associated with the level of resistance to FQs among different resistant phenotypes isolates of *E.coli* from dogs and cats.

Methods. Mechanisms contributing to resistance that were studied included mutations in target DNAgyrase and topoisomerase IV; efflux pump activity and the presence of plasmid-mediated genes. Target genes *gyrA*, *gyrB*, *parC*, and *parE* (including but not limited to the quinolone resistance determining region [QRDR]) were sequenced. The contribution of the AcrB efflux pump gene to resistance was determined by RT-PCR; function was quantitated based on changes in the minimum inhibitory concentration of ENR as the prototypic fluoroquinolone (FQ) in the presence of an efflux pump inhibitor (Phe-Arg-β-naphthylamide). Plasmid-mediated (PMQR) determinants *qnrA*, *qnrB*, *qnrS*, *qnrD*, *aac(6')-1b-cr*, and *qepA* genes were documented. Comparisons were made among different resistant phenotype for canine and feline *E.coli* uropathogens expressing no (NDR; n=7), single (SDR; 7) or multiple (MDR [n=35]) drug resistance. MDR referred to resistance to at least 2 drug classes, including a subset of isolates resistant to all drugs [PDR; n=14]) resistance. MDR isolates included those susceptible (FQ-S) and resistant (FQ-R) to 5 different FQ.

Results. Using enrofloxacin (ENR) as the prototypic fluoroquinolone, (i) the progressive increase in mutation number in targets genes correlated with a parallel increase in isolate MICs in a stepwise manner. The level of resistance could be categorized as low (single mutation [Ser83Leu] in *gyrA* (ENR MIC 0.25-0.5 µg/ml; isolates remained "susceptible"), low (two mutations causing an increase in ENR MIC to 4-16 µg/ml; isolates now "resistant"), moderate (three to four mutations with ENR MICs 32-64 ≥ 128 µg/ml), and high (four to five mutations with ENR MICs ≥ 128 µg/ml). (ii) AcrB efflux pump activity increased with the level of resistance phenotype, and number of drugs included in the MDR phenotype ($MDR_{(\geq 4)}$, including PDR. (iii) *qnrS* and *aac(6')-Ib-cr* genes, were detected in 15 or 5 ENR^R-MDR isolates, respectively. Among of the MDR isolates, 11 of the 14 PDR isolates (78.6%) were positive for *qnrS* gene, and 4 PDR isolates had both the *qnrS* gene and *aac(6')-Ib-cr* gene.

Conclusions. These findings demonstrate that FQs resistance in dogs and cats MDR *E.coli* isolates is associated with a combination of targets genes mutations, enhanced efflux, and plasmid-mediated mechanism, while the point mutation plays only a rudimentary yet critical role among of them.

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Enhanced Transduction of Fiber Modified Ad in Canine Lymphoma Cell Lines

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Introduction. Canine lymphoma represents an excellent model of human non-Hodgkin's Lymphoma: it has a similar etiology and presentation to the human disease, the model has an intact immune system, and the size of the animal model allows for extrapolation to humans. Recombinant Adenoviral (Ad) vectors derived from human serotypes 2 and 5 are among the most promising vehicles for successful *in vivo* gene delivery, based on previously demonstrated efficiency in successful gene delivery in a number of cancer gene therapy applications. However, our analysis of canine lymphoma cell lines and primary lymphoma cells indicates that they are refractory to infection by Ad vectors due to the lack of the primary Ad5 receptor, coxsackie virus and adenovirus receptor (CAR) on the cell surface and low-level expression of the $\alpha_v\beta_3$ integrin. Therefore, the development of an adenoviral vector to target B-cell lymphoma requires the modification of native viral tropism. To this end, a number of fiber-modified ad vectors with broader tropism have been evaluated.

