

PHI ZETA

The Honor Society of Veterinary Medicine
Epsilon Chapter



November 11, 2009

Research Emphasis Day

AUBURN UNIVERSITY
COLLEGE OF VETERINARY MEDICINE

ACKNOWLEDGEMENTS

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

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

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PHI ZETA RESEARCH DAY FORUM

NOVEMBER 11, 2009 - JOY GOODWIN RUDD STUDENT CENTER

MORNING PROGRAM - Lobby

10:00 - 12:00 Poster Session – Presenters are present from 11:00 – 12:00

AFTERNOON PROGRAM - Overton Auditorium

1:00 - 5:45 Platform Presentations and Keynote Lecture

Platform Presentations

Veterinary Students

- 1:00 Jordan Towns Identification of relative adrenal insufficiency in critically ill adult horses
- 1:15 Melissa Tollett Antimicrobial efficacy of vancomycin and amikacin impregnated plaster of paris beads against methicillin resistant *Staphylococcus aureus*

Graduate Students

- 1:30 Payal Agarwal Canine mammary tumor cells: a model to investigate cyclin dependent kinase inhibitor p16/INK4A in cell cycle arrest
- 1:45 Sudhir Ahluwalia Characterization of mastitis caused by natural *Chlamydia* spp. infection of dairy cows
- 2:00 Christopher Alford Evaluation of a transvaginal laparoscopic NOTES (natural orifice transluminal endoscopic surgery) approach to the abdomen of mares
- 2:15 Khristen J. Carlson Optimization of a test protocol to assess aldosterone secretory capacity in dogs
- 2:30 **BREAK** **Refreshments served in Goodwin Center Lobby**
- 2:45 Maninder Sandey Genomic and expression studies of canine melanoma differentiation associated gene-7/IL-24 (*mda-7/IL-24*)

- 3:00 M. F. Chamorro Evaluation of bovine viral diarrhea virus (BVDV) transmission by horn flies (*Haematobia irritans*)
- 3:15 Dori J. Miller Evaluating effects of milk-borne factors on neonatal porcine endometrial gene expression using laser microdissection
- 3:30 Katrin Saile Saline volume necessary to achieve predetermined intraluminal pressures during leak testing of enteric biopsy sites in the dog

Post-graduate/Faculty

- 3:45 Thomas Passler Transmission of bovine viral diarrhea virus among white-tailed deer (*Odocoileus virginianus*)
- 4:00 Chengming Wang A novel tricolor-fluorescence PCR quantitatively detects and differentiates feline immunodeficiency virus and separates infected from vaccinated cats

4:15 **BREAK Refreshments served in Goodwin Center Lobby**

4:30 **KEYNOTE LECTURE**

IMMUNITY OR IMMUNOPATHOLOGY TO VIRUSES - what decides the outcome?

BARRY T. ROUSE, BVSc., Ph.D., D.Sc.

Lindsay Young Distinguished Professor of Microbiology
Department of Pathobiology
College of Veterinary Medicine
The University of Tennessee, Knoxville, TN

Dr. Rouse received his Bachelor of Veterinary Science at the University of Bristol, UK, the Ph.D. at the University of Guelph, Canada, and then conducted research at the Walter and Eliza Hall Institute of Medical Research in Australia. After 5 years at the University of Saskatchewan he joined in 1977 the College of Veterinary Medicine at the University of Tennessee in Knoxville.

He is a worldwide authority in the field of immune mechanisms in diseases induced by herpesviridae, particularly herpes simplex virus-1 (HSV-1), and in protective and vaccine immunology of HSV-1. He has published more than 370 peer-reviewed scientific articles, and has been continuously funded by NIH. Among many other prestigious positions, he is currently Section Editor for the Journal of Immunology.



PLEASE JOIN US FOR THE INDUCTION AND AWARDS BANQUET

Everybody is invited! Tickets \$40/person - Reserve ticket before November 9, 2009 with Dr. Josephson (josepem@auburn.edu, 334-844-5423) or Dr. Kaltenboeck (kaltebe@auburn.edu, 334-844-2665) - Deposit check for ticket with Amelia Pendleton, 106 Greene Hall (ORGS) or at the banquet in the **Auburn Hotel & Conference Center**.

6:30 **BANQUET at the AU Hotel & Conference Center**

6:30 **COCKTAILS at cash bar**

7:00 **DINNER**

7:30 **INDUCTION of new Phi Zeta Members and AWARD CEREMONY to honor winners of the Platform and Poster Student Competitions**

8:00 **DINNER PRESENTATION**

Dr. LEWIS BARKER

Professor of Psychology, Auburn University

PRIMATE RESEARCH IN THE 1960S: CHIMPS AS ASTRONAUTS AND HUMAN SURROGATES

Posters

Veterinary Students

Robert Collins	Alpha-melanocyte stimulating hormone and melanocortin-4 receptor in pancreatic neurons
Adam Breiteneicher	Immunocontraception of cats with peptides identified using phage display
Amie L. Perry	Comparison of expression of Foxp3 and TGF- β in canine and human mammary cancer lines
Contessa Bowman	Target tissues in chicken embryo following <i>in ovo</i> delivery of adenovirus-vectored vaccine
Ashley Hydrick	Reexamination of the infectivity of chicken anemia virus in non-lymphoblastoid cell lines
Christina D. Osborne	Unique structure of the M loop region of beta1-tubulin is associated with size variability of platelets in the family Felidae
Anthony Cordray	National canine and feline parasite prevalence survey
David T. Priest	Dynamic respiratory endoscopy of standardbred race horses during qualifying races
Drew M. Humphries	Identification of potential platelet alloantigens in the Equidae family by comparison of gene sequences encoding major platelet membrane glycoproteins
R. K. Stahl	Development of transdermal phenylbutazone formulations for horses and deer
Regina R. Williams	Novel exon insertions in dystrophin in Laborador Retrievers and Welsh Corgis
E. A. Zimmer	Neuromotor Characterization of Transgenic Mice Allotopically Expressing ATP6
Jerrod C. Johnson	Comparison of a commercial glucometer and a spectrophotometry-based chemistry analyzer in horses and alpacas
Laura Jackson	Profiling the immune response in cats treated with adeno-associated virus gene therapy
Kelli I. McNamara	Sequencing the dystrophin cDNA in a new canine model of Duchene's muscular dystrophy
Marike Visser	The role of efflux pumps in canine pathogenic multi drug resistant <i>E. coli</i>

Patrick Hubka	Pharmacokinetics of fosfomycin for treatment of MDR- <i>Escherichia coli</i> urinary tract infection in dogs
Justin Farris	NADPH oxidase activity in the diabetic heart
Kimberly Elizabeth Reid	Breaking the paradigm: methods of testing for circulating bovine viral diarrhea virus in a beef herd
Meghan S. Kilgore	Association of CTLA4 polymorphisms with IMHA in dogs
Adam W. Cooner	Evaluating soft tissue composition of the equine palmar foot with computed tomography, magnetic resonance imaging, and three-dimensional image reconstruction
Emily Galliers	Thromboelastography (Teg) as a method of monitoring hemostasis in dogs: are Teg tracings from healthy dogs consistent when analyzed over a period of three consecutive days?
Ashley H. Homm	Ultrasound of adrenal glands in horses

Graduate Students

Farruk M. L. Kabir	Induction of cell surface integrins in lymphomas and potential applications to gene therapy
Xiulei Mo	Surface immobilized molecular beacon probes for PCR and DNA microarrays
Cassandra Breedlove	Combined <i>in ovo</i> -vaccination with non-replicating adenovirus-vectored avian influenza and Marek 's disease vaccines
James W. Gillespie	Optimization of a breast cancer specific phage probe into liposomal Doxil®
Manjunatha K. Nanjappa	Perinatal exposures of male rats to the estrogenic chemical bisphenol A (BPA) impacts rat Leydig cell differentiation
Matthew V. Cannon	Characterization of proteins involved in RNA import into mitochondria
Benjamin W. Newcomer	Efficacy of a novel antiviral compound in preventing acute infection by bovine viral diarrhea virus
Chuanling Xu	Landscape phage probes for rapid detection of <i>Staphylococcus aureus</i>
Victoria A. Light	Relationship between steroid hormone action and collagen homeostasis in the male

Océane Aude Aubry	Preparation of bone marrow aspirates from dogs with hematopoietic tumors for immunohistochemistry using Histogel™
Alexis C. Schmidt	Activated murine thymic plasmacytoid dendritic cells alter thymopoiesis
Olusegun A. Fagbohun	Landscape phage probe for cytokeratins 8/18
David A. Dunn	Neuromuscular and motor coordination analyses of a mouse model of mitochondrial complex I disorder
Rebecca A. Funk	Seasonal changes in the combined glucose insulin tolerance test in normal aged horses
Rebecca A. Funk	Seasonal changes in plasma alpha-melanocyte-stimulating hormone and adrenocorticotrophic hormone in response to thyrotropin-releasing hormone administration in normal aged horses
Rodrigo Gallardo	Assessing intraspatial variation of infectious bronchitis virus in the host
Alejandro J. Silva	Defining the porcine colostral proteome

Post-graduate/Faculty

Linda Martin	Dose response of aldosterone to ACTH stimulation in clinically healthy cats
Linda Martin	Effect of low doses of cosyntropin on cortisol concentrations in cats
K. Parameshwaran	D-Galactose administration as a model of aging in mice for testing therapeutic antioxidant compounds
Stuart B. Price	Reduction of <i>Salmonella</i> colonization in chickens following bacteriophage treatment

Veterinary Student Platform Presentations

Identification of relative adrenal insufficiency in critically ill adult horses

Jordan Towns¹, Allison Stewart², Rebecca Funk², Ashley Homm¹, Anne Wooldridge², Amelia Munsterman², Christina Hewes², Erin Groover², Robert Kemppainen.³

¹College of Veterinary Medicine, Auburn University, AL, ²Department of Clinical Sciences, ³Department of Anatomy, Physiology and Pharmacology; Auburn University, AL

Introduction Relative adrenal insufficiency (RAI) has been identified in critically ill humans, dogs and neonatal foals, and is identified by lower-than-expected circulating cortisol concentrations, especially when compared with circulating adrenocorticotrophic hormone (ACTH) concentrations, and/or by a blunted response to an ACTH stimulation test.

Methods Endogenous cortisol and ACTH concentrations were measured by radioimmunoassay from critically ill adult horses on days 1, 2, 4 and 6. A low dose ACTH stimulation test was performed on a subset of horses, with cortisol concentrations determined at T=0 and 30min after administration of 0.1 µg/kg of synthetic ACTH. A Severity-of-Illness-Score (SOIS) on a scale of 1-10 (10 = most severely ill) was assigned to each horse. The ACTH:cortisol ratio and the delta cortisol after ACTH stimulation (cortisol at T=30 - cortisol at T=0) were calculated. **Results** Forty horses were enrolled. 50% of SOIS 7-10 horses had elevated ACTH:Cortisol ratios. 6/26 cases had a poor response to the ACTH stimulation test.

Parameter	SOIS 1-3	SOIS 4-6	SOIS 7-10
Cortisol (µg/dL)	6.4 ± 3.5 (1.5-11)	7.1 ± 4.6 (1.9-18)	12 ± 8.9 (1.5 -33)
ACTH (pg/mL)	15 ± 15 (3.9-65)	32 ± 56 (3.6-241)	81 ± 96 (11-450)
ACTH:Cortisol ratio	2.7 ± 1.8 (1.0-5.7)	5.4 ± 7.9 (1.0-28)	8.2 ± 6.1 (1.1-23)

Conclusions RAI appears to occur in a small number of critically ill adult horses.

Acknowledgements to Michelle Brown², Erin Robbins², Whitney Brown¹, Amy Bley, Qiao Zhong² and Anne Busch³. Funding by MAF, CVM and ACVECC.

Antimicrobial efficacy of vancomycin and amikacin impregnated plaster of paris beads against methicillin resistant *Staphylococcus aureus*

Melissa Tollett³, Dawn Boothe², Harry Boothe³, Jameson Sofge², Dubraska Campos Diaz¹, and Aylin Atilla²

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²Departments of Anatomy, Physiology, and Pharmacology, Auburn University, AL

³Department of Clinical Sciences, Auburn University, AL

Introduction: Local administration of antimicrobials allows for higher drug concentrations at the site of infection while avoiding potential side effects of systemic administration of certain antimicrobials. Plaster of Paris (POP) beads are inexpensive, biocompatible, absorbable, and have been suggested to be a reliable method of local antimicrobial application. Both vancomycin and amikacin are bactericidal drugs with efficacy against *Staphylococcus* spp. and in combination might be a good option to treat methicillin resistant *Staphylococcus* spp. (MRSA). Our hypothesis was that an eluent from POP beads containing both vancomycin and amikacin would inhibit growth of MRSA for at least two weeks *in vitro*.

Methods: Vancomycin and amikacin impregnated POP beads were incubated in saline for three months. Four bead types were used: amikacin alone (A), vancomycin alone (V), amikacin with vancomycin in same bead (AV), and amikacin and vancomycin in separate beads but same tube (A+V). Antimicrobial naïve beads were also incubated in saline as controls. Broth microdilution methods in manually prepared microwell trays were used to assess antimicrobial efficacy using the Vizion imaging system. Antimicrobial eluent for each time point and each drug or drug combination was tested in triplicate series for repeatability. Four bacterial isolates were obtained from canine clinical cases: MRSA, methicillin susceptible *Staphylococcus aureus* (MSSA), methicillin resistant *Staphylococcus intermedius* (MRSI), and methicillin susceptible *Staphylococcus intermedius* (MSSI). Eluent from beads was also tested against quality control organisms ATCC 43300 (MRSA) and ATCC 25923 (MSSA). After inoculation, all plates were incubated at 35°C for 18 hours.

Results: Inhibition of growth for clinical isolates was as follows: A – 12 to 18 hours, V – 56 days, AV – 18 to 24 hours, and A+V – 5 days.

	MRSA	MSSA	MRSI	MSSI	ATCC MSSA	ATCC MRSA
Amikacin (A)	12 hrs	18 hrs	18 hrs	18 hrs	36 hrs	12 hrs
Vancomycin (V)	56 days	56 days	56 days	56 days	21 days	28 days
AV	18 hrs	18 hrs	18 hrs	24 hrs	18 hrs	24 hrs
A+V	5 days	5 days	5 days	5 days	3 days	5 days

Conclusions: Vancomycin and amikacin in combination did not exhibit a synergistic antimicrobial effect against MRSA. Instead, the eluent containing vancomycin alone was the most effective growth inhibitor of all *Staphylococcus* spp. tested. Vancomycin and amikacin in combination failed to inhibit growth beyond 5 days, which was significantly less than that achieved with vancomycin alone. The POP beads containing amikacin only inhibited microbial growth for less than 24 hrs. Therefore, amikacin alone or in combination with vancomycin in POP beads is not an appropriate choice for locally treating MRSA. Furthermore, based on the *in vitro* efficacy of vancomycin in POP beads, this method of antimicrobial delivery warrants investigation in the clinical treatment of resistant *Staphylococcus* spp. infections.

