PHI ZETA

The Honor Society of Veterinary Medicine
Epsilon Chapter

Research Emphasis Day
November 10, 2010

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COLLEGE OF VETERINARY MEDICINE

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welcomes you to our

PHI ZETA RESEARCH DAY FORUM
November 10, 2010

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 10, 2010 - JOY GOODWIN RUDD STUDENT CENTER

8:30: BREAKFAST Buffet - Goodwin Center Lobby

9-11: MORNING Presentations - Overton Auditorium

Veterinary Students

9:00	B.A. Johnson	Identification of Critical Illness-related Corticosteroid Insufficiency (CIRCI) in adult horses

Graduate Students

9:15	Payal Agarwal	INK4A/ARF Multifunctional Regulatory Tumor Suppressor Gene Locus in Canine Mammary Cancer
9:30	Allison M Bradbury	Immunomodulatory Effects of AAV-mediated gene therapy in the treatment of GM2-Gangliosidosis
9:45	Lawrence A. Brown	Comparison of three Magnetic Resonance Imaging sequences for measuring cartilage thickness in the canine stifle
10:00	Matthew Cannon	Mitochondrial RNA import mechanisms - Identification of proteins selectively interacting with RNAs imported into mitochondria
10:15	Ghislaine Dujovne	Effectiveness of a human contraceptive implant (Implanon®) on estrus suppression in mares
10:30	James W. Gillespie	Optimization of Translocation of Phage Major Coat Protein into Lipid Membranes
10:45	Victoria J. Jones	Evaluation of Enzyme Correction Following Gene Therapy in the Cat Brain

11–1: POSTER Presentations- Goodwin Center Lobby

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

11:30-1:15: LUNCH Buffet - Goodwin Center Lobby

1:15-6: AFTERNOON Presentations - Overton Auditorium

Graduate Students

1:15	Xiulei Mo	Towards Electrostatic PCR and Electronic DNA Microarray: Electric Switching and Hybridization of Surface DNA Probes
1:30	B.W. Newcomer	Characterization of an Antiviral Compound Effective Against Several Pestiviruses
1:45	Lauren E. Reid	Comparison of the effect of trilostane and mitotane on serum aldosterone concentrations in dogs with pituitary-dependent hyperadrenocorticism
2:00	Maninder Sandey	Molecular analysis of Canine MDA-7 expression and functions
2:15	Evan Sones	Survival of Dogs Treated with Various Radiation Protocols for Intranasal Sarcomas

2:30–3:00: BREAK Buffet - Goodwin Center Lobby

3:00	Fan Yang	The structural and functional relationship of nine naturally occurring melanocortin-3 receptor mutations

Post-graduate/Faculty

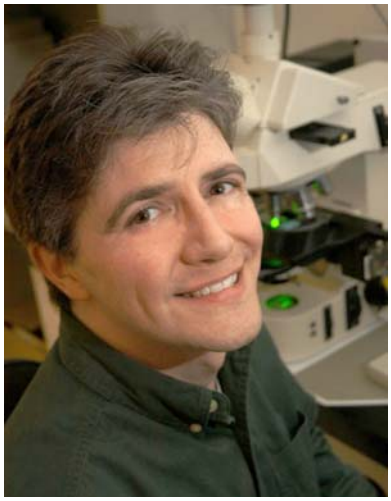
3:15	Sherine A. Aly	A novel *soxS* mutation associated with MultiDrug resistance in Fluoroquinolone resistant *Escherichia coli*
3:30	Christy L. Bratcher	Stability of Bovine Viral Diarrhea Virus in Beef from Persistently Infected Cattle
3:45	Amelia Munsterman	Comparison of gastric and intra-peritoneal pressures as measures of intra-abdominal pressure in the horse

4:00–4:30: BREAK Buffet - Goodwin Center Lobby

4:30: KEYNOTE LECTURE

The Domestic Dog - A Genome with Two Tales

Matthew Breen, BSc., Ph.D.
Professor of Genomics
Department of Molecular Biomedical Sciences
College of Veterinary Medicine
North Carolina State University, Raleigh, NC



Dr. Breen is a worldwide known animal geneticist. After graduation in Genetics/Cytogenetics from Liverpool University, UK, he was post-doctoral fellow at the prestigious Medical Research Council, UK, then Head of the DNA Group at the Australian Equine Blood Typing Research Laboratory, University of Queensland, Brisbane, Australia, and from 1996-2002 Head of Molecular Cytogenetics at the Animal Health Trust, Newmarket, UK. Since 2002 he has been Associate Professor, then Professor of Genomics at NCSU.

The main focus of his work is the genome of the dog, where he was instrumental in the determination of its DNA sequence. He is the Leader of the Clinical Genomics Core and Clinical Studies Core, Center for Comparative Medicine and Translational Research, NCSU, and a Member of the Cancer Genetics Program at the Lineberger Comprehensive Cancer Center, UNC-Chapel Hill. In these functions he directs the use of the canine genome sequence for identification of genetic loci associated with disease and the diagnostic application of this knowledge. His Keynote Lecture and Joy Goodwin Seminar will address genetics-based disease diagnosis and treatment. Understanding these cutting edge issues and implications of these approaches is essential for research as well as clinical practice of Veterinary Medicine.

PLEASE JOIN US FOR THE INDUCTION AND AWARDS BANQUET

Everybody is invited! Tickets $40/person - Reserve ticket before November 8, 2010 with Dr. Josephson (josepem@auburn.edu, 334-844-5423) or Dr. Kaltenboeck (kaltebe@auburn.edu, 334-844-2665) - Deposit check for ticket with Dr. Eleanor Josephson, 109 Greene Hall, 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**

AWARD CEREMONY to honor winners of the Platform and Poster Competitions

Posters

Veterinary Students

Shelley A. Ash	CD28 Polymorphisms and their Association with IMHA in Dogs
Cheryl L. Auch	Urine Enzymes Gamma-Glutamyltransferase and Alkaline Phosphatase in diagnosis of acute tubular injury in canine renal disease
S. Bartlett	Mass IBV serotype vaccine predominates in chickens simultaneously vaccinated with Mass and Ark serotype vaccines
Marcus Bradbury	Evaluation of the Lamin B Receptor Gene in Dogs with Pelger-Huet Anomaly
Jeremy Foote	Canine regulatory T cells display increased resistance to Gemcitabine depletion than naïve CD4+ T cells
K.M. Fuller	A Method for Engineering Point Mutations in Mitochondrial DNA
Michelle D. Karagas	Dystrophin Deficient Myopathy In The Springer Spaniel
Ashley Ladegast	Role of Endotoxemia in the Pathophysiology of *Bovine Viral Diarrhea Virus* Infections
Lisa Mason	An Overview of White Nose Syndrome in Bats
Hannah Matz-Creel	Correlation between external hoof measurements and radiographic parameters of the equine hoof
Hannah Matz-Creel	Evaluation of the effect of a hoof husbandry system on the gait and hoof of horses with foot pain
A. Monday	Tumor Suppressor Gene Expression in Canine Mammary Tumor Cell Lines
Anita Patel	Phage-Based Immunocontraceptive Agents to Control Feral Swine Overpopulation
Brittany L. Powell	Evolutionary Pathways of Infectious Bronchitis Virus in the Immunodeficient Host
Rebecca E. Rifkin	The Effects of Joint Type on Surface Roughness of Equine Carpal Cartilage
JS Suddeth	Investigation of the sparing effects of a native medicinal plant (American skullcap, *Scutellaria lateriflora*) on aflatoxin-contaminated feed in broiler chickens
Suzanne D. Truesdell	Diagnosing Feline Sandhoff Disease Using PCR and Gel Electrophoresis

H.L. Weaver	Assessing Relative Adrenal Insufficiency and ACTH Stimulation in Ill Horses

Graduate Students

Payal Agarwal	Canine Mammary Tumor Cells: A Model to Investigate Cyclin Dependent Kinase Inhibitor p16/INK4A in Cell Cycle Arrest
Michelle Aono	β-defensin Expression in the Canine Nasal Cavity
Megan G. Behringer	Development and Evaluation of a FRET-PCR Assay for Determining Fluoroquinolone Resistance in Canine Urine *Escherichia col*i Isolates
Dubraska V. Diaz	Microbiological and molecular characterization of coagulase positive *Staphylococcus s*pecies isolated from canine clinical specimens
David A. Dunn	Allotopic expression of ATP6 in the mouse as a targeted mtDNA mutation model
Stephen L. Gulley	Adaptive Immunity in Conjunctiva Associated Lymphoid Tissue after Ocular Immunization
Farruk M. L. Kabir	Rapid amplification of cDNA ends (RACE) experimental approach for sequence analysis of canine p16/INK4a tumor suppressor
Madhukar Lohani	Immunomodulatory Properties of Noni (*Morinda citrifolia*)
Eunice Ndegwa	Transmission of IBV Ark serotype type vaccine viral subpopulations to non-vaccinated contact birds
Maninder Sandey	Determining the functional status of canine IL-24 receptors
Kamoltip Thungrat	Trends of small animal *Escherichia coli* antimicrobial resistance in the United States

Post-graduate/Faculty

Natalia Grabchenko	Identification of Brest Cancer Receptors Recognized by Landscape Phage Probes
K. Parameshwaran	An NDUFS4 mutation and neurobehavioral changes in young mice
Vicky L. van Santen	Highly Localized Infections With Ark-type IBV Vaccines

Veterinary Student Platform Presentations

Identification of Critical Illness-related Corticosteroid Insufficiency (CIRCI) in adult horses

B.A. Johnson, BS[1], A.J. Stewart BVSc(hons), MS, DACVIM, DACVECC[1], H.L. Weaver, BS[1], T.J. Towns, BS[1], E. Kwessi[2], BS,MS, PhD[2], Q. Zhong BS[2], R.C Weiss[3], DVM, PhD, DACVP, K.S.[3]Joiner DVM, PhD[3]

[1]Dept. of Clinical Sciences
[2]Dept. of Mathematics and Statistics
[3]Dept. of Pathobiology, Auburn University, Auburn, AL

Introduction. Physiologic stress caused by critical illness provokes an adaptive response by the hypothalamic-pituitary-adrenal (HPA) axis. Disruption of this axis results in lower than normal ACTH and/or cortisol concentration. Inadequate cortisol production in the face of critical illness has been termed relative adrenal insufficiency (RAI) but is now referred to as critical illness related corticosteroid insufficiency (CIRCI).

Methods. We measured basal cortisol and ACTH concentrations on admission and at 48 hour intervals in critically ill horses to determine effects of severe illness on HPA function. A severity of illness score (SOIS) was calculated for each patient based on physical examination findings, bloodwork, and other diagnostic tests. Serum cortisol and plasma ACTH concentrations were measured from batched frozen samples using radioimmunoassays previously validated for horses.

Results. Of 112 patients evaluated there were 36 horses classified as severely ill, 40 as moderately ill and 36 as mildly ill. Endogenous concentrations of ACTH and cortisol were usually higher in the severely ill patients when compared to other patients. However, 19.4% (7) of the severely ill horses had inappropriately low endogenous cortisol concentrations. Pathologic assessment of the adrenal gland was performed in 5 non-surviving horses. Three (60%) of these horses were found to have moderate to marked adrenal hemorrhage.

Conclusions. It appears that a subset of severely ill patients have low cortisol concentrations and may benefit from treatment with a low physiologic dose of hydrocortisone. Evidence of adrenal gland hemorrhage in non-surviving horses may help to explain the low cortisol concentrations.

Acknowledgments. Thanks to equine technicians Michelle Brown, Erin Robbins, Sam Thrower, Julie Watts, Kellie Smith; pathology residents Dr. Jamie Weisman and Dr. Jay Kohler; pathologists Dr. Calvin Johnson; equine faculty Dr. Amy Munsterman, Dr. Anne Wooldridge, Dr. Erin Groover, Dr. Christina Hewes, equine residents Dr. Elizabeth Yorke, Dr. Joaquin Estrada, Dr. Lisa Kivett, Dr. Rebecca Funk and Dr. Chris Alford. Funding was provided by the American College of Veterinary Emergency and Critical Care, Morris Animal Foundation and the Office of the Dean of Research and Graduate Studies, College of Veterinary Medicine.

Graduate Student Platform Presentations

INK4A/ARF Multifunctional Regulatory Tumor Suppressor Gene Locus in Canine Mammary Cancer

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 frequently result in exit from cell cycle. Progression from G1 to S phase is inhibited by two cyclin dependent kinase inhibitors (CKIs); p16 and p21. p16 is a 16kDa protein encoded by the INK4A/ARF gene locus. The same gene locus also encodes a 14kDa protein known as p14ARF (Alternative Reading Frame) in humans with no amino acid homology with p16. Both p16 and p14ARF share the last two common exons but differ in first exons. Both p16/INK4A and p14ARF are tumor suppressor genes but have different functions. p16 inhibits G1/S phase transition by inhibiting the CDK4/6-CyclinD1 complex. p14ARF up-regulates p21 inhibiting G1/S phase transition by stabilizing p53 expression upon disassociation with mdm2. We hypothesize that INK4A/ARF encoded genes have important regulatory roles in canine mammary cancer.
Methods: We developed comparative reverse trancriptase PCR to analyze expression of p16 and p14ARF in canine mammary tumor cell lines (CMT28, CMT27, and CMT12) and normal canine fibroblasts (NCF). Amplicons have been cloned and sequenced to analyze the p14ARF mRNA sequence.

Results: Both NCF and CMT28 cell lines expressed p14ARF and p16/INK4A mRNAs, while CMT12 and CMT27 do not express them. Both the mRNA and the inferred protein sequence of p14ARF have been successfully predicted. Lack of expression of p16 and p14 ARF in CMT cells including CMT28, indicates an important role for the bicistronic INK4A/ARF locus in mammary cancer progression. p14ARF mRNA expression is also defective in CMT12 and CMT27 cells, which also have p16 mRNA expression defects. This suggests that mutation of this locus has a combined effect on both p16/INK4A and p14ARF expression.

Conclusions: This is the first report regarding p14ARF sequence and expression in canine cells. Coincident defects in this extra-ordinary gene complex encoding two out of frame sequences are frequently found in canine mammary tumor cell lines.

Acknowledgement: Author acknowledges: NIH, CVM Interdepartmental Grant, Calvert Cooperation.

Immunomodulatory Effects of AAV-mediated gene therapy in the treatment of GM2-Gangliosidosis

Allison M Bradbury[1,2], Douglas R Martin[1,2]

[1]Department of Anatomy, Physiology, and Pharmacology, CVM, Auburn University, AL
[2]Scott-Ritchey Research Center, CVM, Auburn University, AL

Introduction. GM2 gangliosidosis is characterized by accumulation of GM2 ganglioside in the CNS due to an inherited defect in the gene encoding the lysosomal hydrolytic enzyme β-N-acetylhexosaminidase. The feline model offers clinical and histopathological features typical of the human disease as well as a brain model of intermediate complexity, including ganglioside catabolism, compared to the human brain. GM2 gangliosidosis is caused by a single gene defect of a manageable sized gene, making this disease a good candidate for treatment by gene therapy. The foreign nature of the viral vector and the therapeutic protein delivered could induce an immune response with unfavorable effects on the long-term expression and success of vector-mediated gene therapy.

Methods. Enzyme-linked immunosorbent assay (ELISA) was used to detect antibody presence in GM2 and normal phenotype cats treated with recombinant AAV1 vectors expressing wild type human hexosaminidase (rAAV1-hHEX). 96-well plates were coated with either .1ug/100ul of rAAV1-hHEX or with .1ug/100ul of human hexosaminidase alone. Feline serum samples were added in serial dilutions. Bound primary antibodies were detected by HRP-conjugated goat anti-cat IgG secondary antibody at a dilution of 1:20000. TMB was used as the chromogenic substrate and sulfuric acid was used to stop the reaction. The plates were read at 450nm and positive serum dilutions were defined as a reading ≥ 0.15 after subtraction of background. Serum from untreated GM2 and normal phenotype cats as well as cats treated with AAV2 and feline hexosaminidase were used as controls.