Methods. Ad5 vectors that have chimeric fibers derived from non-human adenovirus serotypes and incorporating a luciferase reporter gene were used to transfect canine lymphoma cell lines. The OSW and 17-71 canine lymphoma cell lines were used to test the efficacy of these vectors in achieving transduction in a CAR-independent manner. These cells were then stimulated with PMA plus ionomycin for 24 hours prior to transduction. These were then further evaluated by incubating with a canine cross reactive monoclonal anti- $\alpha_v\beta_3$ integrin antibody prior to transduction. Transduction efficiency was measured by luciferase expression.

Results. In the OSW cell line, there was low-level transduction achieved with the Ad5.CK1 and Ad5.PK vectors. Following stimulation with PMA plus ionomycin, transduction by both Ad5.CK1 and Ad5.PK was enhanced. In the presence of the anti-integrin antibody, transduction efficiency was reduced to or below the level of that seen in unstimulated cells. In the 17-71 cell line, transduction was achieved only with Ad5.CK1. When these cells were stimulated with PMA and ionomycin the results indicated that transduction efficiency did increase, although this increase was modest – less than 2 fold - with a concurrent reduction seen with the addition of antibody

Conclusions. Adenoviral vectors modified to incorporate either the CAV1 or porcine fiber knob were capable of transducing canine lymphoma cells. This data suggests that stimulation of canine lymphoma cells with appropriate mitogens may enhance transduction by retargeted virus in a CAR-independent manner; however integrin expression remains important in this process.

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Protective effects of a new antioxidant, PMX-500F, against rotenone induced neuromotor decline, ROS generation and cellular stress in mouse brain

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Introduction. Epidemiological studies and animal studies have established that exposure to environmental toxins is a major risk factor for several pathological conditions. Deleterious effects of toxins, particularly pesticides, include changes in neural function and neurodegeneration. Rotenone is a pesticide widely used in agriculture, home gardens, tick control in pets, and in water body management to control unwanted fish. In rats, rotenone exposure results in central nervous system and systemic toxicity. The neurotoxic effects of rotenone are demonstrated to be mediated mainly through its effects on mitochondrial complex I (CI) of the electron transfer chain, microtubules, and proteosomes. A common effect of CI deficiency is the enhanced oxidative stress associated with impaired cellular redox balance. One potential therapy against oxidative stress-induced effects is the intake of enzymatic or non-enzymatic antioxidants. This study reports preliminary evaluation of a new synthetic antioxidant compound; PMX-500F, for treatment of chronic rotenone induced effects in mice.

Methods. Mice (C57BL/6NTac; two months old) received oral administration of rotenone (30 mg/kg/day) or vehicle (0.5% carboxymethyl cellulose) over four weeks. Thirty minutes prior to rotenone administration either PMX-500F (19 mg/kg) or vehicle (Tris, 0.1 M) was injected intraperitoneally. Mice were subjected to Rota-rod and wire hang testing. Following testing, brains were isolated and dissected into forebrain, midbrain and cerebellum, then homogenates were subjected to western blotting and reactive oxygen species (ROS) measurement.

Results. Rotenone administration did not elicit a difference in performance of mice in the Rota-rod test ($P>0.05$). PMX-500F treatment; however, showed a beneficial effect by increasing the latency to fall time ($P<0.05$). In the wire hang test, rotenone treated mice lost grip sooner and thus showed reduced latency to fall than the controls ($P<0.05$). PMX-500F treatment of the rotenone treated mice improved the latency to fall time to control levels ($P<0.05$). Control mice when treated with PMX-500F showed superior performance in the wire hang test ($P<0.05$). Rotenone administration increased ROS generation in the forebrain and midbrain regions, but not in the cerebellum ($P<0.05$). Co-treatment with PMX-500F normalized the ROS levels in forebrain and midbrain regions to that of the control levels. Western blot studies showed that in rotenone administered mice activated pSAPK/JNK levels were higher in the forebrain and midbrain lysates than in control mice ($P<0.05$). pSAPK/JNK levels in the cerebellum were similar in all four groups. Treatment with PMX-500F; however, reduced the pSAPK/JNK levels to that of the controls. Total SAPK/JNK levels were not altered by either rotenone or PMX-500F treatment ($P>0.05$).