Acknowledgements: This data is a component of the manuscript, In vitro Elution of Amikacin and Vancomycin, from impregnated Plaster of Paris beads.

Graduate Student Platform Presentations

Canine mammary tumor cells: a model to investigate cyclin dependent kinase inhibitor p16/INK4A in cell cycle arrest

Payal Agarwal, Patricia DeInnocentes, R. Curtis Bird

Department of Pathobiology, 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 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 Rb protein un-phosphorylated and S phase transcription factor E2F bound and inactive. p16 is frequently mutated in human and canine mammary cancer. 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. There is accumulation of p16 mRNA and protein when many types of cells terminally differentiate or undergo senescence or quiescence. We are developing an in-vitro model to elucidate the role of p16 in differentiation in quiescent cells.

Methods: Well characterized canine mammary cancer cell lines (NCF, CMT28, CMT27, and CMT12) and the p16-transfected clones (CMT27 A and CMT27 H) have been used to investigate the expression of p16 after serum starving cells into quiescence and then re-feeding. Cell cycle arrest and synchronous cell cycle re-entry was confirmed by ³H-thymidine incorporation assay and flow cytometry. Expression of p16, CDK4, CDK6, p27, cyclin D1, and cyclin A mRNA in starved and re-fed conditions was compared by quantitative PCR. Expression of p16 protein in starved and re-fed conditions were compared by western blot reacted with antibodies against p16, CDK4, CDK6, cyclin D1, cyclin A and p27 to bind canine homologous proteins.

Results: We have successfully demonstrated cell cycle arrest and synchronous cell cycle re-entry in CMT28, CMT27 (A)/(H), and normal canine fibroblast. CMT12 and CMT27 (p16 deficient) failed to respond to serum starvation suggesting a role for p16 in transition into/out of cell cycle. Cell cycle arrest and synchronous cell cycle re-entry was further confirmed by ³H-thymidine incorporation and flow cytometry.

Conclusion: All p16 expressing cell lines have demonstrated cell cycle arrest in response to serum-starvation. p16 is accumulated at the quiescent stage, which suggests role in exit from the cell cycle. We will further explore the different roles of p16 in cell differentiation as well as its classical role in cell cycle exit. It may also be possible that p16 may have binding partners other than CDK4/6 during cell quiescence. The same model of serum-starvation and refeeding will be used for the clones of CMT cells transfected with p16 expression constructs.

Acknowledgment: Author acknowledges: NIH, CVM Interdepartmental Grant, Calvert Cooperation, Allison Church Bird for expert flow cytometry analysis.

Characterization of mastitis caused by natural *Chlamydia* spp. infection of dairy cows

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³Department of Mathematics and Statistics, Auburn University, Auburn, AL, USA

Introduction. In this observational study, we aimed to study the yield and composition of milk at the udder quarter level in early lactation (from week 2 to 22 post-calving), to determine the incidence of systemic and localized chlamydial infection, and the changes in milk quality and quantity associated with these infections.

Methods. Milk samples from each mammary gland quarter vaginal cytobrush samples, and serum samples were obtained biweekly from 17 dairy cows. Samples were analyzed by *Chlamydia* 23S rDNA real-time PCR and routine bacteriology. Milk samples were used to determine SCC, milk fat, and milk protein. Serum was used to determine anti-*C. abortus/C. pecorum* serum IgM antibodies and serum amyloid A acute phase protein.

Results. All cows (100%) were positive for anti-*Chlamydia* spp. IgM serum antibodies, indicating endemic infection. Twelve (70%) of the cows were PCR-positive for *Chlamydia* spp. in any of the samples, with 11 cows positive only in vaginal cytobrush specimens, 1 positive only in milk samples, and 2 cows in both types of specimens. Data analysis using principal components resulted in indices "Production loss index", "Anti-*Chlamydia* immune index", and "Inflammatory Index" which delineated the complete dataset in well defined, biologically significant natural clusters. In a multivariate logistic stepwise regression model, cows with chlamydial colonization of the mammary gland had significantly higher milk SCC and lower milk protein. Systemic chlamydial infection highly significantly associated with increased milk yield and milk fat, and decreased milk protein.

Conclusions These data confirm and characterize the negative influence of asymptomatic localized and systemic chlamydial infection on the health of the mammary gland of dairy cows and their milk production.

Acknowledgments. This project was funded by a USDA-CSREES grant from the Alabama Agricultural Experiment Station Foundation to BK and Alabama EPSCoR graduate assistantship to SA.

Evaluation of a transvaginal laparoscopic NOTES (natural orifice transluminal endoscopic surgery) approach to the abdomen of mares

Christopher G. Alford and R. Reid Hanson

Department of Clinical Sciences, Auburn University, AL

Introduction. Natural orifice transluminal endoscopic surgery (NOTES) was first described for use as an experimental swine procedure and has since been further developed to meet the demands of human and small animal medicine. A similar technique, if proven safe and effective, for use in the equine patient would serve as a model in the development of a wide array of diagnostic capabilities and new endoscopic procedures in the wake of current emerging technologies. By combining previously described more invasive surgical techniques of vaginal approaches to the abdomen in horses with more recent descriptions of minimally invasive laparoscopic NOTES procedures in human medicine the researchers intend to develop a viable model for use in horses to both safely and effectively visualize the caudal abdomen.

Methods. Eight healthy adult mares were utilized for this study. A standing transvaginal approach was made in the cranial vaginal vault at the one or eleven o'clock position to the cervix of the mares. A single approach through the vaginal wall was made on the left in four mares, and on the right in four mares. The abdomen was explored and the abdominal viscera evaluated using a 2-meter flexible endoscope followed by a 62-cm 0 degree laparoscope. Images of the abdomen taken with each instrument were saved and compared. The mares were monitored for a week postoperatively for incisional healing and for any signs of intolerance to the procedure.

Results. Exploration of the abdomen was sufficiently performed in all mares through a transvaginal approach using either a left or right sided cervical approach. The endoscope allowed consistent visualization of the left kidney, spleen, nephrosplenic space, stomach, cecum, duodenum, left and right ovaries, diaphragm, and caudal peritoneal reflection. The liver was observed with less consistency. The laparoscope provided similar views of the caudal abdomen, however, lateral mobility was limited and viewing cranially past the nephrosplenic space and the base of the cecum was not possible. Incisional healing occurred rapidly with the vaginal mucosa closed at 3 days. Complications in one mare included mild colic behavior resolving with conservative treatment.

Conclusions. NOTES transvaginal approach appears to be a useful and safe tool in the diagnosis of intra-abdominal disorders encountered in the mare.

Acknowledgements. Funding for this project was made possible through a grant from the Birmingham Racing Commission.

Optimization of a test protocol to assess aldosterone secretory capacity in dogs

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Introduction. Current testing for serum aldosterone requires a 5 µg/kg Cortrosyn (synthetic ACTH) dose with sampling at 0 and 60 min. The goal is to measure peak stimulated concentrations, but preliminary data suggest the dose and/or timing are not optimal. The purpose of our study was to determine the maximally stimulating dose with regard to aldosterone as well as determine the time of peak aldosterone response.

Methods. Ten healthy dogs were given 3 doses (5 µg/kg, 10 µg/kg, and 25 µg/kg) ACTH intravenously in random order with at least a 2-day washout period in between. Blood samples were taken before and at 10, 20, 30, 40, 50, 60, and 90 minutes after ACTH administration. Samples were analyzed by a previously validated radioimmunoassay. Data were analyzed using a repeated measures ANOVA with the Bonferroni test for post hoc comparisons.

Results. All 3 doses caused a significant increase in aldosterone concentration. No difference was detected in aldosterone concentrations between ACTH doses at any time. Peak concentrations were obtained 30 min post-injection for all doses and were significantly greater than those at the 60 min period.

Conclusions. In conclusion, 5 µg/kg Cortrosyn maximally stimulated aldosterone secretion. The time of peak response was at 30 minutes, not the 60-min sampling time currently recommended. Thus, current testing protocols are suboptimal.

Acknowledgments. Financial support provided by ACVIM Foundation.

Genomic and expression studies of canine melanoma differentiation associated gene-7/IL-24 (*mda-7/IL-24*)

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Introduction. Cancer is the leading cause of death in adult dogs. Many of the tumors that afflict our canine companions are very similar to human tumors, creating a situation where improved therapy in one species will directly improve therapy in the other. Melanoma differentiation associated gene-7 was first discovered in human melanoma cells. Expression of cloned human *mda-7/IL-24* results in irreversible growth arrest, cancer reversion and terminal differentiation in a wide variety of cancer cells, including human and canine tumor cells. The MDA-7/IL-24 gene product also possesses immunomodulation, antiangiogenesis and anti-invasiveness properties, in addition to its antitumor activity. Genetic homologs of *mda-7* have been found in the rat and mouse. However, no studies to date have elucidated the role of *mda-7* in dogs.

Methods. The predicted canine *mda-7/IL-24* gene sequence was obtained from National Center for Biotechnology Information (NCBI) sequence database. We designed multiple primer sets to detect the expression of canine *mda-7* by reverse transcriptase-polymerase chains reaction. To ensure that we had an appropriate PCR assay, we then designed a primer set to target a region of *mda-7*, which is conserved in both dogs and humans. These primer sequences were 100% complimentary to canine *mda-7* and 98% to the human *mda-7*. Rapid amplification of cDNA ends (RACE) technique was used to know the full-length cDNA sequence.

Results. Computer analysis predicted that the canine *mda-7* gene has 5 exons and 4 introns with a total cDNA length of 552bp. The exon/intron splice sites for the 2nd, 3rd, 4th and 5th exons of canine *mda-7* were conserved with respect to the human *mda-7* exon/introns splice sites. However, the representative sequences for the human 1st, 2nd and a part of the 3rd exon were missing in both NCBI and GENESCAN predicted canine *mda-7* genes. We also identified a promoter 1389 bps upstream of the first exon, and the sequences around this promoter region was found to be conserved compared to the human *mda-7* promoter. The predicted promoter includes a transcription start site that was 1389 bps upstream of the first predicted exon. With the primer set designed to target the conserve region of *mda-7*, we were able to generate a product from freshly isolated canine keratinocytes, but not from any other tissues. This product was extended by RACE and sequenced. These sequences include two extra exons at the 5' end of mRNA. However, these extra exonic sequences do not have similarity to the human sequence.

Conclusions. We have confirmed that an mRNA is expressed from the canine *mda-7* gene, however, the pattern of expression appears to be far more limited than *mda-7* in other species, and it is not clear if the mRNA encodes a functional protein due to the presence of extra sequences at the 5' end of mRNA of canine *mda-7* which may alter its biological properties.

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Evaluation of bovine viral diarrhea virus (BVDV) transmission by horn flies (*Haematobia irritans*)

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Introduction. Transmission routes for BVDV in cattle have been well described; however, few studies have examined insects as vectors of BVDV. The horn fly (*Haematobia irritans*) is the most prevalent insect affecting cattle in the southern US and this study sought to evaluate the potential for BVDV transmission by horn flies.

Methods. Horn flies were collected from four persistently infected (PI) cattle and distributed into fly cage cylinders, with 25 flies per cage. An additional aliquot of 50 flies per PI animal was tested for BVDV by RT-PCR. Four principal and four control BVDV-naïve calves were housed in individual isolations rooms. Two cages were attached to each animal. Flies were allowed to feed on principal calves, but a plastic barrier prevented flies from feeding on control calves. After 48 hours, cages were removed and fly homogenates from each calf were tested for BVDV by RT-PCR and ultracentrifugation-virus isolation. Blood samples were collected from each calf on days 0, 3, 6-10, 14, 21, and 28 for virus isolation and on days 0, 14, and 28 for virus neutralization.

Results: Fly homogenates from three of four PI cattle were positive for BVDV by RT-PCR at collection. Of the fly homogenates removed after 48 hours, three were positive from principal calves and four from control calves. Virus isolation and serology remained negative in all calves.

Conclusions. These results indicate that BVDV may be detected in horn flies feeding on PI animals, but they do not appear to be an important vector for BVDV.

Acknowledgments. The authors gratefully acknowledge Ms. Patricia Galik and Ms. Yijing Zhang for technical assistance and Department of Clinical Sciences, College of Veterinary Medicine, Auburn University for funding support.

Evaluating effects of milk-borne factors on neonatal porcine endometrial gene expression using laser microdissection

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³Department of Animal Sciences, Endocrinology and Animal Biosciences Program, Rutgers University, New Brunswick, NJ.

Introduction. Based on data for the pig, the lactocrine hypothesis states that patterns of gene expression in the neonate required for developmental programming of uterine tissues are affected by milk-borne factors. A feed-forward mechanism through which lactocrine-acting factors affect expression of genes important for endometrial programming was proposed. Specific targets include the estrogen (ESR1) and relaxin (RXFP1) receptors, and vascular endothelial growth factor (VEGFA). Here, objectives were to use laser microdissection (LMD) to determine effects of the ingestion of colostrum versus milk replacer for 50h from birth (postnatal day = PND 0) on endometrial-specific ESR1, RXFP1 and VEGFA expression on PND 14.

Methods. Newborn gilts were assigned (n=4-6/group) to consume either colostrum or milk replacer for 50 from birth. At 50h all animals were returned to dams and consumed milk normally to PND 14 when uterine tissues were obtained. Tissues were fixed in Xpress Molecular Fixative (Sakura Finetek), embedded in Paraplast Plus (Fischer Scientific), sectioned and mounted on nuclease-free polyethylene terephthalate membrane slides. Endometrium was excised and isolated with an MMI CellCut LMD workstation (Molecular Machines & Industries, Inc.). Endometrial RNA was extracted using RecoverAll (Ambion). A High Capacity Reverse Transcriptase Kit (Applied Biosystems; ABI) was used to generate cDNA. Quantitative-PCR (qPCR) was performed using SYBR Green Plus Master Mix (ABI) to assess treatment effects on ESR1, VEGFA, RXFP1 and S15 (reference gene) expression.

Results. Data indicate that endometrial gene expression on PND 14 in gilts deprived of colostrum for 50h from birth was distinct from that of gilts that consumed colostrum. Specifically, endometrial ESR1 and VEGFA, but not RXFP1 expression was down regulated in replacer-fed gilts on PND 14.