Results. Cats affected with GM2 gangliosidosis and treated with rAAV1-hHEX mounted a significant immune response to the therapy, demonstrated by positive antibody titers at serum dilutions ranging from 1:24576 to 1:305834. Variability in the immune response is multifactorial and due to the age in which the cats are treated, the duration of treatment, and the dosage of rAAV. For all cats analyzed, antibody titers prior to treatment were not detectable at a serum dilution of 1:16, verifying the titers seen in treated cats are not due to prior exposure to AAV. Cats of normal phenotype treated with rAAV-hHEX allowed for long-term studies of the therapy. While short-term studies showed positive antibody titers at 1:12288 and 1:24576, long term studies, >1 year post-treatment, displayed much higher titers at 1:98304 and 1:131072, indicating that the immune response to therapy is not transient but instead long lasting. When analyzed for antibody response to purified human hexosaminidase alone, the immune response was again significant and increased with duration of treatment. However, the titers were not as robust as seen with rAAV+hHEX, suggesting that the immune response is not due solely to the viral vector or the therapeutic protein, but likely a combination of the two.

Conclusions. A pronounced and lasting immune response to rAAV1-hHEX vectors and to purified human hexosaminidase was seen in GM2 gangliosidosis and unaffected cats treated with rAAV-hHEX, but not prior to treatment or in untreated controls. This response could not only hinder the effectiveness of the current treatment, but also the possibility of treating the disease with multiple doses utilizing the same serotype. Additionally, treatment with rAAV could potentially be improved by immunosuppression or tolerization. Recently feline hexasomindase (fHEX) was cloned and inserted into the AAV vector in expectation that species specific fHEX vectors will result in a less robust immune response.

Acknowledgments. This research has been made possible by the mentorship of Dr. Douglas Martin. Scott-Ritchey, my graduate committee, the APP department, and fellow graduate students have also been valuable resources.

Comparison of three Magnetic Resonance Imaging sequences for measuring cartilage thickness in the canine stifle

Lawrence A. Brown

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

Introduction: Osteoarthritis (OA) and other diseases affecting the cartilage in the stifle joint of small animals can dramatically affect their quality of life. Great emphasis has been placed on non-invasive evaluation of cartilage in human medicine, where the disease processes are similar to those in veterinary patients. Evaluation of cartilage thickness is an important indicator for staging arthritic disease in the canine patient. Studies have been conducted on horses to evaluate the accuracy of magnetic resonance imaging for estimating cartilage thickness, however little research has been published for small animals.

This project goal is to assess the diagnostic value of three magnetic resonance imaging sequences, currently used in human medicine, for imaging the canine stifle. The plan is to compare and contrast the ability of these sequences to allow accurate assessment of cartilage thickness in canine stifle cadaver specimens.

Methods: Eleven normal pelvic limbs were disarticulated at the level of the coxofemoral joint from 6 cadaver dogs of various breeds, age and gender, with a body weight >15kg. Each limb was radiographed to evaluate for evidence of gross joint abnormalities that would necessitate exclusion from the study (e.g. neoplastic disease within the joint or presence of a metallic bone plate. Each stifle was imaged using Magnetic Resonance Imaging (MRI) in sagittal and dorsal planes, using three separate sequences; Proton Density Spin Echo (PDSE), 3 dimensional spoiled gradient fat suppression (3D SPGR-FS) and Steady State Free Precision (SSFP).

Cartilage thickness will be measured from these images at the mid sagittal point of the medial and lateral femoral condyles and compared to measurement from histopathological sections of the corresponding limbs.

Results: Preliminary results show these MRI sequences are able to outline the articular cartilage in the sagittal plane. PD and SSFP sequences have been found not to sufficiently outline articular cartilage in the dorsal plane.

Conclusion: There is sufficient representation of cartilage in the sagittal plane for all sequences to assess and compare their accuracy for measuring cartilage thickness in the stifle joint.

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Department of Clinical Sciences
Merrilee Holland, DVM, MS, DACVR
Judith Hudson, DVM, PhD, DACVR
John T Hathcock, DVM, MS, DACVR
Calvin Johnson, DVM, PhD, DACVP

Mitochondrial RNA import mechanisms - Identification of proteins selectively interacting with RNAs imported into mitochondria

Cannon, M.V., [1,2] Pinkert C.A., [1,2]

[1] Auburn University, Auburn, AL
[2] University of Rochester, Rochester, NY

Introduction. Mitochondria are responsible for producing energy for the cell. Deficiencies or alterations in mitochondrial function can cause or exacerbate many human diseases, including metabolic and developmental disorders. Several RNAs are imported into mammalian mitochondria, including the 5S rRNA, RNase P, RNase MRP and tentatively specific tRNAs. Characterization of mitochondrial RNA import mechanisms will allow development of novel methodologies to alter mitochondrial function by gene therapy to treat devastating mitochondrial diseases. To begin uncovering RNA import mechanisms, experiments were undertaken to identify proteins directing RNAs into mitochondria.

Methods. RNA affinity purification isolated proteins selectively interacting with imported RNAs from both cellular and mitochondrial lysates. Proteins precipitated with each RNA were identified by liquid chromatography tandem mass spectroscopy. RNA immunoprecipitation (RIP) aimed to confirm protein:RNA interactions. RIP also tested interaction of imported RNAs with select proteins thought to potentially play a role in mitochondrial RNA import including Tom40, Tim23 and VDAC1.

Results. Both cytoplasmic and mitochondrial proteins were found to selectively interact with mitochondrially imported RNAs. Several cytoplasmic chaperones and mitochondrial proteins were identified by RNA affinity purification. RIP experiments did not confirm protein:RNA interactions due to variability.

Conclusions. Results reveal for the first time a potential role of several proteins in mitochondrial RNA import. Future investigations will confirm protein:RNA interactions and begin to look at potential functions for identified proteins.

Acknowledgments. I gratefully acknowledge Drs. R. Curtis Bird, Paul Brookes, Michael Irwin, Robert Mooney, Jay Reeder and Harold Smith for insightful comments, discussion and assistance and David Dunn, Dr. Kodeeswaran Parameshwaran and Dr. Matthew Schoell for helpful discussion. This work was supported in part by grants from NIH, NSF, AAES, University of Rochester and Auburn University.

Effectiveness of a human contraceptive implant (Implanon®) on estrus suppression in mares

Ghislaine Dujovne[1], Aime Johnson[1], Robyn Wilborn[1], Roberto Palomares[1], Anne Wooldridge[1], Timothy Braden [2]

[1] Department of Clinical Sciences, Auburn University, AL
[2] Department of Anatomy Physiology and Pharmacology, Auburn University, AL

Introduction. Excessive estrus behavior is a commonly reported problem in performance mares. Many animals show temperament changes and become more difficult to handle which reduces their performance during the estrus period. Because of these behavioral problems, many treatments have been evaluated to suppress estrus in the mare, but currently, no single treatment has been shown effective and safe for prolonged suppression. The objective of this study is to evaluate Implanon® (human contraceptive implant) as a reliable method to suppress behavioral estrus in mares.

Methods. Healthy mares between the ages of 6 and 20 years with normal estrous cycles were randomly allocated to 4 groups of 5 animals each. Group 1: negative control (no treatment), Group 2: one Implanon® implant containing 68mg etonogestrel, Group 3: two Implanon® implants, 136 mg etonogestrel; and Group 4: positive control, 0.044 mg/kg altrenogest (Regumate®) orally once daily.

Behavioral estrus response to teasing with a stallion was evaluated twice weekly by an experienced observer who was blinded to the treatments. Estrous cycles were followed for 3 months with weekly progesterone levels and twice weekly examinations via transrectal ultrasonography. The interestrus interval (IEI) was measured based on both behavioral estrus (teasing) and progesterone levels (below 1.0 ng/ml). Data were analyzed using ANOVA (Least Significant Difference) and Correlation tests using the Statistical Analysis System version 9.1 (SAS Institute, Cary, NC).

Results. Mean IEI per group, based on teasing and progesterone levels respectively, were as follows: Group 1 (control) 21.2 and 21.7 days; Group 2: 34.5 and 31.4 days; Group 3: 42.7 and 41 days. For Group 4, the estrus behavior was suppressed during the entire study period, with an IEI of 111.25 days based on teasing and 48 days based on progesterone levels. No statistical difference was found between groups 1, 2 and 3; but group 4 (positive control) was significantly different from all other groups ($P<0.05$). The IEI determined by teasing and progesterone levels were highly correlated with an $R=0.911$, with a lower correlation in group 4 (Regumate®).

Conclusions. No statistical difference was identified between the control group and the Implanon® treated animals; however, a clinically significant difference was seen in Gp 3 (2 implants) with double the IEI compared with Gp 1. This could be explained by the high variability between individuals within the same group, especially in Gp 3.
The high correlation between teasing and progesterone levels validates teasing as a reliable tool to determine estrus in mares by an experienced observer. At this dose etonogestrel was not effective for estrus suppression. Future studies with a higher dose would be necessary to determine whether or not etonogestrel can be used to fully suppress estrus in mares.

Acknowledgments. Thank to US Equestrian Federation for the financial support; Ms. Marti McCoy and Dr. Barbara Schmidt for assistance with the mares.

Optimization of Translocation of Phage Major Coat Protein into Lipid Membranes

James W. Gillespie[1,4], Deepa Bedi[1,4], Olusegun Fagbohun[1,4], Nathan Douthit[2,4], Amanda Mardis[3,4], Valery A. Petrenko[1,4]

[1]Department of Pathobiology, College of Veterinary Medicine
[2]Department of Chemistry and Biochemistry, College of Sciences and Mathematics
[3]Department of Chemical Engineering, Samuel Ginn College of Engineering
[4]Auburn University, AL

Introduction: Over the past few decades many novel types of drug delivery systems have emerged for the treatment of diseases such as cancer and are all typically classified as nanomedicines. These nanomedicines include three major components: 1) pharmaceutical agent, 2) delivery platform, and 3) a targeting moiety. Current research has been focused on targeting different drug delivery platforms to a specific receptor or cell type via synthesized peptides or monoclonal antibodies. These systems often require complex chemical reactions and subsequent purification for conjugation of the targeting moieties to their delivery platform. We have previously shown that an alternative method to obtain these targeting moieties is through the use of phage display-derived peptides. These 55-mer phage peptides contain a random 8- or 9-mer insert in the gene for pVIII, which is then displayed along the entire length of the mature phage. A library of phage display clones can then be used to isolate a specific clone that binds specifically to a specified cell type depending on the selection criteria employed. A unique feature of these peptides is their ease of isolation and insertion into liposomal membranes. We set out to determine the optimum insertion conditions that produce maximal protein insertion, but limits the amount of doxorubicin leakage that could then be used to produce reactions that run to completion and require minimal purification.

Methods: In this study, we isolated major coat protein from three different phage clones (DMPGTVLP, DWRGDSMDS, and wildtype) with different calculated pI and binding properties by standard methods. Coat protein was then incubated with liposomal Doxil at 37ºC at a final sodium cholate concentration of 15mM overnight. Insertion was carried out at a 0.5% (v/v) protein/lipid concentration at varying pH conditions. Samples were then purified by size exclusion chromatography to remove any free protein or unencapsulated doxorubicin. Samples were treated with proteinase K and visualized by western blot to determine the orientation and quantify the inserted protein.

Results: Our experiments show that for the major coat protein DMPGTVLP, insertion at 7.5 yields the highest insertion rate, but does not retain a majority of doxorubicin. Insertion at pH 8.5 yields a reaction that retains a majority of doxorubicin and has almost 100% protein inclusion.

Conclusions: Insertion of major coat protein is highly sensitive to pH, but so far has provided reactions that produce the desired protein orientation with the N-terminal targeting moiety exposed on the surface of the liposomes. A greater understanding of the mechanism of insertion will provide a greater degree of control of the reaction and can hopefully be used to model insertion with different clones from the phage library in a more high throughput platform.

Acknowledgments: We would like to thank the members of the Petrenko lab group for their advise and support throughout the project. This project was funded by an NIH grant R01 CA125063-01.

Evaluation of Enzyme Correction Following Gene Therapy in the Cat Brain

Victoria J. Jones[1, 2] and Douglas R. Martin[1, 2]

[1]Department of Anatomy, Physiology and Pharmacology, College of Veterinary Medicine, Auburn University, AL
[2]Scott-Ritchey Research Center, College of Veterinary Medicine, Auburn University, Auburn, AL

Introduction. GM_1 gangliosidosis is an autosomal recessive lysosomal storage disease (LSD) caused by a defect in the lysosomal enzyme β-galactosidase (β-gal). The enzyme defect causes lysosomal storage of the lipid GM_1 ganglioside in the central and peripheral nervous systems resulting in progressive neurodegeneration and premature death. GM_1 gangliosidosis occurs every 1 in 100,000 live births and is currently incurable. Adeno-associated virus (AAV)-mediated gene therapy in the brain has proven successful in mouse models, leading to correction of defective enzyme throughout the brain and significant therapeutic results. Before human clinical trials can begin, similar therapeutic success must be achieved in a large animal model. The objective of this study was to analyze the extent of AAV vector distribution and β-gal enzyme correction throughout the brain and spinal cord six weeks after bilateral delivery of AAV vector to the thalamus and deep cerebellar nuclei (DCN).

Methods. GM_1 cats ($\beta\text{-gal}^{-/-}$) (n=3) received bilateral injections of AAV2/rh8-fβgal in the thalamus and DCN (total vector dose, 3.2×10^{12} genome copies) at 1.9 months old. Subjects were sacrificed six weeks later, and the brain and spinal cord were cut into coronal sections for analysis. Distribution of β-gal was visually assessed by incubating tissue sections in 5-bromo-4-chloro-3-indol-β-D-galactosidase (X-gal) staining solution. β-gal activity was quantified by 4-methylumbelliferyl (4-MU) fluorimetric assays using the synthetic substrate 4-MU-β-D-galactoside. AAV vector genome copies were determined by SYBR Green quantitative-PCR.

Results. β-gal activity was absent in the brain and spinal cord of untreated GM_1 controls. β-gal activity was widely distributed throughout the brain of treated cats. Activity in coronal sections ranged from 0.6 to 300% of normal β-gal activity. Areas of highest β-gal activity corresponded to areas nearest injection sites. A low level of β-gal activity was also observed throughout the spinal cord. AAV vector genomes were detected in all brain and spinal cord coronal sections. Copy number ranged from 83 to 11,921 vector genomes/40ng genomic DNA. Vector copy number and enzyme activity were positively related within individual cats.

Conclusions. Thalamic and DCN directed gene therapy resulted in extensive vector transduction and corrective levels (>10%) of β-gal enzyme activity in widespread areas of the brain and spinal cord. Enzyme activity was inversely related to distance from the injection site. Future research on the same subjects will assess whether enzyme activity correlates with a reduction in storage material and pathological lesions. Long term studies already underway will enable us to determine the therapeutic effect of thalamic and DCN directed gene therapy on clinical symptoms and lifespan of GM_1 cats.

Acknowledgements. The author acknowledges the National Institute of Health and Department of Cellular and Molecular Biosciences.