Conclusions. These results illustrate that rotenone exposure is associated with selective decline in neuromotor function as well as increased ROS generation and cellular stress in the forebrain and midbrain. Use of the antioxidant PMX-500F elicited beneficial effects and may therefore have potential as a therapeutic strategy against rotenone induced neurological effects.

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Evaluating Transmission of Bovine Viral Diarrhea Virus to Cattle by Exposure to Carcasses of Persistently Infected White-tailed deer (*Odocoileus virginianus*)

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Introduction. Infections with bovine viral diarrhea virus (BVDV) clinically analogous to cattle including persistent infections are described in white-tailed deer (*Odocoileus virginianus*). The epidemiologic role of persistently infected (PI) white-tailed deer is unknown. Persistently infected white-tailed deer shed BVDV, effecting maintenance of the virus in groups of deer. Survival of PI white-tailed deer appears to be reduced, and clinically ill or dead PI deer may be a source of BVDV. This study sought to determine if BVDV transmission could occur when cattle come in contact with carcasses of PI white-tailed deer.

Methods. In two trials, steers were exposed to the carcass of PI fawn A (BVDV 2) or PI fawn B (BVDV 1). Trials were designed with consideration of the influence of contact networks and social hierarchies of cattle herds on disease epidemiology, and only one steer from each group was separated into a pen with the carcass. The number of contacts with the carcass was monitored by time-lapse photography, and every 2 hours surface swabs and muscle biopsy were collected from the carcass. Following 8 hours, the single steer was commingled with four other steers for 28 days. Animals were tested for BVDV infection by virus isolation and virus neutralization. Controls included one steer inoculated intranasally with spleen-homogenate from fawn A, and two steers inoculated intranasally or intravenously with spleen-homogenate from fawn B.

Results. Steers in both trials repeatedly contacted the carcasses, but BVDV transmission did not occur. BVDV was isolated from surface swab and muscle samples from carcass A. In contrast, neither sample type was positive for BVDV from carcass B. The intranasally inoculated control for trial A and the intravenously inoculated control for trial B became viremic and seroconverted.

Conclusions. The observed frequency and proximity of investigative contacts of steers with PI carcasses emphasizes the potential for disease transmission to cattle by this route, but this study suggests little risk of BVDV transmission from contact between cattle and infectious PI carcasses. Complex factors determine the true risk for BVDV infection by contact with a carcass, including BVDV survival in tissues, likelihood of contact with the carcass, and contact network structures of cattle herds. The influence of wildlife on the control of BVDV is currently unknown, and further research is necessary to understand complex interactions at the wildlife-livestock interface.

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Reduction of *Salmonella* Newport Shedding and Disease in Experimentally Infected Calves

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Introduction. Multiple drug-resistant *Salmonella* Newport is associated with cattle and has become the third most common serotype isolated from human infections. Therefore, novel approaches to pathogen reduction are needed to address the continuing threat of this food-borne pathogen. We hypothesize that bacteriophage treatment will successfully reduce shedding of *S*. Newport in cattle.

Methods. Eight 8-10 week-old calves were inoculated orally with 10^9 colony forming units (CFU's) of a spontaneous nalidixic acid resistant mutant of *Salmonella* Newport. Four of the calves were treated at 24 and 48 hours after inoculation with 10^{10} plaque forming units each of a mixture of three *S.* Newport-targeted bacteriophages. Fecal *S.* Newport CFU's, rectal temperatures, and fecal scores were recorded daily for 11 days.

Results. The four bacteriophage treated calves shed significantly less ($P < 0.05$) *S.* Newport than did the untreated control calves on post-inoculation days 4-6. Clinical disease also was reduced in the treated calves as indicated by a significant reduction ($P < 0.05$) in rectal temperature on post-inoculation days 4 and 7 and by the presence of normal fecal consistency throughout the 11-day experimental period.