Conclusions. LMD and related procedures can be used to isolate and capture neonatal endometrium for tissue-specific gene expression analysis. Results support the lactocrine hypothesis and indicate that factors in colostrum (or absent from replacer) affect endometrial programming events as reflected by differences observed on PND 14. The period from birth to PND 14 is recognized as a critical period for uterine development in the pig.

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Saline volume necessary to achieve predetermined intraluminal pressures during leak testing of enteric biopsy sites in the dog

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Introduction. Enteric biopsy, enterotomy, and intestinal resection and anastomosis are commonly performed surgical procedures. Enteric incisional dehiscence, a serious complication, leads to septic peritonitis. The leak test is advocated to evaluate security of enteric incisional closure. The purpose of this study was to determine the volume of saline needed to achieve targeted intraluminal pressures during leak testing of closed enteric biopsy sites in the dog. The hypothesis was that the volume of saline necessary to achieve targeted intraluminal pressures with the intestine occluded digitally would be the same as that with the intestine occluded with Doyen intestinal forceps.

Methods. Jejunal biopsies were performed in 38 dogs and closed in a routine manner. The volume of saline needed to achieve an intraluminal pressure equaling or just surpassing normal peristaltic pressure in the small intestine of the dog (15 to 25 mm Hg [20 to 34 cm water]) in a 10 cm canine jejunal segment containing a closed biopsy site was recorded.

Results. The 95% confidence interval for the volume of saline needed to achieve 20 and 34 cm water intraluminal pressure was 10.9 to 13.6 and 16.3 to 19.0 ml, respectively with digital occlusion and 8.5 to 11.1 and 12.1 to 14.8 ml, respectively with Doyen occlusion. Correlation between volume of saline instilled and pressure achieved was better with Doyen than digital occlusion.

Conclusion. These saline volume guidelines can be used to achieve intraluminal pressures equaling or surpassing peristaltic pressures during leak testing of closed enteric incisions with reasonable accuracy in the dog.

Post-graduate/Faculty Platform Presentations

Transmission of bovine viral diarrhea virus among white-tailed deer (*Odocoileus virginianus*)

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Introduction. Cattle persistently infected (PI) with Bovine viral diarrhea virus (BVDV), a Pestivirus in the family *Flaviviridae*, are an important source of viral transmission to susceptible hosts. Persistent BVDV infections have been identified in white-tailed deer (*Odocoileus virginianus*), the most abundant free-ranging ruminant in North America. As PI deer shed BVDV similarly to PI cattle, maintenance of BVDV within white-tailed deer populations may be possible as a result of contact between susceptible and PI deer. To date, intraspecific transmission of BVDV in white-tailed deer has not been evaluated, which prompted this study.

Methods. Six pregnant white-tailed deer were captured in the first trimester of pregnancy. Under sedation, samples were collected to ensure their BVDV and BVDV antibody free status by virus neutralization, virus isolation on serum and whole blood, RT-nPCR, and immunohistochemistry. Following pregnancy examinations by ultrasound, the does were translocated to a 2.0 ha deer pen at which they were cohabitated with a PI white-tailed deer. Beginning in July, 2008, the deer pen was searched for fawns daily. When a fawn was found, it was evaluated and whole blood, serum, skin biopsy (ear notch) and nasal swab samples were collected for virus isolation, RT-nPCR, ELISA, IHC, and virus neutralization. When a fawn was positive for BVDV at initial testing or appeared unthrifty, further samples were collected approximately 3 weeks after birth.

Results. Cohabitation with the PI deer resulted in BVDV infection in all does, as indicated by seroconversion. All does gave birth to live fawns and no reproductive losses were observed. At birth, evidence of BVDV infection was identified in two singlet fawns, of which one was determined to be PI by repeated serum RT-nPCR, whole blood virus isolation and immunohistochemistry.

Conclusions. This study demonstrates for the first time that BVDV transmission may occur among white-tailed deer. The birth of a PI fawn through contact to a PI white-tailed deer indicates that under appropriate circumstances, BVDV may be maintained in white-tailed deer. Maintenance of BVDV in free-ranging populations likely is influenced by various factors including deer density and movement, sex of PI deer, and human influence in form of wildlife management. Infections with BVDV in white-tailed deer populations may have a negative impact on health and welfare, but should also be considered where BVDV control programs are planned.

Acknowledgments. Funded by Animal Health Research, Auburn University. The authors gratefully acknowledge Mr. Chad Newbolt and Mr. Nathan Worthington for their assistance with the capture of white-tailed deer, and the technical staff of the BVDV laboratories at Auburn University, especially Ms. Yijing Zhang, Mr. Andrew Eason, and Ms. Terri Hathcock.

A novel tricolor-fluorescence PCR quantitatively detects and differentiates feline immunodeficiency virus and separates infected from vaccinated cats

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Introduction. Feline immunodeficiency virus (FIV) is amongst the most common infectious agents of cats with 5 well-characterized subtypes A, B, C, D, and E. Serum antibodies against FIV cannot serve as indicators of infection status since vaccination-inducing antibodies are indistinguishable from antibodies in response to infection.

Methods. We established a *gag* gene-based tricolor fluorescence quantitative PCR that amplifies single target copies of all known FIV strains and differentiates the known FIV subtypes.

Results. All blood samples from experimentally FIV-infected cats were antibody positive in the SNAP[®] assay and highly positive in the FIV PCR. In contrast, all cats became antibody positive after FEL-O-VAX[®] FIV vaccination, but remained negative in the FIV PCR. Sixty one of 101 feline blood specimens submitted for FIV diagnosis were positive. Twenty-three of the positive PCRs identified subtype-A, 11 subtype-B1, 11 subtype-B2/E, and 16 subtype-C. FIV subtype D was not detected in any submitted specimens even though 13 blood specimens were from cats known to have received the FIV-vaccine which contains subtypes A and D.

Conclusions. This PCR quantitatively identifies five FIV subtypes, and unambiguously discriminates between FIV-vaccinated and FIV-infected cats.

Acknowledgments. We gratefully acknowledge the excellent animal care provided by the staff of the division of Laboratory Animal Health.

Veterinary Student Poster Presentations

Alpha-melanocyte stimulating hormone and melanocortin-4 receptor in pancreatic neurons

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Introduction. Alpha-melanocyte stimulating hormone (α -MSH) is a tridecaneuropeptide released by the hypothalamus and the neurointermediate lobe of the pituitary gland from the pro-opiomelanocortin (POMC) polypeptide precursor. The α -MSH protein has many functions, among which are the regulation of energy homeostasis and blood insulin level. We have previously shown that the effect on serum insulin level is mediated via activation of melanocortin-4 receptor (Mc4r) containing neurons in the hypothalamus and pancreas of rats treated via brain infusion with α -MSH analog [Nle⁴, D-Phe⁷]- α -MSH (NDP-MSH). The mechanism by which pancreatic Mc4r is activated is unknown.

Methods. The objective of this study is to investigate the presence of α -MSH protein, the possible ligand for MC4r in the pancreas of rats treated with NDP-MSH via intracerebroventricular route, and the effect treatment with NDP-MSH has on the expression of MC4R at the protein level in the pancreas.

Results. Immunohistochemical analysis showed that α -MSH is present in pancreatic islets associated with islet capillaries, and also in nerves throughout the pancreas. The nerve fibers staining positive for α -MSH were also positive for neuron-specific beta III tubulin. Using Immunohistochemistry we were also able to determine that there is a significant increase in expression of the MC4R at the protein level in response to central administration of an α -MSH analog.

Conclusions. Taken together, these findings suggest that pancreatic α -MSH and MC4r in autonomic neurons are involved in a communication pathway between central melanocortin system and peripheral pancreatic islets to regulate insulin secretion.

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Immunocontraception of cats with peptides identified using phage display

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Introduction. Feral cat overpopulation has been recognized as a large-scale problem for decades. The primary concerns that surround this issue include public health, transmission of diseases to other species, endangerment to native species and ecosystems, public nuisance, and the welfare of the feral cats. The main population control strategies used currently such as surgical gonadectomy implemented by “trap-neuter-return programs” and euthanasia have not reduced the magnitude of the problem. The most efficient solution is to prevent the birth of unwanted animals. This could be achieved using effective and economically sound contraceptive preparations.

Methods. In this study, we are developing peptide-based immunocontraceptives that mimic sperm antigens and upon administration into an animal induce production of anti-sperm antibodies capable of blocking oocyte-sperm binding at fertilization. Using sequential rounds of selection from a landscape phage display library on feline oocytes, several peptides with potential contraceptive properties were identified.

Results. One of the peptides is of particular interest since it has significant homology with nuclear autoantigenic sperm protein (NASP). This is a sperm-specific autoimmune protein known to stimulate production of anti-sperm antibodies that have been implicated in reduced fertility in men.

Conclusions. NASP-homologous peptide as well as other candidate peptides will be further characterized in immunogenicity and fertility studies in cats with the intent of developing virally vectored contraceptive vaccines suitable for commercial production.

Acknowledgments. The author would like to thank Scott-Ritchey Research Center, Alabama Agricultural Experiment Station, and Merk Merial Sponsors for their financial support. The Phage display library and feline ovaries were provided by Dr. Valery Petrenko and Dr. Tracy Land, respectively. Finally, this research could not have been accomplished without the intellectual support and training provided by Dr. Tatiana Samoylova, Dr. Alexandre Samoylov, Ms. Anna Cochran and Dr. Nancy Cox.

Comparison of expression of Foxp3 and TGF- β in canine and human mammary cancer lines

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Introduction. Canine mammary cancer has been used as a model for human breast cancer in previous studies because of its similarities to human disease. Canine mammary cancer, unlike mouse models, is a spontaneous hormone sensitive cancer with no viral involvement. Foxp3 and TGF- β have been investigated in human breast cancer as tumor markers and possible targets for therapy/prognosis. These genes have been implicated in immune system evasion by different pathways. Foxp3 is a transcription factor involved in tumor suppression. When missing or mutated it may contribute to the formation of mammary cancers. TGF- β is a growth factor which acts as an immune modulator, helping cancers evade immune detection by recruiting immune suppressive T regulatory lymphocytes (Tregs).

Methods. Foxp3 and TGF- β were amplified using traditional semi-quantitative PCR from canine and human breast cancer cell lines as well as normal canine fibroblasts.

Results. TGF- β is expressed at higher levels in Canine Mammary Tumor 28 (CMT28) cells and minimally expressed in Normal Canine Fibroblasts (NCF). Somewhat higher expression was also seen in Canine Mammary Tumor lines 12 and 27 (CMT12 and CMT27) as compared to the NCF line. FoxP3 expression was higher in Canine Mammary Tumor 28 (CMT28) and MCF7 (a human breast adenocarcinoma) cell lines.

Conclusions. It appears that expression of FoxP3 and TGF- β is higher in more transformed cell lines like CMT28 and MCF7 than in normal cells. This result suggests that these genes are positively affecting or at least correlated with enhanced survival of tumors. Considering that both genes are related to immune system suppression, and thus may be causing the enhanced survival of the tumors, more studies are needed to determine whether it is in fact a causative relationship, or simply association. There has been some debate as to the exact role of FoxP3 in the process of tumor development and progression, with some proposing it as an oncogene and others believing it to be a tumor suppressor. The clear association of FoxP3 expression in these tumors suggests a role as an oncogene is more likely in this context.

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Target tissues in chicken embryo following *in ovo* delivery of adenovirus-vectored vaccine

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Introduction. In recent years avian influenza (AI) has reemerged as a threat to the human and poultry populations. A replication defective adenovirus-type 5 (Ad-5) vectoring the hemagglutinin gene of AI virus (AdTW68.H5ch) has been shown to protect chickens against highly pathogenic avian influenza challenge after *in ovo* vaccination.

Methods. The aim of this work was to determine how this recombinant virus enters the embryo after *in ovo* inoculation. The site of injection was evaluated by digital radiographs and computed tomography. The presence of the virus in the embryo tissues was evaluated by PCR.

Results. Results show that adenoviral genomes were consistently detected by PCR in the chorioallantoic fluid of embryonated eggs on day 21 of incubation, 3 days post inoculation. The vaccine virus genes were also detected in the skin and upper gastrointestinal tract of embryos. Ad-5 positive PCR amplicons were confirmed by sequencing.

Conclusions. The vaccine adenovirus infected the chorioallantoic membrane, skin, and upper gastrointestinal tract of chicken embryos.

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Reexamination of the infectivity of chicken anemia virus in non-lymphoblastoid cell lines

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Introduction. Chicken anemia virus (CAV) is the causative agent of infectious anemia and immunosuppression in chickens. After its isolation, Yuasa determined that the Gifu-1 strain of CAV could replicate in two chicken lymphoblastoid cell lines: Marek's disease chicken cell line (MDCC)-MSB1 and MDCC-JP2; and lymphoid leukemia chicken cell line LSCC-1104X5. One study indicates that the MDCC-CU147 line is the most useful for propagation of a variety of CAV strains. MDCC lines are currently used worldwide for the propagation and *in vitro* study of CAV. However, few studies have been done regarding the possibility that CAV may infect other cell types. Because CAV-VP3 causes apoptosis in transformed cell lines, we hypothesized that CAV might also replicate in other transformed cell lines. Thus we wished to re-evaluate the infectivity of CAV in non-lymphoblastoid cell lines.

Methods. We inoculated five transformed cell lines with CAV isolate 03-4876, took cell and media samples at 0, 12, 24, and 48 hours post-inoculation, and performed qPCR to monitor CAV genome replication. Cell lines used in this experiment include MDCC-MSB1 (positive control), human lymphoblast (Molt-4), human rhabdomyosarcoma (RD4), canine malignant histiocytosis (DH82), and chemically transformed chicken fibroblasts (CHCC-OU2). We converted all qPCR data points to concentration of viral genome per milliliter of culture, and then represented that data on a log(10) scale.

Results. MSB1 cell lines replicated as expected producing a positive growth curve. The remaining cell lines showed a much lower concentration of viral genome. DH82 showed a fairly constant virus concentration, while the Molt-4 and CHCC-OU2 cell lines showed a decreasing concentration of virus over the course of the experiment. We suspect that the virus, whether it entered the cells or not, could not replicate in these cell lines. RD4 showed an ideal growth curve similar in shape to the MSB1 cells, but lower in concentration. The qPCR results for RD4 were repeatable. No viral cytopathic effect (CPE) was observed in non-MSB1 cells; MSB1 cells showed CPE 5-7 days post-inoculation.

Conclusions. We cannot make any final conclusions at this time. To continue with this project we wish to repeat this experiment with an adjusted protocol. We also wish to attempt to detect the virus by immunofluorescence.