Towards Electrostatic PCR and Electronic DNA Microarray: Electric Switching and Hybridization of Surface DNA Probes

Xiulei Mo, Arnold Vainrub

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

Introduction. DNA microarray technology has revolutionized the area of nucleic acid analysis, including gene expression profiling, SNP screening and pathogen detection. However, the current technology has several limitations, and considerable efforts have been made to improve its sensitivity, specificity and reproducibility. One promising development is electronic DNA microarray, where the applied surface potential is used for active control of target hybridization with surface-attached probe. We develop advanced electronically controlled molecular beacons (MB) to investigate the hitherto unknown fundamental characteristics of electrostatic DNA hybridization, and evaluate feasibility of two high-impact potential applications, the electronic isothermal PCR and DNA microarrays with electric control of hybridization.

Methods. The MB was designed to match a portion of *Staphylococcus aureus femA* methicillin-resistance (*MRSA*) gene. Simple single-step immobilization method is developed to attach the MB to glass and ITO surfaces without surface modification. The performance characteristics of MBs array were first studied without applied potential, where immobilization, salt-induced open-closed transition, hybridization and photobleaching processes were investigated. Next, two novel electrostatic effects, electrostatic open-closed switch and electric control of hybridization for surface attached MBs, were investigated at applied alternative potential.

Results. MBs are stably attached to the glass and ITO surface at desired surface density using developed simple single-step immobilization method. Without applied potential, at surface density $\sim 2\times10^{12}$ cm^{-2}, 75% MBs undergo open-closed transition upon change from low to high ionic strength solvent. Hybridization extent of 53-78% was got for glass surface, and the detection limit is about 1 picomole. For electrostatic switching, at optimized condition (applied ±0.8 V, pH 9, 2 mM $[Na^+]$), 10±4% MBs undergo conformational switch between closed state at negative potential and open state at positive potential. The switching extent increases at lower salt concentration, higher surface potential, and basic pH. For electrostatic hybridization, the hybridization of MBs with complementary target is enhanced at positive potential, whereas the MB-target duplexes denature at negative potential. Experimentally, up to 32% of hybridizable MBs undergo electrostatic hybridization/denaturation upon applied potential cycling, and the extent of electrostatic hybridization increases at lower target concentrations. Theoretical prediction demonstrates that up to 92% DNA hybrids can be denatured at 10^{-17} M target concentration.

Conclusions. The developed electronically controlled MBs array is robust for sensitive DNA detection. More importantly, the results provide better knowledge of the mechanism of on-surface intra- and inter-molecular hybridization, and demonstrate high potential of applications in electrostatic PCR and electronic DNA biochips.

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Characterization of an Antiviral Compound Effective Against Several Pestiviruses

B.W. Newcomer[1], M.S. Marley[2], J.F. Ridpath[3], J.D. Neill[3] and M.D. Givens[2]

[1]Department of Clinical Sciences, Auburn University, 36849, Auburn, AL, USA
[2]Animal Health Research, Auburn University, 36849, Auburn, AL, USA
[3]National Animal Disease Center, USDA, Agricultural Research Service, 1920 Dayton Ave, Ames, IA 50010, USA

Introduction: The *Pestivirus* genus of the *Flaviviridae* family consists of four separate species: bovine viral diarrhea virus (BVDV) type 1 and type 2, classical swine fever virus and border disease virus (BDV). Classification of several other viruses, including HoBi virus, Bungowannah virus (BwV) and pronghorn virus (PhV), as pestiviruses has been proposed due to their genetic and structural relatedness to the current member viruses. An aromatic cationic compound, 2-(2-benzimidazolyl)-5-[4-(2-imidazolino)phenyl]furan dihydrochloride (DB772), has previously been shown to inhibit BVDV 1 in vitro at concentrations lacking cytotoxic side effects. The compound also inhibits BVDV replication in vivo. The aim of this study was to determine the scope of antiviral activity of DB772 among the pestiviruses.

Methods: Isolates of BVDV 2, BDV, BwV, HoBi, and PhV were tested for in vitro susceptibility to DB772. A 24 well plate was seeded with ovine fetal turbinate cells (BDV and PhV), porcine kidney cells (BwV) or MDBK cells (BVDV 2 and HoBi). After incubating for 24 hours, DB772 was added to the wells to achieve a concentration of 25, 12.5, 6.25, 3.13, 1.56, 0.78, 0.39, 0.20, 0.10, 0.05, 0.02, 0.01, or 0.006 μM DB772 except for the positive control well containing no DB772. The plates were then infected with the appropriate virus at a multiplicity of infection of 0.5 and incubated for four days. After incubation, the plates were frozen and wells assayed for the presence of virus by virus isolation and titration (BDV and BVDV 2) or PCR (BwV, HoBi and PhV). Each virus was tested in triplicate. Cytotoxicity of the compound was assayed for each cell type in the study using a commercially available cell counting kit.

Results: Of the pestiviruses examined, BwV appears to be the most sensitive to DB772 with complete inhibition seen at a concentration of 0.01 μM DB772. Complete inhibition of BVDV 2, BDV, and PhV was seen when DB772 was included in the culture media at concentrations of 0.20 μM and higher. In two of three tests, a concentration of 0.05 μM DB772 was sufficient to completely inhibit HoBi virus replication. However, in the third replicate, HoBi virus was not completely inhibited until DB772 concentration reached 3.1 μM. Viral titers of BDV decreased by five log scores when cultured in media containing 0.05 μM DB772. Partial inhibition of BVDV 2 or PhV at lesser concentrations of DB772 was not detected.

Conclusions: DB772 effectively inhibits all pestiviruses studied at concentrations of 0.20 μM or less which is consistent with results obtained in an earlier study with BVDV 1. As cytotoxicity is not seen until concentrations of DB772 exceed 60 μM, a wide therapeutic window exists. This antiviral compound represents a potential new therapeutic and/or preventative for use in pestivirus infections.

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Comparison of the effect of trilostane and mitotane on serum aldosterone concentrations in dogs with pituitary-dependent hyperadrenocorticism

Lauren E. Reid[1], Ellen N. Behrend[1], Robert Kempainen[2], Linda Martin[1], Tanya Civco[1]

[1]Dept of Clinical Sciences, College of Veterinary Medicine, Auburn University, AL
[2]Dept of Anatomy, Physiology and Pharmacology, College of Veterinary Medicine, Auburn University, AL

Introduction. Hyperadrenocorticism, or Cushing's syndrome, is one of the most common endocrine disorders of dogs. The physical and biochemical changes are due to high concentrations of glucocorticoids. In 80-85% of cases, the cause is due to excessive ACTH secretion, usually by a pituitary adenoma. The two most effective medical treatments are trilostane (Vetoryl®) and mitotane (o,p'-DDD, Lysodren®). Treatment with either drug can result in hypocortisolemia, followed by hypoaldosteronemia, which has potentially life-threatening consequences.

Therapeutic monitoring is performed regularly with the adrenocorticotropic hormone (ACTH) stimulation test. The test is commonly performed by collecting a basal blood sample followed by a sample at 60 minutes post-injection of synthetic ACTH to determine adrenal reserve for cortisol secretion. An ACTH stimulation test may also be used to measure aldosterone secretory reserve; however, the time to peak aldosterone secretion appears to be 30 minutes after the 5 μg/kg dose commonly used for the test. Thus, the true effect of either medication on aldosterone secretory capacity is unknown.

The objectives of this study were: 1) to assess and compare the effect of treatment with trilostane and mitotane on aldosterone secretory reserve at 30 minutes post-ACTH stimulation and 2) to assess if changes in aldosterone secretion correlate with changes in sodium and potassium concentrations.

Methods. Forty-six dogs treated for pituitary-dependent hyperadrenocorticism with either mitotane or trilostane had ACTH stimulation tests performed by sampling at 30 and 60 min post-ACTH for monitoring of aldosterone concentration using a previously validated radioimmunoassay. Basal and ACTH-stimulated aldosterone concentrations were compared to 10 historical normal controls. Serum sodium and potassium concentration were also measured. Mann-Whitney Rank Sum test was used to compare values between groups. Linear regression analysis was used to evaluate the relation between serum potassium and aldosterone concentrations. Significance was set at the $p<0.05$ level.

Results. ACTH-stimulated aldosterone concentrations in both trilostane and mitotane treated dogs were significantly lower than that in clinically healthy dogs. There was no difference comparing aldosterone concentrations at 30 and 60 min after ACTH injection in either treatment group. A positive correlation existed between the cortisol and aldosterone concentrations in the trilostane-treated group($R=0.813$), however, a correlation was not present in the mitotane-treated group. Aldosterone concentrations in the mitotane-treated group were significantly lower pre-ACTH and at 30- and 60-min post-ACTH than in the trilostane-treated group. Hyperkalemia and hyponatremia were not associated with aldosterone suppression in either group.

Conclusions. In conclusion, treatment with mitotane and trilostane resulted in decreased aldosterone secretory reserve, but this did not correlate with hyperkalemia or hyponatremia. Thus there is no obvious benefit to measurement of aldosterone concentrations in predicting the development of hypoadrenocorticism.

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Molecular analysis of Canine MDA-7 expression and functions

Maninder Sandey[1,2], R. Curtis Bird[1] and Bruce F. Smith[1,2]

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

Introduction: Cancer is the leading cause of death in adult dogs. The goal of the proposed research is to understand a critical component of the molecular pathway regulating tumor growth and survival, melanoma differentiation associated gene-7 (mda-7), and how it plays a role in the molecular pathogenesis of canine cancers. 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 orthologs of *mda-7* have been found in the rat and mouse. However, no studies have carried to elucidate the role of *mda-7* in dogs. Thus, we planned to elucidate the biological properties of canine MDA-7.

Methods: A partial predicted canine *mda-7/IL-2*4 mRNA sequence was obtained from National Center for Biotechnology Information (NCBI) sequence database. Several primer sets were designed to amplify canine mda-7 mRNA by reverse transcriptase-polymerase chain reaction. Rapid amplification of cDNA ends (RACE) was used to identify the full mRNA sequence of canine mda-7. Canine-MDA-7 was cloned into mammalian expression vectors (pCMV 3TAG3 and pCDNA3.1+/HYGRO). Canine MDA-7 protein was transiently expressed from these recombinant plasmids in HEK-293 cells. Expression of canine Mda-7 was confirmed by RT-PCR and western blot analysis. Antitumor properties of canine MDA-7 were analyzed by cell viability assay (MTT dye), apoptosis assay (Annexin-V-FITC) and cell cycle analysis (Propidium Iodide) using normal canine fibroblasts and various cancer cell lines (CMT28, CML-10, Hela, OSW and 17-71).

Results: Canine MDA-7 expression is restricted to keratinocytes. The full length mRNA consists of 1179bps with eight exons which undergo alternative splicing to yield five different transcripts (901, 964, 1026, 1057 and 1179bps). The IL-10 signature sequence is present in the 3rd exon, and this exon is deleted in one of the transcripts (901). Four of the five transcripts, when analyzed in-silico, yield protein isoforms that are dissimilar at the C-terminus. Western blot analysis detected a 23KDa protein when one of the transcript was expressed from recombinant plasmid. Overexpression of canine MDA-7 from recombinant plasmids inhibited the growth of cancer cells when measured by cell proliferation assay using MTT Dye (CMT28, CML-10, Hela, OSW and 17-71). Similarly, overexpression of canine MDA-7 induced apoptosis of cancer cells, and these canine MDA-7 treated cancer cells accumulate in G2/M phase. Moreover, overexpression of canine MDA-7 does not affect the growth of normal canine fibroblasts.

Conclusions: We have confirmed that canine mda-7 is expressed in canine keratinocytes. In these cells, MDA-7 undergoes extensive alternative splicing that yields five alternate transcripts of varying length (901, 964, 1026, 1057 and 1179bps). When overexpressed, canine MDA-7 has antitumor activities against variety of cancer cell lines. Canine MDA-7 is cytotoxic and induces apoptosis in cancer cells. Moreover, no cytotoxic or apoptotic effects were seen on normal canine fibroblast. Due to the antitumor properties of canine mda-7, it may prove useful as an effective treatment for canine cancers.

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Survival of Dogs Treated with Various Radiation Protocols for Intranasal Sarcomas

Evan Sones[1], Annette Smith[1], Stephanie Schleis[1], William Brawner[1], Gregory Almond[1], Kathryn Taylor[2], Jackie Wypij[3], Terrance Hamilton[4], Jennifer Arthur[4], Michele Keyerleber[5], Jessica Lawrence[6], Casey Cadile[7], Zachary Wright[8]

[1]Dept of Clinical Sciences, College of Veterinary Medicine, Auburn University, AL
[2]Veterinary Medical Care, LLC
[3]College of Veterinary Medicine, University of Illinois, Urbana, IL
[4]Georgia Veterinary Specialists, Atlanta, GA
[5]Cornell University Hospital for Animals, Cornell University, Ithaca, NY
[6]College of Veterinary Medicine, University of Georgia, Athens, GA
[7]Veterinary Medical Specialists, San Mateo, CA
[8]VCA Veterinary Care Animal Hospital and Referral Hospital, Alberquerque, NM

Introduction. Sarcomas comprise one-third of all canine nasal tumors. Canine nasal lymphoma is typically considered a separate disease. Little survival data has been reported for individual nasal sarcomas.

Methods. Records of 95 cases of nasal sarcoma from multiple institutions were reviewed. Histopathologic diagnosis, date of diagnosis, radiation protocol and date of death or last follow-up were collected. Kaplan-Meyer survival analysis generated median survival times (MST) and log-rank tests (significance $p < 0.05$) compared radiation protocols.

Results. Overall MST for all sarcomas, excluding lymphoma, was 428 days. Those treated with daily-fractionated radiation therapy (DRT) had a MST of 539 days, those treated with Monday, Wednesday, Friday fractionated radiation therapy (MWF RT) had a MST of 379 days, and those treated with palliative radiation therapy (PRT) had a MST of 289 days. No significant difference existed between these three groups ($p=0.209$). MST for each type of sarcoma: chondrosarcoma - 463 days, fibrosarcoma - 379 days, osteosarcoma - 539 days, undifferentiated sarcoma – 200 days, and lymphoma – 550 days. There was a significant difference between definitive and palliative treatment for chondrosarcoma (428 vs. 119 days, $p=0.00187$) and osteosarcoma (539 vs. 7 days, $p=0.008$).

Conclusions. The MST generated from this study can be used as prognostic information for canine patients with nasal sarcoma. There appears to be no difference in DRT and MWF RT. There was no difference in survival when lymphoma was compared to other nasal sarcomas ($p=0.835$). Larger case numbers would strengthen these results.

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The structural and functional relationship of nine naturally occurring melanocortin-3 receptor mutations

Fan Yang and Ya-Xiong Tao

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

Introduction. The melanocortin-3 receptor (MC3R) is a G-protein-coupled receptor mainly expressed in the central nervous system including the hypothalamus, cortex, thalamus and hippocampus. It is also distributed in heart, kidney, gastrointestinal tract and placenta as well. MC3R and the related melanocortin-4 receptor (MC4R) play the non-redundant role in the regulation of energy homeostasis. MC4R is considered as a critical regulator of food intake and energy expenditure. Genomic study results showed that the lack of Mc3r results in increased feed efficiency and adiposity. Newly studies show that MC3R was also required for entrainment to meal intake. Until now, the relevance of MC3R mutations in human obesity pathogenesis was not clear. In this study, we performed functional studies on nine naturally occurring human *MC3R* mutations recently reported to investigate whether these alterations on MC3R structure would lead to deficiency in their functions.

Methods. We generated the nine mutants by site-directed mutagenesis. The mutants were S69C, A70T, I87T, M134I, L249V, A260V, M275T, T280S, and L297V. And then we transiently expressed the mutants as well as wild-type MC3R in HEK293T cells. Ligand binding and intracellular signaling were then performed with the high potent MC3R agonist NDP-MSH. We also performed the confocal microscopy to investigate the cell surface expression of these mutants.