Conclusions. These results indicate that treatment with bacteriophages holds promise for reducing *Salmonella* contamination of dairy and beef products, thus decreasing the spread of this pathogen to humans. In addition, these results show that bacteriophage treatment could serve as an alternative to antibiotic treatment of salmonellosis in cattle, thus reducing antibiotic resistance in *Salmonella*. Finally, results from this project could be applied to the use of bacteriophage treatment to reduce *Salmonella* and other food-borne pathogens in other livestock and food crops.

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Management of Acute or Chronic Equine Laminitis with Selected Hoof Care Principles, Dietary Management, and Controlled Exercise

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Introduction. Laminitis is a common debilitating hoof condition of the horse. The clinical outcome of fourteen obese laminitic horses presented to the Auburn University Veterinary Teaching Hospital was evaluated. All fourteen horses were treated by application of the same husbandry: employing specific hoof care principles, dietary management, and controlled exercise.

Methods. Fourteen laminitic horses with similar clinical signs and history were managed using the same hoof care, nutritional and exercise program. Each horse at the time of presentation had a body condition ≥ 8/9, Obel grade lameness ≥ 2/4, and rotation of the third phalanx ≥ 6 degrees. The hoof care principles that were used for treatment minimized weight-bearing by the hoof wall by transfer of weight bearing to the bars, soles, heel buttress and sometimes the frog. Soles were protected with pads, hoof boots, yielding terrain or hoof casts. Exercise in the form of hand-walking was recommended and encouraged once desired hoof loading mechanics were in place and the horse was able to walk with heel first hoof impact while wearing protective hoof coverings. The diet was modified to minimize the intake of non-structural carbohydrates while avoiding periods over one hour without available food. Clinical parameters evaluated included: Obel lameness score, body condition score and five radiographic measurements. Results were compared statistically in SAS 9.2.

Results. Twelve horses were sound at a trot at post-treatment evaluation, and the remaining two horses demonstrated only a mild lameness (Obel score $\leq$ 1). Following treatment, horses were 5.5 times more likely to be sound as compared to pre-treatment examinations. A statistically significant decrease of the third phalanx rotation was detected post-treatment ($p < 0.0001$, 95% CI for difference 3.7532 – 6.4616 degrees). The difference of the dorsal hoof wall thicknesses between the proximal and distal aspect of the distal phalanx (DDHWT) was significantly reduced at post-treatment evaluation ($p < 0.0001$, 95% CI for difference 2.0341 – 3.5019 mm). Although treatment reduced the distance between coronary band and extensor process, this difference was not statistically significant ($p = 0.0651$, 95% CI for difference 2.3695 – 4.0794 mm). The sole depth was significantly increased at post-treatment evaluations ($p = 0.0015$, 95% CI for difference 3.1217 – 5.3743 mm). A statistically significant overall reduction of palmar angle measurements was detected in acutely and chronically affected horses ($p = 0.0001$), however this treatment effect was statistically larger for chronic as compared to acute cases ($p_{interaction} < 0.0001$).

Conclusions. Management of laminitic horses with hoof trimming and solar padding that reduces or eliminates weight-bearing by the hoof wall along with controlled exercise and a decrease in dietary carbohydrates can cause an improvement in radiographic parameters and return some laminitic horses to riding soundness.

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Development of Phage Ligands for targeting Non-Small Cell Lung Cancer

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Introduction. Lung cancer is reported to be the leading cause of cancer related death with poor survival in United States due to the late diagnosis and lack of effective treatment. As one of major types of lung cancer, non-small cell lung cancer dominates about 85% of lung cancer cases compared with small cell lung cancer. We explored the phage display technology to discover the new ligands that can specifically target to non-small cell lung cancer cells. Phage display technology provides high through-put capacity to select targeting ligands against different diseases including cancers. One landscape phage library (f8/8) was applied to screen the ligands that bind to non-small cell lung cancer (Calu-3). We depleted the phages that bind to non-related targets by incubating with flask, normal lung cells (Small airway lung cells) and serum. After depletion of non-specific binding, we identified 9 phage clones that can bind to non-small cell lung cancer cell line (Calu-3). Our results show that these ligands have high specificity and selectivity to Calu-3 cells and could be candidates for nano-drug development in the future.