Unique structure of the M loop region of beta1-tubulin is associated with size variability of platelets in the family Felidae

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Introduction. Platelet size is relatively uniform in mammals except in domestic cats. In most species platelet size ranges from 2–4 microns, however in cats, platelet size may range from 2 – 6 microns or larger. Uniform platelet production by megakaryocytes can be disrupted if microtubule stability is impaired. Cavalier King Charles Spaniels with inherited macrothrombocytopenia have a mutation in the beta1-tubulin gene that results in disruption of microtubule stability and production of large platelets, thus illustrating the importance of microtubules for uniform platelet production. A difference in gene sequence was found in a highly conserved region encoding the M Loop region of beta1-tubulin in cats compared to other species. The M Loop is partially responsible for the lateral interaction between protofilaments within microtubules; structural changes in this region can lead to both lateral and longitudinal microtubule instability. We hypothesize that the altered amino acid sequence in the M Loop region of beta1-tubulin is responsible for microtubule instability, and contributes to the size variability of platelets seen in domestic cats.

Methods. DNA was isolated from blood samples of other members of the Family Felidae and subjected to PCR using primers specific for the area encoding the M loop region of beta1-tubulin. Gene sequences obtained were compared to that of domestic cats. Blood smears were also evaluated to assess for the presence of platelet size variability.

Results. The same variation in the M loop region of beta 1-tubulin was documented in other members of the Family Felidae. Platelet size variation was also present in similar amounts in big cats and domestic cats.

Conclusions. An association between platelet size variation and an altered M loop region in beta1-tubulin was documented in domestic cats and big cats. Evaluation of cat platelet microtubules at the electron microscopic level would be useful to determine if microtubule structure is altered. Possibly the M loop variation documented in cats results in further instability in the seam region and/or a variation in protofilament number. This could further impair microtubule stability and may be of particular importance during times of thrombocytopenic stress.

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National canine and feline parasite prevalence survey

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Introduction. Five-hundred and twenty-five canine and 149 feline fecal samples were obtained from 20 shelters in the US during the period June 1-August 15, 2009.

Methods. Samples were examined for common internal parasites using centrifugal fecal flotation in sucrose (SG=1.26). Fourteen of the 20 shelters were from the southeastern US; 6 shelters (2 each) were from the northeast, midwest and west.

Results. Prevalence of common canine parasites were: *Ancylostoma caninum*, 40.2%; *Toxocara canis*, 11.4%; *Trichuris vulpis*, 18.1%; *Cystoisospora* spp., 13.7%, *Toxascaris leonina*, 0.6%, *Uncinaria stenocephala*, 3.6%. Prevalence of common feline parasites were: *Toxocara cati*, 24.2%; *Ancylostoma tubaeforme*, 14.1%; *Cystoisospora* spp., 23.5%.

Conclusions. These results indicate that internal parasites remain common in shelter animals. This population remains beyond the reach of the veterinarian and continues to serve as a source of infection for pets.

Dynamic respiratory endoscopy of standardbred race horses during qualifying races

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Introduction. Upper respiratory tract (URT) obstruction is a common cause of poor performance in race horses. Until very recently, a diagnosis could only be made using high speed treadmill endoscopy. One limitation of this technique is it does not accurately mimic racing conditions. We hypothesized that a recently developed Dynamic Respiratory Scope (DRS) would be safe and effective for use during racing conditions and would not affect performance.

Methods. Horses were selected at two Standardbred race tracks in New York. In each case any history of URT noise, poor performance and prior URT surgery was recorded. Videoendoscopic images of the URT were recorded during qualifying races. Horse speed over ground and respiratory noise were recorded by a small GPS receiver and by a microphone at nostril level. Preferentially recorded race video was used to determine the degree of head elevation.

Results. Twenty one horses were examined to date. The trainers reported no effect on drivability of the horse. Median of the average race times (all times min:sec) for the current season (median 2:00, range 1:54-2:06) and the median race times during the DRS examination (median 2:00, range 1:54-2:07) were not significantly different. Of the eleven horses who reported no history of abnormal respiratory noise we found one horse with an URT obstruction. Of the eleven horses who reported an abnormal upper respiratory noise we found three horses with an URT obstruction.

Conclusions. We found the DRS to be safe, non-performance limiting and effective for use during Standardbred racing. This study merits further work to correlate URT morphology with driver and horse events.

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Identification of potential platelet alloantigens in the Equidae family by comparison of gene sequences encoding major platelet membrane glycoproteins

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Introduction. Polymorphisms in genes encoding membrane proteins can result in the synthesis of proteins termed alloantigens. While protein function may not be altered, structural differences can elicit an immune response in an individual with an alternate form of the same protein. Identifying alloantigens is particularly important for blood transfusions and for the awareness of potential development of neonatal alloimmune thrombocytopenia (NAIT). NAIT is a condition in which there is an incompatibility between newborn and maternal platelet antigens, resulting in development of maternal antibodies against antigens on the newborn's platelets. In humans, platelet alloantigens have been identified on membrane glycoproteins IIb/IIIa and Ia (integrin subunits α IIb, β 3 and α 2). Very little is known about potential platelet alloantigens in the Family Equidae, although there have been reports of suspected NAIT in foals. Our project focused on evaluating genes encoding major platelet glycoproteins within the Equidae family.

Methods. DNA was isolated from blood samples collected from a foal with suspected NAIT and the dam and sire, a donkey, and a zebra. Primers were designed based on gene sequences encoding equine platelet membrane glycoproteins IIb, IIIa, (integrin subunits α IIb and β 3) and Ia (integrin subunit α 2). PCRs were performed and amplified DNA was separated using gel electrophoresis. Target bands were harvested and sequenced; gene sequences were compared to the equine genome available on GenBank. Foal sequences were compared to the dam and sire when heterozygous polymorphisms were identified.

Results. Several potential alloantigen sites have been identified on membrane glycoproteins α IIb, β 3 and α 2 when comparing amino acid sequences of horse, donkey and zebra.

Conclusions. At this point we have not identified a nucleotide change between the mare and foal that might explain the existence of NAIT, however, data is still being acquired and other platelet membrane glycoproteins should be evaluated before conclusions can be drawn. It is also possible that in spite of the clinical signs observed, NAIT was not the cause of thrombocytopenia in this foal. This project represents the first effort at identifying potential platelet alloantigens in members of the Equidae Family. The data obtained forms the groundwork for identifying platelet alloantigens involved in transfusion reactions and NAIT in horses, donkeys, mules, and zebras.

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Development of transdermal phenylbutazone formulations for horses and deer

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Introduction. Phenylbutazone (PBZ) is a non-steroidal anti-inflammatory that is commonly used in the treatment musculoskeletal lameness and laminitis in horses. The objective of this study was to develop a transdermal PBZ formulation that will reduce systemic side effects, increase local permeation, and provide extended therapy. Transdermal PBZ may improve the drugs local therapeutic effects because it does not rely on blood distribution for delivery of the drug. Many of the areas where PBZ is most effective, such as joints and laminitic hooves, have relatively low perfusion which often results in low therapeutic levels of drugs.**Methods.** A formulation of alkyl urea enhancers and PBZ were studied using the Franz diffusion cells apparatus. Dermatomed equine and deer skin were used as the in vitro animal model and compared for absorption. Absorption was determined using transdermal pharmacokinetic parameters via high performance liquid chromatography analysis.

Results. In both cases, the formulation with the permeation enhancer showea trend of increased flux compared to the control formulation which consisted of the vehicle and PBZ. Also, the alkyl urea formulation showed increased skin retention of PBZ. A preliminary in vivo study was performed by applying the formulation with the enhancer to the tibiotarsal joint of a horse and evaluating the PBZ concentration in the synovial fluid several times over a 24 hour period. The results of the in vivo study are pending.

Conclusions. If efficacy is achieved from the in vitro study, a larger number of horses will receive the topical formulation to obtain statistically significant results.

Novel exon insertions in dystrophin in Labrador Retrievers and Welsh Corgis

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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 both Welsh Corgis and Labrador Retrievers 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.

Methods. Genomic DNA from affected, carrier, and normal Welsh Corgis and Labrador Retrievers were extracted from whole blood and skeletal muscle using conventional methods. Introns 12 and 19 of the canine dystrophin gene were amplified in 1-2 kb fragments and sequenced where possible. Larger fragments, up to approximately 10 kb were amplified when the flanking sequence of the insertions was identified. Complete sequence coverage of the insertions were made by the process of primer walking. Primers were then designed to bracket the insertions for diagnostic assays.

Results. Both insertions were determined to be intronic long interspersed nuclear elements (LINE). These, in combination with the existing intronic sequence, resulted in splicing and inclusion of portions of these elements in the mRNA. The Welsh Corgi insertion was 549 base pairs long, and the Labrador Retriever insertion was 6363 base pairs long. Using this information, diagnostic assays for both mutations were developed and tested on blood derived genomic DNA from carrier, affected, and normal dogs.

Conclusions. We have completely sequenced the introns and associated LINE insertions in both the Welsh Corgi and Labrador Retrievers and have identified the precise mechanism by which these result in novel exons in the dystrophin mRNA. We have also been able to use this data to develop rapid PCR based assays to determine genotypes, which in turn will aid in the development of new therapies.

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Neuromotor characterization of transgenic mice allotopically expressing ATP6

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Introduction. Several mitochondrial diseases in humans are linked specifically with the mitochondrial ATP6 gene that encodes for a subunit of the F₀ subcomplex of ATP synthase; e.g., Neuropathy, Ataxia and Retinitis Pigmentosa (NARP). The ATP6 protein is embedded within the inner mitochondrial membrane forming a proton channel through the membrane. Proton flow through the channel drives ATP production by utilizing other protein components of ATP synthase. Allotopic expression of ATP6 enables an animal model to be created for this human disease. A line of transgenic mice that allotopically expresses the ATP6 gene was created at Auburn University (A6M mice). Motor coordination and neuromuscular measurements of these mice relative to wild-type control animals were performed to assess neuromotor function of this new transgenic disease model.

Methods. Motor coordination tests including wire hang, pole, rotarod, balance beam and footprint tests were performed on A6M and control mice. The wire hang test measured the amount of time the mouse could hang upside down from a wire cage top. Pole test analyses involved a mouse being placed at the top of the pole. Latency to turn around and run down the pole was measured. The rotarod test measured latency to fall from a rod rotating at 32 rpm or accelerating from 4 to 40 rpm (accelerated rotarod). Balance beam testing measured latency to traverse a small beam into an escape box. For gait testing, the feet of mice were marked with nontoxic paint and the mice allowed to run up a ramp where a piece of paper had been put down to record footfalls. Distances between various footfalls were measured.

Results. A6M mice displayed significant deficits in the wire hang, pole and balance beam tests ($p < 0.05$). Footprint analyses detected no gait difference between the A6M and wildtype mice ($p > 0.05$). A6M mice displayed superior performance in comparison to wildtype mice in constant and accelerated rotarod analyses ($p < 0.05$).

Conclusions. Various biochemical and physiological measures will be carried out to confirm neuromuscular analyses. A6M mice serve as an animal model for NARP pathogenesis and as a potential paradigm for future therapeutic modalities.

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Comparison of a commercial glucometer and a spectrophotometry-based chemistry analyzer in horses and alpacas

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Introduction. The accuracy of a commercial glucometer was evaluated in horses and alpacas. There is an increasing awareness among practitioners of the importance of glucose homeostasis to the recovery of critically ill individuals. It is necessary to have not only a rapid, but economical way in which to measure the glucose concentrations of such patients. Equally, if not most importantly, the accuracy of these machines must offer confidence to the user.

Methods. Animals from the Auburn University Equine and Alpaca Teaching Herd were selected for use in this study. The glucometer was also used in the equine intensive care ward, to compare the accuracy on ill and healthy individuals. Blood was drawn from each individual; one drop was used for the glucometer, and the remaining was added to a heparinized tube for centrifugation. Plasma was separated via centrifugation and removed from the cells within 15 minutes of sample collection. Plasma glucose concentration was determined by Auburn University's Clinical Pathology Laboratory using a spectrophotometry-based instrument and a blood gas analyzer.

Results. Results from equine patients, show promise for the accuracy of the glucometer with an average percent bias of 9.44%.

Conclusions. The accuracy of the glucometer in alpacas will be determined as more samples are evaluated and the proper coding for the machine has been determined. The accuracy of the meter is yet to be determined, and conclusions will be made when data becomes available.

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Profiling the immune response in cats treated with adeno-associated virus gene therapy

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Introduction. Currently, there is no treatment for the lysosomal storage disease GM2 gangliosidosis. The non-pathogenic virus, adeno-associated virus (AAV), has been used as a vector to deliver a normal, functional gene directly into the brain, resulting in production of the gene product—in this case, hexosaminidase—throughout the brain. Preliminary therapeutic experiments with gene knockout mice using AAV vectors have shown promising results. The goal of this project was to further characterize the cats' immune response. Understanding what cell types contribute to the response will provide guidance on how to proceed in future long-term experiments with GM2 cats.

Methods. For immunohistochemical staining of tissues, the Avidin-Biotin Complex (ABC) method was used on formalin-fixed, paraffin-embedded cat brain sections. For each cat, one slide received primary antibody and secondary antibody, one slide received only secondary antibody, and one slide received neither antibody. The primary antibodies used were MHC II, CD68, Iba-1, CD11b clone OX-42, and CD20.

Results. The MHC II antibody recognizes cells that express major histocompatibility complex class II, including T and B lymphocytes and all antigen-presenting cells. In GM2 cats treated with the AAV vector, we saw many small positive cells in areas of perivascular cuffing, which were probably lymphocytes, and robust staining of cells with a ramified morphology in the parenchyma. In treated normal cats, staining was mild and limited to scattered small, round cells that may be lymphocytes or possibly macrophages. Untreated GM2 and normal cats showed little to no MHC II staining. CD68 is expressed on macrophages and monocytes, but microglial cells have also been shown to express this antibody. There was little to no staining in treated GM2 cats, untreated GM2 cats, and untreated normal cats. In treated normal cats, there were scattered small round cells that stained positively for CD68, mainly in perivascular cuffing. These cells were most likely from a monocytic lineage. Iba-1 is specifically expressed in macrophages and microglia. The results obtained with this antibody were essentially identical to those obtained with CD68. There was no untreated normal section stained with Iba-1. CD11b, clone OX-42, is mainly expressed in monocytes and granulocytes, but it also stains cells with the morphology of microglia in the brain. However, there was no staining in any of the treated or control cats. CD20 is primarily expressed on B cells. Treated GM2 cats and treated normal cats showed positive cells in areas of perivascular cuffing, but the treated normal cats had a much greater response. Untreated GM2 and normal cats did not display significant staining.

Conclusion. While immune responses are seen in both normal and GM2 affected cats treated by intracranial injection of AAV vectors expressing human hexosaminidase, the type and level of response differ.