Results. Of the nine mutants, four mutants (S69C, T280S, M134I and M275T) had decreased maximal ligand binding, and two mutants (S69C and T280S) displayed significant impairments in responding to NDP-MSH stimulation with increased cAMP generation. The other mutants performed normal binding and signaling properties compared with the wild-type MC3R. The results of confocal microscopy showed that all the mutants had normal cell surface expression.

Conclusions. We identified four loci at the MC3R that may be important for MC3R function. Of these four mutants, two MC3R mutations caused dysfunction in the mutant receptor and therefore could potentially contribute to obesity pathogenesis. According to the results of confocal microscopy, these two mutants are binding defective mutants.

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Post-graduate/Faculty Platform Presentations

A novel *soxS* mutation associated with MultiDrug resistance in Fluoroquinolone resistant *Escherichia coli*

Sherine A. Aly[1], Nipattra Debavalya[1], Omar A. Oryazabal[2], Sang-Jin Suh[3] and Dawn M. Boothe[1]

[1]Department of Anatomy, Physiology and Pharmacology, College of Veterinary Medicine, Auburn University, AL
[2]Department of Biological Sciences, Alabama State University, Montgomery, AL
[3]Department of Biological Sciences, Auburn University, Auburn, AL

Introduction: Resistance to fluoroquinolones (FQ) in *Escherichia coli* requires multiple stepwise mutations in genes that impact target protein structure and drug transport regulation. The objective of this report is to describe a new putative role of *soxS* mutation in the expression of FQ resistance with multidrug resistance (MDR) in *E. coli.*

Materials: Three FQ^R *E. coli* isolates, collected from healthy dogs' feces after treatment with enrofloxacin (5 mg/kg oral once daily) for ten days, were subjected to antimicrobial susceptibility testing, Pulse Field Gel Electrophoresis (PFGE) and tested for mutations in DNA Gyrase (*gyrA* & *gyrB*), Topoisomerase IV (*parC*), *soxR*, *soxS*, *marR and acrR.* Expression of the genes encoding for AcrB and EmrE efflux pumps and global regulators SoxS and MarA were determined by Reverse Transcriptase PCR (RT-PCR). Mutant *soxS* allele from clinical *E. coli* isolates was cloned and the antibiotic resistant phenotype of the cloned *soxS* was assessed by E test.

Results: The three isolates were found to be MDR and belong to two different PFGE groups. Two mutations in *gyrA*, two mutations in *parC* and a single mutation in *soxS* were identified in all MDR isolates with no other mutation in *soxR, marR* or *acrR*. All *soxS* mutations were localized to codon 12 in which a G:C→T:A transversion resulted in alanine (GCA) to serine (TCA) substitution. The three isolates showed elevated *soxS, acrB and emrE* gene transcript levels but subnormal *marA* levels. The cloned soxS protein conferred higher basal resistance to all antibiotics tested.

Conclusion: The newly discovered *soxS* A^{12}-S^{12} mutation appears to play an important role in modulating the function of SoxS with increased expression of AcrB efflux pump resulting in MDR phenotype. To our knowledge, this is the first report to point at a *soxS* mutation as a cause of multidrug resistance.

Stability of Bovine Viral Diarrhea Virus in Beef from Persistently Infected Cattle

Christy L. Bratcher[1], Soren P. Rodning[1], Pat K. Galik[2], Kay P. Riddell[3], M. Shonda Marley[3], YiJing Zhang[2], Leonard N. Bell[4] and M. Daniel Givens[3]

[1]Department of Animal Sciences, College of Agriculture, Auburn University, AL
[2]Animal Health Research, College of Veterinary Medicine, Auburn University, AL
[3]Department of Pathobiology, College of Veterinary Medicine, Auburn University, AL
[4]Department of Poultry Science, College of Agriculture, Auburn University, AL

Introduction. Bovine viral diarrhea virus (BVDV) is a pathogen that causes gastrointestinal, respiratory and reproductive disease in cattle. Based on available research, BVDV is not considered zoonotic. Our research objective was to evaluate the detectable concentration of BVDV in four subprimal cuts of beef from PI animals immediately after slaughter, aging, freezing, and cooking to variable temperatures.

Methods. Six persistently infected beef cattle were humanely euthanized and slaughtered. These six animals were infected with a genotype 1a (n=4), genotype 1b (n=1) or genotype 2 (n=1) strain of BVDV. Five grams of subprimal cuts (chuck, rib, loin, and round) were sampled fresh (Day 0) and after aging for 2, 7, 14, and 21 days. Furthermore, these four subprimal cuts and three ground products (ground chuck, ground round, and ground beef) were prepared from each carcass after two days of aging, frozen, thawed and assayed for BVDV raw or after cooking to 55, 60, 65, 70, 75, and 85 °C. All samples of beef were assayed for detection and titration of infectious BVDV using virus isolation after being minced and blended.

Results. The concentration of BVDV in beef after aging varied significantly depending on the duration of aging ($p = .05$) but not depending on the cut of meat or the animal. The concentration of BVDV in frozen, thawed, uncooked beef varied significantly depending on preparation as a ground product ($p = .01$). The average cell culture infective doses [50% endpoint; $CCID_{50}$]/g of intact meat was $10^{5.85}$ compared to $10^{6.01}$ $CCID_{50}$/g of ground meat. The cut of meat, preparation as a ground product, or animal did not significantly impact detection of BVDV after cooking to any temperature. Results demonstrate that viable BVDV in beef survives aging, freezing, and thawing. To destroy BVDV in beef cuts, internal temperatures must exceed 70 °C during cooking.

Conclusions. This research demonstrates that BVDV maintains viability within beef from persistently infected animals despite aging for up to 21 days, freezing and thawing, and cooking up to 70 °C as the target temperature. These results are consistent with prior research results involving classical swine fever virus, a related pestivirus of swine.

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Comparison of gastric and intra-peritoneal pressures as measures of intra-abdominal pressure in the horse

Amelia S. Munsterman[1], R. Reid Hanson[1]

[1]Department of Clinical Sciences, Auburn University, AL

Introduction. The purpose of this study was to develop an indirect method for measurement of intra-abdominal pressures in the standing horse using measurement of gastric pressures as a less invasive technique, and to compare this method with direct pressures obtained from the peritoneal cavity.

Methods. Gastric pressures were measured in 10 sedated, adult horses using a nasogastric tube with a U-tube manometry technique, while simultaneously measuring intraperitoneal pressures with a peritoneal cannula. Gastric pressures were further assessed using 5 increasing volumes of fluid infused into the stomach (0, 400, 1000, 2000, and 3000 ml). Bias and limits of agreement were determined using Bland Altman analysis and correlation was calculated using Lin's concordance coefficients.

Results. Gastric pressures in all horses were positive, ranging from 0 to 25.8 cm H_2O (mean +/- SD, 14.44+/-4.69 cm H_2O). Intraperitoneal pressure measurements were subatmospheric on average, and ranged from -6.6 cm H_2O to 3.1 cm H_2O (mean +/- SD, -1.59+/-2.09 cm H_2O). Measurements of intraperitoneal pressures were repeatable within and between horses; however, intra-and inter-individual variance was significantly larger for measurements of gastric pressures (95% confidence level). The mean and relative bias for comparison between the two techniques was 15.9 +/- 5.3 cm H_2O and 244.3+/-199.2%, respectively. The Lin's concordance correlation coefficient between gastric and intraperitoneal pressures was -0.003 (P = 0.75).

Conclusions. Due to a lack of statistical correlation between gastric and intraperitoneal pressure measurements, gastric pressures cannot be substituted for intraperitoneal pressures as an indirect method of intra-peritoneal pressure measurement. Direct measurement of intraperitoneal pressures is recommended at this time for comparison of intra-abdominal pressures between horses, due to less variability within and between individuals.

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Veterinary Student Poster Presentations

CD28 Polymorphisms and their Association with IMHA in Dogs

Shelley A. Ash, Marcus Bradbury, Kevin King, Mary K. Boudreaux and Pete W. Christopherson

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

Introduction. Immune-mediated hemolytic anemia (IMHA) is the most common autoimmune disease seen in dogs. The disease occurs when the immune system destroys a patient's red blood cells. Recent studies have focused on T cell activation and its involvement in autoimmune diseases. The CD28 gene encodes the CD28 receptor that is expressed on the surface of most T lymphocytes and is responsible for increasing the efficiency of T cell activation and proliferation. The goal of our study was to determine if polymorphisms in the CD28 gene exist in canine patients diagnosed with IMHA.

Methods. DNA isolated from dogs diagnosed with IMHA was subjected to PCR using primer sets designed around the promoter and coding regions of the CD28 gene. PCR products were separated via electrophoresis on agarose gels, and products were extracted and submitted for sequencing. Sequences were compared to age and breed matched controls and published canine GenBank sequence.

Results. Significant differences in CD28 gene sequences were not detected between IMHA dogs and age and breed matched controls or sequences available on GenBank.

Conclusions. Our results suggest that causes other than polymorphisms in the CD28 gene, such as changes in other T-cell regulatory mechanisms or posttranscriptional alterations of the CD28 gene, may be involved in the pathogenesis of IMHA in dogs.

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Urine Enzymes Gamma-Glutamyltransferase and Alkaline Phosphatase in diagnosis of acute tubular injury in canine renal disease

Cheryl L. Auch, BS, and Elizabeth G. Welles, DVM, PhD, DACVP

Department of Pathobiology, College of Veterinary Medicine, Auburn University

Introduction. Common renal function tests such as serum creatinine (sCr), urea, endogenous creatinine clearance rates and urine specific gravity (USG) have various shortcomings for the diagnosis of acute tubular injury that may be complemented by measurement of urinary enzymes: gamma-glutamyltransferase (GGT) and alkaline phosphatase (ALP). Calculations of ratios of urine enzyme activity to urine creatinine (Cr) concentration adjust enzyme activities for urine concentration and are a more accurate assessment of the enzyme level.

Methods. This retrospective study examined urinary GGT:Cr and ALP:Cr ratios in 459 dogs and compared them against various factors including age, sex/alteration status, urine protein: urine creatinine (UPC) ratios, urine collection method, urine pH, the presence of polyuria/polydipsia (PU/PD), renal failure, bacteruria, squamous or transitional epithelial cells in urine and hematuria. Enzyme stability and effects of storage temperatures were also investigated. Statistical analysis included t-testing, one-way analysis of variance (ANOVA) with Tukey Method testing and correlations.

Results. ALP:Cr ratios were increased in intact male and intact female dogs compared with castrated male and spayed female dogs, GGT:Cr and UPC ratios had a weak correlation, presence of chronic renal failure (CRF) decreased the range of enzyme activity, and hematuria was associated with increased urine enzyme activity. Urine enzymes rapidly degraded after two days when stored at 4ºC and -20ºC. GGT was more stable at -20ºC than ALP. Reference intervals for ALP:Cr and GGT:Cr ratios were established in dogs without evidence of renal disease as (0.00 – 0.95) and (0.00 – 2.07), respectively.

Mass IBV serotype vaccine predominates in chickens simultaneously vaccinated with Mass and Ark serotype vaccines

S. Bartlett and V. van Santen

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

Introduction. Infectious bronchitis virus (IBV) is an RNA coronavirus that causes highly contagious respiratory disease in chickens. This disease has a significant economic impact on commercial egg and broiler production. Because IBV is an RNA virus, it is subject to mutation and recombination resulting in multiple serotypes of the virus world-wide. Current vaccines do not cross-protect against different serotypes. Two commonly used vaccines in the US are Arkansas (Ark) and Massachusetts (Mass), frequently administered together. We previously showed that specific minor subpopulations of live-attenuated Ark-serotype vaccines are selected in chickens.

Methods. We inoculated a group of chicks ocularly with equal doses of Ark and Mass strains of IBV vaccine to determine whether both vaccines would replicate efficiently or if one would outcompete the other. One dose of the Mass vaccine was approximately 80 times the 50% embryo infectious doses (EID_{50}) of the Ark vaccine. We recovered tears from the chicks and isolated RNA from each sample. For each sample of isolated RNA, we amplified a portion of the IBV genome by reverse transcription (RT)-PCR and identified Ark and Mass IBV by restriction fragment length polymorphism (RFLP) analysis. We also performed RT-PCR using Ark-specific primers to amplify any Ark that was present.

Results. Because the Ark vaccine contains minor subpopulations able to more efficiently replicate in chickens, we expected the Ark vaccine to predominate. However, we detected only Mass vaccine genomes in RNA isolated from tears of the vaccinated chickens.

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Evaluation of the Lamin B Receptor Gene in Dogs with Pelger-Huet Anomaly

Marcus Bradbury[1], Barbara Horney[2], Shelley Ash[1], Peter Christopherson[1], and Mary K. Boudreaux[1]

[1]Department of Pathobiology, College of Veterinary Medicine, Auburn University, AL
[2]Department of Pathology and Microbiology, Atlantic Veterinary College,
University of Prince Edward Island

Introduction Pelger-Huet anomaly (PHA) is a trait characterized by ovoid to hypo-segmentation of granulocyte nuclei and modified chromatin distribution. PHA is a benign condition and is not associated with impaired granulocyte function. A linkage between PHA and mutations in the gene encoding the Lamin B receptor (LBR) on chromosome 1 has been reported in humans. Mutations in this gene have also been associated with HEM/Greenberg skeletal dysplasia, in utero death, epilepsy, developmental delay, and other skeletal abnormalities. These findings suggest that mutations in this gene are associated with a broad range of phenotypes ranging from benign to lethal. PHA has been identified in dogs, however, to our knowledge molecular studies have not been performed.

Methods. Our study evaluated the coding sequence for the LBR gene in 15 dogs from 2 PHA families (1 Australian Shepherd family and 1 Basenji family). In the total group of 15 dogs, 2 were normal, 8 were affected and 7 were questionable. DNA was isolated from whole blood samples and subjected to PCR. Primers were designed based on DNA sequence within introns flanking all 13 exons and splice sites encoding the LBR gene. Amplified DNA samples were separated using agarose gel electrophoresis and were harvested for sequencing.

Results. Significant differences in DNA sequences were not been identified in the LBR gene in any of the dogs evaluated.

Conclusions. Our studies suggest that PHA may not be caused by mutations in the gene encoding the LBR protein but may be caused by mutations in a gene in close proximity. This may explain the broad range in phenotypes reported with mutations in the LBR gene.

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Canine regulatory T cells display increased resistance to Gemcitabine depletion than naïve CD4+ T cells

Jeremy Foote Ph.D.[1], Patricia Deinnocentes[1], Bruce Smith Ph.D., VMD[1,2], and R. Curtis Bird Ph.D.[1]

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

Introduction. Invasive breast cancer is the most frequently occurring malignancy in women and canine populations. Both incidence and prevalence of breast cancer are increasing, making development of new strategies for breast cancer treatment, in particular those designed to treat individuals suffering from late state metastatic disease, a priority. Immune modulatory and vaccination strategies capable of boosting patient immunity to breast cancer present promising treatment modalities; however, our ability to surpass self-tolerance, in part enforced by regulatory T cells, remains a critical hurdle. Recent studies using mouse models of human breast cancer provide evidence for the chemotherapeutic, nucleoside analogue gemcitabine in provoking robust humoral and cell mediated immunity to the mammary tumor antigens. Recently utilization of gemcitabine in treatment of canine sarcomas and in our hands as an adjuvant co-administered with autologous CMT-dendritic cell fusion vaccines designed to treat late stage mammary cancer has led us to investigate gemcitabine's mechanism of action on immune suppressor cells. Using an initial pilot study we sought to determine the effect gemcitabine on canine regulatory T cells from healthy laboratory Beagles.