Methods. We used phage display technique to select the best candidates that have high binding affinity and specificity to Calu-3 cells. Before incubation with calu-3 cells, phage library was first treated with empty flask, normal lung epithelial cells and serum for depleting the non-specific phage peptides. Specific phage peptides were collected after incubating with Calu-3 cells. More than 400 Phage clones were obtained and identified by PCR. 9 phage clones that showed highest specificity and binding affinity were chosen for further study. The intracellular distribution of these 9 phage ligands was studied by phage capture assay and confocal microscope.

Results. After four rounds of selection, phage ligands were identified. There are 9 phage ligands show the high specificity and selectivity to non-small cell lung cancer (Calu-3). Two phage ligands both with NGR sequence showed the different intracellular distribution in Calu-3 cells revealed by phage capture assay and confocal microscopy study, which indicates they probably have different mechanisms for phage ligands trafficking in the cells. The trafficking mechanism will be further studied by using different drug inhibitors.

Conclusions. Phage display is a high through-put technique that has been used in drug discovery and development. Here, we found 9 candidates that have high binding capability and specificity to target non-small cell lung cancer (Calu-3). Two of phage ligands have NGR sequence, which has been reported to target aminopeptidase N. Aminopeptidase N was overexpressed by endothelial cells and different cancer cells. However, these two phage peptides showed different intracellular distribution, which may involve different trafficking mechanisms in the cells. For nano-drug development in the future, we need to elucidate the mechanisms of phage ligands trafficking in the cells, which will help us to design anti-cancer drugs and deliver more drugs into the cancer cells.

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DNA Sequencing of Labradoodle Duchenne Muscular Dystrophy

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Introduction. In humans, Duchenne Muscular Dystrophy (DMD) has an incidence of 1 in 3500 males. DMD is caused by a mutation of the dystrophin gene, which is located on the x-chromosome. Absence of the dystrophin protein permits excess calcium to infiltrate the sarcolemma of a cell, causing cell damage and eventual cell death. Necrotic muscle fibers are ultimately replaced with adipose and connective tissue resulting in muscle contracture and fibrosis, pseudohypertrophy of tongue, shoulder, and neck muscles, and generalized muscle wasting and weakness. Multiple animal models of DMD have been identified, including the mouse, cat and dog. A majority of research on this disease has been carried out in mice, which, while useful, do not completely recapitulate the human disease. Thus, a research model that is closer to humans in disease complexity and effects would be an important tool. Canine models are much closer to humans in size and scope of the disease. Previously, we and others identified two new canine models of DMD, in the Welsh Corgi and Labrador Retriever breeds. Both breeds were shown to have insertions at the level of mRNA (cDNA) between exons 13 and 14 and exons 19 and 20 respectively. The aim of this project was to completely sequence the mutations in Labradoodles at the genomic level to determine the origin of the inserted sequence, to develop diagnostic assays and to fully describe these mutations for use in future gene therapy. Different canine breeds manifest disease states in different manners, similar to human populations.

Methods. Eight unique primer combinations and several interfaced primer combinations were incorporated in RTPCR of skeletal muscle derived RNA from DMD affected labradoodles. The resulting DNA templates were purified and submitted to the Massachusetts General Hospital/ Harvard sequencing laboratory. The resulting sequences were analyzed using the DNA Star software and assembled into a contiguous DNA sequence. Comparison to normal muscle derived DNA was then performed using the same software.

Results. The labradoodle has variants in nucleotide sequence that result in at least one stop codon early in the contiguous DNA. Other variations are evident, but pose less of a consequence due to their placement behind the original stop codon.

Conclusions. Repetitive elements in several sections of the gene posed considerable hurdles for correct nucleotide order. The considerable length of this gene, over 10,000 base pairs, also added to the sequencing challenge. Knowledge of the correct sequence aberration is necessary for development of constructs in gene therapy. Adenoviral vectors specific to this sequence can now be made.

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