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Sequencing the dystrophin cDNA in a new canine model of Duchene's muscular dystrophy

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Introduction. Duchenne muscular dystrophy (DMD) is a common X-linked inherited disease affecting many species including humans, dogs, cats, and mice. Using canine models, gene therapy approaches to DMD and DNA testing programs for the disease can be developed. Canine models of DMD have been established for the several breeds, including Labrador Retriever and Welsh Corgi. We have recently identified a Labradoodle (Labrador Retriever and Poodle cross) with DMD, whose disease was not caused by the Labrador Retriever mutation. Our objective was to sequence the dystrophin cDNA from the affected Labradoodle, to identify the mutation, and to establish a new canine model of DMD.

Methods. Messenger RNA was prepared from normal control canine muscle and from the affected dog. The mRNA was validated by optical density and RT-PCR for canine L37, a ribosomal protein. The dystrophin cDNA is >12,000 base pairs long. Using RT-PCR primers, the gene was amplified in four segments that overlapped. The overlapping segments were successfully amplified using RT-PCR, proving that dystrophin cDNA was present in the sample. Once the four segments of the cDNA were amplified, each amplicon was sent to be sequenced and then compared to the normal canine dystrophin sequence.

Results. Preliminary data indicates a putative mutation to be a single base pair substitution resulting in a premature stop codon.

Conclusion. The stop codon terminates the protein early, making it nonfunctional.

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The role of efflux pumps in canine pathogenic multi drug resistant *E. coli*

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Introduction. Cell membrane pumps efflux antimicrobial drugs from bacterial cells. Three super families of proton efflux pumps impact drug resistance: Resistance Nodulation Division (RND), Small Multidrug Resistance (SMR) and the Major Facilitator Superfamily (MFS). The purpose of this study is to describe the impact of these pumps on the type (eg, MDR) and extent (magnitude of minimum inhibitory concentration (MIC) of antimicrobial resistance in pathogenic *Escherichia coli* (n=20) collected from dogs with spontaneous urinary tract infections.

Methods. The isolates have been phenotyped as non-drug resistant (NDR), single drug resistant (SDR) or multi drug resistant (MDR). The minimum inhibitory concentration (MIC) was determined by matching a 0.5 MacFarland standard. The isolates were pipetted into a micro well plates containing increasing amounts of 13 different drugs. Susceptibility was determined via Trek Diagnostics System Vizion. Procedure was repeated with the addition of 100µg/mL Phe-Arg-β-naphthalamide. The expression of isolates was quantitated using real-time PCR. Primers were selected from genetic codes in GenBank, with the exception of 16S rRNA which was selected from primers described by D.J⁴. Real-time PCR was performed using a Roche® Light-Cycler 1.5 using Roche® DNA Master Sybr Green I. Standard curves were created by diluting cDNA ten fold, determining concentration using Thermo Scientific Nanodrop 2000®. LightCycler 4 Software was utilized to calculate standard curve and concentration of expression.

Results. The MIC decreased for several drugs in most isolates. Ticarcillin/ clavulanic acid MIC decreased in all isolates (10/10) indicating that the major RND efflux pump plays a role in its resistance. Other drugs for which accumulation appeared to increase in the presence of the EPI, thus decreasing MIC were ampicillin and chloramphenicol; erythromycin and enrofloxacin were moderately affected. Interestingly, exposure to the EPI actually increased isolate MIC for cephalosporins. All isolates increased MIC for at least one cephalosporin. Surprisingly, MIC increased in 9/10 for the 1st generation drug cephalothin and in 7/10 isolates for the third generation cefpodoxime. For cephalothin and cefpodoxime in particular, non resistant isolates become resistant in the presence of the drug. Pump Expression: Real-time PCR indicates that each of the efflux pumps tested are constantly expressed in all isolates without induction by antimicrobials. However when stressed with the addition of ciprofloxacin (10 µg/mL), all genes were induced to increase expression. The MDR isolates showed the largest change in expression, with a large increase in EmrE and AcrB. The expression of cmr and MacB did not change as radically. Variability precludes identification of these changes as significant.

Conclusions. All major efflux pumps classes were constitutively expressed in non resistant isolates and were expressed in resistant, including multidrug resistant isolates. The EPI decreased MIC for several drugs, but increased MIC for cephalosporins. Stress induced by ciprofloxacin was associated with increased expression of all 5 pump types, including the ABC superfamily. This study indicates that care should be taken when assuming a pump inhibitor will improve antimicrobial efficacy, particularly for cephalosporins.

Pharmacokinetics of fosfomycin for treatment of MDR-*Escherichia coli* urinary tract infection in dogs

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Introduction. Emerging antimicrobial resistance of Gram negative bacterial species such as *Escherichia coli* increasingly is limiting therapeutic options for treatment of infections. This includes urinary tract infections (UTI) in cats and dogs. Fosfomycin tromethamine is broad-spectrum bactericidal antimicrobial approved in humans for oral, single dose treatment of uncomplicated UTI. The purpose of this study was to determine the pharmacokinetics of a commercially available fosfomycin oral preparation (tromethamine salt) in dogs and to establish a dosing regimen that should be effect toward multidrug resistant (MDR) *E coli* based on our previously establish MIC₉₀ of 1 mcg/ml for canine and feline UTI pathogens.

Methods. Fosfomycin was studied using a randomized, cross-over design in 12 adult (8 male, 4 female) clinically healthy client-owned dogs weighing a minimum of 20kg. Procedures were approved by the Institutional Animal Care and Use Committee. Prior to the study each animal was subjected to physical examination, complete blood count, serum chemistry and profile and urinalysis to ensure their health. The day of the study an external jugular catheter was placed using local anesthetic and manual restraint. Kinetics were determined in two phases 24 hours apart (fosfomycin half-life is 2h, 1allowing for non-detectable concentrations by 24 hr). Phase involved administration of either a single oral 80mg/kg dose of fosfomycin tromethamine salt, or 40 mg/kg IV of fosfomycin (di-sodium salt). Dogs were further randomized to receive the drug with or without food. The oral dose for each dog was either administered in 20ml of water or in 6oz of moist food. Blood (4ml) for serum was collected from the indwelling jugular vein catheter at 0, 5,10,20, 30, 40, 60, 75 minutes, and 1.5, 2, 2.5, 3, 4, 6, 8, 24 hours for IV dosing and 0, 15, 30, 45 minutes, 1, 1.5, 2, 3, 4, 6, 7.5, 9, 10.5, 24 hours for oral dosing. After 24 hours the second phase (alternate route) was implemented. Dogs were studied in pairs, each receiving a different route on each day.

Results. For IV administration, C_{max} (mcg/ml), clearance (ml*kg/hr), V_d (L/kg), half-live (hr) and MRT were (mean±sd): 210± 104, 0.23±0.15,0.36±0.19, 1.14±0.35 and 1.7±0.4, respectively. For oral administration, C_{max}, half-life and MRT (units as above) were 66±21, 2.5±1.09 and 5.1 ±1.7, respectively. Drug was detected at concentrations exceeding the MIC₉₀ of fosfomycin for multidrug resistant (MDR) *E. coli* (1 mcg/ml) for 7 hr (2.5 mcg/ml) and 12 hr (9 mcg/ml) following IV and oral administration, respectively. Oral bioavailability was 88± 32%. Food increased oral bioavailability, being 109±31% (95% CI: 84-135) with food and, 66±16% (95%CI:52-79%) without food. Gender and sequence of drug administration had no impact on oral bioavailability.

Conclusions. Fosfomycin tromethamine can achieve and maintain concentrations that should be effective for treatment of UTI associated with MDR *E. coli* following twice daily oral administration at 80 mg/kg in dogs. Administration of the drug in food will enhance absorption.

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NADPH oxidase activity in the diabetic heart

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Introduction. Cardiovascular disease represents the main cause of morbidity and mortality in diabetic patients and increased reactive oxygen species (ROS) production has been implicated as a contributing factor to cardiovascular complications. The major focus of this project was to examine the early effects of diabetes on ischemia/reperfusion injury. The specific aim of this study was to determine NADPH oxidase (NOX) expression and activity in early diabetic hearts following ischemia/reperfusion injury.

Methods. Previously, rats were made diabetic by an injection of streptozotocin (STZ) or its vehicle and implanted with an osmotic minipump containing the SOD mimetic EUK-134 or its vehicle. After 4 weeks, *in vivo* left ventricular (LV) pressure and contractility were similar in all four groups. Hearts were then isolated and perfused in the Langendorff mode for the measurement of LV pressure and contractility before and after a 30 min global ischemia. Following the experiment, hearts were frozen in liquid nitrogen for biochemical and molecular analysis. To determine NOX expression in the various groups, real time PCR was performed using isoform specific primers. NOX activity was measured by a luminescence assay in Krebs-Henseleit Buffer using lucigenin as the electron acceptor and NADH and NADPH as respective substrates for each sample.

Results.. Gene expression of the various NOX isoforms were measured using real time PCR and gene specific primers. Hearts from all groups expressed both NOX2 and NOX4 isoforms. There was no change in NOX2 expression in any of the groups as measured by real time PCR. On the other hand, NOX4 gene expression was significantly decreased in the diabetic hearts. Next we determined NOX activity in heart homogenates. NOX activity was expressed as Relative Light Units (RLU)/ 100 mcg protein and experiments were carried out in the presence of the specific superoxide substrate lucigenin and in the presence and absence of the NOX substrates NADPH and NADH. In control experiments, NADPH (100 μ M) and NADH (100 μ M) caused an increase in NOX activity that was blocked by the NOX inhibitor diphenylene iodonium (DPI 100 μ M). EUK-134 is a superoxide dismutase mimetic that dismutates superoxide into hydrogen peroxide and water. EUK-134 (0.1-100 μ M) caused a dose-dependent decrease in NADPH-stimulated luminescence. These initial studies suggested that the increase in RLU in response to NADPH was due to an increase in NOX-induced superoxide generation. When measuring NOX activity in the hearts from control and diabetic hearts, there was no difference in NOX activity when NADPH was used as the substrate but a significant decrease in NOX activity in the diabetic hearts from both vehicle and EUK-134 treated hearts when NADH was used as the substrate.

Conclusions. Previously performed mechanical function experiments in our lab suggested that during the early diabetic state, the diabetic heart is protected from ischemia/reperfusion injury when compared to the non-diabetic heart. This study suggest that there is also a decrease in the expression of the NOX4 isoform early on in diabetes and a decrease in NADH stimulated NOX activity that may contribute to the protective effect observed in the early diabetic heart.

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Breaking the paradigm: methods of testing for circulating bovine viral diarrhea virus in a beef herd

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Introduction. Bovine viral diarrhea virus (BVDV) is an economically significant disease of cattle. Currently, this disease is present in approximately 10% of U.S. cattle herds, with $\leq 0.4\%$ of animals being persistently infected (PI). Protocols for detecting BVDV in a herd range from inexpensive and least reliable to expensive and most reliable. Due to the high cost of available, reliable diagnostic protocols, the goal of this project was to break this paradigm by developing a reliable yet inexpensive method of testing. The proposed hypothesis for this project was that appropriate sampling and testing of a pool of collected hornflies or a sample collected from the water trough could be validated as a reliable means of determining the presence of BVDV in a beef herd.

Methods and Results. Fly Project: One hornfly from a BVDV-positive cow was added to 5, 10, 50, 100, 200 and 400 hornflies from a BVDV-free cow. RT-nPCR was performed on all samples. Virus was detected in all dilutions. Water trough project: Surface water samples were collected from water troughs provided to PI animals, acutely infected animals, vaccinated pregnant cows that were exposed to BVDV early in pregnancy, PI and non-infected alpacas, and non-infected cows. Virus was detected by RT-nPCR in water troughs from PI animals. QPCR indicated a concentration of 3.95×10^3 viral copies per 5 μl of original sample taken from a PI water trough.

Conclusion. The results of this experimentation have shown that BVDV can be isolated from pooled hornflies and water troughs. However, future field studies involving a PI animal co-mingled with BVDV-negative cattle is necessary.

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Association of CTLA4 polymorphisms with IMHA in dogs

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Introduction. Immune-mediated hemolytic anemia (IMHA) occurs when a patient's immune system attacks the body's own red blood cells. The disease can be primary (also termed autoimmune hemolytic anemia or idiopathic) or secondary to various causes such as medications, neoplasia, or infectious agents. Antibody formation against erythrocytes leads to extravascular and sometimes intravascular hemolysis, and the accelerated destruction and removal of erythrocytes results in a regenerative anemia. Less commonly, antibodies target early red blood cell precursors leading to a non-regenerative anemia. IMHA is the most common autoimmune disorder found in dogs. The CTLA4 gene encodes for the cytotoxic T-lymphocyte antigen 4 molecule, a receptor responsible for the inhibition of self-reactive T-lymphocytes and downregulation of T-cell function and proliferation. Deregulation of self-reactive lymphocytes has been indicated as a major cause for autoimmune disorders. Human studies have found an association between a polymorphism in the CTLA4 gene and autoimmune diseases such as Diabetes and Grave's disease. The focus of our study was to determine if similar or other polymorphisms exist in canine patients diagnosed with IMHA or immune-mediated, non-regenerative anemia.

Methods. DNA isolated from blood samples from affected dogs was subjected to PCR using primer sets designed around the coding regions of the CTLA4 gene. Sequence data obtained from dogs with IMHA or immune-mediated, non-regenerative anemia was compared to sequence from an age-matched control for each breed.

Results. No significant polymorphisms were detected when sequences from IMHA dogs were compared to both age-matched controls and published canine GenBank sequence.

Conclusions. These results suggest that other causes, such as changes in the non-coding region of the CTLA4 gene or alterations in other molecules involved in T-cell regulation may be involved in the pathogenesis of IMHA in dogs.

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Evaluating soft tissue composition of the equine palmar foot with computed tomography, magnetic resonance imaging, and three-dimensional image reconstruction

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Introduction: Previous histological studies of the equine foot by R. M. Bowker (2003) have shown that the soft tissue composition of the palmar foot shows a significant degree of variation among horses. This variability in composition is closely correlated to differences in foot health. Palmar foot health is vital for soundness, athletic capability, and the treatment of laminitis and navicular syndrome. Radiography is inadequate for evaluating soft tissues. We hypothesized that computed tomography (CT) and magnetic resonance (MR) imaging of the equine palmar foot, combined with three-dimensional image reconstruction technology, could be used to evaluate the contrasting soft tissue compositions that exist between feet with varying degrees of development.

Methods: We collected three cadaver forefeet: a reasonably developed foot and two feet in different stages of underdevelopment. Digital photographs and lateral radiographs were taken of each foot. MR and high-resolution CT images of each foot were obtained in a transverse plane perpendicular to the palmar angle of P3. Using Mimics® image processing software, the collateral cartilages and digital cushions were manually extracted from each CT and MR image, respectively, to build three-dimensional models from which volume measurements could be made.