Methods. Peripheral blood from 5 healthy laboratory Beagles before and 7 days after gemcitabine treatment All procedures were IACUC approved and the clinical trial was further approved by the College Committee for Research Involving Client-Owned Animals. Pretreatment blood was sampled and Gencitabine (Gemzar-HCl 200 mg) was diluted in 100 ml injection-grade saline and administered IV by pump over 30 min. Blood was sampled on each of the next 7 days. Peripheral blood mononuclear cells (PBMC's) were harvested using gradient centrifugation. PBMC's were surface stained with anti-canine CD4 followed by fixation, permeabilization and intracellular staining with anti-human Foxp-3 monoclonal antibody. Cells were washed and resuspended for acquisition of 2.5×10^4 cells by Accuri flow cytometry and analyzed with CFlow Plus Accuri software.

Results. Gemcitabine treated animals had a 4 fold reduction in total lymphoid cells (12.24 +/- 8.79% {p = .002}) in comparison to untreated controls (43.84 +/- 12.2%). This effect was primarily mediated by depletion of CD4+ Helper T cells, which displayed a 7-fold reduction in Gemcitabine treated Beagles (3.2 +/- 3.6% {p = .01}) compared to controls (21.9 +/- 10%). Examination of Foxp-3+ CD4+ T cells revealed a 3-fold decrease the total Foxp-3+ CD4+ T cells in gemcitabine treated mice (.89 +/- .7% {p = .014}) compared to control Beagles (2.78 +/- 1.1%), however analysis of Foxp-3 expression in CD4+ T cells from gemcitabine treated Beagles revealed a 2 fold enrichment in Foxp-3 expression (28.8 +/- 7.3% { p = .055}) compared to untreated controls (15.5 +/- 10.8%).

Conclusions. These findings confirm previous studies of gemcitabine mediated depletion of naïve CD4+ T cells established in murine models of mammary cancer and provide evidence that Foxp-3+ regulatory T cells do display sensitivity to gemcitabine-mediated depletion indicating a primary difference between murine and canine immunobiology.

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A Method for Engineering Point Mutations in Mitochondrial DNA

K.M. Fuller[1], M.H. Irwin[2], and C.A. Pinkert[1]

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

Introduction. Conventional trans-genesis with nuclear genes has allowed for the establishment of animal models for disease and production enhancement through the manipulation of expression of specific segments of the genome, however, these advancements have not yet been reproduced within the mitochondrial genome. This project was conducted in order to develop a method that allows for manipulations of mitochondrial DNA (mtDNA) for the purpose of creating animal models of human mtDNA associated diseases and for enhancing the production traits (growth, feed conversion) of food animal species. mtDNA recombination *in vivo* is thought to be rare or, in most cases, non-existent, hampering efforts to modify mitochondrial genes.

Methods. To enhance the potential for recombination of mtDNA, a transgenic mouse over-expressing bacterial recombinase A (*recA*) targeted to mitochondria was produced. Mitochondria were isolated from livers of transgenic (mts-recA) and wild type mice, and were electroporated at varying field strengths with 100ng/□l targeting oligonucleotide (30, 60, or 90mer) with homology to a region of the 16S rRNA mtDNA gene containing an EcoR1 restriction site. Targeting oligonucleotides were constructed to recombine and change this EcoRI site (GAATTC) into an EcoRV site (GATATC). Mitochondria were then encapsulated with liposomes for transfer into cultured 3T3 mouse fibroblasts.

Results/Conclusions. The region of interest was amplified by PRC to yield a 562bp product which was subsequently digested with EcoRI or EcoRV (control = undigested). Digestion of the 562bp product with EcoRI occurs in wt mice; however, recombination in mts-recA mitochondria is demonstrated by a sub-population of EcoRV-sensitive amplification products.

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Dystrophin Deficient Myopathy In The Springer Spaniel

Michelle D. Karagas[1], Caroline Brown[1], Bruce F. Smith V.M.D., PH.D.[1,2]

[1]Scott-Ritchey Research Center
[2]Department Of Pathobiology
College of Veterinary Medicine Auburn University, AL 36849

Introduction. Canine dystrophin deficiency is a degenerative myopathy of both cardiac and skeletal muscle. This canine disease is an excellent model of human Duchenne muscular dystrophy in terms of clinical signs, disease severity and response to therapy. Duchenne muscular dystrophy is a severe recessive X-linked form of muscular dystrophy caused by a mutation in the dystrophin gene. The dystrophin gene is large, containing 13,887 base pairs, and codes for the protein dystrophin. Dystrophin plays an important role in the structural integrity and maintenance of muscle tissue by providing a link between cytoskeleton and extracellular matrix. Absence of the dystrophin protein causes progressive muscle instability, fibrosis, and eventual wasting. The objective of this study is to identify whether dystrophin deficient myopathy occurring in the springer spaniel is due to a mutation within the canine dystrophin gene. Data resulting from this study will be used in experiments evaluating gene therapy treatments in canine models.

Methods. This study was carried out by extracting mRNA from frozen cardiac and skeletal tissue. Reverse transcription polymerase chain reaction (RT-PCR was used to create complementary DNA (cDNA) which was then exponentially amplified. PCR products were detected using agarose gel electrophoresis with DNA dye (gel red) and visualized using ultraviolet light. The significant bands were purified using Fermentas PCR purification kit. The purified DNA was sequenced and compared to the normal canine dystrophin gene sequence.

Results. To date, ten differences from the published canine cDNA sequence have been detected in the springer spaniel cDNA sequence. Four of these are silent mutations that do not change the protein sequence in anyway. Six mutations resulting in changes in the encoded amino acids were detected. Five of these mutations cause conservative amino acid substitution and one caused a non-conservative substitution. Small areas of the cDNA remain to be sequenced completely.

Conclusions. Five mutations have been identified, one of which may be the cause of this disease in the breed. Currently, the cDNA sequencing is being completed. Several previously un-sequenced sections are being addressed. The 5' and 3' prime ends are being sequenced using 5' and 3' RACE (Rapid amplification of cDNA ends). The goal of RACE is to evaluate whether a mutation lies within the 5' region to cause complete loss of the protein. Further study is needed to determine if one of the identified mutations is causative.

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Role of Endotoxemia in the Pathophysiology of *Bovine Viral Diarrhea Virus* Infections

Ashley Ladegast, James L. Sartin, Paul H. Walz, M. Daniel Givens

Dept of Anatomy, Physiology and Pharmacology
College of Veterinary Medicine, Auburn University, AL

Introduction. Bovine viral diarrhea virus (BVDV) and bacterial infections have devastating effects on cattle. We hypothesize that more severe disease results as a consequence of BVDV and subsequent bacterial infection. The objective of this experiment was to determine whether endotoxin administered to BVDV-infected calves alters growth, reproduction and metabolism.

Methods. 24 calves were assigned to treatment groups: 1-control, 2-BVDV infected, 3-endotoxin, 4-BVDV plus endotoxin. On day 0, calves in groups 2 and 4 were oronasally administered a low virulent strain of BVDV. Endotoxin (1µg/kg BW) was intravenously administered to group 3 and 4 calves on day 8. Blood samples were obtained at 10 minute intervals for 8 hours in order to visualize pulse parameters for luteinizing hormone (LH) and growth hormone (GH). Additional blood samples were collected at hourly intervals to measure free fatty acids, glucose, cortisol, insulin-like growth factor-1 [IGF-1], insulin, tumor necrosis factor-α [TNF-α], nitrite/nitrate. Rectal temperature, pulse and respiration were monitored daily in each calf.

Results. Body temperature was elevated in BVDV and endotoxin calves, but dropped in the combined treatment. In the combined treatment, body temperature dropped significantly. There were no effects on free fatty acids. Endotoxin and BVDV increased glucose for the first 2 hours ($P<0.05$) followed by hypoglycemia at 7 hours ($P<0.05$). Responses to endotoxin and BVDV were too severe and the study terminated. The remaining data will be analyzed over the coming months. These data indicate the combination treatments produce more severe responses to the animal which suggests the need for further study.

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An Overview of White Nose Syndrome in Bats

Lisa Mason and Seth Oster

Auburn University, College of Veterinary Medicine

Introduction. White Nose Syndrome (WNS) is an emerging cutaneous fungal disease in bats that causes high mortalities among hibernating bats in the Eastern United States. White filaments of the fungus recently identified as *Geomyces destructans* often obscure the muzzle, but have also been found on the wings (Gargas et al., 2009). The fungus appears to deplete hibernating reserve energy causing high mortality in the hibernating bat colonies as they basically starve to death.

During the winter of 2006-2007, WNS emerged killing over 80% of many colonies of hibernating bats in a small area around Albany, NY. Since then the disease has continued to spread to other hibernating bat colonies along the eastern US and Canada. Most species of bats in the region are susceptible; however, little brown bats (*Myotis lucifugus*), northern long-eared bats (*M. septentrionalis*), and endangered Indiana bats (*M. sodalis*) have the highest prevalence of the disease. Caves have been closed to human traffic to stop the spread of WNS.

Geomyces destructans hyphae penetrate connective tissue of glabrous skin and muzzle of the bats, though there typically is no cellular inflammatory response (Meteyer et al., 2009). Fungal hyphae form cup-like epidermal erosions and ulcers in the wing membrane and pinna with involvement of underlying connective tissue. The hyphae are also present in hair follicles, sebaceous glands, and apocrine glands of the muzzle.

Methods. The fungus has been cultured using cornmeal agar and slab dextrose agar held at a 3 to 14 degrees Celsius for 16 days. No growth has been observed at temperatures above 24 degrees Celsius. Use of periodic acid Schiff stain proved to be useful for histologic examination of the non-pigmented fungus. *G.destructans* has a unique microscopic characteristic due to its curved-shaped conidia.

A rapid PCR for detection of *Geomyces destructans* has been developed (Lorch et al., 2010). Although the test has a high specificity, a diagnosis of WNS as characterized by the National Wildlife Health Center requires clinical signs, positive PCR for *Geomyces destructans*, and confirmation via histopathology.

Results. As of April 20, 2010, 12 states in the US have reported cases of WNS. The disease also has been reported in Ontario and Quebec. Positive bats have also been reported in the White Oak Blowhole Cave, the largest known hibernaculum of the federally endangered Indiana bats in Tennessee. There is evidence of *Geomyces destructans* in sediments from WNS-infected caves indicating the potential for spread of the fungus by mechanical vectors such as humans.

Correlation between external hoof measurements and radiographic parameters of the equine hoof

Hannah Matz-Creel[1], F DeGraves, DVM, PhD[2] , P Ramey[3], I Ramey[3], R Wilhite, PhD[1], D Taylor DVM, MS[1]

[1]Departments of Clinical Sciences and Anatomy and Physiology, Auburn University, College of Veterinary Medicine, Auburn AL
[2] The Ohio State University, Columbus OH, [3]Hoof Rehab LLC, Lakemont, GA

Introduction. Foot pain is a devastating problem of horses often resulting in crippling effects for which there are no effective solutions. It would be ideal if these problems were recognizable even before their effects on the horse were seen. Our hypothesis is that external measurements of the hoof capsule should allow non-invasive prediction of radiographic parameters. The potential for such a correlation between the external measurements of the hoof capsule and the measurements from digital radiographs was investigated.

Methods. Eighteen front cadaver hooves were randomly selected from local necropsy services. The hooves were stored frozen prior to measurement. All hooves were thawed at room temperature prior to data collection. The hooves were cleaned with a standard hoof pick, a necropsy knife and/ or a hemostat. No keratinized hoof material was removed. Excess dirt was removed by washing and scrubbing if needed. Eight different measurements were taken of each hoof by the same person using the same metric measuring devices. The same thawed hooves were marked with barium paste prior to radiography. Lateral and dorso-palmar radiographs were taken of each hoof. Eight radiographic parameters were determined. The data were modeled using linear regression analysis.

Results. Results and potential correlations will be presented.

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Evaluation of the effect of a hoof husbandry system on the gait and hoof of horses with foot pain

Hannah Matz-Creel[1], John Schumacher[1,2], Craig Angle[2], Alex Sperandeo[3], Sherry Johnston[2], Pam Haney[2], Brooke Black[4], Robert Gillette[2], Debra Taylor[1]

[1]Department of Clinical Sciences, College of Veterinary Medicine, Auburn Univ, AL
[2] Animal Health and Performance Program, College of Veterinary Medicine, Auburn Univ, AL
[3] Sperandeo Hoofcare, Cornelia, GA
[4] Equine Sports Massage Therapy and Rehab, Waverly, AL

Introduction: Palmar foot pain continues to be a leading cause of equine lameness. The scientific literature contains little information on methods to change the morphology and overall health of domestic horse hooves. Anecdotal reports of improved gait and hoof health in horses with foot pain after specific hoof husbandry practices are reported. We instituted this study in an effort to further evaluate this practice.

Methods: Horses suffering from foot pain were analyzed by subjective gait analysis, radiographs and computer assisted videographic motion analysis (CAVMA) before and after initiation of a specific hoof husbandry system. The landing pattern of the hoof and the stride length were computer-analyzed for statistical evaluation. Pre- and post- treatment lateral radiographs were obtained for comparison. After initial data collection, a hoof husbandry system was initiated with the following criteria; 1) horses were trimmed to encourage limb extension and heel first hoof impact every 6 weeks, 2) horses were hand walked or ridden at a walk if they exhibited a flat or heel first hoof landing, 3-5 days per week in increasing time intervals wearing flexible rubber hoof boots (exercise was adjusted to the horse's condition and comfort level, they were not exercised when they exhibited a toe first hoof landing) and 3) horses were given access to 5 inch deep pea gravel loafing areas in their environment. Phenylbutazone or asprin were administered when needed for analgesia to establish the desired flat or heel first hoof landing. No phenylbutazone was given for 26 days prior to re-evaluation, no asprin was given 10 days prior to re-evaluation. All data was recollected 5-6 months after onset.

Results: Subjective gait analysis of the horses indicated that horses improved by several lameness grades. Owner satisfaction with horse progress was high. Multiple radiographic parameters are being analyzed for apparent change. Objective gait analysis data has been collected and is being analyzed.

Conclusions: The hoof husbandry system employed elicited a change in radiographic parameters and gait characteristics. More studies are needed to evaluate this hoof husbandry system and its effects on the equine gait and hoof health.

Acknowledgments: Merial, American Hoof Association.

Tumor Suppressor Gene Expression in Canine Mammary Tumor Cell Lines

A. Monday, F.M. Lutful Kabir, P. DeInnocentes and R.C. Bird

Department of Pathobiology, College of Veterinary Medicine, Auburn University, AL 36849

Introduction. Canine mammary cancer is one of the most prevalent cancers in dogs, particularly in unspayed females. Previous studies have shown that defects in tumor suppressor genes are present in many different types of cancer. The major tumor suppressor genes, p16/INK4A, p27, p21 and p53, encode proteins which play critical roles in regulating the cell cycle. The cyclin-dependent kinase inhibitors (CDKI) prevent the cyclin/CDK complex from performing its function and cause the cell to arrest. These tumor suppressor genes have been shown to be abnormally expressed in a few canine mammary tumor (CMT) cell lines. The purpose of this study was to investigate further to determine the prevalence of the expression defects in other independent CMT cell lines.

Methods. Gene expression was analyzed using semi-quantitative rt-PCR using RNA extracted from cultured cells. Expression was compared with normal canine fibroblasts (NCF).

Results. All cell lines showed abnormal expression of at least one tumor suppressor gene.

Conclusions. The results indicate that, similar to human cancers, expression defects of the major tumor suppressor genes are very common in canine mammary cancers. These results support the use of spontaneous canine animal models of human breast cancer.