Results: We found that the volume ratios of the collateral cartilages and digital cushion to the distal phalanx were markedly greater in the reasonably developed foot than in either of the underdeveloped feet; moreover, a greater percentage of its digital cushion was composed of fibrocartilage.

Conclusions: While we cannot claim statistical significance from results obtained from only three feet, we did succeed in developing a unique methodology for quantifying soft tissue structures of the equine palmar foot. An IACUC proposal has been approved for utilizing this methodology in a live horse suffering from navicular syndrome pre- and post-treatment.

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Thromboelastography (Teg) as a method of monitoring hemostasis in dogs: are Teg tracings from healthy dogs consistent when analyzed over a period of three consecutive days?

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Introduction. Thromboelastography (TEG) is a method of measuring blood coagulation and fibrinolysis that is relatively new to veterinary medicine. Unlike standard coagulation tests, which measure isolated components of the coagulation cascade, TEG uses whole blood to evaluate the function of the hemostatic system and is influenced by both cellular and plasma components of blood. Clinically, TEG has been shown to detect both hypocoagulable and hypercoagulable states in dogs and shows great potential for monitoring dogs with hemostatic derangements. The purpose of this project was to determine if TEG produces consistent results when used to monitor hemostasis in a healthy dog over a period of three consecutive days. Additionally, we varied the time at which samples were run after venipuncture (15 min. vs. 1 hr.) and whether or not kaolin activator was added to the samples before TEG analysis.

Methods. Blood was collected from ten healthy dogs, each on three consecutive days, and placed in tubes containing sodium citrate. For TEG, calcium was added to an aliquot of citrated whole blood to initiate clot formation at 15 minutes and 1 hour after venipuncture. Samples were analyzed in duplicate, and kaolin was included as a coagulation activator in one of every two samples. Results were analyzed using one-way ANOVA for repeated measures and a t-test or Mann-Whitney test for group comparisons.

Results. No statistically significant differences were found between TEG parameters of individual dogs measured on three consecutive days. Comparison of blood samples run with or without kaolin activator and run at 15 min. vs. 1 hr. post-venipuncture yielded no statistically significant differences.

Conclusions. Thromboelastography is a consistent and reliable test for monitoring hemostasis in dogs over a period of three days and variation in consecutive TEG measurements is likely due to changes in patient status.

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Ultrasound of adrenal glands in horses

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Introduction. Ultrasonographic visualization of the adrenal glands in the mature horse has previously only been described by rectal and laparoscopic approaches. The purpose of this project was to determine if transabdominal ultrasound imaging of the adrenal glands was feasible in adult horses.

Methods. Transabdominal ultrasound imaging of the kidneys and adrenal glands of 10 horses was performed in a dark, quiet room with minimal restraint and sedation as needed. Using a C5-2 curvilinear probe, the left kidney was identified in the caudo-dorsal abdomen just cranial to the tuber coxae. The left adrenal gland was visualized with the probe in the 17th intercostal space and parallel to the rib cage. The right adrenal gland was visualized in a similar manner, but with the ultrasound probe in the 16th intercostal space. The length, width, and depth of each adrenal gland were measured for comparison. The transabdominal ultrasonographic image obtained was confirmed by imaging a dissected specimen in a water bath.

Results. Both adrenal glands were observed in 9/10 horses and only the right adrenal gland was imaged in one horse. In each horse, a hypoechoic structure was seen deep the cranial pole of the kidney and just cranial to the renal artery as it leaves the hilus of the kidney. Mean length was 6.46 ± 1.43 cm and width was 1.43 ± 0.36 cm.

Conclusions. Although technically difficult, transabdominal ultrasonography of the adrenal glands is possible in adult horses. Knowledge of variability in normal imaged adrenal gland size will allow for identification of adrenal gland lesions such as pheochromocytoma.

Graduate Student Poster Presentations

Induction of cell surface integrins in lymphomas and potential applications to gene therapy

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Introduction. Canine lymphoma accounts for up to 24% of all canine neoplasia, and is considered treatable, but not curable, necessitating new therapeutic approaches. Recombinant adenovirus vectors (Ad) have been recognized as effective at *in vivo* gene delivery and have been utilized as gene therapy agents for a number of cancers. The elucidation of the mechanics of viral entry has allowed the development of recombinant vectors that exploit existing cell surface receptors to achieve viral entry into the cell. B cells are normally resistant to infection by Ad5, likely due to the lack of the Coxsackie and Adenovirus receptor (CAR) on the cell surface and low level expression of the $\alpha\beta 3/5$ integrins necessary for viral internalization. Our analysis of primary lymphoma cells has indicated that these cells resemble lymphocytes in both CAR and integrin expression. Based on this, we are developing a two-fold strategy of retargeting Ad and upregulating integrin expression to achieve efficient transduction of target cells.

Methods. Ad5 vectors carrying the luciferase gene with fiber modified to incorporate an RGD motif, which binds integrin receptors, a pk7 motif, which binds heparan sulfate containing receptors, or double modification to include both were used to infect HEK293, a control cell line, the canine lymphoma cell line OSW and primary canine lymphoma cells. Infection efficiencies were determined by assaying luciferase activity. The level of cell surface expression of $\alpha\beta 3$ integrin was measured on resting OSW cells and primary lymphoma cells. OSW cells were then treated with LPS, ConA or PMA plus ionomycin to evaluate the ability of each to upregulate integrin expression.

Results. Both the Ad5 pk7 and Ad5 RGD vectors were able to efficiently transduce control cells. In contrast, none of the Ad5 vectors was able to transduce unstimulated OSW cells and primary lymphoma cells. Analysis of OSW cells indicated low levels of $\alpha\beta 3$ integrin expression in resting cells. Following stimulation with LPS, PMA or ConA these cells were then re-examined and integrin expression was shown to increase in response to stimulation leading to the potential of enhanced Ad infection rates.

Conclusions. Previous studies have indicated that Ad can be successfully retargeted to cell surface receptors other than CAR, however low level expression of integrins on lymphoma cells appear to limit the efficiency of transduction by retargeted Ad in these cells. Our experiments to date suggest that native viral tropism can be modified. However, canine lymphoma cells express low levels of the integrin that is necessary for virus internalization. Treatment of cells with LPS, PMA or ConA stimulates cells to upregulate expression of $\alpha\beta 3$ integrins. It is predicted that this upregulation will enhance viral transduction in stimulated cells.

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Surface immobilized molecular beacon probes for PCR and DNA microarrays

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Introduction. Molecular Beacons (MB) are modern fluorescent hairpin oligonucleotide probes for highly sensitive and specific label free DNA detection. We develop advanced electronically controlled MB for solid-phase PCR and microarray applications.

Methods. MB with Fluorescein/Dabcyl fluorophore-quencher pair was designed to match a fragment of the *Staphylococcus aureus* FemA methicillin-resistance gene. Siloxane bonding was used for attachment of MB to both regular and conducting (ITO) glass surfaces; silane reactive groups were coupled to 3'-end of MB using epoxy-amine chemistry. Capillary sandwich type cell was constructed that allows change of solution around the surface probes, insertion of DNA analyte, and monitoring of evanescent fluorescence.

Results. MB hairpin attached to glass is open in low ionic strength immobilization solvent, but closes in 1M NaCl hybridization solution and reopens after hybridization with complementary DNA target. These MB conformation changes are reproducibly repeated in several solution exchange cycles. Fluorescence intensity increases 3 fold for dissolved and 1.5 fold for glass attached MB upon hybridization to analyzed DNA. The DNA detection limit is about 1 picomole.

Conclusions. Stable silane modified MB probes were synthesized, attached to surface and exploited for sensitive DNA detection. The sensitivity can be further improved by replacing Dabcyl by efficient BHQ quencher.

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Combined *in ovo*-vaccination with non-replicating adenovirus-vectored avian influenza and Marek 's disease vaccines

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Introduction. Protective immunity to avian influenza (AI) can be elicited in chickens by *in ovo* vaccination using a replication competent adenovirus (RCA)- free recombinant adenovirus (Ad) encoding the hemagglutinin protein of AI virus (AdH). Marek's disease virus (MDV) vaccination is routinely performed by the *in ovo* route in the U.S. poultry industry.

Methods. We evaluated the effects of combined *in ovo* vaccination with AdH and commercially available MDV vaccines [(A) HVT/Rispens; (B) SB-1/HVT]. White leghorn SPF chickens were vaccinated *in ovo* with RCA-free Ad vectoring a codon-optimized H5 gene of A/tk/WI/68 (AdTW68. H5_{ch}) in combination with MDV vaccines A or B. Hatched chickens were tested for AI hemagglutination inhibition (HI) antibodies and for MDV viral DNA by PCR.

Results. HI AI positive birds were detected on day 20 after hatch in all groups. Birds receiving the Ad vaccine only continued to increase antibody levels as well as the percent of antibody positive animals (to ~85%) through day 55 after hatch. Groups vaccinated with AdH5+HVT/Rispens developed similar AI antibody levels but the percent of antibody-positive birds was significantly reduced (to ~40%) compared to AdH5-only vaccinated chickens. Similarly, AdH5 combined with SB-1/HVT also elicited AI antibodies but with reduced coverage (~50%). On the other hand, feather follicles of all MDV *in ovo* vaccinated chickens were positive for MDV DNA by PCR throughout the experimental period.

Conclusions. These results indicate that the MDV vaccines may interfere with AdTW68.H5 *in ovo* vaccination.

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Optimization of a breast cancer specific phage probe into liposomal Doxil®

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Introduction. Doxorubicin has been an effective means of treatment for various types of cancer; it has been shown to be an effective treatment of various breast cancers. Free doxorubicin is however very toxic to many cell types and accumulates in the heart to become lethal. With the invention of stealth liposome and the ability to remote load doxorubicin into these liposomes to create Doxil®, higher doses can be given because these liposomes are long circulating and do not accumulate as traditional free doxorubicin treatments. However, there are still many undesirable side effects experienced as a result of treatment with these drugs and the need for a chemotherapeutic agent that specifically targets cancerous cells is needed to reduce these side effects caused by current treatments. To create a targeted liposomal formulation, we propose the use of a landscape phage from multibillion phage display libraries that has been selected to specifically target MCF-7 breast adenocarcinoma cells. These phage libraries display 55-mer peptides with random inserts in the pVIII gene that give them an ability to bind to specific targets based on the random insert into the gene.

Methods. In this study, we isolated and purified DMPGTVLP coat protein by standard methods. Purified DMPGTVLP coat protein was then incubated with liposomal Doxil at 37°C with sodium cholate at its critical micelle concentration (CMC) to allow spontaneous insertion of the peptide into the liposome after dialysis to remove cholate micelles. Insertion was carried out with a 0.5%(v/v) protein/lipid concentration across a range of pH values and types of buffer systems. DMPGTVLP-Doxil samples were then purified by size exclusion chromatography to remove impurities. Samples were then treated with proteinase K and visualized by western blot to determine the orientation of the coat protein into the liposome. Concentration of doxorubicin contained in the liposomes was determined by lysing with 1% Triton X-100 and determining the absorbance at 500nm.

Results. Our experiments suggest that insertion at pH 8.5 in 1X TBS was found to be the best conditions for insertion of the DMPGTVLP coat protein into Doxil based on the following three main requirements: that liposomes have 1) least amount of doxorubicin leakage, 2) highest concentration of protein inserted, and 3) highest concentration of peptide with the N-terminus exposed to the exterior of the liposome and the C-terminus displayed on the interior of the liposome. Insertion at these conditions yields protein concentrations approaching 100% incorporation with almost no leakage of doxorubicin. Initial MCF-7 cytotoxicity experiments suggest that targeted DMPGTVLP-Doxil has an enhanced effect of toxicity than similar treatments with untargeted Doxil.

Conclusions. These results suggest that proper orientation of coat protein into liposomes is critical for the effectiveness of targeted treatments. The improved insertion of DMPGTVLP into liposomal Doxil has potential to be a more effective chemotherapy drug for the specific treatment of breast carcinomas. Further experiments of cytotoxicity in MCF-7 and other cell lines are in progress to determine the specificity of targeted Doxil to breast cancer cell lines.

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Perinatal exposures of male rats to the estrogenic chemical bisphenol A (BPA) impacts rat Leydig cell differentiation

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Introduction. Leydig cells are localized to the testicular interstitium and produce the male sex hormone testosterone (T), which is required to maintain male fertility. In the rat, postnatal Leydig cell development occurs through three different stages: progenitor, immature, and adult Leydig cells, typified by developmental characteristics at 21, 35, and 90 days of age. Leydig cell development is defined by a gradual decrease in cell proliferation and an increase in steroidogenic capacity. BPA is widely used in the manufacture of polycarbonate plastics and epoxy resins, and is a constituent of dental sealants. BPA is known to activate estrogen receptors (ERs), which are expressed in male reproductive tract tissues, including Leydig cells. Therefore, the present experiment was designed to assess the effect of perinatal exposure to environmentally relevant BPA levels on Leydig cell differentiation.

Methods. Timed pregnant Long Evans rats (n=14) were gavaged with olive oil vehicle (control) or BPA at 2.5 or 25 µg/kg body weight (bw) from gestational day (GD) 12 through nursing to weaning on postnatal day (PND) 21. Leydig cells from male rats at PND 21 and PND 35 were isolated to evaluate proliferation and steroidogenesis, respectively. Proliferative activity was assessed by [³H] thymidine incorporation, whereas expression levels for regulatory proteins were analyzed using the Western blotting technique. Leydig cell T production capacity was evaluated *ex vivo* and T production was determined by radioimmunoassay (RIA).

Results. Perinatal exposures of male rats to BPA (2.5 and 25 µg/kg bw) increased [³H] thymidine uptake (4.1 ± 0.1 and 6.2 ± 0.1 CPM/ 10^3 cells) compared to control (3.6 ± 0.1) at 21 days of age ($P < 0.01$). Increased proliferative activity was associated with enhanced cyclin D3 protein expression, a marker for cell cycle progression. Induction of proliferative activity was also linked to increased kinase activity in Leydig cells affecting protein kinase B and extracellular signal-regulated kinases. At 21 days, Leydig cell T production (ng/ 10^6 cells • 3 h) measured 4.5 ± 0.7 , 1.9 ± 0.5 , and 0.8 ± 0.2 after exposure to 0, 2.5, and 25 µg/kg BPA, respectively ($P < 0.05$). Similarly, T production at PND 35 measured 14.1 ± 3.6 and 21.2 ± 4.4 in BPA groups compared to control (62.3 ± 4.2 ng/ 10^6 cells • 3 h) ($P < 0.01$). The decrease in Leydig cell T production appeared to affect serum T levels. For example, at day 35 postpartum, serum T measured 2.2 ± 0.5 , 1.5 ± 0.5 , and 1.4 ± 0.3 ng/ml, respectively ($P > 0.05$).