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Phage-Based Immunocontraceptive Agents to Control Feral Swine Overpopulation

Anita Patel[1], Alexandre Samoylov[1], Anne Cochran[1], Bettina Schemera[3], Nancy Cox[1,2], Valery Petrenko[2], Tatiana Samoylova[1]

Scott-Ritchey Research Center[1], Department of Pathobiology[2], Division of Laboratory Animal Health[3], College of Veterinary Medicine, Auburn University, AL

Introduction: The overpopulation of feral swine is causing extensive problems for U.S. ecosystems, agriculture, and public health. The numbers of feral swine continue to grow despite implemented methods of overpopulation control (e.g. trapping and hunting). In response, we have developed an avenue to control overpopulation via the creation of immunocontraceptive vaccines. These vaccines are based on the use of peptides, which bind to porcine zona pellucida (ZP). These peptides function as antigens, which mimic sperm cells during fertilization. As a result, the peptides can cause production of anti-sperm antibodies and, thus, serve a contraceptive function.

Methods. Peptide antigens capable of binding ZP proteins were identified with the use of phage display technology. The antigens were tested for immune responses in mice and pigs using peptide or phage ELISA. The effectiveness of those peptide- and phage-based antigens that showed considerable immune responses was tested in fertility trials in mice.

Results and conclusions. Our preliminary data in fertility trails in mice demonstrated up to 40% reduction in the number of pups born from vaccinated animals. Mice were used in these experiments due to the large numbers of animals that are needed to adequately discern the effects of the vaccines. Those vaccines that pass the mouse fertility trials will be tested for contraceptive effects in swine. The peptides/phage clones that will be proved to be effective in swine fertility trials will be formulated into oral vaccines and made available for feral swine control.

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Evolutionary Pathways of Infectious Bronchitis Virus in the Immunodeficient Host

Brittany L. Powell[1], Rodrigo Gallardo[2], and Haroldo Toro[2]

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

Introduction. Avian infectious bronchitis virus (IBV), a coronavirus affecting the respiratory and urogenital systems of chickens, has been shown to undergo intraspatial variation in the immunocompetent host. Viral immunodeficiency is common in poultry populations. We hypothesized that IBV follows a distinct evolutionary pathway in the immunodeficient host.

Methods. Based on IBV S1 genetic and phenotypic changes, we analyzed IBV subpopulations in the tear fluids, tracheas, oviducts and testes, and kidneys during serial passages in immunodeficient (inoculated with chicken anemia virus at 7 days old) or immunocompetent chickens.

Results. The vaccine predominant population was rapidly negatively selected in all tissues both in immunocompetent and immunodeficient chickens. The subpopulation selected in immunodeficient hosts differed from that of immunocompetent hosts. In addition, our data showed that the same predominant population was selected in different tissues of chicken anemia virus immunodeficient chickens.

Conclusions.
-IBV shows increased persistence in CAV immunodeficient chickens.
-IBV ArkDPI replicates more effectively in the paraocular tissue than in the trachea, reproductive tract, and kidneys of chickens.
-Distinct IBV phenotypes (population denominated C2) show increased adaptation in CAV immunodeficient chickens.
-Selection of viral population C2 in the immunodeficient host is associated with IBV establishment in the host population.
-IBV intraspatial variation occurred in CAV immunodeficient chickens during the 1st passage.
-After serial passages, the IBV predominant population became established in all tissues.

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The Effects of Joint Type on Surface Roughness of Equine Carpal Cartilage

Rebecca E. Rifkin[1]*, Patrick A. Smyth[2], Robert L. Jackson[2] PhD, R. Reid Hanson[1] DVM, Dipl ACVS, Dipl ACVECC[1]

[1]Department of Clinical Sciences, College of Veterinary Medicine, Auburn University, Auburn, AL. USA
[2]Department of Mechanical Engineering, College of Engineering, Auburn University, Auburn, AL, USA

Introduction: The performance of equine joint surfaces is influenced by the surface roughness of the joint cartilage. Various joint types with different motions, and regimes of lubrication, have altered demands on the articular surface that may affect cartilage surface properties.

Methods: Micro- and nanoscale profilometry was performed on the carpal cartilage harvested from 16 equine forelimbs. Eighty cartilage surface samples from three different functioning joint types (radiocarpal, midcarpal, and carpometacarpal) were measured by a Veeco Dektak 150 Stylus Surface Profilometer.

Results: The average micrometer surface roughness measurements were different for each joint. The average surface roughness of the radiocarpal cartilage was 1.803 micrometers (SEM 0.086). The average surface roughness of the midcarpal cartilage was 2.245 micrometers (SEM 0.181), and the average surface roughness of the carpometacarpal cartilage was 1.568 micrometers (SEM 0.123).
Comparison of midcarpal to carpometacarpal cartilage showed a statistical difference in surface roughness (P= 0.002). Comparison of the radiocarpal to carpometacarpal cartilage did not show a statistically significant difference in surface roughness (P=0.119).

Conclusions: Knowledge of cartilage micro- and nanoscale roughness will assist the future development, and design of treatments, for intra- articular substances or surfaces to preserve joint integrity and reduce limitations or loss of joint performance.

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Investigation of the sparing effects of a native medicinal plant (American skullcap, *Scutellaria lateriflora*) on aflatoxin-contaminated feed in broiler chickens

[1]JS Suddeth, [1]M Lohani, [1]BT Akingbemi, [1]EG Welles, [1]J Wright, [1]F Hoerr, [1]KS Joiner, [1]FW van Ginkel, [2]JB Hess, [2]WD Berry, [2]A Similien, [2]DA Shannon, [3]GE Rottinghaus, [3]DR Ledoux, and [1]BW Kemppainen

Colleges of [1]Veterinary Medicine and [2]Agriculture, Auburn University, AL
Colleges of [3]Veterinary Medicine and [4]Agriculture, University of Missouri, Columbia, MO.

Introduction
Poultry are very sensitive to the toxic effects (poor performance, immunosuppression, and liver disease characterized by diffuse fatty changes) caused by chronic consumption of feed contaminated with low levels of aflatoxin (AF), a naturally occurring fungal toxin. Skullcap's flavonoids (bioactive compounds) have potent anti-oxidant and anti-inflammatory actions that oppose oxidative damage and inflammation caused by AF. The objective of this research was to determine if skullcap reduces damage caused by AF fed to chickens.

Methods. Three treatment groups of chickens were given feed amended with a range of doses of dried skullcap (50, 250, and 1250 mg/kg body weight, BW) for six weeks; 3 treatment groups consumed feed amended with the same doses of skullcap for one week, followed by five weeks of feed amended with the same doses of skullcap and 1.4 PPM aflatoxin. Control groups received basal diet and basal diet amended with AF.

Results. Only the highest dose of skullcap (1250 mg/kg BW) was associated with a toxic effect [lower ($p \leq 0.05$) body weight after both 14 days and 35 days of exposure]. Fourteen days exposure to AF resulted in minimal liver damage (plasma level of liver specific enzyme glutamate dehydrogenase, GLDH, 3.8 ± 0.9 IU/L, n=12). Thirty five days of exposure to AF resulted in liver necrosis (GLDH 12 ± 9.8; > 10 suggests liver necrosis) that was not observed in chicks consuming AF and the highest dose of skullcap (4.2 ± 0.73). Additionally, the highest dose of skullcap partially protected chickens from the AF-induced increase in liver weight (g liver/100 g BW); and as the dose of skullcap increased, there was a linear ($R^2=0.85$) decrease in the AF-induced fatty liver. Exposure to AF coincided with a significant ($p \leq 0.05$) drop in CD4+ T cells in the spleen.

Conclusions. The highest dose of skullcap was associated with the adverse effect of lower body weight and beneficial effect of partially protecting chickens from AF-induced liver damage. The protective effect of skullcap on AF-induced liver damage could be due in part to skullcap's flavonoids decreasing AF metabolism to its toxic form by the liver (Ueng et al., Biochemical Pharmacology, 62:1653 – 1660, 2001). The decrease in T helper cells caused by AF is consistent with known AF suppression of cell-medicated, and to a lesser degree, humoral immunity.

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Diagnosing Feline Sandhoff Disease Using PCR and Gel Electrophoresis

Suzanne D. Truesdell[1,2], Nancy E. Morrison[1], Doug R. Martin DVM, Ph.D.[1]

[1]Auburn University College of Veterinary Medicine Scott-Ritchey Research Center
[2]Department of Anatomy, Physiology and Pharmacology, Auburn University, AL

Introduction. Sandhoff disease, also known as G_{M2} gangliosidosis variant 0, is an inherited storage disease caused by an accumulation of complex lipids mainly in nervous tissue due to defects in the lysosomal enzyme β-hexosaminidase which breaks down G_{M2} ganglioside. Seemingly healthy babies show decreased motor skills and neurological signs around 3 to 5 months of age, then disease progression increases dramatically after 8 to 10 months. Most patients do not live longer than 5 years. Cats are serving as the model to study the disease and work toward the development of a treatment or cure. Domestic cats are a better model than mice because of their larger, more complex brain and their genetic similarities to humans.

Methods. This project exploited the unique nature of the feline Sandhoff Disease mutation to create a PCR-based, single-step diagnostic assay. Current methods of genotyping are labor-intensive, error prone and expensive, and the PCR that went under development greatly enhanced the diagnostic capabilities (and therefore research efficiency) for the GM2 breeding colony.

Results. This project successfully differentiated between the three feline Sandhoff Disease genotypes with the use of PCR and primers E1387c, INV, and MUT/L and gel electrophoresis.

Conclusions. There is still some work to do to confidently differentiate between carrier and normal genotypes. Future work could include the following: adjust the PCR reagents to get better specificity and a cleaner band pattern, primer redesign would reduce the amount of primer dimers that form, and splitting up the primers to run them side by side by mixing just two of the three primers would identify any specific problems with individual primers.

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Assessing Relative Adrenal Insufficiency and ACTH Stimulation in Ill Horses

H.L. Weaver, BS[1]; A.J. Stewart BVSc(hons), MS, DACVIM, DACVECC[1]; E. Hackett, DVM, MS, DACVS, DACVECC[3]; B.A. Johnson, BS[1]; T.J. Towns, BS[1]; A. Munsterman, MS DACVS, DACVECC[1]; A.A. Wooldridge, DVM, PhD, DACVIM[1]; L.E. Kivett, DVM[1]; J. Estrada, DVM[1]; R. Funk, DVM[1]; C.A. Hewes, MS, DVM[1]; E.S. Groover, DVM, DACVIM[1]; C. Alford, MS, DVM[1]; R.R. Hanson, MS, DACVS, DACVECC[1]; E. Kwessi, BS, MS, PhD[2], Q. Zhong BS[2]

[1] Dept. of Clinical Sciences, and
[2]Dept. of Mathematics and Statistics, Auburn University, Auburn, AL
[3]Dept. of Clinical Sciences, Colorado State University

Introduction: Factors involved with illness and inflammation stimulate the hypothalamic pituitary adrenal (HPA) resulting in an increase in ACTH and cortisol secretion. 30-45% of critically ill humans and 42-51% of septic neonatal foals have inadequately low endogenous cortisol concentrations and a disproportional ACTH:cortisol ratio, indicating dysfunction of the HPA axis which is termed RAI (relative adrenal insufficiency) and results in increased morbidity and mortality. Treatment with physiologic doses of glucocorticoids in humans has increased survival rates by 30%. Little research has been done on the HPA axis in adult horses and it is not known if RAI exists in this population.

Methods: Critically ill medical and surgical colic patients were assigned a severity of illness score (mild, moderate or severe) based on clinicopathologic findings. Endogenous serum cortisol and plasma ACTH concentrations were measured at T=0. Cosyntropin (0.1μg/kg IV) was injected and cortisol concentrations were again measured at T=30 minutes on days 1, 2, 4, and 6. Concentrations of ACTH and cortisol were measured by iodide labeled radioimmunoassays. The presence of RAI was determined by Δ cortisol concentrations <159nmol/mL or endogenous cortisol concentrations <269nmol/mL. Statistical analysis was performed with ANOVA; $P < 0.05$ considered significant.

Results: 10 horses were categorized as mild, 30 as moderate, and 17 as severely ill. 14 of the severely ill horses (82%) were diagnosed with RAI, and 8 did not survive.

Conclusion: 25% of horses were diagnosed with RAI, 14% did not survive. Non-survivors and survivors diagnosed with RAI may benefit from physiologic low dose hydrocortisone treatment.

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Graduate Student Poster Presentations

Canine Mammary Tumor Cells: A Model to Investigate Cyclin Dependent Kinase Inhibitor p16/INK4A in Cell Cycle Arrest

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

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

Introduction: Cancer is the result of accumulating genetic/epigenetic changes/mutations in proto-oncogenes and tumor suppressor genes causing loss of control of cell cycle. Tumor suppressor genes encode proteins that suppress cell growth and most frequently result in exit from the cell cycle. One of the most important tumor suppressor genes, p16/INK4A/CDKN2A, arrests cell cycle in early G1 phase and inhibits binding of CDK4/6 with cyclin D1, which leaves the Rb tumor suppressor protein 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 and in quiescent cells.

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

Results: Both NCF and CMT28 express p16 mRNA, while only NCF expresses p16 protein. We have successfully demonstrated cell cycle arrest and synchronous cell cycle re-entry in CMT12, CMT27, CMT28, CMT27A, CMT27H, CMT28A, CMT28F, and normal canine fibroblasts. Cell cycle arrest and synchronous cell cycle re-entry was further confirmed by ^{3}H-thymidine incorporation and flow cytometry. Expression of p16 mRNA was enhanced as each cell line entered the quiescent phase.

Conclusion: All cell lines have demonstrated cell cycle arrest in response to serum-starvation. p16 is accumulated at the quiescent stage, which suggests a 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 and differentiation.

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β-defensin Expression in the Canine Nasal Cavity

Michelle Aono[1], Jishu Shi[2], John Dennis[1], and Edward Morrison[1]

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

Introduction. Detector dogs are exposed to many environments during the course of their working lifetimes. This leads to an increased exposure to harmful or infectious agents, making it highly desirable to select and train dogs with strong immune systems. The innate immune system includes small cationic peptides, called defensins. *In vitro*, this family of peptides has been shown to exhibit broad-spectrum activity against bacteria, viruses and fungi and to modulate immune responses. *In vivo*, a decreased expression level of defensins correlates with an increased rate of infection in atopic dermatitis. Since the olfactory ability of the detector dog is its most valuable asset, β-defensin expression in the canine nasal cavity was selected as a possible marker for immune system robustness. A three part hypothesis was posed: β-defensins are expressed in the canine nasal cavity; there is a β-defensin which is specific to olfactory tissue; and like humans, dogs have heterogeneous expression levels of β-defensins.

Methods. Canine nasal tissues were collected *post mortem* from 13 dogs at the alar fold, maxiloturbinate, ethmoid labyrinth, vomeronasal organ and olfactory bulb. RNA was extracted using 100 mg of each tissue in Trizol (Invitrogen) and treated with DNAfree (Ambion) to remove DNA contamination.

RTPCR primers were designed to detect canine β-defensin 1, 2, 3, 102, 103, olfactory marker protein and GADPH. Optimal annealing temperature and cycle number were determined empirically for each primer pair. RTPCR products were sequenced to ensure the specificity of the primers and all products were run on a 2% agarose gel and stained with ethidium bromide.

Results. β-defensin 103 RNA was strongly expressed in the rostral portion of the canine nasal cavity. β-defensin 103 was not co-expressed in tissues with olfactory marker protein. The general expression pattern of β-defensin 103 RNA was fairly consistent for all 13 dogs but there was variation in tissue specific expression levels between dogs. Canine β-defensin 1, 2, 3, 102 were not expressed in the tissues samples analyzed.