Conclusions. Low dose exposure of male rats to BPA from GD 12 to PND 21 altered Leydig cell proliferative capacity and T production, indicating that the perinatal period is a sensitive window of exposure to BPA action. The results imply that early-life exposures to BPA have the potential to cause adverse effects on male reproductive tract development.

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Characterization of proteins involved in RNA import into mitochondria

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Introduction. Mitochondria produce energy for the cell through oxidative phosphorylation. Perturbation of this process can cause a host of metabolic diseases. Several RNAs including the 5S rRNA, Th RNA of RNase MRP, H1 RNA of RNase P, 5.8S rRNA are imported into mammalian mitochondria. Mechanisms of RNA import into mitochondria are currently a mystery.

Methods. In order to identify proteins involved in RNA import into mitochondria, an RNA affinity purification protocol isolated specific proteins binding four of the imported RNAs from whole cell and mitochondrial lysates. Isolated proteins were identified by liquid chromatography electrospray tandem mass spectroscopy (LC-MS/MS). Proteins co-precipitating with imported RNAs, but not co-precipitating with a control non-imported RNA were candidates for further study.

Results. The following proteins were identified by LC-MS/MS: 14-3-3 (beta, gamma and zeta), galectin-1, calmodulin, cathepsin D, argininosuccinate synthetase, FK506 binding protein 2, HSP90 alpha, keratin 6A, TCP1 gamma, mitochondrial aldehyde dehydrogenase 2 and RAD23 homolog B. RNA immunoprecipitation (RIP) experiments are ongoing to confirm the interaction of identified proteins with imported RNAs.

Conclusions. Based on initial findings, we propose that proteins identified by RNA affinity purification may be implicated in RNA import into mitochondria as either cytoplasmic chaperones or RNA binding proteins directing RNA import into mitochondria. Further experiments will determine the precise mechanism by which RNA is imported into mitochondria. Such studies will help characterize the role of defective RNA import in metabolic disease pathogenesis.

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Efficacy of a novel antiviral compound in preventing acute infection by bovine viral diarrhea virus

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Introduction. Bovine viral diarrhea virus (BVDV) is a widespread bovine pathogen causing disease affecting the gastrointestinal, respiratory, reproductive, cardiovascular and immune systems. While vaccines can provide effective protection 72 hours after administration, no treatments exist to immediately prevent BVDV infection despite exposure. However, in vitro studies have shown 2-(2-benzimidazolyl)-5-[4-(2-imidazolino)phenyl]furan dihydrochloride (DB772) to effectively prevent BVDV infection in cell culture. The aim of this project was to assess the efficacy of DB772 for the prevention of acute BVDV infection in calves.

Methods. Eight miniature calves were housed two to a room in isolation facilities. One calf in each room was treated with DB772 at 12 mg/kg every 8 hours for 11 treatments beginning on Day 0 (Group 1); the other calf in each room was treated with diluent only on the same dosing schedule (Group 2). After two treatments, each calf was inoculated with 40,000 cell culture infectious doses (CCID₅₀) of BVDV type 2 (strain 1373) by aerosol inoculation. The calves were examined daily for evidence of disease until the end of the study (Day 28). Samples for serum biochemical profiles, complete blood counts, virus neutralization, titration (serum and nasal swab samples) and virus isolation (serum, nasal swab samples, and white blood cells [WBC]) were collected at various time points during the study period.

Results. All calves were clinically normal upon study initiation. All 8 calves were seronegative for BVDV 2 ($\leq 1:4$) before treatment was initiated. Azotemia was exhibited by all calves in Group 1 on Day 4. The azotemia resolved without treatment in two calves, while one was euthanized without treatment on Day 10 and another died on Day 13 despite treatment. From Day 0 to Day 4, the average lymphocyte count in Group 2 decreased by 48% compared to a 12% decrease in Group 1. Virus was isolated consistently from all calves in Group 2 on Days 4 to 8 and 3 of these 4 calves remained positive after passage of WBC at Day 14. Calves from Group 1 remained negative by virus isolation until passage revealed one positive on Day 14 and another on Day 21. On Day 21, both remaining calves in Group 1 had serum anti-BVDV antibody titers of $\leq 1:2$ while all four calves from Group 2 had serum anti-BVDV antibody titers $> 1:256$.

Conclusions. In contrast to prior studies, treatment with DB772 in this study was associated with acute renal toxicity. Treatment with DB772 did appear to prevent infection with BVDV 2 as evidenced by preventing seroconversion by Day 21 in all treated calves in contrast to high antibody titers in untreated calves. The positive virus isolation results on Days 14 and 21 from treated calves represent later infections than detected in untreated calves since the treated calves had not developed anti-BVDV antibodies by Day 21 and virus isolation positives were detected much later. This pilot study indicates that DB772 is an effective antiviral therapy to prevent acute BVDV infection despite the significant concern of renal toxicity. The resulting serum concentrations of antiviral agent in this study were expected to be several-fold higher than required to prevent BVDV infection. Thus, lower dosages may retain antiviral efficacy while eliminating negative side effects.

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Landscape phage probes for rapid detection of *Staphylococcus aureus*

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Introduction. *Staphylococcus aureus* is a Gram-positive bacterium that can cause many different types of infectious diseases, ranging from minor skin infection and food poisoning to life-threatening deep infections. Over 500,000 nosocomial infections are reported per year in the United States. The emergence of drug resistance has made many antibiotics ineffective, which are costing patients and health maintenance organizations lots of money. Development of systems for the routine monitoring of the environment and food for *S. aureus* is a challenge, which requires fast, sensitive, accurate and inexpensive detection and monitoring methods. It was demonstrated that landscape phage libraries contain many potential probes for surface markers of cells, spores and bacteria.

Methods. In this study, we propose an innovative approach for fast, accurate and inexpensive detection of bacterial cells, which is based on the use of phage magnetic beads as substitute immunosorbents, and PCR as identification system. A panel of phage probes from two libraries (f8/8 and f8/9) against *S. aureus* 5250 has been selected and characterized for their binding to the target bacteria.

Results. One of the phage probes was successfully applied to capturing target bacteria and separating by Dynabeads M-280 Streptavidin.

Conclusions. The continuous study is planed to modify the parameters of phage-bacteria interaction to adapt them for detection *S. aureus* in contaminated food. To distinguish the Methicilin-resistant *S. aureus* (MRSA) from Methicilin-sensitive *S. aureus* (MSSA), we plan to apply PCR as a confirmation test.

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Relationship between steroid hormone action and collagen homeostasis in the male

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Introduction. It has been suggested that gonadectomy increases the risk for cranial cruciate ligament (CCL) injury in both sexes. Although androgen is the predominant sex steroid in males and estrogen is the female hormone, males and females synthesize both sex steroids and express androgen (AR) and estrogen (ER) receptors. To date, most studies have investigated the role of estrogen in the female; it is not clear that steroid hormone action affects CCL function in the male. Therefore, experiments were designed to characterize steroid hormone action and collagen homeostasis in CCL of male rabbits.

Methods. New Zealand White male rabbits (approx 5 months of age) were either intact or gonadectomized (castrated). For the castrated animals, one group was not supplemented and other groups were injected subcutaneously with a sterile slow release tablet containing one of the following: testosterone (T, 50 mg), estradiol (E₂, 25 mg) or dihydrotestosterone (DHT, 50 mg). Unlike T, the highly potent androgen DHT is incapable of aromatization to E₂. Each group consisted of 6 animals. Hormone supplementation was for a period of 3 weeks after which animals were euthanized. Blood was collected to obtain serum for measurement of steroid hormones by radioimmunoassay (RIA). CCLs were harvested for determination of collagen concentration using a Sircol dye assay. Protein expression of steroid hormone receptors and collagenases (matrix metalloproteinases, MMP) in CCL were analyzed by Western blotting.

Results. Gonadectomy caused a decrease in serum levels of both T and E₂, which were restored to control levels by T and E₂ replacement, respectively. Furthermore, gonadectomy increased AR protein expression levels in CCL. Supplementation with T and DHT caused further increases in AR protein expression whereas E₂ did not affect AR expression. In comparison, ER α protein expression was not affected by gonadectomy and hormone supplementation. Gonadectomy caused a decrease in collagen concentrations in CCL, which were restored to normal levels by hormone supplementation (T, DHT and E₂). The decrease in collagen concentrations was associated with altered protein expression of the collagenases MMP-1 and MMP-2.

Conclusions. Steroid hormones regulate AR protein expression in the CCL and are associated with altered collagen homeostasis and collagenase (MMP) activity. These observations support the hypothesis that steroid hormones may be a contributing factor to the pathogenesis of CCL injury in domestic species.

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Preparation of bone marrow aspirates from dogs with hematopoietic tumors for immunohistochemistry using HistoGel™

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Introduction. Bone marrow examination is important in the diagnosis, management, and prognosis of hematopoietic tumors. HistoGel™, an aqueous gel which encapsulates histologic and cytologic samples for processing, has successfully been used to process human blood and bone marrow samples. This study's objectives were to validate the use of HistoGel™ for preparation of cell blocks from canine bone marrow aspirates and to authenticate immunohistochemistry performed on HistoGel™ prepared canine bone marrow samples from dogs with hematopoietic tumors.

Methods. Bone marrow aspirates from dogs with hematopoietic tumors were prepared for cytological evaluation as part of routine staging. Cell blocks were made from the same bone marrow samples using HistoGel™. Prepared liquefied HistoGel™ was added to bone marrow aspirates and mixed. Following refrigeration, the solidified mixture was transferred to a tissue processing cassette, fixed in 10% formalin, embedded in paraffin, and routinely processed for histologic and immunohistochemical evaluation. Lymphocytic neoplasms were categorized using immunohistochemical antibodies for CD3, CD20, BLA36, and CD79a. Mast cell tumors were evaluated with Toluidine blue.

Results. Bone marrow aspirates from 21 dogs with hematopoietic tumors were evaluated. Large particles were detected on HistoGel™ preparations and cell morphology was good in all cases. Immunohistochemistry yielded strong positive results comparable to those obtained with tissue biopsies and there was no significant background staining.

Conclusions. Preparation of cell blocks from bone marrow aspirates from dogs with hematopoietic tumors using HistoGel™ is technically simple and relatively inexpensive. Immunohistochemistry can be applied to samples prepared in this way using standard techniques established for immunostaining of tissue biopsies.

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Activated murine thymic plasmacytoid dendritic cells alter thymopoiesis

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Introduction. The pathogenesis of thymus dysfunction during HIV infection is complex and poorly defined. We hypothesized that virus-activated thymic plasmacytoid dendritic cells (pDC) would produce pro-inflammatory cytokines that inhibit thymopoiesis.

Methods. Thymuses from 20-25 day-old ICR mice were enriched for pDC by positive magnetic selection using mAb m-PDCA-1. Enriched cells contained CD11c⁺ myeloid DC (5%) and m-PDCA-1⁺CD11c⁺ pDC (2%), representing 100-fold enrichment of pDC. Enriched cells were stimulated with TLR9 ligand (1 μ M ODN 1585) for 6 or 24 hours. Supernatant and RNA were collected and analyzed for the expression of IFN- α , TNF- α , IL-6 using ELISA and RT-PCR. Murine thymus organ cultures (TOC) were incubated for 96 hours in 70% conditioned medium and analyzed by flow cytometric quantification of CD4+CD8+ thymocytes. Control TOC included 70% unconditioned medium and direct stimulation with 1 μ M ODN 1585.

Results. Enriched thymic pDC secreted higher levels of TNF- α , IFN- α and IL-6 than non-pDC at 24 hours post-stimulation. mRNA for TNF- α and IL-6 peaked at 6 hours. Murine TOC that received conditioned media from stimulated pDC had a significant reduction in the percentage of CD4+CD8+ thymocytes when compared with TOC grown in unconditioned medium or TOC directly stimulated with ODN 1585.

Conclusions. The secreted products of stimulated pDC alter thymopoiesis in a paracrine fashion resulting in a thymocyte phenotype typical of HIV infection.

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Landscape phage probe for cytokeratins 8/18

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Introduction. Identification of cancer biomarkers is paramount for early diagnosis and assessment of remission or progression of the disease sequel to therapy. Tumor-specific biomarkers serve also as molecular “ZIP-codes” for navigation of chemotherapeutics compounds and genes to the sites of the disease. In immunohistochemical or serological tests, carcinoma biomarkers are often revealed using monoclonal antibodies. However, antibodies have some intrinsic limitations that hamper their use as targeting ligands. Cancer cell-specific landscape phage probes evolved recently as a rapid and economic alternative or adjunct to antibodies. They can be selected from multibillion phage display libraries and converted into drug- or gene-delivery constructs, or, in conjugation with specific labels, can be used for tumor imaging. The knowledge of the counterpart receptors for the phage probes can further expand and rationalize their medical applications.

Methods. Here in, we used affinity selected landscape phage to affinity purify proteins from the breast carcinoma cell line, MCF-7 plasma membrane. The phage matrixes were developed by dextran cross-linking of individual highly specific breast phage probes obtained from our previous study. The cross linked phage probes were incubated with MCF-7 cell membrane lysate. Proteins bound to the phage matrixes were eluted with a mild acid buffer, separated on SDS-PAGE and sequenced using chromatomass spectrometry.

Results. The phage matrix DVYSLAYPD was found to isolate cytokeratins 8/18 which currently used as carcinoma biomarkers.

Conclusions. This observation indicates that cancer biomarker selective phage probes are promising biomaterials for cancer diagnostics and therapy.

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Neuromuscular and motor coordination analyses of a mouse model of mitochondrial complex I disorder

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Introduction. NDUF54 is a nuclear encoded protein subunit of mitochondrial NADH dehydrogenase. Several mutations in NDUF54 lead to mitochondrial complex I deficiency and Leigh syndrome in humans. A mouse model of NDUF54-linked Leigh Syndrome was created by knocking in a mutated allele of containing the premature stop codon. This line of transgenic mice displayed diminished mitochondrial function. A series of neuromuscular tests was performed to further characterize the NDUF54 mutant mouse model.

Methods. Mice heterozygous for a mutant NDUF54 allele that encodes a truncated NDUF54 protein were subjected to a battery of neuromuscular and motor tasks including wire-hang, pole, and balance beam tests, constant speed and accelerating rotarod analyses, and gait analysis.

Results. Performance values for NDUF54 mutant mice were not different than those of wild-type control mice in pole test, constant speed rotarod, accelerating rotarod, and gait measurements ($p > 0.05$). Compared to wildtype controls, mice expressing mutant NDUF54 display significant neuromuscular/motor deficiencies in wire hang and balance beam analyses ($P < 0.05$).