Conclusions. Of the five defensins we analyzed, β-defensin 103 is highly expressed in the rostral portion of the canine nasal cavity. This "tip of the nose" expression may ensure that β-defensin 103 is localized to the area of highest pathogenic exposure. However, β-defensin 103 is not specifically expressed in olfactory sensory epithelia. The variation of defensin expression between individual dogs bears investigation into how this may correlate to the dogs ability to ward off infection and disease.

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Development and Evaluation of a FRET-PCR Assay for Determining Fluoroquinolone Resistance in Canine Urine *Escherichia col*i Isolates

Megan G. Behringer[1], Kamoltip Thungrat[1], Bashar W. Shaheen[1], and Dawn M. Boothe[1]

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

Background: Antimicrobial resistance in *Escherichia coli* is an increasing concern in both human and veterinary hospitals' patients. The choice drug for treatment in dogs is enrofloxacin, a second generation fluoroquinolone (FQ) whose activity reflects, in part, ciprofloxacin. Among the difficulties in effective *E. coli* treatment is rapid detection of FQ resistance. The purpose of this study was to determine the specificity and sensitivity of a FRET based assay for the rapid detection of urinary tract infections caused by FQ associated multi-drug resistant *E.coli*.

Methods: 306 clinical canine urine *E. coli* isolates were subjected to susceptibility testing for 14 drugs representing 6 drug classes. Isolates were designated NDR (no drug resistance), SDR (single drug resistance) and MDR (multi-drug resistant) (n=101 MDR, 116 SDR and 89 NDR). Minimum inhibitory concentration (MIC) for enrofloxacin ranged from 0.03 µg/ml to 512 µg/ml, with high MIC generally associated with MDR. Extracted DNA was subjected to FRET-PCR targeting single nucleotide polymorphisms in *gyrA*. Further, to determine the level of detection, microbial free canine urine was inoculated with 106 to 101 CFU/ml of 7 isolates characterized by variable susceptibility to enrofloxacin (MIC_{Enro}=0.03, 0.06, 0.15, 1, 64, 128, 256 µg/ml).

Results: Of 306 isolates, 50 were confirmed positive for enrofloxacin resistance (MIC_{Enro} >4 µg/ml), 43 of which were positively identified by the FRET-PCR assay giving a sensitivity of 86.00%. Only 1 isolate that was resistant was not detected (specificity of 96.66%). However, of the isolates expressing high level resistance (MIC > 8 X breakpoint [64 mcg/ml]), and MDR (n=34), sensitivity = 97.06% , colony dilutions of *E. coli* confirmed the assay able to detect enrofloxacin resistance at as low as 101 CFU/ml. The relationship between CFUs and the peak of the –(d/dt) Fluorescence of the melting curve was R2 =0.988.

Conclusions: These results confirm that the assay designed provides the specificity and sensitivity to accurately predict antimicrobial resistance in clinical *E. coli* isolates.

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Microbiological and molecular characterization of coagulase positive *Staphylococcus* species isolated from canine clinical specimens

Dubraska V. Diaz-Campos[1]; Terry Hathcock[1], Roberto A. Palomares-Naveda[2] Kenny Brock[1]

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

Introduction. Multidrug-resistant *staphylococcus* species in dogs have become a serious challenge in veterinary medicine. The staphylococci most commonly associated with infections in canine are the *Staphylococcus pseudintermedius* (SP), previously recognized as *S. intermedius* (both members of the *S. intermedius* group), *S. schleiferi* subspecies *coagulans* (SC) and *S. aureus* (SA).

Methods. To better understand the magnitude of this problem, a total of 324 *Staphylococcus* sp from canine clinical specimens were analyzed based on the following objectives: 1) characterize canine S*taphylococcus* sp using conventional biochemical tests and molecular techniques, 2) identify epidemiological risk factors and 3) distinguish the most appropriated diagnostic method for recognition of methicillin-resistance (MR). Isolates were characterized by Polymerase Chain Reaction-Restriction Fragment Length Polymorphism (RFLP), latex agglutination for penicillin-binding protein 2a (PBP2a), agar disk diffusion for oxacillin (OX) and cefoxitin (FOX) antimicrobial susceptibility testing, and multiplex PCR for Scc*mecA* typing and detection of Panton-Valentin leucocidin (PVL) genes.

Results. Thirty-nine percent (125/324) of the isolates were MRSP, 9% (28/324) MRSC and 9% (30/324) MRSA. Fifty-one percent (151/297) of the isolates were collected from skin and 34 % (99/294) from dogs ≥ 9 years old. PVL genes were found in 13% (4/30) of the MRSA, all possessing the type IV Scc*mecA* cassette. The type V cassette was present in 66% (113/171) of the isolates, followed by type IV with 13% (22/171). Interestingly, 11% (18/171) of the MRSC were Scc*mecA* type V. The sensitivity and specificity of OX for the detection of MR strains was 83% and 93%, respectively, but for FOX the sensitivity was 20% and the susceptibility 100%.

Conclusions. To our knowledge this is the first time that SCC*mecA* Type V has been reported in MRSC. Our findings confirm that cefoxitin is not appropriate for the detection of MR in canine staphylococci and recommend the use of PCR or the PBP2a latex agglutination test instead.

Allotopic expression of ATP6 in the mouse as a targeted mtDNA mutation model

David A. Dunn[1] and Carl A. Pinkert[1]

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

Introduction. Animal modeling of mitochondrial DNA (mtDNA) mutations has trailed nuclear transgenesis due to a host of cellular and physiological distinctions. mtDNA mutation modeling is of critical importance as mutations in the mitochondrial genome give rise to many pathological conditions. A T to G mutation on nucleotide 8993 of the human mitochondrial genome results in either Neurogenic muscle weakness, Ataxia, and Retinitis Pigmentosa (NARP) or Maternally Inherited Leigh Syndrome (MILS) phenotypes.

Methods. A study was undertaken to develop a mutation model where the mtDNA 8993 mutation was engineered for expression from the cell nucleus. Nuclear localization and transcription of mtDNA genes followed by cytoplasmic translation and transport into mitochondria (allotopic expression) provide an opportunity to create *in vivo* modeling of a targeted mutation in mitochondrial genes. Two murine ATP6 genes were synthesized *de novo* and cloned into a mammalian expression vector. The A6M gene vector coded for the T8993G mutation and the A6W gene vector encoded the wild-type ATP6 protein sequence. Both DNA constructs contained nuclear codon substitutions and a Cox VIII N-terminal mitochondrial transport signal. Transgenic mice generated using these constructs were subjected to a battery of five neuromuscular tasks (wire hang, pole, balance beam, gait analysis, and Rota-Rod testing) and four biochemical measurements (serum lactate and mitochondrial MnSOD protein concentrations, ATP synthesis and O_2 consumption).

Results. Compared to non-transgenic controls, A6M mice displayed neuromuscular/motor deficiencies in wire hang, pole, and balance beam analyses ($P<0.05$), no differences in gait analyses, and enhanced function in Rota-Rod evaluations ($P<0.05$). A6W mice exhibited superior performance in balance beam time-to-cross and constant velocity Rota-Rod analyses ($P<0.05$), inferior performance in the wire hang test ($P<0.05$) and no difference in balance beam footslips, accelerating Rota-Rod, pole test and gait analyses ($P>0.05$) when compared to non-transgenic control mice. Mice of both transgenic lines did not differ from non-transgenic controls in a number of bioenergetic and biochemical tests including serum lactate and mitochondrial MnSOD protein concentrations, ATP synthesis rate, and O_2 consumption ($P>0.05$).

Conclusions. The generalized modest neuromotor impairment seen in A6M transgenic mice mirrors a comparable phenotype observed in human NARP patients. Potentially conflicting functional evidence (increased Rota-Rod performance) together with the surprising lack of a biochemical phenotype provide opportunity for further characterization of the pathways leading from mtDNA mutation, to functional deficit, and eventual disease states. Allotopic expression of a mitochondrial gene *in vivo* supplies a resource for studying mechanisms of pathogenesis in diseases resulting from mitochondrial DNA mutation. This study also provides evidence for the potential utility of allotopic expression as a strategy for gene replacement therapy in patients harboring mitochondrial DNA mutations.

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Adaptive Immunity in Conjunctiva Associated Lymphoid Tissue after Ocular Immunization

Stephen L. Gulley[1], Haroldo Toro[1], Aart Lammers[2], Rucha Gurjar[1], Frederik W. van Ginkel[1]

[1]Department of Pathobiology, College of Veterinary Medicine, Auburn University, Auburn, AL 36849
[2]Adaptation Physiology Group, Department of Animal Sciences, Wageningen Institute of Animal Sciences, Wageningen University, Marijkeweg 40, PG 6709, Wageningen, The Netherlands.

Introduction: Conjunctiva-associated lymphoid tissues (CALT) along with the Harderian glands constitute the chicken's main para-ocular lymphoid system. These tissues are the first to be exposed to pathogens entering by the ocular or nasal routes, provide adaptive immunity and, consequently, influence avian disease resistance. Although considerable knowledge has been accumulated regarding the role of Harderian glands in ocular immunity, the role of CALT in generating antigen-specific ocular immune responses has not been well defined. It is assumed that CALT, like Harderian glands, have a sentinel role protecting mucosal surfaces of the eye.

Methods: To test whether CALT plays an important role in generating protective ocular immunity, chickens were ocularly immunized with a human, replication-deficient adenovirus vector of serotype 5 (Ad5). The T cell composition of CALT was analyzed by fluorescence-activated cell sorting (FACS) using commercially available antibodies (Southern Biotechnology, Birmingham, AL). IFN-γ levels in tears were measured by ELISA (Invitrogen, Carlsbad , CA). Expression of the polymeric immunoglobulin receptor (pIgR) was measured by RT-PCR and immuohistochemistry. CALT was analyzed by H&E stain and by immunohistochemistry using IgA- and IgG-specific antibodies (Southern Biotechnology, Birmingham, AL).

Results: Approximately 37% of the CALT lymphocytes were $CD3^+$ of these $CD3^+$ lymphocytes about half of them were CD4 positive and ~20% CD8 positive. Thus, both T helper and cytotoxic T cells are present in CALT, which increased in numbers after immunization and can drive humoral and cell mediated immune responses after ocular Ad5 immunization. The induction of Ad5-specific T cell-mediated ocular immunity was further indicated by elevated levels of IFN-□ in tears. The B cell ELISPOT assay demonstrated that ocular immunization with Ad5 resulted in increased production of Ad5-specific IgA antibody secreting B cells in CALT. In order for B cell responses to be effective in neutralizing viruses at mucosal surfaces, transport of IgA across the epithelium by the pIgR would be required. Our RT-PCR and immunohistochemistry results indicated that the pIgR were expressed on CALT epithelial cells allowing transport of polymeric IgA.

Conclusions: Thus, CALT are important mucosal inductive sites producing antigen-specific IgA antibodies and contain increasing numbers of cytotoxic T cells and T helper type cells after ocular Ad5 immunization which may contribute to elevated levels of IFN-γ in tears after ocular Ad5 immunization. The CALT epithelium expressed the pIgR allowing active transport of IgA across the epithelium. The prevalence of IgA producing cells in CALT combined with the presence of pIgR demonstrates that CALT contribute to mucosal immune protection of the eye.

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Rapid amplification of cDNA ends (RACE) experimental approach for sequence analysis of canine p16/INK4a tumor suppressor

Farruk M. L. Kabir[1], Payal Agarwal[1], Patricia DeInnocentes[1], Maninder Sandey[1, 2], and R. Curtis Bird[1]

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

Introduction: Endogenous tumor suppressors are important components for elucidation of the regulatory mechanisms of many mammalian tumors. p16 or INK4a, a cyclin-dependent kinase (CDK) inhibitor, is one of the key regulators in cell cycle G1/S phase transition and cell proliferation through the retinoblastoma (Rb) pathway. It has been reported that p16/INK4a is differentially expressed in several canine mammary tumor cell (CMT) lines suggesting that defects in this tumor suppressor promote transformation of canine tumors. Though a partial sequence has been obtained from previous studies, the exact or complete sequence of canine p16/INK4a has not yet been revealed. p16 is a peculiar cell cycle regulatory gene since it shares its reading frames with another tumor suppressor gene called p14ARF. RACE is widely used to amplify and clone full-length cDNA when only a partial cDNA sequence is available. The objective of this study was to design an experiment applying RACE to sequence the full length transcript of canine p16.

Methods: Initially two canine cell lines, CMT28 and normal canine fibroblast (NCF), were grown in culture medium and total RNA was isolated from cells by phenol-chloroform extraction. Designing of the gene specific primers was a critical step since the sequence of p16 is highly repetitive and GC-rich. The primers were designed by Vector NTI and Primer-Blast tools. The RACE reactions began with the trimming of the 5'-end of primary RNAs obtained from cells, removing the cap structure or free 5'-phophates and adding an RNA adapter to 5'-end (5'RACE). In the case of 3'RACE, the first step involved reverse transcription with the 3'RACE adapter. The subsequent steps were reverse transcription of processed RNA to make cDNA, nested PCRs using the gene specific and RACE primers and gel analysis of the products. The specific RACE products were identified and sliced from the gel and cloned into the TOPO TA cloning vector. After cloning, only cloned vectors that contained DNA inserts were isolated from bacterial culture, identified by gel analysis and then DNA sequencing.

Results: From 5' RACE, a 250-300 bp product was found after nested (inner RACE) PCR for both cell lines. 3'RACE produced similar products but also with weak multiple bands on the gel probably due to some nonspecific amplifications. Successful clones were observed on the gel as distinct bands (~300 bp) and identity of amplicons were confirmed by DNA sequencing.

Conclusions: NCBI BLASTing of the nucleotide sequences obtained, showed surprisingly different sequence information suggesting that redesigning of primer sets and optimization of the RACE reactions might be required to reach the correct sequence information for canine p16. Indeed, newly designed primers showed larger specific amplicon from RT-PCR compared to previously designed primers and this might be helpful to optimize the RACE and to obtain full length sequence of the gene.

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Immunomodulatory Properties of Noni (*Morinda citrifolia*)

Madhukar Lohani[1], Barbara W Kemppainen[1], Brandon Moore[2], Denzil V Maurice[3], Joe E Toler[4], Rucha Karve[2] and Thomas R Scott[3]

[1]Department of Anatomy, Physiology and Pharmacology, Auburn University, Auburn, AL
[2]Department of Genetics and Biochemistry, Clemson University, Clemson, SC
[3]Department of Animal and Veterinary Sciences, Clemson University, Clemson, SC
[4]Department of Applied Statistics and Economics, Clemson University, Clemson, SC

Introduction. Noni (*Morinda citrifolia*) is a popular medicinal plant of family Rubiaceae. Its fruit is rich in various phytochemicals and polysaccharides. Anecdotal evidence indicates that it is used in folk medicine to promote health and prevent disease. Limited *in vitro* studies also support the immunomodulatory role of Noni, however only two studies used an animal model. The purpose of this study was to examine the immunomodulatory properties of Noni fruit in chickens.

Methods. In two experiments, different concentrations of dietary Noni were fed to day-old broiler chickens for 3 to 6 weeks. Gut tissue and blood were sampled to determine the expression of selected genes, concentrations of immunoglobulins and α1-acid glycoprotein using qRT-PCR, ELISA and α1-acid glycoprotein measurement kit assay, respectively.

Results. Dietary Noni at 6% increased the expression of Toll-like receptor (TLR)-4 and TLR-5; interleukins (IL)-8 & IL-12, and decreased the expression of IL-6 and TLR-7. At 4%, dietary Noni stimulated the expression of TLR-3. Increased expression of TLR-3, TLR-4 and TLR-5 indicate antiviral and antibacterial activities in chickens fed Noni fruit that are further enhanced by increased expression of IL-12 and IL-8. Decreased expression of IL-6 indicates anti-inflammatory inducing properties of Noni. However, dietary Noni did not have effects on serum and gut immunoglobulins or α1-acid glycoprotein concentrations.

Conclusion. This study showed that dietary Noni has immunomodulatory properties in chickens via modulation of gene expression in gut tissue.

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Transmission of IBV Ark serotype type vaccine viral subpopulations to non-vaccinated contact birds

Eunice Ndegwa and Vicky van Santen

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

Introduction. There has been high incidence of isolation of IBV Ark serotype from commercial flocks in the southeastern U.S. in spite of intensive vaccination programs. Among the factors that may lead to this scenario is presence and consequent selection of residual virulent subpopulations in attenuated vaccines as has been shown for mildly pathogenic avian influenza (H5N2), live attenuated turkey rhinotracheitis and live attenuated infectious bursal disease vaccines. Selected and persisting vaccine viruses may also result in increased virulence through mutation and/or recombination. We previously reported that minor viral subpopulations within commercial avian infectious bronchitis (IBV)-Ark serotype vaccine were positively selected in chickens while others are negatively selected over time. We hypothesize that IBV Ark-vaccine subpopulations more readily transmitted to non-vaccinated contact chickens may be more fit than those that are not transmitted. These subpopulations may be better at inducing immunity or may have ability to persist in the flock with possible unwanted consequences.

Methods. We inoculated duplicate groups of five fourteen day-old SPF chickens ocularly with 1.6 X 10^3, 3.2 X 10^4, or 3.2 X 10^5 EID_{50} IBV-Ark vaccine to simulate the field vaccine dose variability during mass administration. Three days post vaccination (DPV), we introduced five non-vaccinated birds to each of the isolators with vaccinated chickens. We collected tears from each vaccinated chicken 3, 6, and 8 DPV, and tracheal swabs at 8 DPV and also tears at 6 and 12 days post exposure (DPE) and tracheal swabs at 12 DPE from each non-vaccinated contact bird. The sequence of a portion of the spike (S) gene of the IBV present in each sample was determined to identify the subpopulation(s) present both in vaccinated and contact birds.

Results. We observed that different vaccine subpopulations were selected depending on the dose of vaccine applied. Results suggest that not all viral subpopulations present in vaccinated birds are capable of being transmitted to contact non-vaccinated birds. In particular, the major vaccine viral subpopulation and another minor subpopulation detected in the low-dose vaccinated group were not detected in any of the contact birds in the same groups. Two specific minor vaccine subpopulations were more frequently detected in contact birds than any of the other vaccine subpopulations irrespective of the vaccine dose group.

Conclusions. Selection of vaccine viruses in vaccinated chickens depends on vaccine dose and only a few of the selected viruses are transmitted to non vaccinated contact birds, suggesting differences in fitness of selected vaccine viruses. Ability for some vaccine viruses to be transmitted to contact birds but not others may reflect variability in fitness of these viruses to replicate in the upper respiratory tissues of the chicken and/or sensitivity to host's innate immune response. Further research on the significance of transmitted viruses may offer information on designing of more safer and effective IBV vaccines.

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Determining the functional status of canine IL-24 receptors

Maninder Sandey[1,2], R. Curtis Bird[1] and Bruce F. Smith[1,2]

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

Introduction: Interleukin-10, 19, 20, 24 and 26 are a group of cytokines that belong to IL-10 family of class II cytokines. Most of these cytokines are involved in regulation of inflammatory and immune responses. However, human MDA-7/IL-24 has potent antitumor, immunomodulation, antiangiogenesis and anti-invasiveness properties. IL-24 also has a potent bystander apoptosis-inducing effect on adjacent tumor cells through class II cytokine receptors. Class II cytokine receptors consist of classical transmembrane-receptor proteins that heterodimerize to form a functional receptor. There are two alternative receptor complexes for IL-24 composed of three different receptor chains. Type I receptor complexes consists of IL-20 R-alpha /IL-20 R-beta, while type II is composed of IL-22 R-alpha/IL-20 R-beta receptor chains. Binding of IL-24 to these receptor complexes results in activation of the JAK-STAT pathway. Preliminary findings suggested that the human MDA-7/IL-24 did not show bystander apoptosis-inducing activity on canine cancer cell lines. Thus, we decided to investigate the functional status of these two receptor complexes in dogs.

Methods: The predicted mRNA sequences for canine IL-20 R-alpha and IL-22 R-alpha receptor chains were obtained from the NCBI sequence database. Primer sets were designed to amplify these receptors chains by reverse transcriptase-polymerase chain reaction. The sequence for canine IL-20 R-beta was predicted by using softwares and primer sets were designed to amplify this predicted sequence. Rapid amplification of cDNA ends (RACE) was used to identify the full length mRNA sequence of canine IL-20 R-beta. Expression of IL-20 R-alpha was confirmed by western blot analysis. Immunocytochemistry studies were performed to localize the expression of IL-20 R-alpha in HACAT (Human keratinocyte cell line) and canine keratinocytes.

Results: PCR-amplified canine IL-20 R-alpha and IL-20 R-beta ORFs had 70.6% and 76.1% similarity to their respective human orthologs. We also detected the expression of canine IL-20 R-alpha protein (~65KDa) by western blot analysis. Human IL-20 R-alpha protein was found in both membrane and in cytoplasm of HACAT cell lines by IFAT. However, expression of the canine IL-20 R-alpha protein was not detected at cell membrane of canine keratinocytes. We predicted an mRNA sequence of 730bps for canine IL-20 R-beta. We identified an mRNA sequence of 828bp for canine IL-20 R-beta. When translated, canine IL-20beta protein is made up of 235 amino acids compared to 311 in human IL-20 R-beta. The extracellular domain of canine and human IL-20 R-beta has 80.4% similarity. However, canine IL-20 R-beta does not have transmembrane and cytoplasmic domains.

Conclusions: Our studies have confirmed that canine IL-20 R-alpha (mRNA and protein), IL-20 R-beta and IL-22 R-alpha (mRNA) are expressed in canine keratinocytes. Although, canine IL-20 R-alpha protein was detected in the cytoplasm of canine keratinocytes, it is not expressed at the surface. Canine IL-20 R-beta protein contains only the extracellular domain. No amino acid sequences representing transmembrane and intracellular domains were detected.

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Trends of small animal *Escherichia coli* antimicrobial resistance in the United States

Kamoltip Thungrat[1], Jameson Sofge[1], David M Carpenter[2], and Dawn M Boothe[1]

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

Introduction. No national surveillance system exists for monitoring emergent resistance in companion animals. However, *E. coli* resistance is an increasing therapeutic and public health concern in these animals. The purpose of this study was to describe current resistance patterns of canine and feline pathogenic *E. coli* throughout the United States and identify risk factors of antimicrobial resistance.

Methods. Isolates (n=1512) of clinical *E. coli* collected from dogs or cats from May 2008 through May 2010 located in 6 different regions. Susceptibility was determined to 15 drugs (6 drug classes) by broth microdilution methods. Pharmacodyamaic statistics were described regionally. Phenotypes were determined and type of resistance was based on the number of drug classes to which resistance was expressed: none (NDR), single (SDR) and multi (MDR). To identify risk factors of antimicrobial resistance, information will be collected from veterinarians regarding their antimicrobial preferences.

Results. The majority of isolates were from urinary tract (71.5%) and dogs (75.7%). The proportion of resistance type for each drug was: NDR (17.5%), SDR (56.4%) and MDR (26.12%). The proportion of MDR was greatest in the Southwest (25.29%) and least in the Northwest (9.19%) (*P<0.05*). For all regions, the proportion of resistance was: cephalothin (CPH, 65.2%) > amoxicillin-clavulanic acid (AMX, 59.4%), ampicillin (AMP, 52.9%), tricarcillin-clavulanic acid (TCX, 21.8%), doxycyline (DXY, 15.9%) > cefoxitin (CFX, 15.2%), cefpodoxime (CPX, 13.7%), chloramphenicol (CHP, 13.2%), enrofloxacin (ENR, 13.0%) , ciprofloxacin (CIF,12.3%), trimethoprim-sulfamethoxazole (TMX, 10.7%), ceftazidime(CFZ, 10.0%), gentamicin (GTM, 10.0%), cefotaxime (CFT, 9.2%) > meropenem (1.1%) (*P<0.05)*. The MIC_{90} exceeded the resistant breakpoint for AMP, AMX, CPX, CPH, CIF, CFX, DXY and ENR whereas MIC_{50} did not surpass the susceptible breakpoint. Beta-lactams (96.37%) was the most and aminoglycosides the least (0.12%) SDR. The drug class most frequently involved in MDR was beta-lactams (97.72%) and least, GEN (30.13%). Multivariate analysis identified female [odds ratio (OR) 1.035] and age (3-5 years) (OR 1.432) as associated with *E. coli* MDR infection.

Conclusions. Resistance differs regionally, being greatest in the Southwest. CPH is the most and meropenam is the drug least associated with resistance; these patterns are consistent with current drugs used by veterinarians.

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Post-graduate/Faculty Poster Presentations

Identification of Brest Cancer Receptors Recognized by Landscape Phage Probes

Natalia Grabchenko, Olusegun Fagbohun, Deepa Bedi, Valery Petrenko

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

Introduction. Biopanning of phage displayed peptide libraries on intact cancer cells allowed to develop a new generation of tumor-targeting agents. Discovery of counterpart receptors for the targeting phage ligands may help in elucidation of mechanisms of cancer cell targeting and development of novel diagnostics and advanced targeted cancer medicines. The aim of this work was to identify surface cellular receptors for a group of landscape phages selected previously against breast cancer cells MCF-7.

Methods. Breast cancer-selective phages with guest peptides DMPGTVLP, or DWRGDSMDS on the pVIII major coat have been used as ligands in affinity matrixes for isolation of cell surface receptors. The breast cancer membrane receptors captured by the phage-based affinity matrixes were eluted and separated by SDS-PAGE. Their structures were identified by nano-liquid chromatography tandem mass spectrometry (Nano-LC MS/MS). Specific interaction of the identified receptors with phage was demonstrated in phage competition assay with polyclonal affinity-purified antibody.

Results. Nucleolin was identified as a receptor for phages displaying peptides DMPGTVLP and DWRGDSMDS.

Conclusion. Identification of nucleolin as a receptor for selected phages allows proposing a new strategy for their use as tumor-targeting agents and enhance efficacy of already approved cancer nanomedicines.

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An NDUFS4 mutation and neurobehavioral changes in young mice

Kodeeswaran Parameshwaran[1], Michael H. Irwin[1], Kosta Steliou[2] and Carl A. Pinkert[1]

[1]Department of Pathobiology, College of Veterinary Medicine, Auburn University, AL
[2]Cancer Research Center, Boston University School of Medicine, Boston, MA

Introduction. Mitochondrial dysfunction is associated with age dependent declines in neurological function. Severe neurodegenerative diseases such as Alzheimer's and Parkinson's disease are characterized by pathophysiology in which mitochondrial dysfunction plays a critical role. Complex I of the mitochondrial respiratory chain is a major determinant of respiratory efficacy and a recent mouse model harboring a complex I NDUFS4 gene mutation resulted in both decreased Complex I activity and increased lactate concentration in brain samples. Studies were undertaken to analyze whether these metabolic deficits in the brain were discernible in terms of selected neurobehavioral tasks. In addition, therapeutic efficacy of an antioxidant, PMX-500F (PhenoMatrix, Boston MA), was also tested.

Methods. Two month old control and NDUFS4 heterozygous mutant mice were injected with either vehicle (0.1 M TRIS) or PMX-500F (23.8 mg/kg) daily for two months. Mice were then subjected to three neurobehavioral tests (Rota-Rod, wire hang, and Y maze). One week after the completion of all behavioral tests, mice were anesthetized and hippocampus, cortex, and cerebellum were obtained under approved IACUC protocols. Levels of reactive oxygen species and lipid peroxidation were then measured.

Results. NDUFS4 mutants did not exhibit differences from controls in terms of limb strength, motor coordination and spatial memory as assessed by the three analyses ($P>0.05$). Lipid peroxide and ROS levels were similar in mutant and control mice in all three brain regions tested ($P>0.05$). Additionally, administration of PMX-500F did not influence tested parameters ($P>0.05$). In the Rota-Rod test, control mice demonstrated a stronger improvement in performance on the third day of testing ($P<0.01$) compared to PMX-500F treated mutants ($P<0.05$) suggesting a decline in motor learning in the mutants when compared to controls.

Conclusions. Young NDUFS4 mutants do not show neurobehavioral defects or oxidative stress in the brain despite a possible decline in complex I activity. Due to heterozygosity of genotype coupled with endogenous compensatory mechanisms, we hypothesize that an increase in metabolic throughput occurs downstream of complex I activity; influenced by alterations in mitochondrial abundance and subcellular trafficking.

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Highly Localized Infections With Ark-type IBV Vaccines

Vicky L. van Santen and Eunice N. Ndegwa

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

Introduction. Infectious bronchitis virus (IBV) is a coronavirus of chickens that has economic consequences in the poultry and egg industry worldwide. In commercial broilers, it is controlled by vaccination with live-attenuated virus strains of various serotypes. The viral spike (S) protein is responsible for attachment to host cells and also determines the serotype. In the southeastern United States, IBV with S genes similar to Arkansas (Ark) serotype are the most frequently isolated, in spite of intensive Ark-IBV vaccination programs. We previously found that minor viral subpopulations within a commercial IBV-Ark vaccine were positively selected in chickens, and that distinct subpopulations were frequently found in the trachea and tears of a single chicken. These subpopulations were identified by distinct S sequences, differing from the major attenuated vaccine population in four to eight amino acid positions. We further explored the dynamics of selection of specific vaccine subpopulations in vaccinated chickens.

Methods. We inoculated groups of 10 14 day-old SPF chickens ocularly with 1.6×10^3, 3.2×10^4, or 3.2×10^5 EID_{50} IBV-Ark vaccine divided between two eyes. These doses corresponded to 2, 40, and 400 vaccine doses as stated on the vaccine vial. We collected tears from each eye of each chicken 3, 6, and 8 days post inoculation (DPI), and tracheal swabs at 8 DPI. The sequence of a portion of the S gene of the IBV present in each sample was determined in order to identify the subpopulation(s) present.

Results. We found that in chickens inoculated with low or intermediate vaccine doses, distinct vaccine subpopulations were frequently found in the two eyes of the same chicken. In addition, we observed that the vaccine subpopulations selected differed depending on the dose of vaccine applied. Certain vaccine subpopulations were most frequent in chickens vaccinated with low doses and were more rapidly eliminated from chickens in all dosage groups. One specific vaccine subpopulation was strikingly more frequent in chickens vaccinated with the highest dose, and increased in frequency with time in the other dosage groups.

Conclusions. Results suggest that IBV present in tears collected from separate eyes of individual chickens resulted from independent selection events, and that infections with IBV-Ark vaccine virus can remain highly localized. Restriction of specific subpopulations to the site of their selection is likely due to innate immune responses at each inoculation site. The relationship of subpopulations selected to vaccine dose might be explained by differences among subpopulations in both relative abundance in the vaccine and relative fitness in chickens. Thus, specific subpopulations of higher abundance, but lower fitness, would be selected in chickens inoculated with the lowest dose. In chickens inoculated with higher doses, those more abundant subpopulations would be out-competed by specific rarer, apparently more fit subpopulations. Consequently, the outcome of vaccination with Ark-IBV vaccine might be highly variable, depending on the dose received by individual chickens, which is difficult to control in commercial operations.

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