Conclusions. Human patients with NDUF54 mutations often present with neuromuscular deficiencies. A mouse model of an NDUF54 truncation mutation was previously established and exhibited mitochondrial dysfunction on a biochemical level. Here, a neuromuscular functional deficit was identified. The battery of neuromuscular and motor coordination tests used in this study assist in developing a greater understanding of the pathways by which energy metabolism can influence disease pathogenesis.

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Seasonal changes in the combined glucose insulin tolerance test in normal aged horses

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Introduction. Insulin resistance (IR), a problem of adult horses, can occur alone or secondary to pituitary pars intermedia dysfunction (PPID). Affected horses are often obese and are predisposed to developing laminitis, especially in the spring and summer while the adrenal axis shows seasonal variation in the autumn. The purpose of this study was to evaluate seasonal changes in the combined glucose insulin tolerance test (CGIT) for IR.

Methods. Ten healthy aged horses were administered the CGIT in February, May, June, August, September, and November. Horses were administered dextrose (150 mg/kg) and insulin (0.1 U/kg) intravenously. Glucose concentrations were measured at 0, 1, 5, 15, 30, 45, 60, 75, 90 and 150 minutes. Insulin concentrations were analyzed at 0, 5, and 75 minutes. Results of the CGIT were compared across months using 2-way analysis of variance ($P < 0.05$).

Results. No significant differences in mean glucose or insulin concentrations, mean baseline glucose to insulin ratios or mean [insulin AUC]*[glucose AUC] across months were identified. However, glucose AUC was lower in August and November compared to February and lower in November compared to June, and [insulin AUC]/[glucose AUC] was higher in August versus February, May or June.

Conclusions. The majority of horses were considered insulin sensitive throughout the study. Seasonal changes do not appear to greatly affect the results of the CGIT in normal aged horses, but subtle changes may exist.

Acknowledgments. Thank you to our laboratory assistants and technicians for their dedication and hard work. The Birmingham Racing Commission and the Auburn University Department of Clinical Sciences provided financial support.

Seasonal changes in plasma alpha-melanocyte-stimulating hormone and adrenocorticotrophic hormone in response to thyrotropin-releasing hormone administration in normal aged horses

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Introduction. Diagnostic tests for equine pituitary pars intermedia dysfunction (PPID) have recently been demonstrated to be affected by season. However, adrenocorticotrophic hormone (ACTH) response to thyrotropin-releasing hormone (TRH), a new and potentially more sensitive diagnostic test for equine PPID, has not been evaluated for seasonality. The purpose of this study was to evaluate seasonal changes in plasma ACTH and alpha-melanocyte stimulating hormone (α -MSH) responses to TRH administration.

Methods. Ten healthy aged horses with a normal response to a dexamethasone suppression test (indicating absence of PPID) were administered synthetic TRH (1 mg) intravenously. Plasma ACTH and α -MSH concentrations were measured at 0, 5, 10, 15, 20, 25, 30, 45, 60, and 180 minutes. Testing was performed in February, July, August, September, October, and November. Mean ACTH and α -MSH concentrations at each time point were compared across months using a repeated measures analysis of variance ($p < 0.05$).

Results. Concentrations of ACTH at baseline and in response to TRH were significantly higher in July, August, September, October and November compared to February. Concentrations of α -MSH post-TRH administration were significantly higher in August, September, October and November compared to February.

Conclusions. Plasma ACTH and α -MSH responses to TRH administration experience seasonal variation, with TRH-stimulated ACTH and α -MSH concentrations increasing from summer through fall.

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Assessing intraspatial variation of infectious bronchitis virus in the host

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Introduction. The spike (S) protein, responsible for viral attachment, shows genetic and phenotypic variability among infectious bronchitis coronavirus (IBV) populations. We previously found different degrees of genetic heterogeneity among four commercial Ark-DPI-derived IBV vaccines before passage in chickens, reflected in the genes encoding the S1 subunit of the S protein. For three vaccines, a single subpopulation with an S gene sequence distinct from the vaccine predominant consensus was found in tears, trachea, and/or Harderian glands of chickens within 3 days after ocular vaccination. This finding suggests that a distinct virus subpopulation was positively selected by the chicken upper respiratory tract.

Methods. We hypothesized that the dominant genotype/phenotype further changes during host invasion as the environment of distinct tissues exert selective pressure on the replicating virus population. To address this hypothesis, we inoculated chickens with an Ark-type IBV commercial vaccine via the ocular and nasal routes. Each bird received 25 μ l in each eye and 25 μ l in each nostril of the Ark-vaccine containing 10⁶ egg infectious doses 50%/ml. A portion of the S1 gene of IBV contained in the tissues or fluid samples were amplified by RT-PCR. We examined the S1 gene sequences of IBV obtained from lachrymal fluid, trachea, and oviduct/testis of individual chickens at different times post-inoculation.

Results. Based on the S1 consensus sequences obtained from the chicken tissues, six distinct predominant IBV populations were selected as nucleotide changes resulted in non-synonymous changes. Consistent with our previous results (Van Santen and Toro, 2008), the predominant IBV population contained in the vaccine (prior to inoculation), became a minor population in all tissues and at all times after replication in the host. Interestingly, we observed significant differences in the incidence of IBV predominant populations in tears, trachea, and the reproductive tract in chickens. While population named component 1 (C1) showed increased incidence in the tears and reproductive tract (oviduct/testis) of inoculated chickens, this population was found in significantly fewer chicken tracheas ($P < 0.05$). On the other hand, C5 was more frequent ($P < 0.05$) in the reproductive tract of chickens than in either tears or trachea. C4 was highly selected in tears, trachea and reproductive tract without significant differences among these tissues ($P > 0.05$).

Conclusions. These results corroborate previous observations that predominant IBV population contained in the vaccine is rapidly negatively selected in the host. These results also indicate that intraspatial variation indeed occurs in the host and thus the dominant genotype/phenotype further changes during host invasion as the environment of distinct tissues exert selective pressure on the replicating virus population.

Defining the porcine colostrum proteome

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Introduction. The importance of colostrum (first milk) for immunological and nutritional support of newborn mammals is well known. Many bioactive peptides are present in milk at higher concentrations than in maternal circulation. Evidence indicates that such milk-borne, lactocrine-acting factors affect patterns of gene expression in neonatal somatic tissues, including the female reproductive tract. Thus, it is important to understand the biochemical nature of colostrum. Data for relaxin, a prototypical lactocrine-acting peptide in porcine colostrum, indicate that transmission of such factors is significant prior to gut closure in the neonate. The array of proteins/peptides constituting the porcine colostrum proteome has not been defined. Objectives of this study were to employ two-dimensional gel electrophoresis (2DE) and image analysis to: (1) define the porcine colostrum proteome on lactation day (LD) 0; and (2) determine if and how this proteome changes from LD 0 to LD 6.

Methods. Colostrum (LD 0) and milk (LD 6) samples were obtained from six lactating sows. Protein was extracted from individual samples, total protein concentrations were determined (DC assay, Bio-Rad Laboratories) and standard amounts of protein were subjected to SDS-PAGE (10% total monomer) and 2DE. For 2DE (Criterion, Bio-Rad Laboratories) first dimension separations were carried out using immobilized pH gradient strips (pH 3-10) followed by SDS-PAGE in polyacrylamide gradient gels (10-20% total monomer). Digital images of individual Sypro RUBY (Bio-Rad) -stained gels, run in duplicate, were analyzed using PDQuest 2D Analysis Software (Bio-Rad).

Results. Total protein concentrations for colostrum (LD 0) and milk (LD 6) were 8.5 mg/ml and 8.3 mg/ml. Standard SDS-PAGE analyses revealed distinct differences in the distribution of protein bands between LD 0 and LD 6. PDQuest analyses identified 197 spots on 2DE gels that defined the colostrum/milk proteome. Of these, 50 were unique to LD 0 and 47 were unique to LD 6. Differences in relative spot intensity were also identified for spots common to both LD 0 and LD 6.

Conclusions. The proteome for colostrum (LD 0) is distinct from that of milk (LD 6). Neonates consuming colostrum obtain a complex mixture of proteins and peptides from birth that changes qualitatively and quantitatively with time, particularly with respect to those elements of the proteome defined by isoelectric point (pH) x molecular weight ($\times 10^{-3}$) coordinates 6.0-7.0 x 15-25.

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Post-graduate/Faculty Poster Presentations

Dose response of aldosterone to ACTH stimulation in clinically healthy cats

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Introduction. The aldosterone response to ACTH administration, the dose of ACTH which stimulates maximal aldosterone secretion, and the time of maximal response has not been evaluated in cats. The purpose of this study was to determine the aldosterone response to 5 doses of cosyntropin in cats.

Methods. Seven cats were randomly given 5 doses (125 µg/cat, 10 µg/kg, 5 µg/kg, 2.5 µg/kg, 1 µg/kg) of cosyntropin IV with a 2-week wash out period between doses. Blood samples were obtained before and at 15, 30, 45, 60, 75, and 90 minutes after cosyntropin administration. Samples were analyzed by a previously validated radioimmunoassay. Data were analyzed using a repeated measures ANOVA; post hoc comparisons were made using the Least Squared Means method.

Results. Higher dosages of cosyntropin resulted in more sustained elevations in serum aldosterone and a later time of peak response. The peak serum aldosterone concentration subsequent to 125 µg/cat was similar to all other doses, except the 1 µg/kg dose.

Conclusions. Highest aldosterone concentrations occurred at 60 minutes subsequent to the 125 µg/cat and 10 µg/kg cosyntropin doses. Doses of 5 and 2.5 µg/kg also produced maximal aldosterone secretion in cats, but time of peak concentration occurred at 45 minutes.

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Effect of low doses of cosyntropin on cortisol concentrations in cats

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Introduction. The ACTH stimulation test utilizing a standard dose of cosyntropin (125 µg/cat, IV) is used to evaluate feline adrenocortical function. However, lower doses of cosyntropin will stimulate maximal cortisol secretion in cats. The purpose of this study was to determine the lowest dose of cosyntropin that will produce maximal cortisol secretion in cats.

Methods. Seven cats were randomly given 5 doses (125 µg/cat, 10 µg/kg, 5 µg/kg, 2.5 µg/kg, 1 µg/kg) of cosyntropin IV with a 2-week wash out period between doses. Blood samples for determination of serum cortisol concentrations were obtained before and at 15, 30, 45, 60, 75, and 90 minutes after cosyntropin administration. Samples were analyzed by a previously validated radioimmunoassay. Data were analyzed using a repeated measures ANOVA; post hoc comparisons were made using the Least Squared Means method.

Results. Higher dosages of cosyntropin appeared to result in more sustained elevations in serum cortisol and a later time of peak response. However, the peak serum cortisol concentrations subsequent to the 125 µg/cat, 10 µg/kg, and 5 µg/kg doses were not significantly different.

Conclusions. Cosyntropin administered at a dose of 5 µg/kg IV produces maximal cortisol secretion in cats.

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D-Galactose administration as a model of aging in mice for testing therapeutic antioxidant compounds

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Introduction. Accumulating evidence suggests that mitochondrial dysfunction and oxidative stress play a major role in aging. Chronic administration of D-galactose was reported to cause deterioration of cognitive and motor skills that are similar to symptoms of aging and therefore regarded as a model of accelerated aging in mice. Since enhancing the endogenous antioxidants is now widely regarded as an attractive therapy for conditions associated with mitochondrial oxidative stress, in the present study we tested the effects of β -lipoic acid, L-carnitine and PMX-500F (a proprietary compound produced by PhenoMatrix, Inc.) on D-galactose treated mice.

Methods. Female C57BL/6J mice, 8 weeks of age, were injected with (100 mg/kg, i.p.) D-galactose daily for six weeks and some groups were treated with a daily dose of α -lipoic acid (5 mg/kg), L-carnitine (7.8 mg/kg), PMX-500F (11.9 mg/kg) or the vehicle (0.1 M TRIS) for weeks 4-6 of treatment. Control mice were treated with physiological saline. We performed accelerating rotorod, open field test, Y maze test and analyzed serum lactate levels.

Results. In contrast to some of the previous reports, results of this study did not demonstrate any significant impairment in motor coordination, open field activity or spatial memory. In addition, serum lactate levels in D-galactose treated mice were not elevated when compared to those of controls. Treatment with the three compounds also did not result in any significant changes in the behavioral and biochemical parameters tested.

Conclusions. Results of this study suggest that chronic D-galactose treatment may not represent a suitable model for inducing mitochondrial-related age-associated symptoms in mice. We are currently evaluating a mouse model of a complex I respiratory chain disorder (NDUFS4 mutant) for use in testing therapeutic compounds for treatment of mitochondrial disorders.

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Reduction of *Salmonella* colonization in chickens following bacteriophage treatment

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Introduction. *Salmonella*-contaminated poultry products continue to cause a significant number of human food-borne infections in the U.S. each year. Novel intervention strategies to reduce entry of this pathogen into the food chain are needed. We hypothesize that treatment of chickens with *Salmonella*-targeted bacteriophages (phages) reduces *Salmonella* colonization in chickens.

Methods. *In vitro* characterization of 4 previously isolated lytic phages was performed by examining lysis patterns of *Salmonella* in liquid cultures inoculated individually or in combination with varying quantities of each phage.

Results. Cultures inoculated with a combination of all 4 bacteriophages showed decreased time to lysis and no recovery of *Salmonella* growth by 6 h, compared to cultures inoculated with single bacteriophage. *In vivo* experiments followed using a mixture of all 4 bacteriophages to treat experimentally infected chickens. Thirty-six day-old chicks were inoculated with $\sim 10^6$ colony forming units (CFU's) of *S. Typhimurium* (ST). Eighteen of the chicks were treated with 10^7 plaque forming units (PFU's) of each of the 4 phages on days 0-6. Cecal culture for ST of 6 treated and 6 untreated chicks was performed on days 3, 5, and 7. Phage-treated birds showed a decrease in cecal ST numbers compared to untreated chicks on day 3, but not on days 5 or 7. Importantly, phage could be detected in the ceca of only 4 of the 18 treated birds. In a second *in vivo* experiment, 18 chicks were inoculated with $\sim 10^4$ CFU's of ST. Twelve of these chicks were treated with 10^9 PFU's of each of the 4 phages on days 0-10. Cloacal culture was performed on all 18 chicks on days 3, 5, 7, and 10. Phage-treated birds showed decreased numbers of ST in the cloaca on days 3 and 5 compared to untreated birds, and 7/12 treated birds contained phage in the cloaca from all 4 culture days.

Conclusions. These results indicate that phage treatment is most effective for short-term reduction of ST in chickens, and higher doses of phage are most effective in pathogen reduction. Thus, phage treatment could be used by producers a few days prior to broiler harvest to reduce the risk of human infection.

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